The rearing system modulates biochemical and histological differences in loin and ham muscles between Basque and Large White pigs

L. Lefaucheur1 and B. Lebret1

*1 INRAE, Agrocampus Ouest, PEGASE, 35590 Saint-Gilles, France*

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**Supplementary Material S1**

**Materials and methods**

*Pork quality traits*

Ultimate pH was determined in the longissimus muscle (LM, first lumbar vertebra level), semimembranosus (SM, external side, 3 cm deep), red (RSTM) and white (WSTM) portions of semitendinosus, on samples taken 24 h post mortem, cut into small pieces, frozen in liquid and stored at ‑80°C until analyses. Ultimate pH was measured after 2 g of muscle sample was homogenized in 18 ml of 5 mM iodoacetate (Inglod Xerolyte electrode, Metrohm pH-meter, Berlin, Germany) after calibrating the pH meter with buffers of pH 4.0 and 7.0 and correcting the pH measurement for temperature. For each muscle, samples from one replicate were analyzed by single determination in a single series measurement; buffer values were checked regularly during series measurement. The intra- and inter-assay CVs of pH determination were 0.80% and 3.03%, respectively, at pH = 6.50.

Color coordinates lightness (L\*), redness (a\*) and yellowness (b\*) were measured on the LM (first lumbar vertebra level) and SM (internal side of a 3 cm deep slice on the external side of the muscle) 24 h post mortem. The LM and SM slices were exposed to artificial light for 90 min at 4°C for blooming. Color coordinates were then determined using a Minolta Chromameter CR300 (Osaka, Japan) with a D65 illuminant and a 1 cm diameter aperture, after calibrating the chromameter with its white standard calibration plate. Color was measured at three locations representative of each muscle slice, and mean values were calculated per muscle sample. Hue angle (h°) was calculated from a\* and b\* values as h° = atan(b\*/a\*)×180/ $π$.

Muscle glycolytic potential (GP) was determined from LM (last rib level), SM (external part, 2 cm deep), and RSTM and WSTM (central portions) samples taken within 40 min post mortem, cut into small pieces, frozen in liquid nitrogen and stored at -80°C. GP was determined according to Monin and Sellier (1985), as GP = 2[(glycogen) + (glucose) + (glucose-6-phosphate)] + (lactate). After 1 g of muscle was homogenized in 10 mL of 0.55 Mperchloric acid, glucose and glucose-6-phosphate were determined together using an enzymatic method (glucose HK, ABX Diagnostics kit, Montpellier, France) using an automatic spectrophotometric analyzer (Konelab20, Thermo Scientific, Waltham, MA, USA). Muscle lactate content was determined enzymatically (Lactate PAP kit, Biomerieux, Marcy l’Etoile, France) using the same spectrophotometric analyzer. Muscle glycogen content was determined from glucose determination after hydrolysis by amyloglucosidase, as described by Talmant *et al.* (1989). For each replicate and muscle, GP components were determined in two series measurements, each including five samples of each treatment. In each muscle sample, lactate; free glucose and glucose-6-phosphate; and glucose from glycogen hydrolysis were determined in duplicates from a single muscle homogenate. Means per component and sample were calculated, and results were expressed as micromol per g of wet tissue. GP was then calculated and expressed as micromol of equivalent lactate per g of wet tissue. The intra- and inter-assay CVs of GP determination were 5.3% and 10.2%, respectively, at 192 µmol eq. lactate/g.

Intramuscular fat (IMF) content was determined in the LM (first lumbar vertebra level), SM (internal side of a 3 cm deep slice on the external side of the muscle) and central portions of RSTM and WSTM taken 24 h post mortem, minced and stored under vacuum at -20°C until analysis. IMF content was determined from single chloroform/methanol (2:1 v/v) lipid extraction of each muscle sample according to Folch *et al.* (1957) and expressed as percentage of fresh muscle weight. The inter-assay CV of the IMF content was 4.60% at 3.30% IMF content (the intra-assay CV could not be determined because each lipid extraction was an independent process for each sample).

*Images of Semitendinosus muscle sections*

At 24h post mortem, images of the mid-belly cross-section of *Semitendinosus* (STM) muscle were captured using a reflex digital camera (Nikon D90, Tokyo, Japan). Images were acquired at similar magnification conditions using a 2 x 3 cm area transparent scale placed on each section. They were used for illustrations in Figure 1 as well as to determine STM transverse cross-sectional area (Visilog 6.0 imaging software, Noesis, Courtaboeuf, France).

