Effect of sire and sex on the intramuscular fatty acid profile and indices for enzyme activities in pigs

M. Ntawubizi, K. Raes, N. Buys, S. De Smet

Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium
Research Group EnBiChem, Department of Industrial Engineering and Technology, University College West-Flanders, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium
Department of Biosystems, KULeuven, Kasteelpark 30, 3001 Heverlee, Belgium

Article history:
Received 5 February 2008
Received in revised form 1 September 2008
Accepted 12 September 2008

Abstract

In this study, the effect of sire, sex and intramuscular fat content on the intramuscular fatty acid profile, in particular the long chain polyunsaturated fatty acids (PUFA) was investigated. Therefore, pork samples of the Longissimus thoracis were taken from 121 females and castrates that were the progeny of 5 boars. All animals had been fattened on the same diet and were slaughtered at a live weight of approximately 110 kg. Indices for the activities of Δ9, Δ6 and Δ5 desaturase, as well as elongase activity were estimated from ratios of product to precursor fatty acids. Intramuscular fat content was positively related to the total saturated fatty acid proportion \( r = 0.376; p < 0.01 \) and the total monounsaturated fatty acid proportion \( r = 0.579; p < 0.01 \), and inversely correlated with the total PUFA proportion \( r = -0.637; p < 0.01 \). A significantly higher index for Δ5 and Δ6 desaturase and elongase activity for PUFA metabolism was observed in females compared to castrate males. Sire had a significant effect on the intramuscular fatty acid profile, notably on the total \( n-3 \) PUFA, and on most individual long chain \( n-6 \) and \( n-3 \) PUFA. The \( \text{cis}-9 \) C18:1/\( \text{cis}-9 \) C16:1 elongase activity index, as well as the combined desaturase and elongase enzyme activities in both the \( n-6 \) and \( n-3 \) PUFA chains were significantly influenced by sire.

© 2008 Elsevier B.V. All rights reserved.

Keywords:
Fatty acids
Pork
Sex
Sire variance
Desaturase
Elongase

1. Introduction

Fatty acid (FA) composition in meat producing animals has, for many years, received considerable interest due to its implications for human health and for meat quality (Wood et al., 2004; Givens, 2005; Valsta et al., 2005). Many efforts have been made to improve the nutritional value and sensory quality of meat, by controlling the intramuscular fat (IMF) deposition and its FA composition. Particularly long chain (LC) polyunsaturated fatty acids (PUFA), the major constituents of intramuscular phospholipids, are nowadays the focus of much research as they are of particular relevance to human health. It is well established that the FA composition of pork is determined by genetic factors, e.g. breed, sex and genotype, and environmental factors of which diet is by far the most important one (Gatlin et al., 2002; De Smet et al., 2004; Raes et al., 2004).

Evidence for breed differences in FA composition and in the activities of several enzymes involved in fat synthesis and FA metabolism (malic enzyme, acetyl-CoA carboxylase and glucose-6-P dehydrogenase) has been cited in several studies (Cameron and Enser, 1991; Mourot et al., 1999; Van Laack and Spencer, 1999; Pérez-Enciso et al., 2000; Wood et al., 2004). Within-breed genetic variation in the proportion of the major FA in backfat and intramuscular fat is also considerable. Average heritabilities higher than 0.5 have been reported for the proportion of linoleic acid (LA, C18:2n – 6) and stearic acid (C18:0) in backfat (Sellier, 1998). Similarly, heritabilities ranging between 0.24 and 0.73 were found for intramuscular...
FA by Cameron and Enser (1991). Hereby, carcass leanness is inversely related to the proportion of saturated FA (SFA) and monounsaturated FA (MUFA) in subcutaneous and intramuscular fat, whereas it is positively related to the content of most PUFA (Malmfors et al., 1978; Cameron and Enser, 1991; Sellier, 1998). Interaction of genetic with dietary factors may further contribute to differences in intramuscular FA composition. An increasing PUFA proportion was reported in pigs, when fat deposition was reduced by restricted feeding compared to ad libitum feed intake (Wood, 1984; Cameron et al., 2000).

