RESEARCH REPORT

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PORK QUALITY

Title: Frequency and effects of the Napole Gene in the U.S. Pork Industry
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Ohio State University Pork Quality Study - Phase I

Abstract

Improvement of meat quality is one of the primary goals of the pork industry. Many genetic and environmental factors contribute to the quality of fresh and processed meat products. The dominant Rendement Napole gene (RN) has been found to have both positive and negative effects on pork quality. Currently, the best method for classification of animals as RN positive (RN^-RN^-, RN^-rn^+) or RN negative (rn^+rn^+) is the glycolytic potential test (GP). High glycolytic potential indicates that the animal is a carrier of the RN gene. This study investigates the effect of GP on pork quality traits for a population of 523 post-mortem *longissimus dorsi* samples from the 1998 National Barrow Show Progeny Test. Animals were classified as RN positive (n=23), or RN negative (n=500), based on a GP threshold of 160 □moles lactate equivalents per gram for the population bimodal distribution. Objective muscle quality measurements, subjective taste panel characteristics, carcass measurements and performance traits were collected on each animal. All animals utilized for this analysis were free of the stress gene. Residual correlations between GP and ultimate pH, Instron tenderness, water holding capacity, cooking loss, and Minolta color score were -.54, .12, .22, .25 and .29, respectively. RN positive pigs (n=23) had significantly ($p \leq .001$) higher glycolytic potential (182.3 vs 109.5 □mole/g) than RN negative pigs (n=500). RN positive pigs also had significantly ($P < .01$) lower pH (5.4 vs 5.6), poorer water holding capacity (.054 vs .037 mg), greater cooking loss (22.7 vs 19.3%) and paler Minolta color (25.0 vs 23.3) than RN negative pigs. No statistical differences were found between normal and RN carrier pigs for Instron tenderness, juiciness and tenderness scores. No differences were also observed for backfat, loin muscle area, or average daily gain between the two groups. Breed was a significant source of variation for all traits evaluated. Berkshire and Chester White breeds exhibited significantly ($p < .001$) lower GP values than Hampshire samples. The results of this study agree with previous research indicating that high GP values are associated with lower pH, poorer water holding capacity, higher cooking loss, and paler color. The differences in GP across breeds warrant future studies to determine the relationship of GP with muscle quality and sensory traits.

Introduction

In order to strengthen consumer acceptance, meat quality improvement has recently become one of the top priorities of the pork industry. Many different environmental and genetic factors can influence the quality of fresh or processed pork products. Most of the past genetic research relating to pork quality has focused on the Halothane, "stress" gene, which is associated with pale, soft and exudative (PSE) pork. Recently, the Rendement Napole (RN), "acid meat" gene, has been shown in European and U.S. research to have both negative and positive effects on pork quality.

Carriers of the dominant RN gene have been shown to exhibit paler color, reduced pH and water holding capacity, as well as increased drip and cooking losses. Positive effects of increased tenderness, juiciness, growth and carcasses advantages have also been reported for Napole carriers.

While PSE pork is attributed to a combination of higher temperature and a rapid rate of pH decline in muscle, the effects of the RN gene are the result of lower ultimate muscle pH. U.S. researchers, Sayre et al. (1963), were the first to report that Hampshire pigs had lower ultimate pH and paler color. However these differences were not further investigated until Monin and Sellier (1985) reported that this lower ultimate pH was the result of high "glycolytic potential". Glycogen is the major source of energy in the muscle. When needed, glycogen is broken down through the glycolytic pathway into lactic acid in post-mortem muscle tissue (Figure 1).

The RN gene is mainly associated with lines of Hampshire breeding, with reports of the gene frequency being as high as .627 in U.S. Hampshire populations. (University of Illinois, 1998). Given this high frequency within a popular terminal sire breed, there is great potential for increased economic losses from the undesirable pork quality effects of the RN gene. Few studies on pigs with diverse genetic backgrounds have been completed for U.S. populations, which leaves open the possibility of the presence of RN in other breeds.

While the search for a DNA marker continues, the best method of classifying animals as either RN positive (RN^-RN^- or RN^-rn^+) or RN negative (rn^+ rn^+), is the glycolytic potential (GP) assay. Individuals that carry the dominant RN gene exhibit higher glycolytic potential. RN classifications are based on a bimodal distribution threshold of GP values unique to each population.

