Pork quality traits and associated muscle metabolic changes in pigs under chronic prenatal and postnatal heat stress

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Lay Summary

Pig thermal environment and especially chronic heat stress (HS) can affect animal growth and physiology, with potential impacts on carcass and pork quality. The effects of chronic HS during postnatal growth (GHS) on pork quality are controversial, but the effects of chronic HS during the pig prenatal period (PHS) or of combined PHS and GHS on carcass and meat traits are even less characterized. However, a better understanding of HS consequences on pork quality is of high economic importance for the pork sector, as the frequency and severity of heat waves are likely to increase with global warming. This research focused on the effects of PHS and GHS on pork carcass and meat quality from various muscles and primary cuts (loin, ham), and the underlying muscle biochemical properties. Prenatal HS did not affect growth and carcass traits. Compared with pigs grown in a thermoneutral environment (GTN), GHS pigs had reduced gain resulting from lower feed intake and had lighter carcasses, but similar carcass lean meat content. PHS had little effects on pork quality, whereas GHS induced specific metabolic effects in the loin and ham muscles, leading to higher meat pH and water holding capacity and thereby improved pork technological quality.

Teaser text

- Chronic heat stress in growing pigs induced muscle-specific metabolic responses, leading to improved pork technological quality in the loin and ham.
- Prenatal heat stress had much less impact on carcass composition, muscle metabolism and pork quality.

Abstract

Chronic heat stress (**HS**) is a major concern affecting pig growth performance and metabolism, with potential consequences on carcass and meat quality traits. The objective of this study was to assess the influence of prenatal (**PE**) and growing (**GE**) thermal environments, and their combination, on muscle metabolism, carcass characteristics, and pork quality. From 6 to 109 d of gestation, 12 sows (1 per block) were kept under thermoneutral (TN) conditions (cyclic 18 to 24 °C; **PTN**) and 12 sows under chronic HS (cyclic 28 to 34 °C; **PHS**). Two female offspring per sow were selected based on body weight at weaning, for a total of 48 female pigs (12 blocks of 2 sisters from each PE) and one sister was placed in each GE. Gilts were housed from 82 to 140 d of age under cyclic **GTN** (18 to 24 °C; n = 24) or **GHS** (28 to 34 °C; n = 24) environments. Data were analyzed using a mixed model including PE, GE and PE × GE interaction as main effects, and sire, sow within PE, pen within PE × GE, and slaughter day (for plasma, muscle and meat traits) as random effects. No significant PE × GE interaction was found on any trait under study ($P \geq 0.05$). Prenatal HS did not affect growth performance and carcass traits ($P \geq 0.05$). Compared with GTN, GHS pigs had lower average daily feed intake, average daily gain and hot carcass weight ($P < 0.01$), but similar carcass lean meat content ($P \geq 0.05$). Prenatal HS hard scarce effects on pork quality, with only higher a^* and C^* values ($P < 0.05$) in the Gluteus superficialis. Growing HS led to higher pH 24 h ($P < 0.05$) in the Longissimus thoracis et lumborum **(LTL)** and ham muscles, and higher meat quality index in the ham muscles. In contrast, quality traits of the Semispinalis capitis (**SC**) were not affected by either PE or GE ($P > 0.05$). Except a tendency for a higher citrate synthase activity in the SC ($P = 0.065$), PHS did not affect muscle metabolism. Growing HS induced muscle-specific metabolic responses, with reduced glycolytic potential ($P < 0.01$) and metabolic enzyme activities ($P < 0.05$) in the

glycolytic LTL, but not in the oxidative SC ($P > 0.05$). Plasma glucose content at slaughter was lower in the GHS compared with GTN pigs ($P = 0.002$), indicating an altered energy metabolism in pigs under GHS. Altogether, growing HS altered growth without affecting carcass traits, but improved technological quality of loin and ham. Prenatal HS, alone or combined with GHS, had limited or even no effect on carcass and pork quality.

Keywords: prenatal heat stress; chronic heat stress; carcass traits; muscle composition; energy metabolism; technological quality

List of abbreviations

BW	body weight
CS	citrate synthase
G2	backfat thickness measured with lean fat sensor device
GE	growing environment
GHS	growing heat stress
GM	Gluteus medius
GP	glycolytic potential
GS	Gluteus surperficialis
GTN	growing thermoneutral
HAD	beta-hydroxy-acyl-CoA dehydrogenase
HS	heat stress
IMF	Intramuscular fat
LDH	lactate dehydrogenase
LTL	Longissimus thoracis et lumborum
M2	muscle thickness measured with lean fat sensor device
p.m.	post mortem
PE	prenatal environment
PHS	prenatal heat stress
PTN	prenatal thermoneutral
RH	relative humidity
SC	Semispinalis capitis
SM	Semimembanosus
TN	thermoneutral
WHC	water holding capacity

Introduction

Chronic heat stress (**HS,** lasting for several weeks) is a major environmental factor compromising pork production efficiency as it impairs reproductive performance of sows and productive performance of growing pigs by reducing growth rate. This loss of performance is mainly due to reduced feed intake as an adaptive response to limit metabolic heat production, but also to specific metabolic adaptations (reviews by Rhoads et al., 2013; Ross et al., 2015; Liu et al., 2022). In addition, HS can alter carcass composition and value by increasing fat and/or decreasing lean deposition, even though this effect is not systematic. The severity of the impact depends on the intensity and duration of HS, feeding management (ad-libitum or restricted) and the physiological stage the of pigs (prenatal, or postnatal growing or finishing phases) (Boddicker et al., 2014; Johnson et al., 2015; Ma et al., 2019; Liu et al., 2022). Subsequent changes in carcass composition result from the decreased feed intake combined with metabolic changes. Pigs experiencing HS have reduced energy metabolism resulting from lower thyroid hormone activity and altered insulin circulation, all indicating disturbed endocrine homeostasis (Rinaldo and Le Dividich, 1991; reviews by Ross et al., 2015; Gonzalez-Rivas et al., 2020). Because skeletal muscle with different metabolic properties exhibit different responses to thermal growing environment (hot, 28°C vs cold, 12°C, Lefaucheur et al., 1991; cold, 12°C vs thermoneutral (**TN**), 23°C, Faure et al., 2013) one can hypothesize that HS could affect muscle metabolism and, thereby, pork quality traits in a muscle-type dependent manner. Indeed, increased glycogen stores and glycolytic metabolism were observed in the glycolytic Longissimus muscle whereas increased oxidative metabolism was observed in the oxidative Semispinalis capitis muscle of pigs exposed to cold vs hot or TN environments (Lefaucheur et al., 1991; Faure et al., 2013).

