A DNA based test for evaluating and improving pork colour in Canadian pigs

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Project Team

The project team consists of members from Western Swine Testing Association (WSTA), Canadian Centre for Swine Improvement (CCSI), University of Guelph and Agriculture and Agri-Food Canada (AAFC) Lacombe Research Centre (LRC).

The project was led by WSTA. WSTA is a non-profit association based in Alberta providing swine testing and evaluation services in Western Canada. WSTA offers technician service or owner tester programs for commercial or seedstock swine operations, carcass evaluation programs and genetic evaluations. More information about WSTA is available at www.wsta.ca. WSTA co-ordinated the research activities with the producers and other project team members.

CCSI is a national non-profit organization created by the industry to provide leadership and services related to genetic improvement in Canada. Its members include Canadian Pork Council (CPC), Canadian Meat Council (CMC), Canadian Swine Breeders Association (CSBA) and regional swine improvement centers in Western Canada, Ontario, Quebec and Atlantic Canada. More information about CCSI is available at www.ccsi.ca. CCSI geneticists helped in the project design, analyses of the data and developed recommendations and guidelines as well as tools for effective use of the technology.

The Laboratory Services, University of Guelph is a multi-faceted analytical and diagnostic laboratory which serves a broad range of agri-food and veterinary clients within government, commercial and academic sectors on a fee-for-service basis. It is a state-of-the-art ISO 9001:2000 registered facility. The lab lab performed the DNA extraction and genotyping on all the animals of this project.

AAFC Research Centre, Lacombe is well known for the research work on meat quality traits. The centre conducted the meat quality evaluations for this project.

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ABSTRACT

Significant differences in consumer choice of pork chops are a factor that the pork industry should consider to be able to meet consumer demands. One of the most important characteristics of pork is the meat colour. Quantitative selection for meat colour, however, requires the sacrifice of the animals. Meat colour and genotype of SLC44A3 gene of 500 pigs consisting of Duroc, Landrace, Lacombe, Yorkshire and crossbreds were used in this study to examine the association of this gene with meat colour and change of colour from slaughter to retail. Significant differences ($p<0.01$) were found among breeds for subjective colour (NPPC), with Duroc (4.45±0.07) and Lacombe (4.49±0.09) having significantly darker loin colour compared to other breeds. Landrace (3.94±0.08) and crossbreds (3.98±0.06) had significantly paler loins, while Yorkshire (4.16±0.07) colour was intermediate. Females (4.25±0.05) averaged darker colour than castrates (4.16±0.06) but the difference was not quite significant at $p<0.05$. Thirteen single nucleotide polymorphisms (SNPs)were found in SLC44A3 gene of this sample of pigs. From the 13 SNPs, three of these were used in the analysis of SNP effects on pork colour. Other SNPs had very low frequency of the minor allele (less than 5%) or were totally confounded with one of these three SNPs. Four of the 13 SNPs are in the coding section of the gene and results in some amino acid changes. In addition to looking at the SNPs independently, the various combinations of these three SNPs showed that four haplotypes existed in this sample of pigs. Overall the minor allele frequencies were quite high at 35%, 45% and 39% for these 3 SNPs, respectively, but within a breed, some alleles had much lower frequencies. These small sub-class sizes limited the ability to accurately test for the gene effects and interactions with breed. Nevertheless, a significant association was found for one SNP with NPPC colour ($p<0.05$), and significant effect of haplotypes was also found within breeds ($p<0.05$). Loin reflectance estimated by Minolta L* at days one, four and seven post-mortem was also measured to evaluate the change of colour as pork ages from slaughter to retail. Change in L* was mainly observed between days one and four during storage in the dark. Significant difference between L* and also change in L* was observed among breeds ($p<0.05$) and gilts tended to retain dark colour more than barrows. Although SLC44A3 gene SNPs and haplotypes showed significant effect on NPPC colour, a larger number of samples is needed to be able to provide specific guidelines for the commercial application of this gene test. Breed and sex differences, however, could be exploited to help produce darker or lighter pork to match different market needs.

BACKGROUND INFORMATION

Visual characteristics are considered to be the main factors governing consumer choice of pork. In an international cross-cultural comparison of consumer preferences, Ngapo *et al.* (2007a) highlighted significant differences in consumer choice of pork chops based on four appearance characteristics (colour, amount of fat cover, marbling and drip loss). Among them, colour preferences were the most consistently chosen. More recently, a survey of consumer preferences by Canadian Pork International (CPI) has revealed that pork colour is one of the most important characteristics for gold markets such as Japan (CPI, 2006), where meat colour appears to be more important in purchasing fresh pork, before origin of pork and price. Pork colour was mentioned by 92% of surveyed Japanese female consumers asked to provide the three first criteria for their choice of pork. Pork quality attributes are quite highly correlated among each other. As an example, Huff-Lonergan *et al.* (2002) showed in an exhaustive study that darker meat had a greater propensity to be firmer, have less drip loss, be more tender, and have a higher ultimate pH. Darker meat colour is then favorably correlated with fresh and processed meat characteristics.

Meat quality traits including pork colour have been identified as a special group of traits where DNA information is especially useful for the Canadian swine industry (Mathur, 2003). So far, there has been limited use of selection on meat quality traits in Canadian swine mainly because the measurements are collected on slaughtered animals which are therefore not available for breeding.

Comparative mapping with human and pig genome has indicated that the SLC44A3 gene is associated with meat colour in Berkshire×Yorkshire crosses (Yu and Rothschild, 2006). This provides an opportunity for genetic evaluation of pork colour on live pigs using blood samples, tissues such as ear notch or even hair root. The authors found six Single Nucleotide Polymorphisms (SNPs) in SLC44A3 gene where the most polymorphic SNP was located on exon 14, within the *Nla* III restriction enzyme cutting position. After restriction enzyme digestions by *Nla* III, three different genotypes (11, 12 and 22) were compared in the three-generation resource family. The “22” genotype was associated with lighter colour, lower pH and more drip loss, indicating that allele 2 was undesirable for meat quality in the population under study. The frequency of allele 1 in the base Yorkshire population was 0.61. However, these results are based on an experimental trial and need to be verified on a larger base under Canadian conditions. If similar or larger effects are found in Canadian pigs, the gene can be useful for Canadian pork production.

An important aspect of the market research on meat quality shows that the preference for light or dark red colour depends on the country (Ngapo *et al.*, 2007b). This makes it very difficult to choose the breeding objectives for meat colour through quantitative selection. Yet, the potential use of SLC44A3 gene might provide an opportunity such that the pigs with the allele associated with dark colour could be marketed to one country while those pigs with the light allele are marketed to another country according to their preferences.

OBJECTIVES

The ability to adjust meat colour according to consumer preferences using a DNA test rather than sacrificing the pig can be highly valuable for the Canadian swine industry. The SLC44A3 gene has been found to be associated with meat colour in crosses between Berkshire and Yorkshire pigs in the United States (Yu and Rothschild, 2006). If this gene has similar effects in Duroc, Yorkshire, Lacombe and Landrace pigs used in Canada, the gene test could potentially be used by Canadian producers to select pigs and adapt the meat colour to the desired levels. For the reasons mentioned, the project had the following objectives:

1) Estimate the current levels of pork colour in Canadian pig breeds
2) Estimate the frequency of the potential SNPs within the SLC44A3 gene
3) Evaluate the effect of SLC44A3 gene on pork colour in Canadian pigs.