The first phase of the OSU Napole gene research involves the classification of a diverse population by GP testing, followed by statistical analysis of all correlated production, carcass, meat quality and sensory traits. Correlations between GP and pork quality traits of interest, as well as a study of progeny groups and pedigree information have been completed.

Objectives

1. Improvement of pork quality through an understanding of diverse genetic resources.
2. Use the glycolytic potential assay to classify animals into two groups: Napole positive (RN^-RN^-, RN^-rn^+) or Napole negative (rn^+rn^+).
3. Measure genetic correlations between the Napole gene and other production and carcass traits including muscle quality and processing characteristics.
4. To determine the effects of the Napole gene on pork quality, carcass and performance characteristics.

Procedures

Samples. Five hundred twenty three frozen loin muscle samples from animals previously characterized for growth, carcass and meat quality characteristics in the 1998 National Barrow Show Progeny Test were obtained. Berkshire (n=177), Chester White (n=90), Duroc (n=76), Hampshire (n=44), Landrace (n=50), Poland China (n=13), Spots (n=10), and Yorkshire (n=63) were represented in the test. All animals were subjected to similar on-test management, carcass evaluation and sensory analysis.

Glycolytic Potential Test. Glycolytic potential of the loin muscle is the estimated sum of the compounds that have the potential to be transformed into lactic acid in the post-mortem muscle. GP (□mole/g) is calculated as: 2([glycogen] + [glucose-6-phosphate] + [glucose]) + [lactate]. Loin samples were subjected to a preparation procedure developed by Dalrymple and Hamm (1973), which allows for the simultaneous extraction of metabolites of interest from the muscle. The concentration of glycogen, glucose and glucose-6-phosphate was determined by a procedure developed by Keppler and Decker (1972), while the amount of lactate present in the muscle was determined according to the procedures of Bergmeyer (1974). Animals were classified as high glycolytic potential (n=23) or low glycolytic potential (n=500) based on a GP threshold of 160 □moles lactate equivalents per gram for the population bimodal distribution.

Statistical Analysis. Muscle quality and sensory traits were analyzed in a mixed model analysis of SAS (1988) with fixed effects of day off test, breed and GP status and a random sire(breed) effect. Partial correlation coefficients were calculated among quality traits using the MANOVA statement in SAS.

Results

Residual correlations ($p<.01$) between glycolytic potential (GP) and ultimate loin pH (pH), Instron tenderness (INS), water holding capacity, cooking loss, and Minolta color were -.55, .15, .20, .29, .29 respectively (Table 1). As expected, pH had the strongest relationship and was negatively correlated with GP. This is in agreement with Miller (1998) who also found a negative correlation coefficient of -.49 for GP and longissimus ultimate pH. Given these results, as glycolytic potential increases**,** tenderness and cooking loss also increases, while pH and water holding capacity decrease.

Napole positive pigs had a significantly ($p\leq.001$) higher glycolytic potential (182.3 □mole/g) than the normal pigs (109.5 □mole/g) (Table 3). Loin muscle glycogen concentration and lactate concentration also differed for the two groups. Breed differences were observed for GP, with Berkshire and Chester White samples having the lowest values at 134.8 and 133.3 □mole/g, respectively. Hampshire samples were intermediate for GP (152.0□mole/g) and were similar to all but Berkshire and Chester White samples. Yorkshire samples, traditionally thought to be “Napole free”, had the highest LS mean for GP at 155.5 □mole/g. Hampshire samples had the highest loin muscle glycogen concentration (31.5 □mole/g). However, the Yorkshire samples had the highest loin muscle lactate concentration (99.2 □mole/g), which contributed to the higher GP values observed for this breed.

RN positive pigs had significantly ($P<.01$) lower ultimate pH (5.4 vs 5.6), poorer water holding capacity(.054 vs .037 mg), greater cooking loss (22.7 vs 19.3 %) and paler Minolta color (25.24 vs 23.02) than RN negative pigs (Table 4). These results are

also in agreement with previous research showing that the high GP samples lose more water than RN negative samples. RN positive pigs were paler in color according to subjective scores (2.4 vs 2.7) and Hunter reflectance values (49.7 vs 47.9). Off flavor scores were also significantly higher for the RN pigs, and a lower quality index indicates the less desirable loin pH, Minolta and Instron measurements for the Napole pigs. No statistical differences were found between normal and RN pigs for Instron, juiciness score or tenderness score, indicating that there is not an advantage in tenderness or juiciness for the Napole positive group, as had been reported previously. No advantages in carcass characteristics or growth were observed for this population of RN pigs.

