The rearing system modulates biochemical and histological differences in loin and ham muscles between basque and large white pigs

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Abstract

Conventional pork production, based on highly selected breeds for growth efficiency and carcass leanness, is generally considered to decrease pork quality. In contrast, non-selected breeds produced in extensive systems are associated with high pork quality, which is generally attributed to higher intramuscular fat (IMF) content and less glycolytic muscle metabolism. The present study aimed to determine biochemical, histological and quality traits of loin and ham muscles of pigs from selected Large White (LW) and local French, non-selected Basque (B) breeds. Pigs were reared in a conventional indoor (C, slatted floor), alternative (A, indoor bedding and outdoor area) or extensive system (E, free range, B pigs only). A total of 100 castrated males were produced in two replicates, each containing 5 groups of 10 pigs based on breed and system: LWC, LWA, BC, BA, and BE. The glycolytic longissimus (LM) and semimembranosus (SM) muscles, and the deep red (RSTM) and superficial white (WSTM) portions of semitendinosus muscle (STM), were studied at 145 kg body weight. Overall, breed induced stronger effects on muscle traits than the rearing system, among which the E system induced greater changes. The lower muscle growth of B pigs was associated with fewer muscle fibers and a smaller cross-sectional area (CSA) of glycolytic fibers ($P < 0.01$). The SM was less glycolytic and more oxidative in B than in LW pigs ($P < 0.001$). The WSTM followed a similar trend, with a larger relative area of type I fibers in B pigs. In contrast, the LM and RSTM were more oxidative in LW pigs. B pigs had higher IMF content and ultimate pH in all muscles, along with lower glycolytic potential, less light and redder meat in the LM and SM ($P < 0.001$). Compared to the C system, the A system induced only a shift towards a more oxidative metabolism in the LM and a smaller fiber CSA in the RSTM of LW pigs ($P < 0.05$), without influencing pork quality traits. Compared to BC pigs, BE pigs had a more oxidative and less glycolytic muscle metabolism, along with higher ultimate pH, lower lightness and redder meat ($P < 0.01$), but similar IMF content. Overall, results indicate that influences of breed and rearing system on muscle properties depend on muscle type, and that IMF content and fiber type composition are unrelated traits that can be modified independently by genetic or rearing factors.

Keywords: muscle fiber type, muscle energy metabolism, intramuscular fat, pork quality, breed

Implications

Compared to conventional production of high-performing pig breeds, non-selected local breeds, often produced in outdoor or extensive systems, generally have better pork quality. Muscle biology (fiber type composition, metabolism and lipid content) and pork quality traits differed greatly between selected Large White and local

Basque pig breeds. Moreover, compared to conventional systems, the extensive system further improved Basque pork quality, while the alternative system (bedding and outdoor area) had less influence on muscle and pork properties. Independence between fiber type composition and lipid content allows each to be controlled by genetics or pig breeding to optimize pork quality.

Introduction

In the context of consumers' increasing expectations for improved sustainability of production systems, animal welfare and eating quality of meat products (Font-i-Furnols and Guererro, 2014), pork products from local pig breeds reared in extensive systems have an excellent image among consumers (Bonneau and Lebret, 2010). Several local pig breeds have been maintained as a genetic-diversity reservoir; they generally have a lower growth rate and lean meat content, as well as higher fatness and eating quality, than selected breeds (Pugliese and Sirtori, 2012). Moreover, their phenotypic traits resulting from their genetic potential can be modulated by rearing factors such as final slaughter weight and age, feeding, climatic conditions, space allowance and physical exercise (Rosenvold and Andersen, 2003; Lebret, 2008). The local Basque breed reared in extensive conditions and slaughtered at ca. 145 kg body weight (BW) in southwestern France is an example of a diversified pork chain that results in high-quality and typical products recognized by the Protected Designation of Origin label (Mercat *et al.,* 2019). Among muscle biological characteristics that confer high quality, intramuscular fat (IMF) content is an important trait that should reach 2-3% of fresh muscle to achieve satisfactory pork tenderness and juiciness and acceptable visual perception by consumers (review by Listrat *et al.,* 2016). However, the association between IMF content and pork quality is not always reported due to the influence of many other factors, such as the post-mortem decrease in pH, aging conditions and cooking methods. Muscle fiber type composition also influences pork quality through its effects on pH, water holding capacity, color and muscle microstructure (review by Lefaucheur, 2010). Increasing the percentage of fast-twitch glycolytic fibers in pig longissimus muscle (LM) increases the rate and extent of the pH decrease and meat lightness, and decreases protein solubility and water holding capacity. In contrast, a higher percentage of slow-twitch oxidative fibers increases pork flavor, tenderness and redness (Kang *et al.,* 2011). In addition, increasing the cross-sectional area (CSA) of muscle fiber is widely reported to decrease water holding capacity and pork tenderness (Lefaucheur, 2010). Fiber-type composition and IMF content differ greatly among muscles and are influenced by genetic, animal (e.g. age) and environmental factors, but relationships between the two traits remain controversial (Lefaucheur, 2010). It is commonly stated that oxidative muscles contain more IMF than glycolytic muscles (Bereta *et al.,* 2014; Jeong *et al.,* 2017); however, other studies do not support this assessment (Kang *et al.,* 2011).

Lebret *et al.* (2015) observed that pigs of the local, non-selected Basque (B) breed had higher quality pork than pigs of the conventional Large White (LW) breed. The objective of the present study was to determine the biological properties of muscle that explained this difference. We hypothesized that B pigs have higher muscle oxidative metabolism and IMF content and lower fiber CSA than LW pigs. Since outdoor or free-range rearing of pigs can increase muscles' oxidative metabolism and influence their energy stores (i.e. IMF content and glycolytic potential (GP), which can influence pork quality (Bee *et al.*, 2004; Gentry *et al.*, 2004)), we also hypothesized that biological properties of B and LW pig muscles would differ depending on their rearing conditions. Therefore, we aimed to determine the biochemical, metabolic and histological traits of loin and ham muscles that influence the pork quality of B and LW pigs reared in a conventional indoor, alternative (indoor bedding and outdoor area) or extensive free-range system (B pigs only), with the ultimate aim to identify breeding and rearing practices that improve pork quality.