Gender is also known to affect fat deposition, e.g. castration is associated with increased fat deposition (Mersmann, 1984; Mourot et al., 1999; Peinado et al., 2008). Hence, some studies reported an effect of sex on the FA composition in pigs (Lebret and Mourot, 1998). Studies in humans have suggested that gender is also associated with differences in the capacity for conversion of α-linolenic acid (LNA, C18:3n−3) to the long chain n−3 FA eicosapentaenoic acid (EPA, C20:5n−3), docosapentaenoic acid (DPA, C22:5n−3) and docosahexaenoic acid (DHA, C22:6n−3) (Burdge and Wootten, 2002). The conversion of LNA to EPA, DPA and DHA in the n−3 PUFA series, and 1A to arachidonic acid (AA, C20:4n−6) and C22:4n−6 in the n−6 series is dependent on the sequential activities of Δ6 and Δ5 desaturase and on elongation of the carbon chain (Sprecher, 2000). There is competition for these enzyme activities between n−6 and n−3 PUFA, with a preference for the n−3 PUFA metabolism (Mohrhauser and Holman, 1963).

The effects of gender and genetic variation on the muscle LC PUFA metabolism of food producing farm animals have been studied to a lesser extent than influences on subcutaneous fat tissue. Therefore, the aim of this study was to investigate the effect of sex and sire differences on the intramuscular FA profile in slaughter pigs. Particular attention was paid to differences in the indices for enzyme activities (desaturases and elongases) involved in the n−3 and n−6 PUFA metabolism.

2. Materials and methods

2.1. Experimental design

Samples for this study originated from the progeny of 5 boars and 180 sows (Rattelow Seghers hybrid lines crossed for the purpose of a QTL-search experiment (Harmegnies et al., 2006). Female and castrate male piglets (n=1671) were all fattened on the same diet, and were slaughtered at a live weight of approximately 110 kg. A sub-sample of 121 animals was taken to analyse the intramuscular FA profile of the Longissimus thoracis. The animals were equally distributed according to sex (n=60 and 61 for castrated males and females respectively) and sire (n=26, 23, 25, 23 and 24 for the 5 boars respectively), and sexes were almost equally distributed within sires. The sub-sample was taken uniformly across the distribution of carcass lean content. The carcass lean content was determined with a reflectance probe as approved for use in Belgian abattoirs (CGM apparatus, Sydel, France). At 1 day post mortem, a sample of the L. thoracis (±10 cm width) at the height of the 3th/4th last rib was taken, vacuum packed and stored at −20 °C until analysis.

2.2. Fatty acid analyses

After homogenisation of the minced meat samples, the total lipids were extracted using chloroform/methanol (2/1, v/v), adapted from the method of Folch et al. (1957), and FA were methylated as described by Raes et al. (2001). The FA methyl esters (FAME) were analysed on a HP6890 gas chromatograph (Agilent, Brussels, Belgium), with a CP-Sil88 column for FAME (100 m×0.25 mm×0.2 μm; Chrompack, Middelburg, The Netherlands) using the following temperature program: 150 °C during 2 min and an increase of 1 °C/min to 200 °C, followed by an increase of 5 °C/min to 215 °C (Raes et al., 2001). Peaks were identified on the basis of their respective retention times, corresponding to their FAME standards (Sigma, Bornem, Belgium). All data are expressed as g/100 g of FAME. The IMF content (g/100 g meat) was determined by Soxhlet (ISO 1444–1973). The indices for the activities of Δ9, Δ6 and Δ5 desaturase, as well as the elongase activity, were estimated by the ratios of product to precursor fatty acids.

2.3. Statistical analysis

Animal performance and carcass data were analysed using a general linear model with sex (gilts and castrates) and sire (n=5) as fixed effects. FA data were analysed using a general linear model with sex and sire as fixed effects and IMF content as a covariate. IMF was included as a covariate to account for variation in FA composition due to differences in the level of fatness, hence to detect differences in intramuscular FA metabolism that are independent of the level of fatness. The sex×sire interaction term was never significant and was therefore not included in the model. Comparison of means between sires was done using the Bonferroni test (p<0.05). To assess relationships between IMF content and carcass traits, FA proportions or desaturase/elongase indices, Pearson correlation coefficients were calculated between IMF content and the residuals of a linear model with only sire and sex as fixed effects. The analyses were performed using S-Plus for Windows (version 6.0).

3. Results

The mean (RSD, residual standard deviation) cold carcass weight and carcass lean content was 86.5 (7.03) kg and 57.6 (4.84) % respectively (Table 1). Carcass yield was on average 79.0 (1.67) %. The mean Soxhlet IMF content was 1.31 (0.482) %.