METHODS AND MATERIALS

Animals and meat samples

Representative pigs from major Canadian breeds, including Duroc, Landrace, Lacombe and Yorkshire were contributed by breeders and producers on the Canadian Swine Improvement Program. A total of 500 pigs were used in this project. The final data set included 95 Duroc, 46 Landrace, 43 Lacombe, 107 Yorkshire and 204 crossbred pigs (Table 1). Five animals were excluded because of inconsistencies in SNP genotypes. The pigs were slaughtered at the Olymel plant in Red Deer and carcasses cooled under blast chill conditions. The loin samples were extracted from the carcasses at 24 h post mortem and sent to the Lacombe Research Centre (LRC) for subjective and objective evaluation of meat colour. Meat tissue samples from the pigs were sent to the University of Guelph for genotyping.

Meat Colour

Meat colour was evaluated using the Minolta Colorimeter as well as subjectively using the NPPC and the Japanese colour score charts. More detail on the measurement is given in the report from Lacombe Research Centre in Appendix 1. Table 2 shows correlations among the various colour measurements that were taken and correlations of colour with other meat quality measurements. Associations with NPPC colour are reported in this analysis, but similar results were found for the Japanese colour, as can be expected given the high correlation between these two measures. Meat colour was also evaluated using the Minolta Colorimeter 1, 4 and 7 days post-mortem to assess change in colour from slaughter to retail. Meat was stored in the dark from days 1 to 4 and then in light from days 4 to 7. Results of these measurements are presented in Appendix 1. Effects of breed, sex and the SLC44A3 gene on NPPC colour were investigated.

Animal genotyping

All the genetic analyses were conducted in Dr. Shu Chen's Lab at the University of Guelph, a multifaceted analytical research laboratory dedicated to agriculture and livestock research. DNA was extracted from the pig tissue samples using ABI 6100 Nucleic Acid Prep Station with the NucPrep™ Chemistry (Applied Biosystems) or the Qiagen spin column method (Qiagen). The potential SNPs within the SLC44A3 gene were detected using the method of Yu and Rothschild (2006). Briefly, the target region was amplified using the GeneAmp PCR System 9700 (Applied Biosystems) with the primers 5'ACCAACCAACGACTGATG 3' (forward) and 5'GAATCTCCACACGGTCAA 3' (reverse). The PCR products were purified and then sequenced using ABI Prism® BigDye Terminator Cycle Sequencing Ready Reaction kit v3.1 (Applied Biosystems). Cycle sequencing was performed using a GeneAmp 9700 (Applied Biosystems). Dye terminator was removed using Performa V3 Plate (Edge BioSystems). The reactions were electrophoresed on an ABI 3730 or ABI 3100 automated DNA Analyzer (Applied Biosystems). The chromatograms were analyzed using ABI Prism® DNA Sequencing Analysis Software (Applied Biosystems). The SNPs were identified from high quality target sequences using ABI Prism® SeqScape® Software within the Software's clear confidence range. SNPs within the gene were documented for association analysis (Table 3).

Choosing SNPs for analysis

From 13 discovered SNPs on 500 genotyped animals (Table 3), seven SNPs with Minor Allele Frequency (MAF) of less than 5% were excluded from the analysis (SNPs 1, 2, 5, 7, 10, 12 and 13). The excluded SNPs were at base pair (bp) positions 122, 133, 314, 330, 389, 410 and 473 respectively. Looking at the frequency of SNPs within population, there were some inconsistencies in five records which were excluded from the data decreasing the number of records to 495. From the six chosen SNPs (3, 4, 6, 8, 9, and 11), SNPs 3, 4 and 6 at positions 215, 234 and 316, respectively, were found to have segregated together which would result in any one of them having the same solutions for the association study. Therefore SNPs 3 and 6 at positions 215 and 316 were also excluded from the analysis. The same was also seen between SNPs 8 and 11 at positions 381 and 396, and SNP 11 was also excluded from the analysis. The remaining SNPs were 4, 8 and 9 at bp positions 234, 381 and 386, respectively. The frequency of the remaining SNPs across and within breeds is shown in Table 4. Also shown in Table 4 are the frequencies of minor alleles (MAF) within different breeds. Since crossbred animals are a mixture of at least two breeds, MAF of crossbreds becomes closer to the population MAF which is expected to be higher than in individual breeds. In addition to individual SNPs, four different haplotypes were found in this sample of pigs. A Haplotype (*Haplo*id Geno*type*) (Zhao *et al.*, 2003), is the combination of two or more linked marker alleles on a chromosome (Zhao *et al.*, 2003; Schaid, 2004). Some studies have shown that haplotypes carry more information than do the SNPs that comprise them (Judson *et al.*, 2000; Judson and Stephens, 2001). In addition to looking at each SNP independently, the association of SLC44A3 gene haplotypes with meat colour was also investigated in this study. The distribution of haplotypes within breed is shown in Table 4.

Data analysis

The data was analyzed by CCSI using the genotyping data, meat colour data and pedigree records of the pigs tested. The meat colour traits studied were NPPC colour and change in L^* from day one to seven post-mortem. Details of the measurements are given in Appendix 1. The MIXED procedure of SAS software (2002-2003) was used for the analysis. Analysis was performed by studying effects of breed and sex, and the association of each single SNP and the effects of haplotypes.

Analysis of SLC44A3 gene SNPs in Purebred Animals. Three separate analyses were conducted for SNPs 4, 8 and 9. The model included contemporary group (defined as farm by slaughter date) as a random effect. Sex, breed, SNP and interaction between SNP and breed were included as fixed effects. Least Square Means (LSM) were used to show the magnitude of each genotype effect and if there were any differences among genotypes of a SNP.

Analysis of SLC44A3 gene SNPs in Crossbred Animals. The analysis of SNPs in crossbred animals was conducted on the same SNPs as in the analysis for purebreds. The model was the same as for the purebred analysis, except that the breed effect was replaced by breed percentage (as a covariate) and interaction of breed and SNPs was excluded from the model. For breed percentage, five variables were defined for the proportion of Duroc, Landrace, Lacombe, Yorkshire and unknown breed composition of each animal, as in Schenkel *et al.* 2006. The interaction between breeds and SNPs is not expected to be significant because crossbred animals represent a mixture of the purebreds that were studied.