Materials and methods

Animals and experimental design

Lebret *et al.* (2014, 2015) described the experimental design in detail. Briefly, 100 castrated male pigs from the pure local B (n = 60) or LW breed (n = 40) were used. The B pigs came from two breeding farms of the Basque pork chain (Mercat *et al.*, 2019), while the LW pigs came from the INRA experimental herd. All animals were free of the RYR1 and PRKAG3 mutated alleles. Pigs were produced in two experimental replicates (R1 and R2), each including 30 B and 20 LW pigs. In each replicate, at a mean BW of 35 kg, 20 B littermates (from 10 B litters each in R1 and R2) and 20 LW littermates (from 10 and 7 LW litters in R1 and R2, respectively) were placed in a conventional (C, one pen per breed, indoor slatted floor, 1.0 m^2/pig) or alternative housing system (A, one pen per breed, indoor bedding and free access to an outdoor area, 2.4 m^2/pig), until slaughter at ca. 145 kg BW. LWC, LWA, BC and BA pigs (n = 10 (one pen) per treatment and replicate) were fed the same standard growing (2.29 Mcal/kg net energy, 18.0% CP, 3.40% crude fat, 0.83% digestible lysine; from 35 up to 75 kg BW) and finishing (2.14 Mcal/kg net energy, 14.7% CP, 1.60% crude fat, 0.75% digestible lysine ; from 75 kg BW to slaughter) diets. Pigs were fed *ad libitum* until the average feed intake of 2.5 kg/d and per pig was reached, corresponding approximately to 75 kg BW in each treatment. Then, the daily feed allowance was progressively increased up to 3.0 kg/d and per pig up to 110 kg BW and maintained at 3.0 kg/d and per pig until 145 kg BW. Pigs were weighed at the start, during and at the end of the experiment, and average daily gain (ADG) was calculated individually. Feed consumption per pen was recorded weekly, and average daily feed intake (ADFI) and feed efficiency were calculated per pen for each treatment and replicate. LWC, LWA, BC and BA pigs were slaughtered at the INRA slaughterhouse in four sessions, each including pigs from the four treatments. In addition, in each replicate, 10 B pigs, half-littermates of BC and BA pigs, were placed at 35 kg BW in an extensive production system (E, outdoor free-range system, 2.5 ha/pig) on a Basque chain farm, until slaughter at ca. 145 kg BW. BE pigs had free access to natural food resources (acorns and chestnuts) and were fed a standard supplementary growing-finishing diet (2.15 Mcal/kg net energy, 15.5% CP, 1.95% crude fat, 0.70% digestible lysine) according to local farming practices (allowance of 1.4 kg/d up to 2.2 kg/d and per pig from 35 kg up to 75 kg BW; 2.2 kg/d up to 2.6 kg/d and per pig from 75 kg up to 110 kg BW; 2.6 kg/d to 2.3 kg/d and per pig from 110 kg up to 130 kg BW; and 2.0 kg/d and per pig from 130 kg until slaughter at around 145 kg BW; Lebret *et al.*, 2014; Mercat *et al.*, 2019). BE pigs were weighed individually to calculate ADG. The ADFI and feed efficiency of BE pigs could not be calculated because their feed consumption, including both the supplementary diet and the natural food resources, could not be determined. BE pigs were included in the experiment 5 months, and BC and BA pigs 3 months, before LWC and LWA pigs in order to slaughter all pigs at 145 kg BW during winter in January (R1) or February (R2). BE pigs were slaughtered in a commercial slaughterhouse (Saint-Jean-Pied-de-Port, France). Slaughtering conditions of the two slaughterhouses used in the experiment were standardized as much as possible.

Carcass and pork quality traits

According to the handling practices of the Basque pork chain, all pigs were slaughtered after 36 h of fasting by exsanguination after electrical stunning (350 V, 4 A), in compliance with national regulations for slaughterhouses. Weights of the hot carcass and cold half-side carcass, as well as the loin and back fat primal cuts, were recorded. Pork quality traits were measured for all animals in the loin (LM, first lumbar vertebra level), as presented by Lebret *et al.* (2015), and in the ham semimembranosus muscle (SM). Measurements included IMF content, GP, ultimate pH (pHu, 24 h post mortem), lightness and hue angle. The semitendinosus muscle (STM) was completely excised from the LWC, LWA, BC and BA pigs of R1 (n = 40) and weighed. Since the STM is heterogeneous, with a deep red (RSTM) and a superficial white (WSTM) portion (Figure 1; Listrat *et al.,* 2016), the IMF content and pHu of both portions were measured. See Supplementary Material S1 for details of the methods.

Metabolic enzyme activities

Enzyme activities were measured in the LM and SM of all R1 and R2 pigs, and in the RSTM and WSTM of LWC, LWA, BC and BA pigs of R1. Within 40 min post mortem, muscle samples were taken from the LM (last rib level), SM (external part, 2 cm deep) and central portions of the RSTM and WSTM; cut into small pieces; promptly frozen in liquid nitrogen and stored at -80°C. 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 described by Lebret *et al.* (2002) and detailed in Supplementary Material S1.