Effects of HS on homeostasis and metabolism in growing pigs suggest potential consequences on muscle traits and thereby meat quality. However, the reality of these consequences is somewhat controversial. Reduced muscle glycogen stores in growing pigs subjected to chronic growing HS is often reported (Gonzalez-Rivas et al., 2020). All pig pre-slaughter fasting, handling (loading/unloading and transport) and slaughtering conditions (including stunning method: electrical or CO_2, carcass chilling…) that markedly affect the rate and extent of post mortem (**p.m.**) muscle pH decline (reviewed by Lebret and Čandek-Potokar, 2022a) being equal, a lower muscle glycogen resulting from HS during pig rearing may lead to higher meat ultimate pH (i.e., 24 h p.m.). This was observed in some studies (Rinaldo and Mourot, 1991; Ma et al., 2019; Pardo et al., 2022). In contrast, others reported no significant differences in ultimate pH of pork from pigs under HS (Witte et al., 2000; Liu et al., 2021; Mun et al., 2022), whereas some report a lower ultimate pH in the loin muscle of pigs submitted to growing HS (Yang et al., 2014). The consequences of chronic growing HS on water-holding capacity and color are also controversial, with either improvement (Rinaldo and Mourot, 1991; Pardo et al., 2022), lack of effect (Witte et al., 2000; Ma et al., 2019; Mun et al., 2022) or impairment (i.e., greater lightness; Yang et al., 2014) reported. Potential effects of prenatal HS on subsequent pork quality have been less studied. Similar pH 24 h, drip loss and color coordinates were found in slaughter pigs raised in TN conditions but submitted to HS vs TN environment during their prenatal period (Tuell et al., 2021), however this remains to be confirmed. Moreover, whether in utero HS interacts with postnatal chronic thermal stress to alter muscle properties and subsequent meat quality has yet to be documented. Thus, the effects of prenatal or postnatal HS and/or their combination on muscle metabolic changes and subsequent meat quality traits need to be clarified. These relationships will not only improve knowledge on

pork quality development and its determining factors, but will help inform future production decisions, as more frequent and intense heat waves are highly likely to occur due to global warming (IPCC, 2021).

Consequences of prenatal or growing chronic heat stress, and of their interactive effects on pig growth performance, carcass composition and physiological responses were recently assessed by Serviento et al. (2020). They observed decreased average daily feed intake, daily weight gain and final body weight, and increased backfat thickness (when assessed at similar carcass weight) in pigs submitted to postnatal HS, the increased backfat thickness being exacerbated when pigs were also submitted to prenatal HS. They also observed an altered thyroid function following growing HS, especially in pigs submitted to prenatal HS (Serviento et al., 2020). Therefore, the objective of this study based on the same animal experiment as Serviento et al. (2020) was to evaluate the effects of prenatal and postnatal HS on the metabolic properties in two muscles of contrasting metabolic types, as well as carcass composition and meat quality in loin and ham.

Material and methods

Institutional animal care and use and authorization for animal experiment

The experiment was performed in the INRAE experimental facilities (UE3P, 35590 Saint-Gilles, France doi.org/10.15454/1.5573932732039927E12) in compliance with European directive 2010/63/EU for animal experiments and French legislation. The technical and scientific staffs had individual accreditation from the French Minister to experiment on living animals. The methods for animal experiment were approved by the local Committee on Ethics in animal experimentation and the present animal experimentation was authorized by the French Ministry

of Higher Education, Research and Innovation (APAFiS #11016-2017080718212019).

Experimental design

The experimental design, already presented in detail by Serviento et al. (2020), is based on a 2 × 2 factorial design, with two prenatal (**PE**) and two growing (**GE**) thermal environments. In brief, the experiment involved a total of 48 female crossbred pigs, issued from 24 litters of Large White × Landrace sows (12 primiparous and 12 multiparous, i.e., 12 blocks of two primiparous full-sisters, or of two multiparous half-sisters) inseminated with semen from four Pietrain NN boars (non-carriers of the hal mutation at the RYR1 gene). Sows were distributed in two batches (corresponding to two prenatal treatments) balanced for parity and litter origin, and for the multiparous sows, for body weight (**BW**) and backfat thickness at weaning of their respective litters. Sows batches were placed at the same time in one of two identical rooms from day 6 to day 109 of their pregnancy corresponding to two prenatal environments : one HS room with chronic cyclic thermal challenge (diurnal variation from 28 to 34°C from 06:00 to 18:00, daily average relative humidity (**RH**) of 34.1 %; **PHS** batch) and one TN room (18 to 24°C from 06:00 to 18:00, RH of 50.7 %; **PTN** batch). On day 110 of gestation, sows were moved in individual farrowing crates of two similar farrowing rooms (balancing for prenatal temperature batch) and maintained in constant TN conditions of 25°C until weaning (d 28 of lactation). At weaning, in each of the 24 litters, 10 littermate piglets (three entire males, four castrated males, and three females) with BW closest to the average BW of each prenatal batch, were selected and placed in the same post-weaning pen (one litter per pen). The 24 litters were allocated to similar two rooms (each including six PTN and six PHS litters) that were maintained in constant TN conditions (27°C) throughout the six weeks of post-weaning.

At 70 d of age, 48 females i.e., two females from each PTN and PHS litters were randomly selected and allotted within litter to one of two similar growing rooms with two different growing thermal environments: thermoneutral (**GTN**) or heat-stress (**GHS**). Each room was equipped with 8 pens (2 × 2 m) designed for housing three pigs each. To balance out the experimental design, the pigs were blocked according to litter origin so that in one pen, all pigs were half-sisters (same sire). Moreover, each pig of one pen in the GTN room has a full sister in the corresponding pen of the GHS room. After five d of adaptation, all pigs were kept under cyclic GTN conditions (18 to 24°C, RH of 76.0 %) from 75 to 81 d of age. From 82 d of age, temperature in the GHS room was gradually changed at a rate of 1°C/hour from 06:00 to 18:00 and was thereafter maintained in a cyclic HS conditions of 28 to 34°C until 140 d of age (RH of 61.1 %). The GTN room was maintained in cyclic GTN conditions from 82 to 140 d of age. Thus, combining PE and GE there were four experimental treatments: PTN-GTN, PTN-GHS, PHS-GTN, and PHS-GHS.

Growth performance

Throughout the growing period, all pigs received *ad libitum* a standard growing-finishing diet (16.3% crude protein; 2,495 kcal net energy /kg) and had free access to water. Pigs were weighed individually at 70 d (distribution in GTN and GHS rooms), 82 d (start of the experiment), and on the day before slaughter. Feed consumption was measured daily (feed offered minus refusals) for each pen during the experimental period. Average daily feed intake and feed efficiency (G:F ratio) were calculated per pen and average daily gain was calculated per pig over the experimental period (82-140 d).