3.1. Intramuscular FA profile

The effects of IMF content, sex and sire on the intramuscular FA profile are presented in Table 2. It is obvious that the IMF content had a highly significant effect on the FA composition (p<0.001), i.e. on all summed and on most individual FA proportions. Positive relationships between IMF content and SFA and MUFA and inverse relationships with PUFA were found. No effect of IMF was observed on the proportion of C18:0, C20:0, cis-11 C18:1 and LNA. A trend for a lower total SFA and MUFA proportion (p<0.1), and a significantly higher total PUFA, total n−6 PUFA and total n−3 PUFA (all p<0.05) proportion was observed in gilts.
compared to barrows. Sex had a significant effect on the EPA, DPA and DHA proportions with higher values for gilts compared to barrows (p<0.05). There was a significantly higher proportion of AA (p<0.01) and a trend for a higher proportion of LA (p<0.1) in gilts compared to barrows, but no effect of sex on other important FA proportions, i.e. C16:0, C18:0, cis-9 C18:1 and LNA was observed. Intramuscular FA profiles were also significantly affected by sex. Results showed a highly significant effect of sire on the total n−3 PUFA as well as on the individual n−3 PUFA LNA, EPA and DHA (p<0.01).

No effect of sire was observed on the total PUFA, n−6 PUFA, MUFA, and SFA proportions (p>0.05), however the individual long chain n−6 PUFA were significantly affected by sire (p<0.05).

3.2. Indices for Δ9 desaturase and elongase activity in MUSA muscle metabolism

Mean values for different indices of Δ9 desaturase and elongase activity in Longissimus muscle are given in Table 3. It is
clear that the Δ9 desaturase and elongase activity in Longissimus muscle was significantly related to the IMF content (p < 0.05). On the contrary, there was no effect of sex on these indices. A significant effect of sire was observed for the cis-9 C18:1/C18:0 Δ9 desaturase activity index (p < 0.01) and for the cis-11 C18:1/cis-9 C16:1 elongase activity index (p < 0.001).

3.3. Indices for desaturase and elongase activity in long chain PUFA muscle metabolism

Table 4 shows mean values of the indices for Δ5 desaturase, Δ5 desaturase and elongase activities as affected by the IMF content, sex and sire in the n−6 and n−3 PUFA metabolism. Results displayed a significant positive relationship of IMF with the n−6 PUFA Δ5 and a negative relationship with Δ5 desaturase activity index respectively. IMF had also an effect on the indices for the elongation of LA to C20:3n−6 (p < 0.01) and of AA to C22:4n−6 (p < 0.001), with opposite sign however. IMF was not related to the index for the elongation of EPA to DPA. On the other hand, IMF was significantly and consistently negatively related to all indices for the combined effects of desaturation and elongation in both the n−6 and n−3 PUFA pathways (p < 0.05).

Females seemed to present higher enzyme activity indices both in the n−6 and n−3 PUFA chains compared to castrate males, but the differences were mostly not significant except for the Δ5 desaturase index (p < 0.01) and the AA/LA index (p < 0.01). An effect of sire was observed for the Δ6 desaturase index in the n−6 series (p < 0.01) and the EPA to DPA elongase activity index (p < 0.001), as well as for all indices reflecting the combined effect of desaturase and elongase activities in both the n−6 and n−3 PUFA chains (p < 0.001).
4. Discussion

4.1. Intramuscular fatty acid profile

Since the dietary FA composition in the current study was similar for all animals, the observed variation in IMF content and intramuscular FA composition could be related to genetic variation in the expression or activity of the enzymes involved in fatty acid metabolism. Pig breed or genotype differences in the (long chain) PUFA profile have been reported earlier for both the neutral lipids and phospholipids fraction of muscle tissue (Van Laack and Spencer, 1999; Cameron et al., 2000; Wood et al., 2004). However, within-breed genetic variation in the muscle long chain PUFA composition and metabolism is much less documented, except for the study of Cameron and Enser (1991). Data on the intramuscular PUFA profile may allow detecting underlying differences in FA metabolism, but interpretation is hampered by confounding effects of variation in the IMF content.

Very similar to the results reported by Cameron and Enser (1991) for Duroc and Landrace pigs, the IMF content was in the present study positively related to the total and most of the individual SFA and MUFA proportions, while it showed a negative relationship with all PUFA proportions, except for LNA. Independent of possible underlying differences in FA metabolism, these relationships are partly a result of the unequal distribution of FA among triacylglycerols and phospholipids and the increasing proportion of triacylglycerols with increasing IMF content. In addition, pigs with a lower IMF content do have lower de novo fatty acid synthesis and, consequently, show greater relative incorporation of the strictly essential dietary fatty acid LA into their tissues. The absence of relationship between the IMF and LNA contents may be explained by the more equal distribution of LNA among triacylglycerols and phospholipids compared to the other PUFA (De Smet et al., 2004; Wood et al., 2004). In subcutaneous fat on the other hand, the LNA proportion was negatively related to carcass fat content similar to LA in the study of Wood et al. (1989).