Haplotype Analysis of SLC44A3 gene. Since markers of a haplotype should be linked together (Zhao *et al.*, 2003; Schaid, 2004), before defining the phase of haplotypes and using SNPs in a haplotype, the non-random association of SNPs, called the Linkage Disequilibrium (LD) was estimated. To avoid the dependency of LD measurement on allele frequency (Hayes, 2007), r^2 (Hill and Robertson, 1968) was measured between SNPs 4, 8 and 9. High levels of LD were observed between the SNPs of SLC44A3 gene from 0.26 between SNPs 4 and 9 to 0.37 between SNPs 4 and 8. The r^2 between SNPs 8 and 9 was 0.32. The observed r^2 values imply acceptable levels of LD between markers (Hill and Robertson, 1968) which can be used together in haplotype analysis. Haplotypes were defined using the PHASE program (Stephens, *et al.* 2001). To estimate the substitution effect of each haplotype, one variable was defined for each haplotype with values 0, 1 and 2 for animals having 0, 1 and 2 copies of each haplotype (Ciobanu, *et al.* 2004; Sharma, *et al.* 2006). The breed percentage was defined as explained above for the analysis of SNPs in crossbred animals. The model was the same as for the crossbred analysis described above except for the addition of an interaction between haplotype and breed percentage, since purebreds were also included with the crossbred data.

RESULTS AND DISCUSSION

Description of population

Table 1 shows the number of animals in different breed composition classes. The number of animals is higher for Duroc and Yorkshire (95 and 107, respectively) in comparison to Landrace and Lacombe (46 and 43, respectively). There were 204 observations of crossbred animals, and 58 of these had unknown breed composition. The crossbreds with known breed composition included various combinations of Yorkshire, Landrace and Duroc, but not Lacombe. There were relatively few observations in the individual breed composition classes for the crossbreds.

Breed and sex effects

Least Square Means of NPPC colour by breed and sex are shown in Figures 1 and 2, respectively. In purebred animals, Lacombe (4.49±0.09) and Duroc (4.45±0.07) had the darkest meat colour compared to other breeds. These two darker breeds were not significantly different from each other. Colour for these two breeds was significantly darker than Yorkshire, Landrace and crossbreds ($p<0.01$). Crossbreds, (3.98±0.06) and Landrace (3.94±0.08) had the palest colour but they were not significantly different from each other. Yorkshire (4.16±0.07) had an intermediate colour. It was significantly darker than Landrace and crossbreds and paler than Lacombe and Duroc ($p<0.01$). These differences are in agreement with an earlier study (CCSI, 2007) for Duroc, Yorkshire and Landrace, but that study did not report on Lacombe or crossbreds. Females (4.25±0.05) had on average darker colour than castrates (4.16±0.06), but this difference was not significant. Although not significant in this study, the tendency is consistent with CCSI (2007) which showed loin colour of barrows was significantly lighter than that of gilts and boars.

SNP and haplotype frequencies

Table 4 shows the frequency of each genotype and minor allele for SNPs 4, 8 and 9. SNPs 4, 8 and 9 showed higher frequencies of alleles T, A and T, respectively in Duroc. In all three SNPs, alleles that showed higher frequency in Duroc showed lower frequency in the other three breeds. Crossbreds showed the same direction as Duroc for SNPs 4 and 8 and opposite direction for SNP 9. The minor alleles in Duroc were not the same as in Lacombe, even though both have darker meat (Figure 1). One possible explanation for this could be that other genes affecting colour are having a larger effect than this gene. Another possible explanation could be that these SNPs are actually markers for another QTL affecting colour, and the linkage in Lacombes is opposite to that in Duroc.

Table 4 also shows the number of animals with each haplotype within different breeds. All four haplotypes were found in each breed and the crossbreds, but with different haplotypes having relatively low frequency within individual breeds. The small numbers in many subclasses within breed limit the ability to detect specific haplotype

effects in this data set. Nevertheless, the fact that all four haplotypes are found in each breed presents an opportunity to make use of this genetic variability if there is a true association with meat colour. It is interesting to note the relatively high frequency of haplotypes in each breed in relation to the average colour for the breed. In particular, the Duroc and Lacombe have similar meat colour but very different frequencies of the different haplotypes. Possible reasons for this are the same as mentioned above for the differences in individual SNP frequencies.

Analysis of SLC44A3 gene SNPs in Purebred Animals

The interaction between breed and SNP was found to be significant for SNP 9. SNPs 4 and 8 were not significant either as an interaction with breed or as a main effect. A significant difference between LSMs of genotypes for SNP 9 was shown in Landrace pigs, with a significant increase in NPPC colour for the TT genotype compared to CC and CT genotypes (Table 5). Possibly the T allele of SNP 9 is a recessive causing an increase of NPPC colour. However, it should be noted that there were relatively few Landrace pigs with CT and TT genotypes. Other breeds tended to show a lower adjusted mean (lighter NPPC colour) for the TT genotype of SNP 9. This is opposite to the effect found for Landrace, but none of those LSMs were significantly different from each other. Given the small number of Landrace swine having CT and TT genotypes (Table 4), further study is necessary to verify this significance. Genotyping more animals could potentially resolve any uncertainty related to the effect of this SNP.

If the TT genotype is associated with a significant increase in the NPPC colour, we might expect to see a lower frequency of TT genotypes in breeds having lighter colour (Figure 1). The observed frequency of TT genotypes was 9% in Landrace which has the lowest NPPC colour LSM value. There is also a relatively high frequency of TT genotypes in Duroc (56%) which is a breed with darker meat colour. The lower frequency of the TT genotype in breeds with lighter meat colour, and higher frequency of TT genotype in breeds with dark colour, provides indirect evidence supporting a darkening of meat colour with this genotype. However, the Lacombe breed, which also has darker meat colour, had a low frequency of the TT genotype (only 1 of 43 Lacombes). Again, genotyping of more animals could potentially resolve the uncertainty regarding the effect of this SNP on meat colour.

Analysis of SLC44A3 gene SNPs in Crossbred Animals

No significant interaction was found between breed percentage and SNPs in crossbreds. This was expected since crossing of the breeds will mask interactions. The main effect of SNPs was not significant but an increase of 0.76 (±0.38) points in NPPC colour attributed to the TT genotype of SNP 9 was significant ($p<0.05$). This same trend was observed in the analysis of SNPs on purebred animals. Although different breed compositions were included in this analysis (Table 1), the number of observations for each breed composition was not large enough to reveal the effect of SLC44A3 gene in the crossbred animals. Fifty-eight animals with unknown breed composition (~12% of

animals) may have a confounding on the effect of breeds, interaction of breeds and genotype.

Haplotype Analysis of SLC44A3 gene

After defining the haplotypes of SLC44A3 gene, four different combinations were found in the population under study. Different frequency of haplotype alleles were found within and across the different breeds (Table 4 and Figure 3). Haplotypes were designated as A, B, C and D ranging from A as the most frequent to D as the least frequent haplotype. The analysis of haplotypes revealed a significant effect of haplotypes within different breeds on NPPC colour. Significant effects were found overall for haplotype effects but the sample sizes are too small to quantify individual effects between specific haplotypes within breeds.