Breed was a significant source of variation for all traits evaluated (tables 3 & 4). Berkshire and Chester White breeds exhibited significantly ($p<.001$) lower GP values than Hampshire samples. However, Hampshire GP was not different ($p>.05$) from Duroc, Landrace, Poland China, Spots or Yorkshire. Berkshire and Chester White ultimate pH were also significantly higher than the Hampshires. Berkshires exhibited the most desirable water holding capacity (.037 mg) and cooking loss (18.5 %) when compared with the other breeds represented. Hampshires were only different in water holding capacity from Berkshire and Landrace. Landrace had the least desirable water holding capacity (.054 mg), but were not significantly different from Spots and Yorkshire. Landrace also had the highest cooking losses but were only different from Hampshire, Chester White and Berkshire. Hampshire samples tended to have the darkest color, according to Minolta and Hunter scores. Landrace, Spots and Yorkshire had the highest reflectance values, indicating paler color. Yorkshire samples had the poorest quality index and Berkshire, Chester White and Duroc had the highest.

Trends within the breeds for the analysis closely follow those previously reported in past investigations. However, the limited number of high GP Hampshires in this analysis may lead to results that are not characteristic of the actual breed differences attributed to effects of the RN gene. Another factor to consider, is that Hampshires did not contribute all of the 23 individuals classified as RN positive, which suggests evidence of the presence of the RN gene in other breeds (Table 2). Specific Chester White and Landrace sire groups were found to have samples testing positive for the Napole gene. Future research focusing on the presence/absence of the RN^- allele in all breeds is needed to further validate these findings.

Summary Phase I

The results of this phase of the study agree with previous research indicating that high glycolytic potential values are associated with lower loin ultimate pH, poorer water holding capacity, higher cooking losses and paler color. No differences were identified among carcass measures or growth performance between RN positive and negative groups for this analysis. Berkshire and Chester White samples were higher in pH and lower in glycolytic potential than the Hampshire samples. However, the differences across breeds for the other quality traits studied warrant further investigations to determine the relationship of GP with muscle quality and sensory traits. Low incidence of the Napole gene was detected in the Landrace and Chester White breeds, within specific sire groups.

To date, the use of the glycolytic potential assay is the only method to classify breeding stock for the Napole gene. A European research consortium has confirmed the development of the RN molecular genetic test that should be available for commercial

use in the near future. The test will improve efficiency of classifying pigs for the RN gene, and will allow the industry to rapidly assess the status of the U.S. pig populations.

Ohio State University Pork Quality Study - Phase II

Abstract

Improvement of meat quality has become one of the top goals of the pork industry in recent years. However, many genetic and environmental factors play a role in the final quality of both fresh and processed meat products. Many studies have found correlations between traits associated with fat metabolism and differences in meat quality. Therefore the objective of this study was to analyze the effects of meat quality candidate genes associated with fat metabolism on quality traits in pigs. Two candidate genes were genotyped in a population of pigs representing the Berkshire, Duroc, Hampshire and Landrace breeds. *Heart Fatty Acid Binding Protein 1* (*HFABP1)* is associated with fatty acid transport and has been found to be associated with intramuscular fat (marbling) in pigs. *Peroxisome Proliferator Activated Receptor* □ (*PPAR*□) is a transcription factor involved in regulating both adipocyte differentiation and fat deposition. Statistical analyses for the total population and within individual breeds were completed using SAS mixed model procedures. Fixed effects for the total population analysis were breed, genotype, sex, off date and Napole gene status. The random effect for total population was sire(breed). Within breed analysis for the *PPAR*□ marker showed significant associations with tenderness score in Berkshires; loin muscle area and off flavor score in Durocs; average daily gain in Hampshires; backfat and Instron tenderness in Landrace. Results of the *PPAR*□ total population analysis reveal an association with off flavor score and average daily gain. Within breed analysis for the *HFABP1* marker showed significant ($p \leq .05$) associations with intramuscular fat, Instron tenderness, loin muscle area, loin glycogen content, and quality index (which includes Instron, Minolta color and loin pH) for Berkshires; loin glycogen content for Durocs; backfat and water holding capacity for Hampshires; backfat and intramuscular fat for Landrace. Results of the *HFABP1* marker total population analysis indicate an association ($p \leq .05$) between loin pH and a quality index. Intramuscular fat and flavor score ($p < .1$) warrant further investigation. Allelic frequencies differ among the breeds for both markers. Further investigation with larger population sizes is need to fully

characterize the effects of these molecular markers. However, these results indicate the potential use of genetic markers for improvement of meat quality within the pork industry.