Slaughter and carcass measurements

Pigs were slaughtered at the INRAE experimental slaughterhouse (UE3P, 35590 Saint-Gilles, France), at 140 ± 0.7 d of age on two slaughtering days (two days apart). In each experimental treatment, the three pigs from two pens were slaughtered on each slaughtering day (i.e., 24 pigs per slaughtering day). All pigs were fasted for 24 h before slaughter. The day before slaughter, pigs from the same experimental treatment were mixed (leading to four groups of six pigs) loaded onto a lorry, transported together to the slaughterhouse (five min) and kept in lairage in the same pen without mixing them with the other groups. All pigs had free access to water. The next day, pigs were taken alternately from each group and slaughtered by electrical head-stunning (320 V, 6 A, 10 s duration) followed by jugular exsanguination in compliance with the current national regulations applied in slaughterhouses.

Just after slaughter, the hot carcass (trimmed of digestive, reproductive and respiratory tracts and perirenal fat) was weighed. Backfat (**G2**) and muscle (**M2**) depths were measured on one dorsal spot between the third and fourth last ribs at 6 cm of the spinal canal axis, using the Lean Fat Sensor (Capteur Gras Maigre) device (Fives Syleps, Lorient, France). Carcass lean meat content was calculated using the G2 and M2 measurements according to the equation developed by Daumas et al. (2010): Lean meat content (%) = 62.19 − 0.729 × G2 + 0.144 × M2.

Tissue sampling and measurements of meat quality traits

Blood samples were collected at exsanguination in 10 ml heparinized tubes, centrifuged immediately (2500 × g; 10 min; 4°C) and plasma stored at −20°C (for a maximum of 4 mo.) before analyses. Plasma concentrations of glucose (Glucose HK, Thermo Fisher Scientific Oy, Vantaa, Finland) and lactate (ABX Pentra lactic acid, Horiba, Montpellier, France), as well as

creatine kinase activity (CK IFCC, Thermo Fisher Scientific), were determined using a spectro-photometric analyzer (Konelab20, Thermo Scientific).

Thirty minutes after slaughter, samples of Longissimus thoracis et lumborum (**LTL**) muscle (last rib level) and Semispinalis capitis (**SC**) muscle (neck level) were taken from the carcass's right side, cut into small pieces and immediately frozen in liquid nitrogen. Samples were stored at -76°C until determination of glycolytic potential, metabolic enzyme activities, and pH at 30 min p.m. The latter was assessed with a pH meter equipped with a dedicated electrode (Ingold Xerolyte electrode, Metrohm pH-meter, Berlin, Germany) after homogenization of 2 g of muscle in 18 mL of 5 mM sodium iodoacetate as described previously (Lebret et al., 2018).

Twenty-four hours after slaughter, on the right carcass side, pH was measured directly in the LTL (between 13^{th} and 14^{th} ribs), in the SC, and in the ham muscles, namely Semimembranosus (**SM**), Gluteus medius (**GM**), Gluteus superficialis (**GS**) and Adductor, using the same apparatus as above and with an automatic temperature compensation.

The same day, a transversal section of LTL muscle (second to last ribs, 3.0 cm depth) was taken and oxygenated for 15 min at 4°C under artificial light before measurement of color coordinates CIE L^*: lightness, a^*: redness, b^*: yellowness, C^*: saturation (chroma) and $h°$: hue (average values of 3 different determinations) using a chromameter Minolta CR 400 (Osaka, Japan) with a D65 illuminant, a 1-cm diameter aperture and a 2° observer angle.

Then, the LTL slice was trimmed of external fat, minced and homogenized. A sub-sample was freeze dried and powdered before determination of protein and water contents. The remaining part was vacuum packaged and stored at -20°C before determination of intramuscular fat (**IMF**) content. Another transversal section of LTL muscle (100 $\pm$ 10 g) was taken consecutively (cranial side) to determine drip loss at 3 days *post mortem* (**p.m.**) (plastic bag method; Lebret et

al., 2018).

The SC muscle was removed from the right carcass side, transversally cut and the fresh muscle surface used for determination of color coordinates as described above. Then, the SC muscle was trimmed of external fat, minced and homogenized, and divided in two fractions prepared as described for LTL, before determination of protein, water, and IMF contents.

On the right ham of each carcass, the day after slaughter, color coordinates were also measured at one site of the GM and GS muscles, as described above. The GM water-holding capacity (**WHC**) was assessed from the time needed to moistening a calibrated piece of filter paper when put on the freshly cut muscle surface, as described previously (Lebret et al., 2018). This assessment allowed for calculation of the meat quality index, which is a predictor of the technological yield of cooked ham (Tribout and Bidanel, 2000), using the following equation: meat quality index = − 41 + 11.01 × [pH 24 h SM] − 0.231 × [*L** GM] + 0.105 × [WHC GM].

Muscle biochemical composition and metabolic enzyme activities

Biochemical composition (protein, water and IMF proportions, and glycolytic potential) of LTL and SC muscles was determined as previously described (Lebret et al., 2018). Protein [= 6.25 × nitrogen] and water contents were determined from freeze-dried samples and expressed as percentage of fresh muscle considering water loss of each muscle sample during freeze-drying. IMF content was determined on LTL and SC samples kept at -20°C by chloroform-methanol (2:1 v/v) extraction. Glycolytic potential, defined as **GP** = 2 × [(glycogen) + (glucose) + (glucose-6-phosphate)] + [lactate] and expressed as µmole equivalent lactate/g of fresh tissue, was determined in LTL and SC muscles samples taken 30 min p.m.

Metabolic enzyme activities were assessed on LTL and SC muscle samples taken 30 min p.m. Activities of lactate dehydrogenase (**LDH**), citrate synthase (**CS**) and β-hydroxy-acyl-CoA dehydrogenase (**HAD**) were determined as markers of glycolytic metabolism, oxidative capacity (tricarboxylic acid cycle) and lipid β-oxidation potential, respectively, as detailed by Lefaucheur and Lebret (2020). Briefly, approximately 100 mg of muscle powder (obtained from muscle pulverization at -70 °C) was thawed in ice-chilled 0.1 M phosphate buffer containing 2 mM EDTA, and homogenized 10 s in ice. The homogenate was ultrasonicated (1 min, in ice) and centrifuged (1500 × g; 10 min; 4°C). The supernatant, which contained soluble enzymes and mitochondrial material was kept on ice and used on the same day to measure the activities of LDH, CS and HAD at 30°C using the same automatic spectrophotometric analyzer as described for plasma analyses. Enzyme activities were expressed as micromoles of substrate degraded per minute and per gram of fresh tissue.