The single SNP analysis of purebred animals showed that SNP 9 had a significant effect on NPPC colour. The genotype which was associated with darker colour was homozygous for the T allele. From the four observed haplotypes in the population (TAT, CGC, TAC, TGC), only haplotype A has a T allele in its third nucleotide base whereas the other three haplotypes have a C as their third base. Figure 1 shows the darkest colour was in Duroc and Lacombe and Figure 3 shows a high frequency of haplotype A in Duroc (0.74) but very low in Lacombe (0.03). Haplotype B had the highest frequency in Lacombe (0.63). Haplotype B has a G allele in the second base (TGC) and GG genotype is showing a trend in darkening the colour in Lacombe. Duroc and Lacombe both showed darker colour (Figure 1) while different haplotypes (haplotype A in Duroc and haplotype B in Lacombe) were associated with this effect. This difference suggests that there are likely other genes that may have an important effect on meat colour. Haplotype D was showing high frequency in Landrace (0.21), but with relatively few animals having this haplotype, no significant effects due to it were detected.

Changes in colour from slaughter to retail

Meat colour shortly after slaughter is an important characteristic, but an ability to maintain colour to the consumer point-of-purchase is also important. Figure 4 and Table 6 show the LSM of L* at one, four and seven days post-mortem in different pig breeds. In all the days, Lacombe and Duroc had the lowest LSM, which means the darkest colour. Landrace and crossbreds had lightest colour on all days, while Yorkshire remained intermediate. Figure 5 shows the change in colour over time. Most of the change occurred from days 1 to 4. There is some indication of breed differences with respect to these colour changes over time, but there are not enough data to quantify specific breed differences. Gilts, which tended to have darker meat colour to start, also showed a tendency to retain this darker colour more than barrows.

Search for causative mutations

The swine population studied by Yu and Rothschild (2006) was a Berkshire x Yorkshire F2 cross. The sequence of exon 14 of the SLC44A3 (meat colour gene) was

published by Yu and Rothschild on the National Center for Biotechnology Information (NCBI) database (www.ncbi.org). This sequence (Accession # DQ981511) was used as a reference to study the polymorphisms observed in different breeds in the present study. Based on the partial coding sequence (cds) from the reference, a comparison between the amino acid sequences from the reference to the sequences with the polymorphisms observed in our populations was undertaken. The coding sequence from exon 14 ranges from position 45 to 281. SNPs 1-4 at positions 122, 133, 215 and 234 are therefore included in the coding sequence but only two SNPs (3 & 4, at nucleotide positions 215 and 234) were included in the SNP analysis. The two others were excluded due to low MAF in the studied swine population.

Table 7 summarizes the findings of the observed polymorphisms in comparison to the reference sequence published on the NCBI database (Yu and Rothschild, 2006) and translated using a tool from ExPASy Proteomics Server at http://ca.expasy.org/. All swine for SNP 1 had a T at position 122 except for one animal. That animal had two alleles which were both different than the one observed in the rest of the population. Due to a possibility of an error, more swine should be studied to verify if the two other alleles do exist in the swine breeds as well as to eliminate uncertainty. At nucleotide position 133 (SNP 2), an A nucleotide in the coding sequence of the color gene would result in a stop codon being generated This is in contrast to the T nucleotide found in most of the population as well as in the reference sequence. A stop codon ends translation for the protein sequence in question. Due to the low MAF of the observed allele, further genotyping should be undertaken to verify the frequency of this allele in a larger sample of the swine populations.

When compared to the reference sequence by Yu and Rothschild (2006), one other nucleotide at position 215 was found (C). Since this mutation in the nucleotide sequence did not result in any change from the reference amino acid sequence it is considered a silent mutation. Interestingly, the nucleotide polymorphisms observed at SNP number 4 at bp position 234 code for changes in an amino acid (functional mutation). No A nucleotide, as observed in the reference population, was found in the populations under study. Instead, only C and T SNPs were found at position 234. Although further investigation is needed, possibly the A SNP at position 234 could be a Berkshire specific SNP. In the analysis, SNP 4 had similar DNA base change and effect as SNP 6 at position 316. SNP 6 did not show any significant effect on NPPC colour. Further investigation using larger number of genotyped animals might reveal more information about the possible effect of this mutation on NPPC colour variation. The effect of the amino acid substitution from SNP 4 on meat colour still needs to be investigated.

The association analysis shows that allele T in SNP 9 could potentially be a favourable allele for selection. This polymorphic site located at nucleotide position 386 of SLC44A3 gene was investigated to see if it was a causative mutation. SNP 9, when looking at the sequence, is not located in the referenced coding region of exon 14 of SLC44A3 gene. The entire SLC44A3 gene has yet to be completed sequenced, but has been mapped to *sus scrofa* chromosome 4. More studies on SNPs found in different

exons of the SLC44A3 gene and in the complete coding sequence of exon 14 should be studied.

CONCLUSIONS

The observed variation of loin meat colour one day post-mortem and changes in colour up to seven days post-mortem in different breeds and sexes provides the opportunity to select the desired breeds and sexes based on the market demands for meat colour. Having knowledge about the genes underlying meat colour variation can help to use Marker Assisted Selection (MAS) within breeds for optimal crossing to produce market hogs with regard to demand for different colours. Application of MAS can be especially useful for evaluation of Artificial Insemination (AI) boars which can produce many progeny.

This study found significant differences among breeds with regard to loin meat colour, with Duroc and Lacombe having darker meat. Yorkshire had intermediate colour, while Landrace and crossbreds had the lightest colour. There also appear to be significant differences among breeds in the change in colour from slaughter to retail, but more testing would be needed to confirm differences and quantify the effects on specific breeds and crosses. Females have shown a tendency to have darker meat and also to maintain this darkness more than barrows. Additional testing and further analysis could help to confirm and quantify the breed and sex effects, but these results are consistent with an earlier study on Canadian purebreds.

SLC44A3 gene has been recommended as one of the possible genes having significant effect on the colour of meat in pigs. After genotyping this gene on 500 animals, thirteen SNPs were discovered, of which three SNPs were useful to study the association of individual SNPs and haplotypes (combinations of SNPs) of this gene with meat colour. The other SNPs may also be of interest for further study, but were not specifically useful for this study due to very low frequency of the minor allele or because they were segregating with another SNP. The three SNPs and four distinct haplotypes were discovered to exist in all four breeds and the crossbreds that were sampled. This genetic variability is important for potential marker assisted selection using a DNA test for this gene.

Analysis of the effects of SNPs and haplotypes of the SLC44A3 gene showed a significant association with loin meat colour. There was a significant interaction found between breed and variations in the gene, and further study is required to better understand the nature of this interaction. Some of the subclasses of SNPs that were included in this study had relatively few observations, especially in Landrace and Lacombe, but also in certain breed crosses. More testing in these two breeds and targeted breed crosses could help to learn more about this gene. The highest frequencies of SNPs and haplotypes varied among the breeds, even when meat colour was similar for different breeds. This implies that there are very likely other genes that have a significant effect on meat colour. Tools are becoming available with the sequencing of the pig genome and

high density SNP panels which could help to capitalize on additional genetic variability for meat colour.