A second objective of this research was to determine the physical assignment of two potential meat quality candidate genes not previously characterized in the pig. *Adipocyte determination and differentiation factor-1* (*ADD1*) and *pyruvate dehydrogenase E1-alpha* (*PDHA1*) were physically mapped in the pig for study as potential candidate genes for pork quality. *ADD1* is a transcription factor believed to play a role in encoding enzymes of lipid biosynthesis and may also be involved in the control of plasma cholesterol levels. *PDHA1* has been found to catalyze the conversion of pyruvate into acetyl-CoA. A deficiency of the enzyme pyruvate dehydrogenase is one of the most commonly defined genetic defects of mitochondrial energy metabolism resulting in lactic acidosis. Primers were designed using porcine cDNA sequence. Results of a pig-rodent somatic cell hybrid panel indicated that *ADD1* was located on pig chromosome 12 with 100% probability. *PDHA1* was determined to be on pig chromosome X (SSCX) with 100% probability. These results are similar to the mapping locations predicted from human-pig comparative mapping studies. Currently, linkage analyses to confirm these results are being conducted for both genes, as well as association studies to characterize their effects on meat quality in the pig.

Introduction

Improvement of meat quality has become one of the top goals of the pork industry in recent years. The main objective of this study was to gain more insight into the genetic control of pork quality and to investigate the potential role of molecular genetic markers in pork quality improvement.

Many studies have found correlations between traits associated with fat metabolism and differences in meat quality traits. Therefore, it was our primary objective for this phase of the project, to analyze the effects of meat quality candidate genes associated with fat metabolism on quality traits in pigs. Many molecular genetic markers are currently mapped in the pig. However, very few association studies have been conducted in order to determine the practical use of these markers in swine selection schemes. Analysis of two genetic markers will be reported.

Peroxisome Proliferator Activated Receptor □ (*PPAR*□) is a transcription factor involved in regulating both adipocyte differentiation and fat deposition. Given this marker's function in human and mice studies, we would expect it to also be involved in fat deposition in the pig. *Heart Fatty Acid Binding Protein 1* (*HFABP1)* is associated with fatty acid transport and has been found to be associated with intramuscular fat (marbling) in pigs. No known association studies with this marker have been previously reported for U.S. commercial swine breeds and this marker

A second objective was to investigate new candidate genes that have not been previously characterized in the pig and physically map their location in the swine genome. With only two "major genes" that have been characterized for pork quality in the pig (Halothane & Napole) there are many genetic controls of meat quality that have not been identified. By comparing the physiological roles of genes characterized in human and mouse populations, we can choose "candidates" that may effect the pig's metabolism in the same manner and contribute to the ultimate quality of fresh and processed pork products.

Adipocyte determination and differentiation factor-1 (*ADD1)* is a transcription factor believed to play a role in encoding enzymes of lipid biosynthesis and may also be involved in the control of plasma cholesterol levels. This gene may also play a role in fat deposition in the pig. *Pyruvate dehydrogenase E1-alpha PDHA1* has been found to catalyze the conversion of pyruvate into acetyl-CoA. A deficiency of the enzyme pyruvate dehydrogenase is one of the most commonly defined genetic defects of mitochondrial energy metabolism resulting in lactic acidosis. According to previous studies of this gene's function, we hypothesize it could also play a role in determining muscle pH and water holding capacity in meat products.

Objectives

1. Improvement of pork quality through an understanding of diverse genetic resources.
2. Determine the association between molecular genetic markers and pork quality traits.
3. Identify and physically map meat quality candidate genes not previously characterized in the pig.