Castration of piglets is responsible for increased fat deposition (Mersmann, 1984; Mourot et al., 1999; Peinado et al., 2008), and one might thus expect differences in FA composition due to sex. In the recent study of Peinado et al. (2008) both female and male castrates were compared to intact females. Backfat and intramuscular fat levels were similar for female and male castrates and were higher than for intact females. The LA proportion in subcutaneous fat in that study was significantly higher for the intact females compared to the female and male castrates, which has similar LA levels. This supports the hypothesis that sex and castration effects on FA composition are mainly the indirect result of differences in fat level. Unfortunately, these authors did not investigate the intramuscular FA composition. In line with our study, Zhang et al. (2007) also cited a higher IMF content and higher proportions of total SFA and MUFA in intramuscular fat in barrows than in gilts, and lower proportions of total n−6 PUFA. In contrast, Ramirez and Cava (2007) did not find a sex effect on subcutaneous and intramuscular FA composition, but it should be mentioned that there was also no difference in IMF content between the sexes. Interestingly in our study, the difference in total n−3 and n−6 PUFA between sexes was mainly due to the long chain PUFA, since the proportion of their precursors LA and LNA was hardly influenced by sex. This clearly suggests an additional effect of sex on the specific enzyme activities involved in the LC PUFA metabolism, independent of fat level.

In a review on genetics of carcass and meat quality, Sellier (1998) cited a high heritability of the intramuscular lipid content (r² around 0.50) and of subcutaneous fat firmness traits related to FA composition. In a single study, high heritabilities around 0.5 were found for the proportions of intramuscular fatty acids by Cameron and Enser (1991). The current study confirms that there is also considerable genetic variation for the proportion of most individual FA in muscle tissue, in view of the significant sire differences that were observed, particularly notable for the long chain PUFA. Sire had no effect on the total n−6 PUFA proportion and its main constituent LA, but the total and individual n−3 PUFA and the long chain n−6 PUFA were all affected by sire. Values for the most extreme sires differed approximately 1.5-fold for AA, LNA and EPA, and more than 2-fold for DHA. It should be mentioned that the highest proportions of total and individual PUFA were found for sire 4, that also had the lowest IMF content and highest carcass lean content. Since IMF was included as a covariate in our model, the sire effect on the FA proportions should be independent from the sire differences in IMF content. As mentioned before, the unequal distribution of most FA among intramuscular triacylglycerols and phospholipids may lead to false inferences about FA metabolism when based on interpretation of total intramuscular PUFA profiles. In future studies, it would be more appropriate to analyse FA profiles of separate intramuscular lipid fractions. Nevertheless, the size of the sire effect in this study after correction for IMF content demonstrates that there is considerable genetic variation in intramuscular long chain PUFA deposition and metabolism independent of the level of IMF content.

4.2. Δ9 desaturase and elongase activity in MUFA muscle metabolism

The Δ9 desaturase catalyses, in both microsomes and peroxisomes, the conversion of SFA in MUFA, e.g. C16:0 and C18:0 to cis-9 C16:1 and cis-9 C18:1 respectively (Sprecher, 2000; Siebert et al., 2003). The elongation of FA is catalyzed, predominantly in the endoplasmic reticulum membrane, both by a mitochondrial elongation system, using fatty acyl-CoA substrates in the range of C10–C14, and a microsomal elongase, acting on C16 and longer chain fatty acids. In the present study, a positive relationship between IMF content and Δ9 desaturase indices (cis-9 C16:1/C16:0, cis-9 C18:1/C18:0) was found on the one hand, and a negative relationship with the elongase activity index (cis-11 C18:1/cis-9 C16:1) on the other hand. No reports are available in the literature to our knowledge to compare these findings with.

No sex effect was found on enzyme activity indices involved in MUFA metabolism in the present study. Reports on sex differences in FA composition are inconsistent, probably partly due to confounding effects of e.g. age, dietary regime (Malau-Aduli et al., 2000; Gatlin et al., 2002) and castration (Monteiro et al., 2006). Malau-Aduli et al. (2000), in a beef cattle study, argued that sex-related hormones are a
possible cause of sex variation in FA metabolism. On the contrary, in pigs, Zhang et al. (2007) reported that the indices for Δ9 desaturase, thioesterase (on C14) and elongase activity did not differ between barrows and gilts. This is in line with our study.