IMPLICATIONS OF PROJECT RESULTS FOR INDUSTRY

The results of this study have confirmed breed differences observed in earlier studies that showed Duroc producing a darker meat compared to other breeds, especially Landrace, with Yorkshire having an intermediate meat colour. This study also found that meat colour in Lacombes was similar to the Duroc. Crossbreds included in this study had relatively light colour, but a large proportion of them included Landrace and Yorkshire. None of the crossbreds included Lacombe. Targeted use of Duroc and Lacombe sire lines and tracking of these hogs in packing plants could help to produce pork for higher value markets requiring darker meat. The tendency for gilts to have darker meat and to retain this colour better than barrows could also be exploited to further enhance the darkness of pork loins when they reach the consumer. A field trial to confirm the use of breed and sex as tools to produce darker pork would be useful, and could be combined with collection of additional DNA samples for further genetic studies using emerging tools such as high density SNP panels.

The current amount of information might not be sufficient to provide a clear message for the application of a colour gene test examined in this study. The overall significance found for the effects of this gene suggest that additional genotyping would be worthwhile in order to quantify the usefulness of such a gene test. This could lead to additional tools and specific recommendations to help target meat colour to match different market needs. The results look promising but more animals genotyped for SLC44A3 and further study are required for a better understanding about the effect of this gene on pork colour and other important meat quality measurements.

LIST OF PUBLICATIONS AND PRESENTATIONS

CCSI. 2008. Variation of SNPs of the SLC44A3 gene in Canadian pig population. CCSI Genetics Committee presentation. October 23, 2008. Quebec City, QC, Canada.

CCSI. 2008. A Discussion and report on the polymorphism of the SLC44A3 gene in Canadian pig population. Swine Genetics Forum. October 24, 2008. Quebec City, QC, Canada.

CCSI and Lacombe Research Centre. 2009. A DNA based test for evaluating and improving pork colour in Canadian pigs. Western Swine Testing Association Meeting. January 19, 2009. Calgary, AB, Canada.

The 13 SNPs discovered in the SLC44A3 gene will be submitted to the NCBI database.

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TABLES

Table 1. Distribution of pigs by breed composition and sex

Breed Composition					Number of Pigs			Frequency (%)
Duroc	Lacombe	Landrace	Yorkshire	Unknown	Females	Castrates	Total	
100%					58	37	95	19.19
	100%				40	3	43	8.69
		100%			36	10	46	9.29
			100%		90	17	107	21.62
				100%	10	48	58	11.72
50%		50%			13	21	34	6.87
50%			50%		6	10	16	3.23
		50%	50%		61	11	72	14.55
50%		25%	25%		5	7	12	2.42
		25%	75%		0	1	1	0.20
75%				25%	3	3	6	1.21
25%		50%		25%	3	2	5	1.01
Total					325	170	495	100

Table 2. Correlations among various meat colour and other meat quality measurements.

	Japanese Colour	Minolta L*	Minolta a*	Minolta b*	Minolta C	Minolta h
NPPC Colour	0.82	-0.61	0.40	-0.32	0.03	-0.52
	<.0001	<.0001	<.0001	<.0001	0.55	<.0001
Japanese Colour		-0.67	0.36	-0.34	0.01	-0.52
		<.0001	<.0001	<.0001	0.82	<.0001
Minolta L*			-0.27	0.69	0.31	0.77
			<.0001	<.0001	<.0001	<.0001
Minolta a*				0.09	0.64	-0.47
				0.05	<.0001	<.0001
Minolta b*					0.79	0.82
					<.0001	<.0001
Minolta C						0.34
						<.0001

	pH_{24}	Drip Loss (4d)	Drip Loss (7d)	Marbling	IMF (%)
NPPC Colour	0.49	-0.36	-0.37	0.32	0.04
	<.0001	<.0001	<.0001	<.0001	0.73
Japanese Colour	0.50	-0.40	-0.41	0.29	-0.01
	<.0001	<.0001	<.0001	<.0001	0.92
pH_{24}		-0.61	-0.62	0.26	0.12
		<.0001	<.0001	<.0001	0.25
Drip Loss (4d) (%)			0.80	-0.14	-0.02
			<.0001	0.002	0.84
Drip Loss (7d) (%)				-0.13	0.08
				0.004	0.44
Marbling (1-10)					0.88
					<.0001

	Fat Firmness	Lean Firmness
NPPC Colour	0.04	0.33
	0.36	<.0001
Japanese Colour	0.03	0.30
	0.44	<.0001
Fat Firmness		0.22
		<.0001

Table 3. Distribution of identified SNP alleles within the SLC44A3 gene

SNP	Base pair position	Number of pigs by genotype			MAF[1] (%)
1	122	?	AG (1)[2]	TT (499)	?
2	133	AA(0)	AT (1)	TT (499)	0.10
3	215	CC (232)	CT (184)	TT (84)	35.20
4	234	CC (84)	CT (184)	TT (232)	35.20
5	314	CC (499)	CT (1)	TT(0)	0.10
6	316	CC (232)	CT (184)	TT (84)	35.20
7	330	CC(0)	CG (3)	GG (497)	0.30
8	381	AA (171)	AG (207)	GG (122)	44.90
9	386	CC (216)	CT (182)	TT (102)	38.60
10	389	CC (499)	CT (1)	TT(0)	0.10
11	396	CC (123)	CT (206)	TT (171)	45.20
12	410	CC(497)	CG (1)	GG (2)	0.50
13	473	AA (2)	AG (27)	GG (471)	3.10

[1] MAF: Minor Allele Frequency

[2] There were unexpectedly three different bases found in the first SNP: A, G and T. As there was only the one animal with the A and G bases, this SNP was excluded from further analysis.

Table 4. Frequency of selected SNP alleles of the SLC44A3 gene by breed

Number of Pigs with each Genotype										
SNP	4			8			9			
	CC	CT	TT	AA	AG	GG	CC	CT	TT	Total
Duroc	2	10	83	77	14	4	8	34	53	95
Landrace	17	14	15	7	11	28	33	9	4	46
Lacombe	14	26	3	3	22	18	41	1	1	43
Yorkshire	31	50	26	23	50	34	54	42	11	107
Crossbreds	18	82	104	60	109	35	76	96	32	204
Total	82	182	231	170	206	119	212	182	101	495

Minor Allele Frequencies (MAF)			
SNP	4	8	9
Duroc	7% C	12% G	26% C
Landrace	48% T	27% A	18% T
Lacombe	37% T	33% A	3% T
Yorkshire	29% T	44% A	39% T
Crossbreds	48% C	45% G	30% T

	Haplotypes[1]			
	A (TAT)	B (CGC)	C (TAC)	D (TGC)
Duroc	140	14	28	8
Landrace	17	48	8	19
Lacombe	3	54	25	4
Yorkshire	64	112	32	6
Crossbreds	160	118	69	61

[1] The letters in brackets indicate the bases at SNPs 4, 8 and 9, respectively for each of haplotypes A, B, C and D.