Procedures

Association Study

The population utilized for this phase of the project consisted of animals from the 1998 National Barrow Show Progeny Test and the 1999 Hampshire Sire Progeny Test. All animals were managed by identical protocols and performance, carcass, quality and sensory panel measurements were taken on each animal. Individuals from each of the four major U.S. breeds were chosen for DNA extraction from frozen loin chops. Berkshire (n=180), Duroc (n=77), Hampshire (n=160), and Landrace (n=55) sire breeds were represented. Genotypes were obtained using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) procedures unique for each gene analyzed. Statistical analyses were performed within each breed separately, and across breeds in the total population to increase the number of animals in each genotype category. Genotype groups with only 1 or 2 individuals represented were eliminated from the within breed analysis in order to clarify any significant trends in the data. The number of pigs for each analysis differs based on the success of each genetic test. Only Porcine Stress Syndrome free pigs were utilized in these analyses. SAS mixed model procedures were utilized. Fixed effects included breed, genotype, sex, off date (depending on the trait) and Napole status (depending on the breed), with a sire(breed) random effect for the total population analysis. The within breed analysis included a random effect of sire, and fixed effects of genotype, sex, off date (depending on the trait), and Napole status (depending on the breed). Allelic frequencies were calculated within breed, and for the entire population based upon observed genotype classifications.

Physical Mapping

Two genes (*ADD1* & *PDHA1*) were chosen as possible candidate genes for meat quality based on their observed physiological functions in other species. Primers for use in PCR amplification were designed based on partial porcine cDNA sequence available for each gene in GENBANK. PCR conditions were tested and optimized for

each gene separately. Physical mapping was achieved by use of a pig-rodent somatic cell hybrid panel.

Results

Association Study

PPAR

Allelic frequencies for *PPAR* (Table 5) were similar in all breeds except for Berkshire. However, no 22 genotypes were observed in the Hampshire and Landrace breeds. Allele 1 was the most frequent in the total population (.81) compared to allele 2 (.19).

The within breed analysis (Table 7) for *PPAR* revealed associations ($p \leq .05$) with loin muscle area and off flavor score in Durocs. Durocs with two copies of the 1 allele were also leaner at the last rib backfat measurement. Average daily gain was significant ($p \leq .05$) for the Hampshire analysis, with the *11* genotype pigs being slower growing (.77 vs .81 kg). Landrace had an association ($p \leq .01$) with backfat and Instron tenderness. Landrace *11* animals were fatter (2.4 vs 1.8 cm) and more tender (4.6 vs 5.9 kg) than the *12* genotype group. An interesting trend in tenderness score exits in the Berkshires for this marker, with the *22* animals being scored the most tender (7.7 vs 6.7). The general trends of the within breed analysis suggest that *PPAR* *11* animals are leaner, have larger loin muscle area, are slower growing and less tender than the *12* animals in the Berkshire, Hampshire and Duroc breeds. Therefore, if *PPAR* is associated with fat deposition in the pig, the allele frequencies observed in this population could be the result of the selection for leaner animals (allele 1) in U.S. populations (Table 5). Curiously, the Landrace breed had the opposite trend, with the *11* animals being fatter and more tender than the *12* animals.

Results of the *PPAR* total population (n=446) analysis (Table 8) indicate an association ($p \leq .05$) with off flavor score, and an interesting trend among genotypes for average daily gain. Animals genotyped *11* tend to grow slower (.81 vs .85 kg) and have a stronger off flavor (4.9 vs. 4.7) than *12* animals.

HFABP1

Allelic frequencies for *HFABP1* (Table 6) were different for each breed, with the allele 1 being the most frequent in the population.

The within breed analysis (Table 9) for *HFABP1* revealed associations ($p \leq .05$) with intramuscular fat, Instron tenderness, loin muscle area, loin glycogen content and quality index for the Berkshire breed (n=170). Berkshires genotyped *11* had more intramuscular fat (2.7 vs 2.3 %), were more tender (4.5 vs 4.9 kg), had smaller loin muscle areas (33.5 vs 35.3 cm^2), and a higher quality index (59.6 vs 50.9) than the *12* animals. The quality index is calculated utilizing loin pH, Minolta color and Instron tenderness. High standard errors for the *22* Berkshire genotype is likely to explain the trends observed, compared to the other genotype groups in this breed. Loin glycogen content was also significant ($p \leq .05$) in the Duroc breed (n=70), along with other interesting trends in backfat, juiciness score and cooking loss. Due to the limited numbers available in the *22* genotype group of both the Hampshire and Landrace breeds (n=2 and 1, respectively) they were excluded from the within breed analysis. The Hampshire (n=146) *11* animals were significantly ($p \leq .05$) fatter (1.98 vs 1.84 cm) and had less desirable water holding capacity (.036 vs .048 mg) than the *12* genotype group. The Hampshires also showed numerical trends for Minolta color, Hunter color