Statistics

Statistical Analysis System software (version 9.4; SAS Institute, Cary, NC, USA) was used to analyze the data. Except for average daily feed intake and G:F ratio for which the pen was the experimental unit (Serviento et al., 2020), the pig was considered as the statistical unit for all traits. Data of live weight, growth rate and carcass traits were analyzed using a PROC MIXED model with PE, GE and their interaction (PE × GE) as fixed effects, and sire, sow (within PE) and pen (within PE × GE) as random effects. Data of plasma parameters, meat quality traits, muscle biochemical and metabolic traits were analyzed using a PROC MIXED model with PE, GE and their interaction (PE × GE) as fixed effects, and sire, sow (within PE) and pen (within PE × GE) and slaughter day as random effects. For each trait, the normality of distribution of

residues from the MIXED model was checked. When necessary, data were log-transformed to reach a normal distribution of residues from the MIXED model (i.e., plasma lactate and creatine kinase). Effects were considered as significant for $P < 0.05$ and as a trend for $P < 0.10$. Least-square means were calculated and are presented in Tables according to PE (PTN and PHS) and GE (GTN and GHS), because no significant PE × GE interaction was found ($P \geq 0.05$).

Results

Growth performance and carcass traits

Effects of prenatal and growing thermal environments on growth performance were already reported by Serviento et al. (2020). Briefly, growth performance was influenced by GE but not PE and there was no significant interaction between PE and GE on these traits (Table 1). Compared to GTN, GHS pigs had a lower average daily gain ($P = 0.002$). This was driven by lower average daily feed intake in GHS compared to GTN pigs (2.24 vs 2.64 kg/d, $P < 0.01$) whereas G:F ratio was not affected by GE (0.44 and 0.43 for GHS and GTN pigs, respectively $P > 0.10$; Serviento et al., 2020). Therefore, GHS pigs had lower final BW and hot carcass weight than GTN pigs ($P < 0.01$). Carcass dressing percentage was not significantly affected by PE or GE (Serviento et al., 2020). Backfat and muscle thickness, as well as lean meat content, were not significantly affected by PE, GE or by PE × GE interaction ($P > 0.05$).

Plasma parameters

Plasma glucose and lactate concentrations were modified according to PE or GE, without significant PE × GE interaction (Table 2). Compared to PTN, PHS pigs tended to have lower ($P = 0.075$) lactate, whereas plasma glucose concentration did not differ. GHS pigs had a lower glucose ($P < 0.01$) but similar lactate concentration compared to GTN pigs. Activity of creatine

kinase in plasma was not modified by PE or GE thermal treatments.

Meat quality traits

Some quality traits determined in the LTL were affected ($P < 0.05$) by GE while PE had no significant effects, with the exception of drip loss which tended ($P < 0.10$) to have a PE × GE interaction (Table 3). The pH 30 min p.m., an indicator of the rate of p.m. pH decline, was not affected by GE, whereas the pH 24 h p.m. was higher in the LTL of GHS compared with GTN pigs (average of + 0.06 pH unit, $P < 0.05$). PTN-GHS pigs tended to have reduced ($P = 0.088$) drip loss compared to PTN-GTN pigs (2.62 and 4.03 %, respectively), with PHS-GHS and PHS-GTN pigs being intermediate. Growing HS affected some color traits of the LTL. Compared with GTN, GHS pigs had lower a^* coordinate value ($P < 0.05$) leading to a trend for a lower C^* ($P = 0.061$) whereas b^*, $h°$ (hue angle) and L^* (lightness) did not differ.

Most of the quality traits determined in the ham muscles were influenced by GE ($P < 0.05$) and much less by PE, whereas no significant PE × GE interaction was found (Table 4). As found in the LTL, pH 24 h was higher ($P < 0.05$) in the GM, GS, SM and Adductor muscles of the GHS compared with GTN pigs with, on average, + 0.08 up to + 0.18 pH unit. Meat color was affected by GE, with lower ($P < 0.05$) L^* and trends ($P \leq 0.081$) for lower b^* and C^* values but similar a^* and $h°$ values found in the GM of GHS compared with GTN pigs. GHS reduced GS a*, b* and C* compared to GTN pigs ($P < 0.05$), while L^* and $h°$ did not differ. WHC assessed in the GM was higher for GHS compared with GTN pigs ($P < 0.01$). This result, combined with the lower L^* in the GM and pH 24 h in the SM, led to a higher value of the meat quality index (+ 2.5 points) for GHS compared with GTN pigs ($P < 0.01$). PTN pigs had higher ($P < 0.05$) GS a^* and C^* values compared to PHS pigs, while L^*, b^* and $h°$ did not differ. GM b* tended to be lower

(P = 0.089) in PTN-GHS pigs compared to PTN-GTN pigs (7.37 vs. 8.83), with PHS-GHS and PTN-GHS pigs being intermediate.

In the SC muscle, none of the quality traits (pH 30 min and 24 h p.m., color coordinates) were influenced by PE, GE or PE × GE (P > 0.10) (Table 5).

Muscle biochemical composition and metabolic enzyme activities

Biochemical composition and metabolic enzyme activities of the LTL muscle were influenced by GE (P < 0.05), but not by PE or PE × GE interaction effects (Table 6). GHS pigs had higher water (+ 0.7 percentage point, P < 0.05) and lower protein contents (- 0.6 percentage point, P < 0.05) than GTN pigs, whereas IMF content did not differ. Glycolytic potential was reduced in the LTL of GHS compared with GTN pigs (average of - 23 µmole equivalent lactate/g, P < 0.01) mainly due to lower glucose issued from glycogen hydrolysis (P = 0.070) and lower lactate (P = 0.099) contents in GHS pigs. The LDL and CS activities were decreased in the LTL of GHS compared with GTN pigs (P < 0.05) whereas HAD activity did not differ.

Similar to the LTL, the composition of the SC muscle was affected only by GE, with higher water (+ 1.1 percentage point, P < 0.01) and lower protein (- 0.5 percentage point, P = 0.055) but similar IMF content for the GHS compared with GTN pigs (Table 7). In contrast with LTL, GP and its components did not differ in the SC of GHS compared with GTN pigs. Activities of enzymes of energy metabolism were not affected by GE in the SC, but CS activity tended to be higher in PHS compared with PTN pigs (P = 0.065), whereas LDH and HAD activities did not differ.

Discussion

The present study aimed at evaluating the effects of chronic, cyclic prenatal and growing heat stress on meat quality traits in loin and ham and on chemical and metabolic traits of two muscle metabolic types, and whether combined prenatal and growing HS could affect muscle and meat traits. In the present study, pre- and postnatal heat stress, either alone or combined, altered growth performance, metabolic responses, and thyroid function (Serviento et al., 2020). These changes have the potential to affect muscle metabolism and meat quality. GHS pigs had a lower final BW and growth rate compared to GTN pigs, mainly driven by reduced feed intake. This adaptive response serves to reduce metabolic heat production and is well-documented (Renaudeau et al., 2008; Johnson et al., 2015; Ross et al., 2015; Gonzalez-Rivas et al., 2020; Liu et al., 2022). Backfat and muscle thickness and carcass lean meat content were not affected by GE, indicating that the decreased average daily feed intake of the GHS pigs, which usually leads to lower fat deposition and leaner carcasses in thermoneutral conditions (Lebret, 2008), did not affect the composition (i.e., lean and fat) of their weight gain in the present HS conditions. However, when adjusted for the same slaughter BW, backfat thickness was affected by GE and by PE × GE interaction, with the PHS-GHS pigs having the thickest ($P < 0.05$) backfat compared to the other three experimental treatments, which did not differ ($P > 0.05$). A recent review reported controversial effects of chronic GHS, independently on PHS, on carcass fatness (Liu et al., 2022). Indeed, increased backfat thickness in GHS pigs has been observed (review by Ross et al., 2015; 35°C vs 22°C during 30 days, Ma et al. 2019), whereas other authors found no difference (32 vs 18 °C for 7 weeks, Witte et al., 2000; 33 $\pm$ 2 vs 22 $\pm$ 2 °C for four weeks, Liu et al., 2021) or reported a decrease in backfat thickness in pigs under HS compared with TN