Histological analyses

Muscle fibers were typed in the LM of all R1 and R2 pigs and in the RSTM and WSTM of LWC, LWA, BC and BA pigs of R1. From the samples taken to measure enzyme activities, sub-samples parallel to the muscle fiber axis were promptly frozen in isopentane cooled by liquid nitrogen and stored at -80°C until analysis, performed as described by Larzul *et al.* (1997) and detailed in Supplementary Material S1. Briefly, 10 µm thick transverse serial sections were cut in a cryostat (-20°C), mounted on glass slides and stained for acto-myosin ATPase after preincubation at pH 4.35 to distinguish contractile types I, IIA and IIB, or stained for succino-dehydrogenase to identify metabolic red oxidative (R) and white glycolytic (W) fibers. Combining both stains, fibers were classified as type I (slow-twitch oxidative), IIA (fast-twitch oxido-glycolytic), IIBR (fast-twitch oxido-glycolytic) or IIBW (fast-twitch glycolytic). From each sample, four fields containing ca. 250 fibers each were randomly chosen to determine the percentage, CSA and relative area of each fiber type using digital image analysis. The total number of fibers (TNF) in the RSTM and WSTM was determined by multiplying the mean number of fibers per unit area (calculated from three fields per sample using a projection microscope) by the transverse area of each portion (determined as described in Supplementary material S1).

Statistical analyses

Statistical Analysis System (SAS) software (version 9.4, 2013, SAS Institute, Cary, NC, USA) was used to analyze the data (details in Supplementary Material S1). Data for the ADFI and feed efficiency of LWA, LWC, BA and BC pigs, for which the pen was considered the statistical unit, were analyzed using ANOVA (GLM procedure), considering the treatment (4 levels) and replicate (2 levels) as fixed effects. Contrasts between breeds and between the A and C systems for a given breed were determined (Lebret *et al.,* 2014). For all other traits, the animal was considered the statistical unit. First, data were analyzed using ANOVA (GLM procedure), considering the treatment (LWC, LWA, BC, BA and BE) and replicate (R1 and R2) as fixed effects to calculate residues. The normality of residues was checked (Shapiro-Wilk test, $P \geq 0.05$). When necessary, data were log- or square-root transformed to obtain a normal distribution of residues, which were checked using the same test. Subsequently, contrasts between breeds were determined from the ANOVA using only the LWC, LWA, BC and BA pigs, to balance the production systems between breeds. Within each breed, contrasts between rearing systems were determined to evaluate effects of the A vs. C system for the LW breed, and the A vs. C and E vs. C systems for the B breed. Means were calculated by treatment. When residues could not be normalized, a non-parametric method (NPAR1WAY procedure, Kruskal-Wallis test) was used to determine effects of the breed and of the rearing system within a given breed; their medians were calculated by treatment.

Results

Growth performance and carcass traits

As previously reported in more detail (Lebret *et al.*, 2014), B pigs had lower ADG than LW pigs (Table 1). ADG did not differ between LWC and LWA pigs, but was higher for BA pigs and lower for BE pigs than for BC pigs. Consequently, B pigs, especially BE, were older than LW pigs at slaughter. LW pigs had higher ADFI and feed efficiency than B pigs. LWA pigs had similar ADFI as, but lower feed efficiency than, LWC pigs, while BA pigs had higher ADFI than, but similar feed efficiency as, BC pigs. B pigs had fatter carcasses, with higher back fat and lower loin proportions, than LW pigs. Compared to the C system, the A system increased the loin proportion in LW pigs, but did not influence proportions of carcass cuts of B pigs. In contrast, the E system decreased the carcass fatness of B pigs.

Biochemical and quality traits of longissimus and semimembranosus muscles

B pigs had higher IMF content in both the LM and SM than LW pigs (Table 2). No significant differences in IMF content between A and C systems were observed within each breed, or between E and C systems within the B breed. B pigs had higher pHu and lower GP, lightness and hue angle (indicating redder meat) in both muscles than LW pigs. Within each breed, the A vs. C system did not influence pHu, GP, lightness or hue angle. In contrast, BE pigs had higher pHu and lower lightness in both muscles than BC pigs. BE pigs had lower GP in the SM than BC pigs, but they did not differ in the LM.

Breed effects on metabolic enzyme activities differed between muscles (Table 3). B pigs had lower CS and HAD activities in the LM than LW pigs, while the opposite was observed in the SM. LDH activity in the LM did not differ between breeds but was lower in the SM of B than LW pigs. Within each breed, compared to the C system, the A system increased CS activity in both muscles and HAD activity in the SM of LW pigs, but did not influence LDH activity in either muscle. BE pigs had higher CS and HAD activities than, but similar LDH activity as, BC pigs in the LM. In contrast, BE pigs had lower LDH activity than, but similar CS and HAD activities as, BC pigs in the SM.

Histological properties of the longissimus muscle

B pigs had a lower percentage of IIA fibers and a higher percentage of IIBW fibers in the LM than LW pigs, but they did not differ in the percentages of I and IIBR fibers (Table 4). B pigs had lower mean fiber CSA than LW pigs, but differences varied among fiber types. B pigs had smaller IIBW fibers and larger I fibers than, and similar CSA of IIA and IIBR fibers as, LW pigs. Only the relative area of I fibers was higher in B pigs than in LW pigs. The influence of the A vs. C system on fiber types varied by breed. LWA pigs had higher IIBR and lower IIBW percentages than LWC pigs, while BA pigs had a higher IIA percentage than BC pigs. The A vs. C system did not influence fiber CSA in either breed, but resulted in a lower relative area of IIBW fibers in LW pigs and a higher relative area of IIA fibers in B pigs. Compared to BC pigs, BE pigs had a larger CSA of I fibers and a higher percentage and larger relative area of IIA fibers at the expense of IIBW fibers.