and cooking loss. Intramuscular fat and last rib backfat was also significant ($p \leq .05$) for Landrace (n=48), with similar trends in observed average backfat measurements between the *12* and *22* genotypes. Significant trends in the within breed analysis indicate that *HFABP1* animals classified as *11* tend to be fatter have more intramuscular fat and are more tender than *12* animals. The Berkshire population had the highest frequency of the allele 1 (.83) when compared to the other breeds analyzed (Table 6).

Results of the *HFABP1* total population (n= 437) analysis (Table 10) indicate an association ($p \leq .05$) with loin pH and quality index, suggesting that *11* animals have higher pH and more desirable overall quality as measured by color, tenderness and pH. Intramuscular fat percentage and flavor score also show interesting results in this analysis. Similarly, trends show *11* animals have a higher intramuscular fat percentage and better flavor scores than *12 HFABP1* animals. Differences observed for breed, Napole status and sex differences follow the typical trends normally seen for each trait. Berkshires and Durocs have higher pH and a better quality index than both Hampshire and Landrace animals. Barrows have more intramuscular fat (2.9 vs 2.2%) and a higher quality index (51.8 vs 43.7) than gilts.

Physical Mapping

Physical assignment of a gene to the pig cytogenetic map with the pig-somatic hybrid panel allows for a varying degree of resolution, depending on the success of PCR amplifications in each hybrid cell line. Results are calculated as a probability of the gene being included in certain regions of a particular chromosome.

ADD1 was mapped to pig chromosome 12 with 100% probability (Figure 2). Results indicate that is it likely to be located in region p11-p13 with 48% probability, and in region q11-15 with 48% probability. *PDHA1* was determined to be on pig chromosome X (SSCX) with 100% probability. Results indicate that it is 81% probable that *PDHA1* lies within the region of p22-23.

These results are similar to the mapping locations predicted from human-pig comparative mapping studies. Currently, linkage analyses to confirm these results are being conducted for both genes, as well as association studies to characterize their effects on meat quality in the pig.

Summary Phase II

Results of this study indicate the potential use of genetic markers for improvement of meat quality within the pork industry. However, further investigation with larger populations would be helpful to in order to fully characterize the effects of these molecular markers and to aid in the explanation of the numerical trends observed for some of the traits. We must also keep in mind that the markers used for these association studies represent just one change in each gene of interest. Through further discovery research, there are potentially many more markers (possibly with greater effects), which could be characterized in the genes. By contributing two new genetic markers to the physical pig map we are increasing the information we have available on the pig genome, as well as characterizing potential genes for future use to improve pork quality through genetic methods.

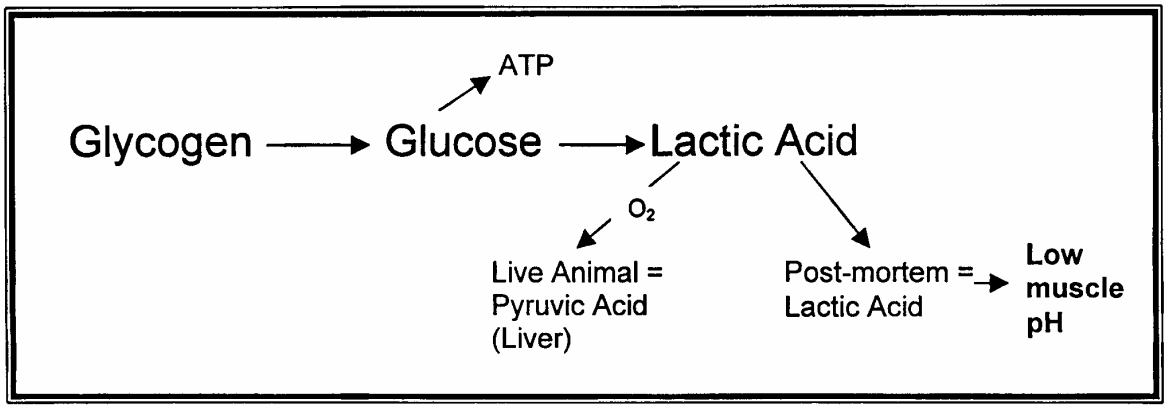

Figure 1. Simplified diagram of glycogen conversion in the muscle.