Table 5. Least square means (±standard errors) for NPPC colour score for three different SNPs within the SLC44A3 gene

	SNP 4[1]			Breed
	CC	CT	TT	Main effect
Duroc	4.25(0.30)	4.43(0.14)	4.48(0.06)	4.39(0.12)
Landrace	3.90(0.11)	3.73(0.12)	4.01(0.12)	3.88(0.08)
Lacombe	4.40(0.12)	4.54(0.10)	4.08(0.24)	4.34(0.11)
Yorkshire	4.21(0.09)	4.14(0.08)	4.04(0.09)	4.13(0.06)
SNP Main Effect	4.19(0.09)	4.21(0.07)	4.15(0.08)	

	SNP 8[1]			Breed
	AA	AG	GG	Main effect
Duroc	4.46(0.06)	4.45(0.12)	4.63(0.21)	4.52(0.09)
Landrace	3.95(0.16)	3.81(0.14)	3.90(0.09)	3.89(0.09)
Lacombe	4.07(0.24)	4.48(0.11)	4.52(0.11)	4.36(0.11)
Yorkshire	4.04(0.10)	4.16(0.08)	4.18(0.09)	4.13(0.06)
SNP Main Effect	4.13(0.09)	4.22(0.07)	4.31(0.08)	

	SNP 9[1]			Breed
	CC	CT	TT	Main effect
Duroc	4.63(0.15)	4.47(0.09)	4.43(0.07)	4.51(0.07)
Landrace	3.89(0.09)[a]	3.65(0.15)[a]	4.37(0.21)[b]	3.97(0.10)
Lacombe	4.48(0.08)	4.05(0.41)	3.99(0.41)	4.18(0.20)
Yorkshire	4.15(0.08)	4.19(0.08)	3.97(0.13)	4.10(0.07)
SNP Main Effect	4.29(0.06)	4.09(0.12)	4.19(0.13)	

[1] The overall p-values for the SNPs and breed interaction were 0.13, 0.71 and 0.03 for SNPs 4, 8 and 9, respectively.

[a, b] Different breeds of animals in each day with different superscripts are different from each other at $p<0.05$.

Table 6. Least Square Means of L^* (±standard errors) in days zero, one, four and seven in different breeds[1]

	Duroc	Landrace	Lacombe	Yorkshire	Crossbreds
Day 1	48.22(0.33)^{a}	49.71(0.40)^{bcd}	47.84(0.45)^{a}	49.02(0.32)^{b}	49.48(0.30)^{c}
Day 4	52.34(0.29)	52.86(0.35)^{b}	51.75(0.39)^{a}	52.62(0.28)^{b}	52.95(0.27)^{bc}
Day 7	52.59(0.32)^{cd}	53.33(0.39)^{d}	52.35(0.43)^{a}	53.18(0.32)^{bcd}	53.39(0.30)^{bd}

$^{a, b, c, d}$ Different superscripts within a row indicate significant differences at $p < 0.05$

Table 7. Comparison of nucleotide and amino acid sequence of reference (Yu and Rothschild, 2006) to SNPs observed in this study

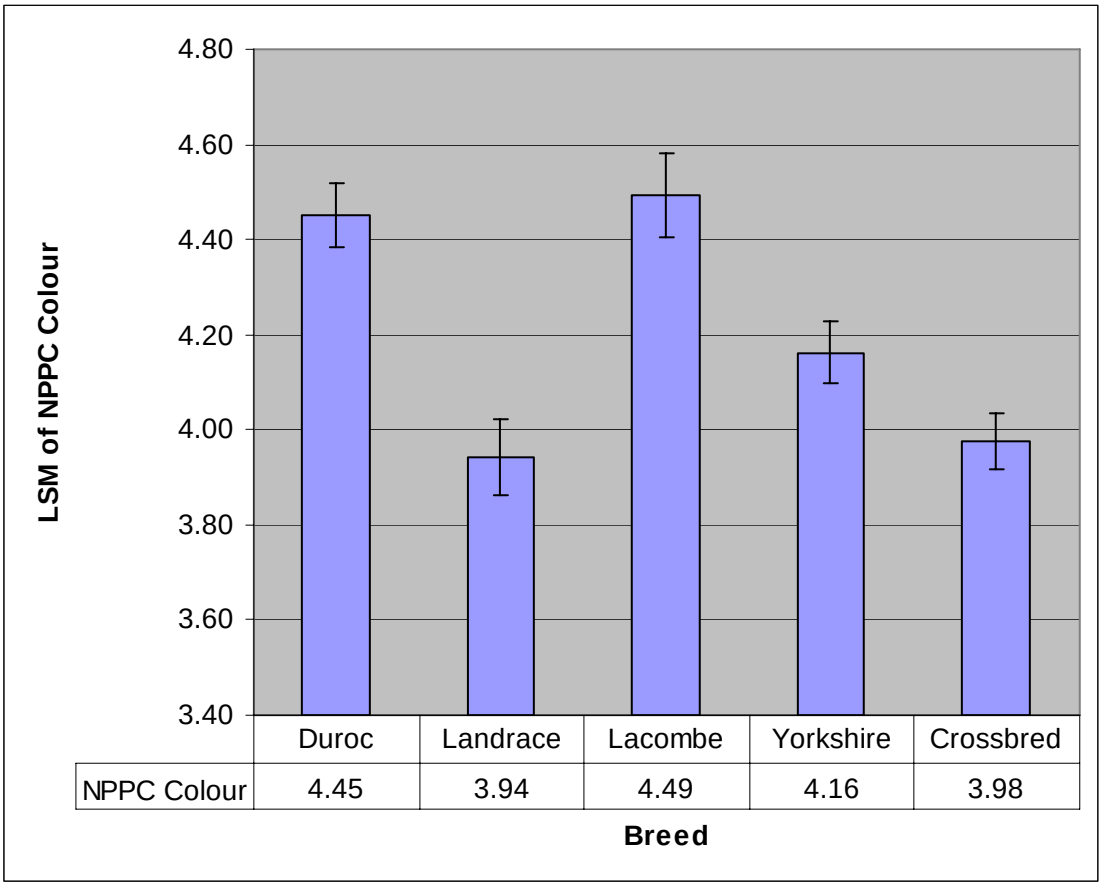
SNP	bp[1]	Reference amino acid	Reference Nucleotide	Observed Nucleotide	Observed Polymorphism
1	122	Isoleucine	C	T	Methionine
2	133	Leucine	T	A[2]	Stop codon
3	215	Phenylalanine	T	C	Silent mutation
4	234	Asparagine	A	C	Histidine
			A	T	Tyrosine

[1] base pair (bp) position of the nucleotide

[2] only one animal was found to have the A nucleotide at bp 133. The rest were T, so some caution is advised.