Biochemical, quality traits and histological characteristics of the semitendinosus muscle

B pigs had a lower proportion of STM in the half-carcass than LW pigs, but the proportion of STM did not differ between A and C systems within each breed (Table 5). B pigs had higher IMF content (Table 5) and visual marbling (Figure 1) in both the RSTM and WSTM than LW pigs, especially in the WSTM. B pigs had higher pHu in both STM portions than LW pigs, with generally higher pHu in the RSTM than in the WSTM. Breed did not influence CS or HAD activities in the RSTM or WSTM, but B pigs had higher LDH activity in the RSTM than LW pigs. Regardless of the breed, the A and C systems did not differ in enzyme activities, IMF content or pHu in the RSTM or WSTM.

Fiber type composition of the semitendinosus muscle

B pigs had 16% smaller TNF in the STM than LW pigs, exclusively due to a smaller TNF in the WSTM (Table 6). TNF did not differ between the A and C systems in either breed. Breed differences in fiber types varied by STM portion. B pigs had a lower percentage of I fibers in the RSTM than LW pigs but higher percentages of IIBR and IIBW fibers. In contrast, B pigs had a higher percentage of I fibers in the WSTM than LW pigs but similar percentages of IIA, IIBR and IIBW fibers. B pigs had smaller mean fiber CSA in the RSTM and WSTM than LW pigs, due to a smaller CSA of all fiber types in the RSTM, but only of IIB fibers in the WSTM. Compared to LW pigs, B pigs had a larger relative area of IIBR at the expense of IIA fibers in the RSTM, and of I fibers at the expense of IIB fibers in the WSTM. No effect of the A vs. C system was observed on fiber type percentages in either the STM portion or breed. The A system resulted in smaller CSA of all fiber types in the RSTM of LW pigs, with no effect in B pigs. Fiber CSA in the WSTM did not differ between A and C systems regardless of fiber type or breed. Overall, relative areas of fiber types did not differ between A and C systems in the RSTM or WSTM.

Discussion

Unlike the local B breed, which has not experienced genetic selection, the LW breed has been selected for a high growth rate, feed efficiency and carcass leanness, which likely indirectly resulted in selecting animals with a larger adult body size. Consequently, at a given BW, B pigs are older and fatter (Lebret *et al.,* 2014), and closer to their adult size, meaning that they are physiologically more mature than LW pigs. Muscle mass depends on the TNF, CSA and length of fibers. Since the TNF in pigs is established by the end of the fetal period (review by Lefaucheur, 2010), post-natal muscles grow exclusively by increasing both the CSA and length of fibers. In our study, the lower muscle mass of B pigs than of LW pigs was associated with both smaller TNF and mean fiber CSA, indicating that the smaller TNF in B pigs was not compensated by larger fiber CSA at the same slaughter weight. Similarly, smaller TNF and fiber CSA were reported in Meishan, a Chinese breed with low muscle growth potential, than in the LW breed (Lefaucheur *et al.*, 2004). Gil *et al.* (2008) made the same observation when comparing other local pig breeds to selected pig breeds. Smaller CSA of IIB fibers and larger relative area of I fibers have been associated with higher pork quality, especially water holding capacity and tenderness (Lefaucheur, 2010). These histological properties observed in the LM of B pigs but not LW pigs may contribute to the higher eating quality of B pork (Lebret *et al.*, 2015). Within each breed, the lack of difference in the mean fiber CSA in the LM and WSTM between the A and C systems agrees with the systems' relatively weak effects on body composition. Similarly, the mean CSA in the LM and loin proportion in B pigs did not differ between the E and C systems. However, the smaller CSA of all fiber types in the RSTM of LWA pigs than of LWC pigs was not expected and requires further study. Unfortunately, the STM characteristics of BE pigs could not be assessed in the present study.

Pork quality traits are influenced by genetic factors, environmental factors and slaughter conditions (Rosenvold and Andersen, 2003), and higher quality is generally reported in local rather than “conventional” selected pig breeds (review by Bonneau and Lebret, 2010). The LM and SM of B pigs had lower GP, higher pHu and IMF content, lower lightness and a redder color than those of LW pigs, which explains their higher eating and technological quality (Lebret *et al.*, 2015). Based on descriptions of differences in muscle metabolism between wild or non-selected pigs and selected pigs (Wimmers *et al.*, 2008; Lefaucheur, 2010), B pigs were expected to have lower glycolytic and/or higher oxidative metabolism than LW pigs. These differences were observed in the SM, in which B pigs had higher CS and HAD activities and lower LDH activity than LW pigs, which is consistent with their lower GP, higher pHu and redder meat. In contrast, the LM of B pigs had less oxidative metabolism, probably due to their older age and greater maturity at a given BW, since fast-oxido-glycolytic fibers in the LM are converted into fast-glycolytic fibers as pigs age (Lefaucheur and Vigneron, 1986). Because type I fibers have the lowest glycogen content (Lefaucheur, 2010), their larger relative area in the LM of B pigs than in LW pigs can partly explain their lower GP. The redder meat in the LM and SM of B pigs could be due to their higher muscle myoglobin content with age (Mayoral *et al.*, 1999). The percentage of I fibers was similar in the LM of B and LW pigs, in agreement with lack of difference for this trait between Meishan and LW pigs (Lefaucheur *et al.*, 2004). However, other studies reported a higher percentage of I fibers in the LM of wild pigs or other local breeds (Ruusunen and Puolanne, 2004; Wimmers *et al.*, 2008). These discrepancies could be explained by differences in physical exercise, climatic conditions, BW, age and physiological maturity between the animals in specific studies. As in the LM and SM, B pigs had higher pHu in the

RSTM and WSTM than LW pigs. This difference was associated with a higher percentage of I fibers in the WSTM, and, surprisingly, with a higher glycolytic metabolism in the RSTM of B pigs than LW pigs, suggesting that different portions of the same muscle can be influenced differently by breed. The relative areas of the two STM portions may also have differed between breeds, but they could not be studied due to the difficulty in delineating the portions (Figure 1).