environment (27.9 ± 3.0 vs 20.0 °C during whole growing-finishing, Rinaldo and Mourot, 2001; 32 vs 21 °C for 10 weeks, Cruzen et al., 2015). In our experimental conditions, PHS did not affect carcass traits at slaughter stage. In contrast, Boddicker et al. (2014) reported increased backfat thickness in slaughter pigs from dams submitted to HS (cyclical, 28-34 °C vs 18-22 °C) during their first half of gestation, regardless of the growing thermal environment (constant, 35°C or 21°C). Johnson et al. (2015) also found that, independently of the thermal environment during growing (HS: 34.4 ± 1.8 °C or TN: 22.7 ± 2.5 °C), HS during the entire prenatal period (cyclical, 27-37 °C vs 15-22 °C) favored body lipid accretion at the expense of protein accretion in pigs between 60 and 80 kg BW. Discrepancies between studies on the effects of PHS and GHS on carcass traits may be partly explained by sex and physiological stage of the pigs, but also by differences in HS conditions: duration, magnitude, constant or cyclic, like in our study, which limited the decrease in feed intake thereby providing more energy for fat deposition, than encountered under constant HS. In any case, present results indicate that chronic cyclical (28 to 34°C) HS during prenatal and/or growing period did not affect the subsequent composition of weight gain in finishing female pigs. Similarly, no significant effect of chronic cyclical HS (28 to 34°C) was found on the carcass composition of intact male pigs submitted to PHS vs PTN and kept thereafter in GTN environment (Renaudeau et al., 2022).

From the present study, HS during growing influenced many quality traits assessed in loin and ham muscles towards higher ultimate pH values in all muscles and higher water holding capacity in the GM and LTL to a lower extent, whereas pH 30 min was not modified in the LTL or SC. These results led to higher meat quality index in the GM and, altogether, clearly indicate an improvement in the technological quality (i.e., processing ability) of meat from pigs under GHS treatment, that would be favorable for processing of both cooked and dry processed pork

products (Lebret and Čandek-Potokar, 2022b). Meat color was also moderately modified by GHS, with slightly lower chroma (i.e., color intensity) in loin and ham muscles, and lower GM lightness. These color changes, associated to the trend for lower loin drip loss, suggest an improvement in meat appearance (i.e., less light and exudative), however their magnitude would probably be too low to be perceived by consumers and influence their purchase decisions (Mancini and Hunt, 2005). In contrast with effects of GHS on the LTL and ham muscles that are mostly of glycolytic type, meat quality traits in the oxidative SC were not affected. These discrepancies may be related to the different muscle metabolic responses to GHS according to their metabolic type.

The improved technological quality traits of loin and ham muscles as a result of GHS in our experiment is in agreement with previous studies. When comparing meat quality traits of pigs reared in warm season of tropical climate (27.9 ± 3.0 °C) or in control climatic conditions (20 °C), Rinaldo and Mourot (1991) reported increased ultimate pH in the LTL and ham Biceps femoris (BF) muscle, but not in the SC. Further, HS pigs had increased WHC in BF, without any effect on pH 45 min p.m. in LTL, BF or SC muscles. Growing HS (30 °C vs 20 °C) for 4 weeks during the pig growing period (44 kg BW onwards) was also associated to lower drip loss and lightness in LTL and Gluteus muscles (Pardo et al., 2022). Ma et al. (2019) reported that LTL muscle from pigs submitted to GHS (35 °C vs 22 °C) for 30 days during their finishing period (78 kg BW onwards) had higher pH 24 h, and similar pH 45 min L^* values, but without any effect on drip loss, a^* and b^* values. Interestingly, cold stress has been reported to have the opposite effect, with lower pH 24 h in the LTL but not in the SC of pigs in cold (12 °C) vs hot (28 °C) conditions (Lefaucheur et al., 1991). This effect could be explained by the increased glycogen stores (GP) in the LTL but not in the SC muscle of pigs placed in cold vs TN or hot

conditions (Lefaucheur et al., 1991; Faure et al., 2013). In contrast with the aforementioned studies, no significant variations in loin pH 24, WHC and *a** and *b** coordinates were found in pigs submitted to constant GHS (32 vs 18 °C) for 5 to 7 weeks during the finishing period (from approximately 90 to 126 kg BW) (Witte et al., 2000) or cyclic GHS (25 to 31°C, or 31 to 37 °C vs 19 to 25 °C) for 10 weeks from 38 kg BW up to slaughter at 97 to 116 kg BW (Mun et al., 2022). Liu et al. (2021) did not either report any difference in pH 24 h and drip loss, but did report a lower pH 45 min and a higher lightness of LTL from pigs submitted to GHS (33 $\pm$ 2 vs 22 $\pm$ 2 °C) for four weeks from 50 to 72-77 kg BW. Contradictory to this, a marked decrease in pH 24 h together with higher drip loss was found in the LTL of pigs under GHS (30 vs 22 °C) for three weeks (Yang et al., 2014). Discrepancies on the effects of GHS on meat quality traits between studies may be explained by different effects of HS on muscle energy metabolism (discussed below), considering its major role in the biochemical and structural peri- and post-mortem transformations of muscle into meat. In contrast with growing HS, prenatal HS had scarce impacts on meat quality traits, with only increased *a** value leading to higher chroma in the GS, whereas LTL color as well as pH or drip loss of loin and ham muscles remained unaffected. Accordingly, Tuell et al. (2021) did not report any difference in pH 24 h, color or drip loss of loin from pigs issued from dams submitted to cyclic PHS (28.4 to 35.8 °C) vs TN environment (17.5 °C) during the first half of gestation. However, processed products issued from these same PHS pigs had higher *a** and *c**, and lower *h*° values, in pork patties compared to PTN pigs (Xue et al., 2022). Combined effects of prenatal and growing thermal environments on meat quality traits were scarce and limited to drip loss and b* of the GM.