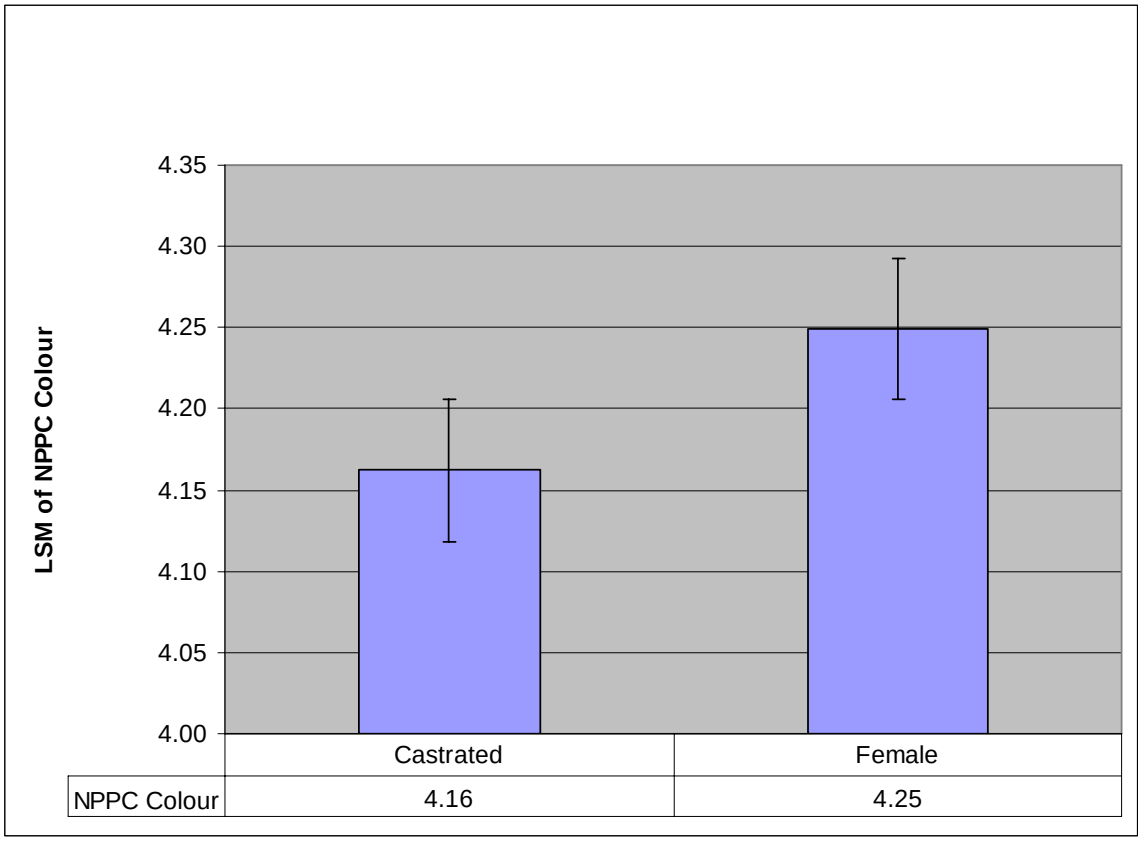
FIGURES

Figure 1. The Least Square Means of NPPC colour within different breeds[1]



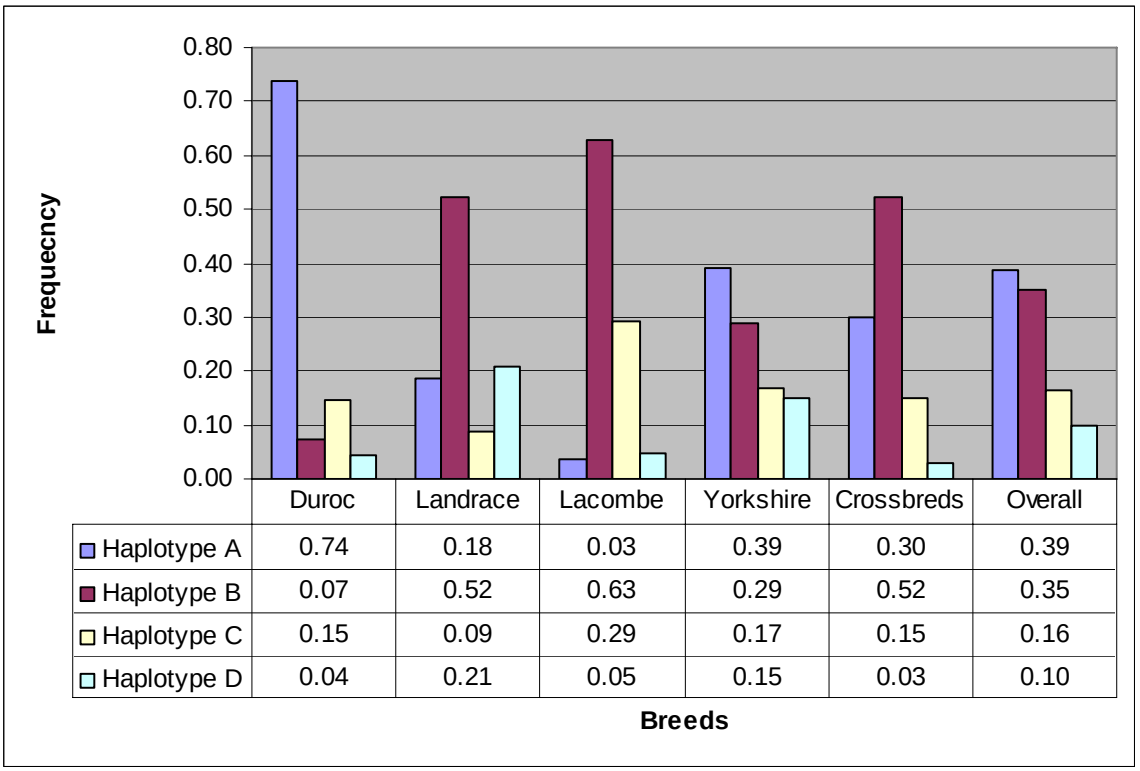
[1] Bars on the graph are showing the standard errors (SE) of LSM estimation of different breeds.