Overall, breed influenced the biological properties of muscle and pork quality traits more than the rearing system. Within each breed, the A and C systems did not differ in the IMF content, GP, pHu, lightness or hue angle in the LM and SM, in agreement with studies that showed no consistent effect of indoor bedding or free access to a small outdoor area on these traits (Lebret *et al.*, 2002; Millet *et al.*, 2005). Although the rearing system yielded similar quality traits, it influenced the muscle metabolism of LW pigs, with higher oxidative metabolism in the LM and SM of LWA pigs than of LWC pigs. These results agree with the shift from fast glycolytic to fast oxido-glycolytic fibers in the LM of pigs offered access to an outdoor area (Lebret *et al.*, 2002) and in the SM of free-range pigs during winter, unlike those of pigs housed indoors (Gentry *et al.*, 2004). No such effects were observed in the LM and SM of B pigs, or in the RSTM and WSTM of either breed, suggesting greater robustness of muscle properties of B pigs to environmental factors. Comparing winter free-range to indoor housing of Swiss LW pigs, Bee *et al.* (2004) also observed higher oxidative metabolism in the LM but no differences in the RSTM or WSTM. Petersen *et al.* (1998) observed higher oxidative metabolism in the STM of pigs physically trained or reared in large pens, but they did not distinguish the RSTM and WSTM portions. Both physical exercise and cold exposure, like for pigs in the A system (Lebret *et al.*, 2014), have been shown to increase muscle oxidative metabolism (Lefaucheur, 2010). In the present study, however, they were not sufficient to modify the metabolic or histological traits of the RSTM or WSTM in either breed.

Among the rearing systems considered, the E system of the Basque pork chain induced the strongest effects on muscle and pork traits. The higher pHu and redness and lower lightness of the LM and SM of BE pigs than of BC pigs agree with the higher oxidative metabolism in the LM and lower glycolytic metabolism in the SM. These results are consistent with the increased IIA:IIB fiber ratio found in the LM of free-range pigs (Petersen *et al.*, 1998; Bee *et al.*, 2004; Gentry *et al.*, 2004). Because BE pigs were older, have more physical activity and encounter colder temperatures, higher oxidative capacity was also expected in their SM, which is involved in locomotion, but this increase was not observed. The redder meat of BE pigs would thus be more likely explained by their greater age at slaughter, which induces higher myoglobin content (Mayoral *et al.*, 1999). The higher pHu in BE pigs than in BC pigs was logically accompanied by a lower GP in the SM, but not in the LM, confirming that muscle properties other than GP determine the pHu (Scheffler *et al.*, 2013).

Unlike our results, extensive pig rearing during winter has been shown to increase GP, especially in muscles involved in movement, such as the SM (Lebret, 2008). This difference may be due to higher pre-slaughter physical activity of BE pigs than BC pigs, despite the high standardization of pre-slaughter handling in the experiment (Lebret *et al.*, 2015).

B pigs had a higher IMF content in all muscles, regardless of their contractile and metabolic profiles, than LW pigs, in agreement with the positive correlation between the IMF content in different muscles in pigs (Quintanilla *et al.*, 2011). Interestingly, the IMF content in both breeds was higher in the glycolytic WSTM than in the oxidative RSTM, with stronger breed differences in the WSTM. Differences in IMF content between Duroc, a breed with high IMF content, and the LW breed were also stronger in the LM than in the more oxidative psoas major muscle (Wood *et al.*, 2004). Assessing relationships between IMF content and metabolic enzyme activities, the percentage of type I fibers or mean CSA in the LM, WSTM and RSTM, revealed no significant correlation between IMF content and these traits, either between or within muscles (Figure 2). Similarly, IMF content has been shown to be genetically unrelated to fiber type composition in the LM (Larzul *et al.*, 1997), suggesting that IMF content and muscular contractile and metabolic characteristics are unrelated traits that can be manipulated separately by breeding or rearing factors.

Conclusion

Overall, effects of breed on pork quality and muscle biochemical and histological characteristics were greater than those of the rearing system, among which the E system induced the greatest changes. Both smaller TNF and CSA of glycolytic fibers were associated with the lower muscle mass of B pigs than of LW pigs. In all muscles, B pigs had higher IMF content and pHu and smaller mean fiber CSA than LW pigs, which likely explains the former's higher pork quality. The expected shift towards a less glycolytic and more oxidative muscle metabolism in B pigs than in LW pigs was observed in the SM and, to a lesser extent, the WSTM, but not in the LM or RSTM, which were more oxidative in LW pigs. Interestingly, the glycolytic WSTM contained more IMF than the oxidative RSTM, especially in B pigs, supporting the independence between IMF content and contractile and metabolic muscle properties.

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Declaration of interest

None.

Ethics statement

All experimental procedures complied with European Union (Directive 86/609/CEE) and French legislation (Décret no. 2001-464 29/05/01). INRA UEPR held the pig experimentation agreement C-35–275–32. All technical and scientific staff involved in the experiment had an individual agreement for experimenting on living animals, provided by the Veterinary Services of the French Ministry of Agriculture.

Software and data repository resources

None of the datasets were deposited in an official repository.