Muscle biochemical composition and metabolic traits were modified by HS during growing, especially in the LTL, whereas prenatal HS had much less effect. Increased water and decreased protein contents without changes in IMF content were found in both LTL and SC muscles of GHS vs GTN pigs. In contrast, GHS affected glycolytic potential and its components in a muscle-type dependent manner. The LTL had reduced glycolytic potential due to trends for lower glycogen and lactate contents, whereas GP was not modified by GHS in the more oxidative SC. These muscle GP differences explain the higher pH 24 found in the LTL and ham muscles (mostly of glycolytic metabolic type), but not in the SC of pigs submitted to GHS (Terlouw et al., 2021). In addition to reduced muscle glycogen stores, GHS induced a decrease in glycolytic and terminal oxidative capacities (assessed by LDH and CS activities, respectively) in the LTL, whereas these metabolic pathways were not modified in the SC. The potential for fatty acid oxidation (assessed by HAD activity) was not modified by GHS in either LTL or SC muscle. Thus, GHS induced specific metabolic responses according to muscle metabolic type, with an overall reduced energy metabolism in the glycolytic LTL, but no effect in the oxidative SC muscle. In agreement with our results, higher muscle water content has been reported in pigs submitted to HS vs TN growing environment (Rinaldo and Mourot, 1991; Cruzen et al., 2015; Pardo et al., 2021; Mun et al. 2022). The lower muscle protein content of GHS pigs is in agreement with Rinaldo and Mourot (1991) and would be explained by the reduced muscle protein synthesis generally observed in HS animals (review by Rhoads et al., 2013). Accordingly, a downregulation of genes involved in muscle structure and development was observed in the LTL of pigs under HS, as both direct effect of temperature and indirect effects of reduced feed intake (Ma et al., 2019). An increased muscle protein breakdown, as suggested by the higher plasma creatine content found in the GHS compared with GTN pigs in our experiment

(Serviento et al., 2020) could also contribute to explain the lower muscle protein content in GHS pigs (Rhoads et al., 2013). Other studies reported lower even non-significant differences in LTL protein content of pigs submitted to HS vs TN growing environment (Cruzen et al., 1995; Pardo et al., 2021; Mun et al., 2022). The lack of GHS effect on IMF content agrees with previous findings in the LTL (Rinaldo and Mourot, 1991; Cruzen et al., 2015; Liu et al., 2021), whereas others reported decreased IMF content in the LTL (Ma et al., 2019; Pardo et al., 2021; Mun et al., 2022) or SC muscle (Rinaldo and Mourot, 1991) of pigs submitted to GHS. In contrast with growing HS, prenatal HS did not affect muscle water, protein or lipid contents, in agreement with Cruzen et al. (2015).

In agreement with present findings, decreased glycogen stores and activities of energy metabolic enzymes (LDH, CS) have been reported in the LTL of pigs submitted to growing HS (e.g., 31.5 vs 18.5 °C for five weeks, Rinaldo and Le Dividich, 1991; 35.3 vs 21.0 °C for one week, Zhao et al., 2018; 33 $\pm$ 2 vs 22 $\pm$ 2 °C for four weeks, Liu et al., 2021; review by Gonzalez-Rivas et al., 2020). Muscle transcriptome studies also showed down-regulation of gluconeogenesis and tricarboxylic acid (citrate) cycle pathways in LTL from pigs under GHS compared with GTN conditions (Ma et al., 2019). Conversely, increased GP and metabolic enzyme activities (LDH, CS and HAD) activities were observed in the LTL of finishing pigs submitted to cold vs hot (28 vs 12 °C; Lefaucheur et al., 1991) or cold vs thermoneutral conditions (12 vs 23°C, Faure et al., 2013). Altogether, these results indicate a decreased activity of energy metabolic pathways in the LTL of pigs under chronic GHS, as part of their adaptive metabolic response to heat. In contrast with our results and the abovementioned studies, Hao et al. (2014) reported a trend for higher LDH activity and similar glycogen content in the LTL of pigs under HS (30°C vs 22 °C for three weeks, same animal experiment as considered by Yang

et al., 2014). In this study, a noticeably high pig rectal temperature still occurred after 21 d of HS (average of 40.3 °C vs 39.4 °C for the control pigs, $P < 0.01$; Hao et al., 2014) suggesting that these HS pigs were not able to adapt to chronic HS. In contrast, in our experiment the rectal temperature of GHS pigs was higher compared to that of GTN pigs after 3 d of HS (average of 40.0 °C and 39.4 °C, respectively, $P < 0.001$), but gradually decreased to an average of 39.3 °C (vs 39.2 °C for the GTN pigs) after 53 d of HS ($P < 0.05$), indicating an adaptation to chronic HS (Serviento et al., 2020). In addition, Hao et al. (2014) found higher plasma activities of creatine kinase and lactate dehydrogenase in HS vs TN pigs at slaughter. These results, associated to their higher rectal temperature, indicate that these HS pigs were actually under acute stress. In support to these results, an increased LDH mRNA abundance was found in the predominantly glycolytic tibialis anterior muscle of rats submitted to acute HS (Sanders et al., 2009). Altogether, this suggests that the different effects of HS on LTL metabolism found in the study by Hao et al. (2014) and the present one or other studies cited above, would rely on differential metabolic responses to acute vs chronic HS. Thus, an increased reliance on glycolysis for energy production would occur as response to acute HS (Rhoads et al., 2013), whereas a reduced muscle glycolysis as adaptive response to long-term heat exposure would occur in pigs under chronic HS.

The differential metabolic response to HS observed between the glycolytic LTL and the oxidative SC muscles in our study is in agreement with Rinaldo and Le Dividich (1991) who reported only a decrease in LDH but no significant changes in CS or HAD activities in the oxidative Trapezius muscle of piglets under GHS. Faure et al. (2013) also observed a weaker metabolic response to thermal environment in the SC compared with LTL in 115 kg BW pigs, with similar GP and LDH activity but higher HAD and CS activities in the SC of pigs under cold

vs thermoneutral environment. Contrary to growing HS, prenatal HS affected only one muscle metabolic trait with a tendency for a higher oxidative capacity (CS) in the SC of PHS compared with PTN pigs at slaughter stage. This result, that remains to be confirmed, suggests that oxidative muscle metabolism is sensitive to HS from the prenatal period. When submitting piglets from 7.7 (weaning) to 25 kg BW to cold conditions (gradual decrease in ambient temperature from 23 to 15 °C) compared to thermoneutral conditions (gradual decrease in ambient temperature from 28 to 23°C), Faure et al. (2013) observed different metabolic responses according to muscle type, with increased activities of oxidative metabolic enzymes in the SC but no significant effect on energy metabolism in the LTL. These results and present data indicate an earlier metabolic adaptation to ambient temperature of the oxidative SC compared to the glycolytic LTL muscle. The muscle-specific changes found in response to chronic GHS (decreased glycogen stores and energy metabolism in the LTL but not in the SC) and PHS (trend for increased oxidative metabolism in the SC but not in the LTL) likely rely on specific physiological/cellular changes in energy production and regulation pathways according to muscle metabolic type. Indeed, the activity of the adenosine monophosphate-activated protein kinase (AMPK), a key sensor of cellular energetic status involved in energy balance in skeletal muscle (Hardie and Sakamoto, 2006) was decreased in the SC muscle of pigs submitted to cold vs TN conditions, whereas differences did not reach significance in the LTL (Faure et al., 2013). Complementary assessment of energy metabolic pathways including AMPK activity and mitochondrial functions would contribute to a better understanding of the specific metabolic adaptation to chronic HS according to muscle type.