The effect of sire on the different Δ9 desaturase activity indices was not consistent with only an effect on the cis-9 C18:1/C18:0 index. On the other hand, sire affected the cis-11 C18:1/cis-9 C16:1 elongase activity. Several reports are in line with these observations. Breed was reported to be a significant source of variation for both the thioesterase, stearoyl-CoA conversion of 20:3 and liver of female compared to male rats, suggesting that the effects of sire also significantly affected the long chain FA metabolism. On the other hand, sire affected the Δ9 desaturase activity indices was not consistent with only an effect on the Δ5 fatty acid desaturases or elongases are not available to our knowledge. In addition, it should be taken into account that muscle is not the major site of lipogenesis and that muscle fatty acid profiles are affected by uptake of fatty acids formed in other adipose tissues. Hence, the present study is only an approach and the outcome should be confirmed by measurements of enzyme activities or gene or protein expressions on a large number of animals. We are nevertheless convinced that the present interpretation of variation in muscle fatty acid profiles allows to conclude that there is considerable genetic variation in muscle long chain fatty acid metabolism.

4.3. Desaturase and elongase activity in long chain PUFA muscle metabolism

The Δ6 and Δ5 fatty acid desaturases and a malonyl-CoA-dependent chain elongase are critical enzymes in the pathway for the biosynthesis of the physiologically active PUFA, i.e. AA from LA and EPA and DHA from LNA (Bézard et al., 1994; Sprecher, 2000). In contrast to the absence of sex differences in the MUFA metabolism, females seemed to have a greater potential for the synthesis of long chain PUFA compared to barrows, although the differences were not consistently significant. The rather small differences compared to studies in humans that have shown a higher capacity of females to convert LNA to long chain n − 3 PUFA (Burdge and Wootton, 2002) might be due to the fact that the animals in the present study were sexually still immature, and that barrows were examined instead of entire males. In the current study, no sex effect was observed on either Δ6 desaturase nor on elongase activity and their combined activity in n − 6 PUFA pathway. Only Δ5 desaturase activity (C20:4 n − 6/C20:3 n − 6) was significantly higher in females, which also explained the significant difference for the overall index for AA synthesis (C20:4 n − 6/C18:2 n − 6) in Longissimus muscle. In line with these findings, after feeding gamma-linolenic acid to essential fatty acid depleted rats, Huang and Horrobin (1987) reported a smaller response in C20:3 n − 6 and a larger response in C20:4 n − 6 in plasma and liver of female compared to male rats, suggesting that the conversion of 20:3 n − 6 to 20:4 n − 6 was more active in female than in male rats.

In line with the sire differences in the PUFA proportions, sire also significantly affected the desaturase and elongase enzyme activity indices in both the n − 6 and n − 3 PUFA chains. IMF content was negatively related to these indices, meaning that animals with low IMF content would have higher potential for desaturation and elongation in the PUFA metabolism. As mentioned above, the sire effect was corrected for differences in IMF content. In view of the important role of the PUFA composition of muscle lipids, this genetic variation suggests there is potential for genetic selection on improved FA composition independent of IMF content warranting further investigation.

A weak point of the present study is that inference on variation in enzyme activities is based on ratios of fatty acids and not on measured activities or expression levels. In a feeding response study, Smith et al. (2002) concluded that, whereas the Δ9 desaturase activity index was not an indicator of absolute enzyme activity in subcutaneous adipose tissue, it reliably predicted the depression of enzyme activity in response to a dietary challenge. Doran et al. (2006) reported a significant relationship between the total amount of fatty acids and measured Δ9 desaturase activity in muscle tissue. Analogous comparisons for the correlation between measured activities and indices for Δ6 and Δ5 fatty acid desaturases or elongases are not available to our knowledge. In addition, it should be taken into account that muscle is not the major site of lipogenesis and that muscle fatty acid profiles are affected by uptake of fatty acids formed in other adipose tissues. Hence, the present study is only an approach and the outcome should be confirmed by measurements of enzyme activities or gene or protein expressions on a large number of animals. We are nevertheless convinced that the present interpretation of variation in muscle fatty acid profiles allows to conclude that there is considerable genetic variation in muscle long chain fatty acid metabolism.

5. Conclusion

Strong relationships between the IMF content and Δ9, Δ5, Δ6 desaturase and elongase activity indices in pork Longissimus muscle are apparent. Sex and sire had no or moderate effects on Δ9 desaturase and elongase activity indices in MUFA metabolism, but both animal factors significantly affected the long chain n − 6 and n − 3 PUFA metabolism. This warrants further investigation in the genetic determination of muscle long chain fatty acid metabolism.

References