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Table 1. Growth performance and carcass traits by pig breed (Large White, LW; Basque, B) and rearing system (Conventional, C; Alternative, A; Extensive, E) (Lebret *et al.,* 2014)

	Treatment: breed × rearing system[1]						Significance[2]			
							Breed	Rearing system within breed		
	LWC	LWA	BC	BA	BE	RMSE	B vs. LW	LW A vs. C	B A vs. C	B E vs. C
N of pigs	20	19	20	20	20					
N of pens	2	2	2	2	2					
Initial live weight (kg)[3]	38.5	37.9	34.8	35.3	35.7	5.55	0.017	0.71	0.81	0.64
Initial age (d)[4]	85	85	106	106	106		<0.001	0.79	0.87	0.91
Final live weight (kg)[3]	148.0	144.8	139.9	146.3	141.8	8.81	0.10	0.24	0.024	0.50
Final age (d)[4]	228	230	320	312	423		<0.001	0.84	0.24	<0.001
ADG (g/d)[4,5]	772	755	498	544	335		<0.001	0.61	0.040	<0.001
ADFI (kg/d)[3,5]	2.88	2.88	2.39	2.67	-	0.047	0.002	0.99	0.009	-
Feed efficiency (kg/kg)[3,5]	0.27	0.25	0.21	0.20	-	0.003	<0.001	0.033	0.14	-
Hot carcass weight (kg)[3]	118.2	113.8	114.2	118.7	113.5	7.29	0.75	0.065	0.052	0.84

Loin (%)[3,6]	22.8	23.7	17.7	18.0	17.7	0.89	<0.001	0.002	0.34	0.84
Back fat (%)[3,6]	7.9	8.0	15.2	15.7	11.3	1.37	<0.001	0.68	0.45	<0.001

[1]Mean, or median when a non-parametric test was performed.

[2]*P*-values of contrasts between breeds (determined using A and C pigs of both breeds, i.e. n=39 LW and n=40 B) or rearing systems within each breed and RMSE obtained from ANOVA or *P*-values of a non-parametric test when data could not be normalized.

[3]ANOVA of raw data.

[4]Non-parametric test.

[5]ADG: Average daily gain; ADFI: Average daily feed intake; Feed efficiency: weight gain:feed intake

[6]Proportion of carcass right side weight.

Table 2. Biochemical characteristics and meat quality traits of the longissimus (LM) and semimembranosus (SM) muscles of pigs by breed (Large White, LW; Basque, B) and rearing system (Conventional, C; Alternative, A; Extensive, E)

	Treatment: breed × rearing system[1]						Significance[2]			
							Breed	Rearing system within breed		
								LW	B	B
	LWC	LWA	BC	BA	BE	RMSE	B vs. LW	A vs. C	A vs. C	E vs. C
N	20	19	20	19	20					
Intramuscular fat (%)[3]										
LM	2.32	2.14	3.79	4.07	3.28	0.117	<0.001	0.34	0.60	0.11
SM	2.21	1.98	3.91	4.00	3.80	0.126	<0.001	0.24	0.78	0.76
Glycolytic potential (µmol eq. lactate/g)										
LM[4]	164	173	136	138	136	18.9	<0.001	0.16	0.69	0.99
SM[5]	164	166	137	145	128		<0.001	0.86	0.57	0.015
pH 24 h[5]										
LM	5.47	5.48	5.58	5.54	5.67		<0.001	0.94	0.40	0.005
SM	5.50	5.48	5.55	5.59	5.75		<0.001	0.41	0.51	<0.001
Lightness[4]										
LM	53.6	53.8	51.2	51.6	48.1	2.74	<0.001	0.86	0.68	<0.001

SM	52.2	51.9	47.3	47.0	44.7	2.90	<0.001	0.80	0.76	0.006
Hue angle[4]										
LM	37.7	38.3	34.5	35.3	27.8	2.80	<0.001	0.53	0.36	<0.001
SM	36.1	35.7	29.6	29.2	24.8	2.50	<0.001	0.66	0.58	<0.001

[1]Mean, or median when a non-parametric test was performed.

[2]*P*-values of contrasts between breeds (determined using A and C pigs of both breeds, i.e. n=39 LW and n=39 B) or rearing systems within each breed and RMSE obtained from ANOVA or *P*-values of a non-parametric test when data could not be normalized.

[3]ANOVA of log values to fit a normal distribution.

[4]ANOVA of raw data.

[5]Non-parametric test.

Table 3. Metabolic enzyme activities[1] of lactate dehydrogenase (LDH), citrate synthase (CS) and β-hydroxy-acyl-Co-A dehydrogenase (HAD) in longissimus (LM) and semimembranosus (SM) muscles of pigs by breed (Large White, LW; Basque, B) and rearing system (Conventional, C; Alternative, A; Extensive, E)

	Treatment: breed × rearing system[2]					RMSE	Significance[3]			
							Breed	Rearing system within breed		
	LWC	LWA	BC	BA	BE		B vs. LW	LW A vs. C	B A vs. C	B E vs. C
N	20	19	20	20	20					
LDH										
LM[4]	2663	2645	2603	2605	2595	0.1	0.24	0.77	0.97	0.90
SM[4]	2608	2556	2321	2269	2191	0.1	<0.001	0.49	0.43	0.047
CS										
LM[4]	5.63	6.34	4.94	5.33	6.22	0.067	<0.001	0.025	0.14	<0.001
SM[4]	8.72	9.95	10.67	11.36	10.66	0.067	<0.001	0.010	0.21	0.98
HAD										
LM[4]	3.38	3.62	2.97	3.12	3.55	0.074	<0.001	0.23	0.37	0.002
SM[4]	4.77	5.35	6.04	6.42	6.26	0.071	<0.001	0.030	0.24	0.49

[1]Expressed as micromol of substrate per min per g of fresh muscle.

[2]Mean of treatment groups.

[3]*P*-values of contrasts between breeds (determined using A and C pigs of both breeds, i.e. n=39 LW and n=40 B) or rearing systems within each breed and RMSE obtained from ANOVA.

[4]ANOVA of log values to fit a normal distribution.