The decreased plasma glucose content but similar creatine kinase activity in GHS vs GTN pigs at slaughter stage, without effect of PHS on these traits, fully agrees with data on

glucose concentration and creatine kinase activity determined on plasma collected in vivo on the same animals at the end of their finishing period (around 130 d of age), i.e., after long term heat exposure (Serviento et al., 2020). The similar plasma creatine kinase activity (indicator of muscular activity) between PTN and PHS pigs, and GTN and GHS pigs, suggests that physiological responses of pigs to pre-slaughter handling did not differ according to their thermal environment (Guise et al., 1998). Plasma lactate content, an indicator of anaerobic metabolism, was not affected by GHS but tended to be reduced by PHS. The decreased intensity of anaerobic metabolism in pigs submitted to PHS is in line with their higher oxidative metabolism (i.e., CS activity) in the SC muscle. The lower plasma glucose content in pigs under GHS is consistent with the reduced circulating glucose observed by Boddicker et al. (2014) in pigs under chronic postnatal HS. Increased basal insulin concentration has been reported pigs under HS (Ross et al., 2015), even though this effect is not always reported. Similar insulin but decreased glucagon serum contents have thus been found in pigs under chronic HS (Shi et al., 2016). The altered thyroid function of GHS pigs, demonstrated by the lower T3 and T4 plasma levels in the same GHS vs GTN pigs at 85 and 130 d of age (Seviento et al., 2020), in agreement with review by Gonzalez-Rivas et al., (2020), likely serves to the decreased glycaemia in GHS pigs. Altogether, this confirms the altered energy metabolism in pigs under HS probably to reduce heat production. Our results show that this adaptive response also involves the glycolytic LTL, but not the oxidative SC muscle. Indeed, the LTL being the largest pig muscle, its reduced energy metabolism could significantly contribute to reduce body heat production in pigs submitted to HS.

Conclusion

Chronic, cyclical HS during the growing phase decreased the voluntary feed intake and growth rate of pigs, without affecting carcass lean meat content. Growing HS influenced pork quality, with improved technological meat quality traits of loin and ham. These effects were associated to reduced energy metabolism and glycogen stores in the glycolytic-type LTL muscle, without changes in the oxidative SC, highlighting a differential adaptive response to HS according to muscle metabolic type. In contrast, prenatal HS had little effect on subsequent pig growth, carcass composition, muscle biochemistry, and meat quality, with the exception of higher a^* and C^* in the GS muscle and a trend for reduced general anaerobic metabolism. Combining PHS and GHS did not significantly modify pig responses to GHS in our experimental conditions. Therefore, submitting pigs to HS, as can be encountered with the increased frequency and severity of heat waves due to global warming, affects growth performance without modifying carcass composition. However, HS has positive impacts on technological quality of pork as a result from metabolic changes specifically in glycolytic muscles.

Acknowledgements

The authors thank the staff of INRAE experimental farm and slaughterhouse (F. Le Gouevec, A. Chauvin, M. Genissel, J. Georges, J. Delamarre, H. Demay, D. Boutin, F. Guerin, Y. Surel, P. Touanel, M. Alix, J. Liger, J. F. Rouault; UE3P, 35590 Saint-Gilles, France). They are also grateful to PEGASE laboratory staff (S. Daré-Michelot, N. Bonhomme, C. Perrier) for their excellent technical assistance.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Table 1. Effects of prenatal and growing thermal environments on pig growth performance and carcass traits

	Thermal environment							
	Prenatal[1]		Growing[1]				*P*-value[2]	
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
Growth performance[3]								
Initial (82 d) BW, kg	42.7	42.0	42.2	42.6	2.9	0.629	0.666	0.141
Final (140 d) BW, kg	101.4	100.5	104.8	97.1	5.6	0.706	**0.003**	0.185
Average daily gain, g/d	1,047	1,047	1,119	975	91	0.990	**0.002**	0.505
Carcass traits								
Hot carcass weight, kg	78.7	77.8	81.0	75.5	4.4	0.611	**0.005**	0.176
Backfat thickness (G2), mm	10.4	11.1	10.9	10.6	1.8	0.493	0.735	0.439
Muscle thickness (M2), mm	60.5	59.6	60.7	59.3	4.4	0.524	0.329	0.782
Lean meat content, %	63.3	62.7	63.0	63.0	1.4	0.405	0.992	0.600

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE) and pen (within PE × GE) were included as random effects.

[3] Data already published by Serviento et al. (2020).

Table 2. Effects of prenatal and growing thermal environments of pigs on plasma components determined at slaughter

	Thermal environment							
	Prenatal[1]		Growing[1]			*P*-value[2]		
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
Glucose, µmol/ml	5.98	5.72	6.26	5.44	0.48	0.176	**0.002**	0.933
Lactate, µmol/ml	8.05	5.47	6.71	6.82	0.22	**0.075**	0.938	0.521
Creatine kinase, U/ml	8.68	7.51	6.37	9.82	0.29	0.335	0.271	0.528

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE), pen (within PE × GE) and slaughter day were included as random effects, and applied to raw data (glucose) or to log values to fit a normal distribution of residues (lactate, creatine kinase).

Table 3. Effects of prenatal and growing thermal environments of pigs on quality traits of the Longissimus thoracis et lumborum muscle

	Thermal environment							
	Prenatal[1]		Growing[1]				*P*-value[2]	
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
pH 30 min p.m.	6.60	6.59	6.57	6.62	0.16	0.834	0.330	0.782
pH 24 h p.m.	5.55	5.52	5.51	5.57	0.07	0.186	**0.028**	0.385
Drip loss, %	3.33	3.15	3.59	2.89	1.19	0.626	**0.088**	**0.085**
Color								
*L**	50.8	50.8	50.9	50.7	1.7	0.963	0.795	0.310
*a**	6.60	7.37	7.50	6.48	1.17	0.114	**0.039**	0.490
*b**	5.68	5.87	6.02	5.53	0.61	0.595	0.190	0.258
*C**	8.73	9.46	9.65	8.54	1.15	0.181	**0.061**	0.360
h°	41.1	39.0	39.2	41.0	3.6	0.281	0.194	0.796

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE), pen (within PE × GE) and slaughter day were included as random effects.