Figure 2. The Least Square Means of NPPC colour in castrated and female pigs



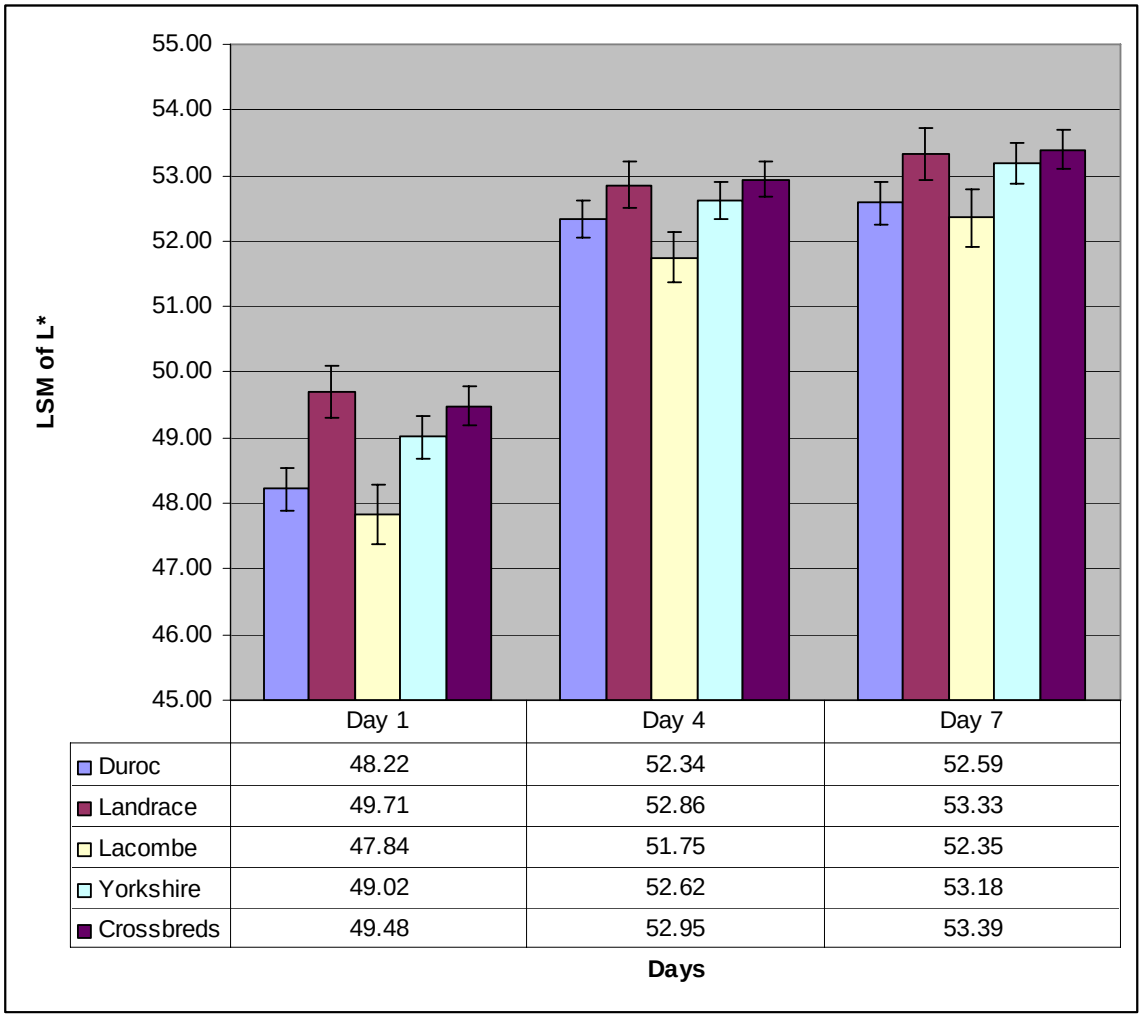
[1] Bars on the graph are showing the standard errors (SE) of LSM estimation in castrated and female pigs.

Figure 3. Frequency of SLC44A3 gene haplotypes within and across breeds



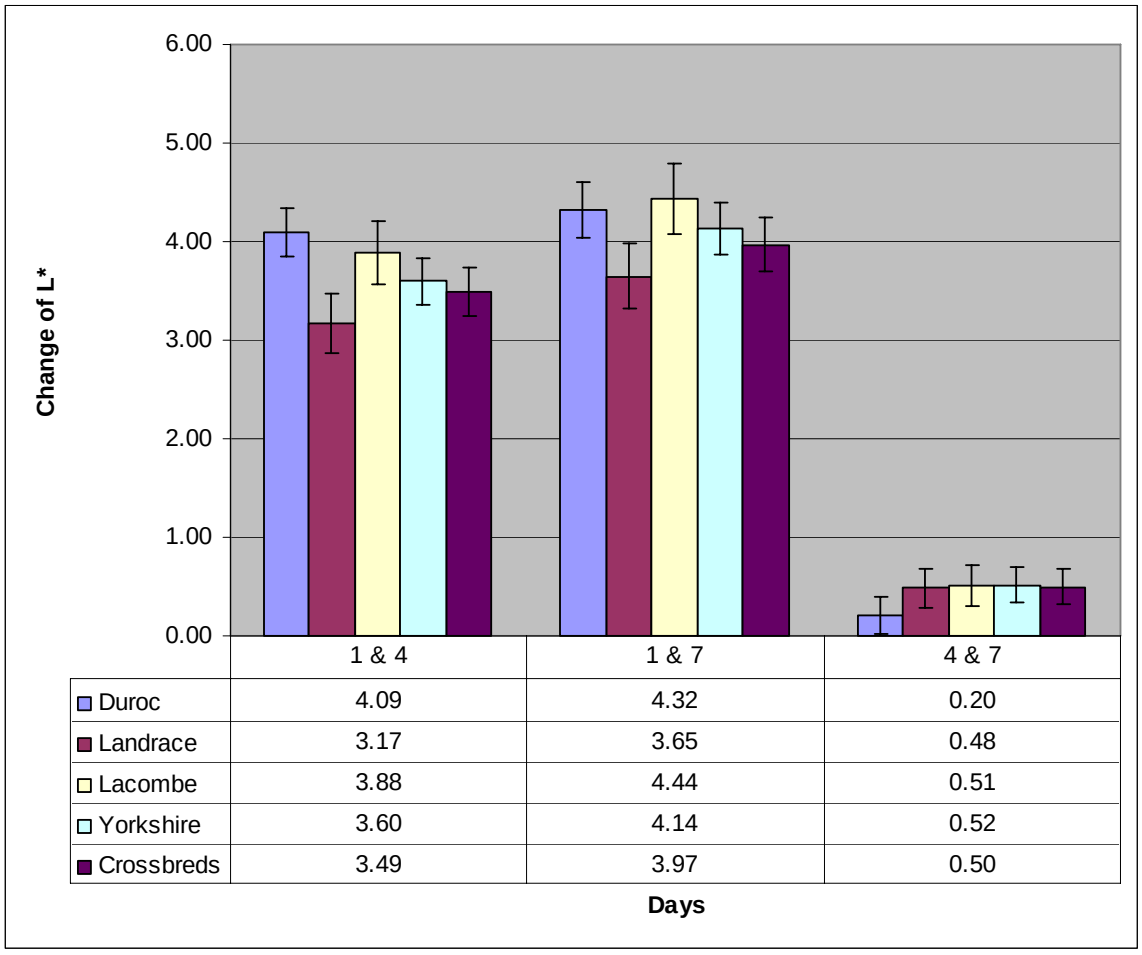
	Duroc	Landrace	Lacombe	Yorkshire	Crossbreds	Overall
Haplotype A	0.74	0.18	0.03	0.39	0.30	0.39
Haplotype B	0.07	0.52	0.63	0.29	0.52	0.35
Haplotype C	0.15	0.09	0.29	0.17	0.15	0.16
Haplotype D	0.04	0.21	0.05	0.15	0.03	0.10

Figure 4. Least Square Means (LSM) of L* from slaughter to retail



	Day 1	Day 4	Day 7
Duroc	48.22	52.34	52.59
Landrace	49.71	52.86	53.33
Lacombe	47.84	51.75	52.35
Yorkshire	49.02	52.62	53.18
Crossbreds	49.48	52.95	53.39

Figure 5. L* Least Square Means (LSM) change of L* from slaughter to retail



	1 & 4	1 & 7	4 & 7
Duroc	4.09	4.32	0.20
Landrace	3.17	3.65	0.48
Lacombe	3.88	4.44	0.51
Yorkshire	3.60	4.14	0.52
Crossbreds	3.49	3.97	0.50

APPENDIX 1

A final report to Western Swine Association (WSTA) on Lacombe Research Centres's portion of the study