547 **Table 4.** Histochemical characteristics of longissimus muscle by pig breed (Large White, LW; Basque, B) and rearing

548 system (Conventional, C; Alternative, A; Extensive, E)

	Treatment: breed × rearing system[1]					Significance[2]				
							Breed	Rearing system within breed		
	LWC	LWA	BC	BA	BE	RMSE	B vs. LW	LW A vs. C	B A vs. C	B E vs. C
N	20	19	20	20	20					
Fiber percentage (%)[3, 4]										
I	8.7	10.1	9.0	8.0	10.1	3.04	0.20	0.15	0.30	0.28
IIA	7.8	8.8	4.6	6.7	6.6	2.18	<0.001	0.19	0.005	0.006
IIBR	5.7	7.4	6.1	6.3	6.6	2.66	0.54	0.045	0.86	0.56
IIBW	77.7	73.6	80.2	79.0	76.6	3.43	<0.001	<0.001	0.27	0.001
Cross-sectional area (μm^2)[3, 5]										
I	3026	2767	3161	3529	3586	0.1	0.002	0.17	0.085	0.049
IIA	2257	2122	2270	2583	2513	0.1	0.066	0.43	0.093	0.18
IIBR	3115	2863	2859	3168	2947	0.1	0.91	0.39	0.29	0.76
IIBW	5514	5300	4343	4565	4823	0.1	<0.001	0.50	0.40	0.076

Mean	4841	4500	4029	4238	4389	0.1	0.003	0.20	0.37	0.13
Relative area (%)[3, 6]										
I	5.1	6.0	6.8	6.5	8.0	0.39	0.017	0.17	0.61	0.087
IIA	3.6	3.9	2.5	4.0	3.6	0.32	0.055	0.43	<0.001	0.002
IIBR	3.5	4.6	4.2	4.5	4.4	0.53	0.52	0.12	0.63	0.81
IIBW	87.3	85.0	85.9	84.5	83.3	0.14	0.12	0.007	0.12	0.003

[1]Mean of treatment groups.

[2]*P*-values of contrasts between breeds (determined using A and C pigs of both breeds, i.e. n=39 LW and n=40 B) or rearing systems within each breed and RMSE obtained from ANOVA.

[3] Fibers I : slow-twitch oxidative, IIA : fast-twitch oxido-glycolytic, IIBR : fast-twitch oxido-glycolytic, IIBW : fast-twitch glycolytic.

[4]ANOVA of raw data.

[5]ANOVA of log values to fit a normal distribution.

[6]ANOVA of square root values to fit a normal distribution.

Table 5. Physicochemical characteristics and metabolic enzyme activities of lactate dehydrogenase (LDH), citrate synthase (CS) and β-hydroxy-acyl-CoA dehydrogenase (HAD) in the red (RSTM) and white (WSTM) portions of semitendinosus muscle (STM) by pig breed (Large White, LW; Basque, B) and rearing system (Conventional, C; Alternative, A; Extensive, E)

	Treatment: breed × rearing system[1]				RMSE	Significance[2]		
						Breed	Rearing system within breed	
	LWC	LWA	BC	BA		B vs. LW	LW A vs. C	B A vs. C
N	10	10	10	10				
STM (%)[3,4]	1.14	1.13	0.68	0.69	0.040	<0.001	0.71	0.93
RSTM								
Intramuscular fat (%)[4]	3.71	4.14	5.58	5.37	0.087	<0.001	0.23	0.67
pH 24 h[5]	5.85	5.70	5.91	6.08	0.198	0.001	0.089	0.069
LDH[4,6]	815	791	927	899	0.1	0.004	0.61	0.60
CS[4,6]	19.6	19.7	21.1	19.7	0.06	0.37	0.92	0.27
HAD[4,6]	14.6	15.2	15.7	14.4	0.07	0.86	0.57	0.24
WSTM								

Intramuscular fat (%)[4]	6.28	5.20	16.0	15.2	0.11	<0.001	0.10	0.66
pH 24 h[5]	5.55	5.49	5.75	5.72	0.083	<0.001	0.13	0.41
LDH[4,6]	1463	1365	1534	1421	0.1	0.18	0.13	0.097
CS[4,6]	5.45	5.55	6.16	5.37	0.093	0.51	0.86	0.16
HAD[4,6]	3.07	3.01	3.49	3.09	0.091	0.25	0.82	0.20

[1]Mean of treatment groups.

[2]*P*-values of contrasts between breeds or rearing systems within each breed and RMSE obtained from ANOVA.

[3]Proportion of right carcass side weight.

[4]ANOVA of log values to fit a normal distribution.

[5]ANOVA of raw data.

[6]Expressed as micromol of substrate per min per g of fresh muscle.

Table 6. Histochemical characteristics of the red (RSTM) and white (WSTM) portions of semitendinosus muscle by pig breed (Large White, LW; Basque, B) and rearing system (Conventional, C; Alternative, A)

	Treatment: breed × rearing system[1]					Significance[2]		
						Breed	Rearing system within breed	
	LWC	LWA	BC	BA	RMSE	B vs. LW	LW A vs. C	B A vs. C
N	10	10	10	10				
TNF (10^{-3})[3,4]	610	642	520	526	0.1	0.004	0.54	0.90
RSTM								
TNF (10^{-3})[3,4]	231	248	229	225	0.1	0.53	0.54	0.88
Fiber percentage (%)[5, 6]								
I	45.4	46.1	39.0	40.8	6.51	0.007	0.80	0.54
IIA	23.1	22.1	19.3	20.4	4.82	0.080	0.63	0.60
IIBR	10.7	9.8	17.5	14.2	6.87	0.013	0.77	0.33
IIBW	20.8	22.0	24.2	24.3	4.37	0.042	0.54	0.95
Cross-sectional area (μm^2)[4, 5]								
I	5719	4427	4391	4261	0.1	0.005	<0.001	0.67
IIA	4314	3848	3251	3186	0.1	<0.001	0.071	0.74
IIBR	5467	4516	3583	3651	0.1	<0.001	0.020	0.81
IIBW	7565	6284	4745	4746	0.1	<0.001	0.026	0.99
Mean	5457	4484	3940	3917	0.1	<0.001	0.003	0.93
Relative area (%)[5, 7]								
I	45.0	43.3	41.4	42.5	0.41	0.19	0.49	0.64
IIA	17.4	17.9	15.1	15.7	0.51	0.10	0.78	0.74
IIBR	10.0	7.8	14.2	12.3	0.91	0.028	0.38	0.51