Table 4. Effects of prenatal and growing thermal environments of pigs on quality traits of ham muscles

	Thermal environment							
	Prenatal[1]		Growing[1]				*P*-value[2]	
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
pH 24 h p.m.								
Semimembranosus	5.60	5.60	5.55	5.66	0.12	0.982	**0.017**	0.548
Adductor	5.73	5.68	5.62	5.79	0.17	0.400	**0.012**	0.371
Gluteus medius	5.50	5.50	5.46	5.54	0.10	0.946	**0.023**	0.967
Gluteus superficialis	5.60	5.58	5.51	5.67	0.11	0.566	**0.002**	0.694
Color								
Gluteus medius								
*L**	53.4	52.7	54.0	52.1	2.7	0.433	**0.047**	0.121
*a**	9.03	9.72	9.78	8.97	1.35	0.330	0.135	0.464
*b**	8.10	8.31	8.60	7.81	1.07	0.634	**0.054**	**0.089**
*C**	12.2	12.8	13.1	11.9	1.5	0.428	**0.081**	0.231
h°	42.3	40.6	41.5	41.4	3.8	0.248	0.951	0.474
Gluteus superficialis								
*L**	49.7	49.2	49.9	49.0	2.3	0.469	0.229	0.788
*a**	9.96	11.3	11.3	9.97	1.57	**0.029**	**0.031**	0.916
*b**	7.47	8.10	8.24	7.33	1.28	0.142	**0.049**	0.580
*C**	12.5	13.9	14.0	12.4	1.9	**0.041**	**0.029**	0.893
h°	36.9	35.7	36.3	36.4	3.4	0.249	0.899	0.394
Technological quality traits, Gluteus medius								
WHC, point[3]	12.2	14.9	9.7	17.4	5.5	0.233	**0.003**	0.538
Meat quality index, point[4]	9.6	10.1	8.6	11.1	2.0	0.517	**0.005**	0.282

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE), pen (within PE × GE) and slaughter day were included as random effects.

[3] Water Holding Capacity, assessed on a scale from 0 (extremely low) to 20 (high).

[4] Determined from Tribout and Bidanel (2000) with Meat Quality Index = − 41 + 11.01 × (pH 24 h Semimembranosus) − 0.231 × (*L** Gluteus medius) + 0.105 × (WHC Gluteus medius).

Table 5. Effects of prenatal and growing thermal environments of pigs on quality traits of the Semispinalis capitis muscle

	Thermal environment							
	Prenatal[1]		Growing[1]			*P*-value[2]		
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
pH 30 min p.m.	6.54	6.54	6.52	6.56	0.15	0.912	0.453	0.963
pH 24 h p.m.	6.10	6.00	6.03	6.07	0.29	0.254	0.612	0.787
Color								
*L**	45.2	45.4	44.9	45.7	1.5	0.812	0.138	0.106
*a**	17.1	18.2	18.2	17.7	1.4	0.253	0.242	0.862
*b**	9.19	9.33	9.24	9.28	0.91	0.603	0.877	0.267
*C**	19.9	20.5	20.4	20.0	1.6	0.303	0.373	0.662
h°	27.4	27.1	26.9	27.7	1.6	0.501	0.140	0.146

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE), pen (within PE × GE) and slaughter day were included as random effects.

Table 6. Effects of prenatal and growing thermal environments of pigs on biochemical composition and metabolic traits of the Longissimus thoracis et lumborum muscle

	Thermal environment							
	Prenatal[1]		Growing[1]				*P*-value[2]	
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
Biochemical composition, on wet basis								
Water, %	75.5	75.6	75.2	75.9	0.8	0.896	**0.033**	0.734
Protein, %	22.6	22.5	22.9	22.3	0.6	0.542	**0.047**	0.797
Intramuscular fat, %	1.46	1.58	1.47	1.58	0.31	0.480	0.257	0.496
Lactate, µmol/g	54.0	53.8	57.7	50.1	13.7	0.970	**0.099**	0.984
Free glucose + G6P, µmol/g	5.35	5.41	5.50	5.26	1.73	0.927	0.651	0.733
Glucose (glycogen), µmol/g	47.1	47.7	51.1	43.7	11.6	0.880	**0.070**	0.352
Glycolytic potential, µmol eq. lactate/g	159	160	171	148	17	0.874	**0.003**	0.185
Metabolic enzyme activities, µmol substrate/min and per g of wet muscle								
LDH[3]	2,224	2,204	2,288	2,141	153	0.713	**0.016**	0.696
HAD[3]	4.21	4.34	4.37	4.18	0.51	0.484	0.254	0.745
CS[3]	7.19	7.35	7.68	6.86	0.84	0.687	**0.015**	0.909

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE), pen (within PE × GE) and slaughter day were included as random effects.

[3] LDH: lactate dehydrogenase, HAD: β-hydroxy-acyl-Co-A dehydrogenase, CS: citrate synthase.

Table 7. Effects of prenatal and growing thermal environments of pigs on biochemical composition and metabolic traits of the Semispinalis capitis muscle

	Thermal environment							
	Prenatal[1]		Growing[1]			*P*-value[2]		
	PTN	PHS	GTN	GHS	RMSE	PE	GE	PE × GE
n	24	24	24	24				
Biochemical composition, on wet basis								
Water, %	72.7	72.7	72.2	73.3	0.9	0.993	**0.007**	0.207
Protein, %	19.1	19.1	19.4	18.9	0.7	0.998	**0.055**	0.168
Intramuscular fat, %	7.07	7.00	7.35	6.72	1.22	0.889	0.130	0.673
Lactate, µmol/g	49.2	49.3	49.9	48.6	8.4	0.973	0.592	0.482
Free glucose + G6P, µmol/g	3.83	4.12	3.95	4.00	1.50	0.516	0.905	0.266
Glucose (glycogen), µmol/g	5.43	7.84	6.24	7.04	4.66	0.124	0.574	0.387
Glycolytic potential, µmol eq. lactate/g	67.9	73.3	70.6	70.6	17.9	0.331	0.992	0.359
Metabolic enzyme activities, µmol substrate/min and per g of wet muscle								
LDH[3]	643	621	652	613	79	0.496	0.248	0.718
HAD[3]	10.4	11.1	10.9	10.6	1.6	0.159	0.627	0.966
CS[3]	12.9	14.2	13.8	13.3	1.6	**0.065**	0.462	0.766

[1] Least square means estimated from raw data. PTN: prenatal thermoneutral environment, PHS: prenatal heat stress, GTN: growing thermoneutral environment, GHS: growing heat stress.

[2] *P*-values of the fixed effects of prenatal thermal environment (PE), growing thermal environment (GE) and their interaction (PE × GE), and RMSE (root mean square error of the model) obtained from mixed model in which sire, sow (within PE), pen (within PE × GE) and slaughter day were included as random effects.

[3] LDH: lactate dehydrogenase, HAD: β-hydroxy-acyl-Co-A dehydrogenase, CS: citrate synthase;