*Metabolic enzyme activities*

To determine the activities of lactate dehydrogenase (LDH), citrate synthase (CS) and β-hydroxy-acyl CoA dehydrogenase (HAD), ca. 500 mg of muscle sample was pulverized at -70°C using a hammer and a metal block cooled with dry ice. Approximately 100 mg of muscle powder was thawed in 50 volumes (wt:vol) of ice-chilled 0.1 M phosphate buffer (pH = 7.5) containing 2 mM EDTA and homogenized (Polytron, Bioblock) for 10 s in ice. The homogenate was then ultrasonicated for 1 min in ice and centrifuged at 1 500 g for 10 min at 4°C. The supernatant, which contained soluble enzymes and mitochondrial material, was kept on ice and used on the same day to measure the LDH, CS and HAD activities at 30°C with an automatic spectrophotometric analyzer (Konelab20, Thermo Scientific, Waltham, MA, USA). LDH activity was assessed following the transformation of pyruvic acid added as a substrate into lactic acid, through the kinetics of NADH decrease determined at 340 nm every 25 s for 5 min. HAD activity was assessed following the transformation of aceto-acetyl-CoA added as a substrate, into hydroxyl-acyl-CoA, through the kinetics of NADH decrease determined at 340 nm every 25 s for 5 min. CS was assessed through the kinetics of transformation of dithiobis-2 nitrobenzoic acid (added in the homogenate sample) into nitrothiophenol at 405 nm every 25 s for 5 min, with the nitrothiophenol being produced during the transformation of oxaloacetic acid and acetyl-CoA, added as substrates, into citric acid. In each muscle sample, activity of each enzyme were determined in duplicates from single supernatant preparation and means of duplicates of each enzyme were calculated. For each experimental replicate and muscle, enzyme activities were determined in three series measurements, each including 4-5 samples of each treatment). Intra- and inter-assay CVs of metabolic enzyme activities per g of fresh muscle were 7.5% and 14.8% respectively, for LDH at 2188 µmol substrate degraded/min ; 13.3% and 18.4%, respectively, for HAD at 4.42 µmol substrate degraded/min; and 11.3% and 19.2%, respectively, for CS at 8.10 µmol substrate degraded/min.

*Histological analyses*

Digital image analysis was used to classify muscle fiber types and determine their cross-sectional area, as detailed by Lefaucheur et al. (1992). The interfiber network was extracted, from a 14 µm thick transverse serial section stained with azorubine.

For each experimental replicate and muscle, histological staining and analyses were performed in batches of 10 samples balanced among treatment groups (2-3 samples of each treatment per batch).

Statistical analyses

Mathematical models and codes used in Statistical Analysis System (SAS) software (version 9.4, 2013, SAS Institute, Cary, NC, USA) to analyze the data.

*The ANOVA – GLM procedure* that included the treatment (5 levels: Large White (LW) in conventional (C) system: LWC, LW in alternative (A) system: LWA, Basque (B) in C system (BC), B in A system (BA) and B in extensive (E) system (BE)) and replicate (2 levels: R1 and R2) as fixed effects to calculate residues and check normality of residues (results of univariate procedure present Shapiro-Wilk test P values; P ≥ 0.05 indicates normality of data)

libname rep '.';

data rep.datafile;

infile 'datafile.asc';

input numpig treatm replicat var1 (…) varn ; run ;

proc glm plot=none data=rep.datafile;

class treatm replicat;

model var1 (…) varn = treatm replicat/ss3;

output out=residu p=pred1-predn

R= rvar1 (…..) rvarn;

proc univariate normal;

var rvar1 (…) rvarn; run;

Data for IMF content; LDH, HAD and CS of the LM, SM, RSTM and WSTM; cross-sectional area (CSA) of fiber types I, IIA, IIBR, IIBW and mean CSA of LM muscle; and the STM proportion were log-transformed into to fit a normal distribution of residues.

Data for the relative area types I, IIA, IIBR, IIBW fibers of the LM, RSTM and WSTM were square-root-transformed to fit a normal distribution of residues.

*ANOVA – GLM procedure* that included the treatment (5 levels: LWC, LWA, BC, BA and BE) and replicate (2 levels: R1 and R2) as fixed effects and determination of contrasts between breeds (B vs. LW) and rearing systems for a given breed (A vs. C; or E vs. C) effects on growth, carcass, muscle and meat traits (with the treatment codes 1 for BA, 2 for BC, 3 for BE, 4 for LWA and 5 for LWC). For data of average daily feed intake and feed efficiency, the same test was used but with the BE treatment excluded. Least-square means were calculated by treatment.

data rep.datafile;

proc glm plot=none data=rep.datafile;

class treatm replicat;

model var1 var2 = treatm replicat/ ss3;

contrast 'B vs LW' treatm 1 1 0 -1 -1;

contrast 'LWA vs LWC' treatm 0 0 0 1 -1;

contrast 'BA vs BC' treatm 1 0 -1 0 0;

contrast 'BE vs BC' treatm 1 -1 0 0 0;

means treatm; run;

*Non-parametric method* (NPAR1WAY procedure in SAS, Kruskal-Wallis test) to determine effects of breed (considering BC, BA and LWC and LWA pigs – as detailed above) and rearing system for a given breed (LWA vs. LWC, BA vs. BC or BE vs. BC) when residues of raw or transformed data could not be normalized (i.e. final age, average daily gain, GP of the SM, ultimate pH of the LM and SM). Breed or rearing system were significant when P < 0.05 for the Kruskal-Wallis test. Medians were further calculated by treatment (Microsoft Excel software).

data rep.datafile;

proc npar1way anova Wilcoxon plots-none data= rep.datafile;

class breed;

var var1…varn; run;

**References**

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