IIBW	26.7	29.2	28.0	28.2	0.45	0.93	0.24	0.93
WSTM								
TNF (10^{-3})[3,4]	374	390	289	297	0.08	<0.001	0.61	0.74
Fiber percentage (%)[5, 6]								
I	1.5	1.3	4.1	4.7	2.03	<0.001	0.83	0.54
IIA	8.4	7.9	8.2	8.5	4.28	0.88	0.82	0.86
IIBR	12.1	14.5	12.0	12.2	4.75	0.42	0.27	0.92
IIBW	78.0	76.3	75.7	74.6	5.19	0.23	0.46	0.63
Cross-sectional area (μm^2)[4, 5]								
I	3526	3773	3412	3421	0.10	0.42	0.57	0.98
IIA	3532	3563	3617	3455	0.10	0.96	0.93	0.65
IIB[8]	4282	4254	3673	3592	0.07	0.003	0.93	0.76
Mean	4194	4195	3668	3569	0.07	0.004	0.99	0.69
Relative area (%)[5, 7]								
I	0.8	1.0	3.6	4.1	0.55	<0.001	0.76	0.63
IIA	6.4	5.9	7.9	8.1	0.74	0.14	0.77	0.92
IIB[8]	91.8	92.0	87.8	87.3	0.23	0.004	0.94	0.80

[1]Mean of treatment groups.

[2]*P*-values of contrasts differences between breeds or rearing systems within breed and RMSE obtained from ANOVA.

[3]Total number of muscle fibers.

[4]ANOVA of log values to fit a normal distribution.

[5] Fibers I : slow-twitch oxidative, IIA : fast-twitch oxido-glycolytic, IIBR : fast-twitch oxido-glycolytic, IIBW : fast-twitch glycolytic.

[6]ANOVA of raw data.

[7]ANOVA of square root values to fit a normal distribution.

[8]Cross-sectional areas of type IIBR and IIBW fibers could not be determined for technical reasons.

Figure 1. Mid-cross-trans sectional section of semitendinosus muscle (STM) from representative Large White (A) and Basque (B) pigs (adapted from Listrat *et al.,* 2016). R: red portion of STM; W: white portion of STM; IMF: intramuscular fat content (%). Scale bar: 2 cm.

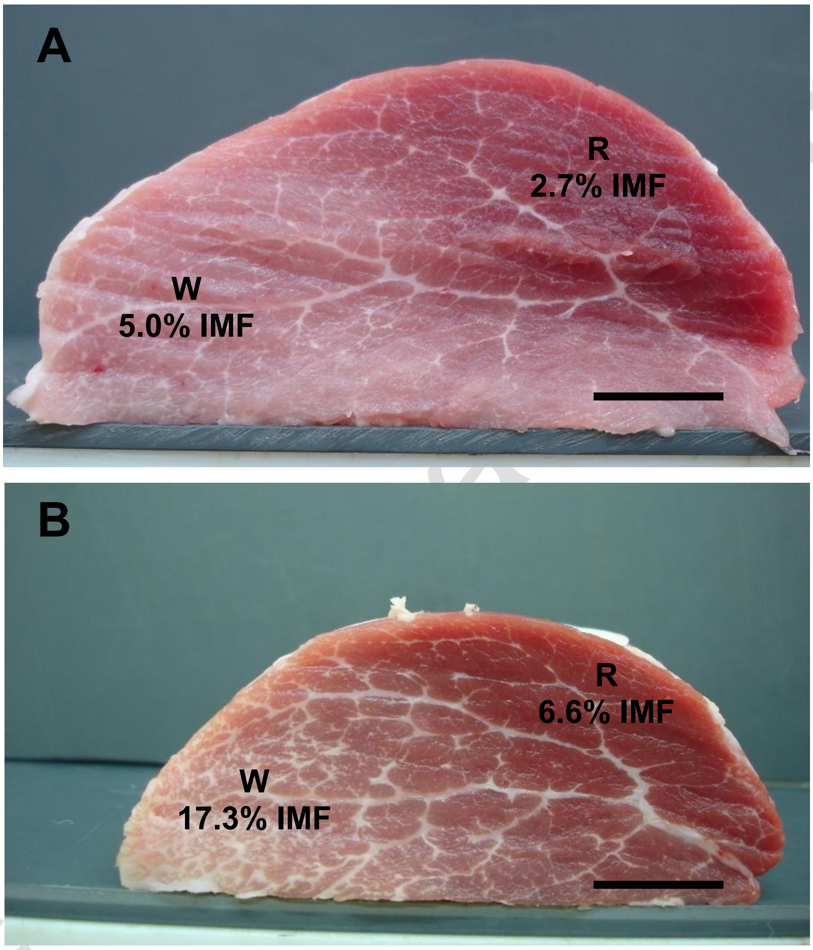

Figure 2. Co-variation of intramuscular fat (IMF) content and lactate dehydrogenase (LDH), citrate synthase (CS) activities, percentage of type I (slow-twich oxidative) fibers and mean fiber cross sectional area (CSA) in longissimus (LM) and red (RSTM) and white (WSTM) portions of semitendinosus muscles (STM) of Large White (LW) and Basque (B) pigs. Only pigs from the conventional (C) and alternative (A) rearing systems, represented by both breeds, are shown.