Table 1. Correlations between glycolytic potential and pork quality characteristics[1].

	pH_u	WHC	INS	CL	MIN
Glycolytic Potential	-.55	.20	.15	.29	.29

[1]pH_u =ultimate loin pH, WHC = water holding capacity (mg of exudate), INS = Instron tenderness (kg), CL = cooking loss (%), MIN = Minolta color reflectance

Table 2. Napole gene[1] distribution within breed populations.

Breed	n	Normal rn^+rn^+	Napole RN^-RN^-/RN^-rn^+
Berkshire	177	177	
Chester White	90	84	6
Duroc	76	76	
Hampshire	44	29	15
Landrace	50	48	2
Poland China	13	13	
Spots	10	10	
Yorkshire	63	63	
Total Pop.	523	500	23

[1]rn^+rn^+ = normal, RN^-RN^- = homozygote for Napole allele, RN^-rn^+ = heterozygote Napole carrier

Table 3. Least squares means and standard errors for glycolytic potential (GP).

Napole Class[2]	n	GP***	GLYC CON***	LAC CON***
RN Positive	23	182.3 ± 4.6[b]	41.09 ± 1.4[b]	99.41 ± 3.0[b]
RN Negative	500	109.5 ± 1.7[a]	11.65 ± .52[a]	86.18 ± 1.1[a]
Breed[3]				
Berkshire	177	134.8 ± 3.0[a]	23.05 ± .91[a]	88.43 ± 1.9[ab]
Chester Wh.	90	133.3 ± 3.4[a]	22.85 ± 1.1[a]	87.33 ± 2.2[a]
Duroc	76	143.5 ± 3.8[b]	25.13 ± 1.2[ac]	92.86 ± 2.5[bc]
Hampshire	44	152.0 ± 4.0[bc]	31.53 ± 1.3[d]	88.88 ± 2.6[ab]
Landrace	50	152.4 ± 4.2[bc]	26.94 ± 1.3[bc]	98.21 ± 2.8[cd]
Poland Ch.	13	144.5 ± 6.9[abc]	24.67 ± 2.1[ab]	94.93 ± 4.5[abd]
Spots	10	150.9 ± 8.2[bc]	28.87 ± 2.5[bcd]	92.81 ± 5.3[abd]
Yorkshire	63	155.5 ± 3.9[c]	27.92 ± 1.2[b]	99.20 ± 2.6[d]

***Significant at $p \leq .001$

[1]GP=loin muscle glycolytic potential (µmole/g): [2 (glyc con) + lac con], GLYC CON = loin muscle glycogen concentration (µmole/g), LAC CON = loin muscle lactate concentration (µmole/g)

[2]RN positive ≥ 160 µmole/g (High GP), RN negative < 160 µmole/g (Low GP); RN class LS means with common superscripts differ at $p \leq .001$

[3]Breed LS means with common superscripts are not different ($p \geq .05$)

Table 4. Least squares means and standard errors for the effect of Napole gene status on pork quality characteristics[1].

Napole Class[2]	n	pH_U***	WHC ***	CL***	COLOR*	MIN**	HUNT**	OFF FL*	Q Index**
RN Positive	23	5.40 ± .04[a]	.054 ± .003[b]	22.75 ± .89[b]	2.4 ± .15[a]	25.0 ± .65[b]	49.7 ± .68[b]	5.7 ± .46[b]	36.0 ± 3.6[a]
RN Negative	508	5.57 ± .02[b]	.037 ± .001[a]	19.32 ± .28[a]	2.7 ± .05[b]	23.3 ± .20[a]	47.9 ± .21[a]	4.6 ± .15[a]	47.8 ± 1.4[b]
Breed[3]									
Berkshire	177	5.60 ± .03[d]	.037 ± .002[a]	18.50 ± .54[a]	2.8 ± .09[b]	23.0 ± .40[ab]	47.8 ± .42[ab]	4.5 ± .29[ab]	50.5 ± 2.4[d]
Chester Wh.	90	5.63 ± .03[d]	.039 ± .002[ab]	19.94 ± .59[b]	2.7 ± .10[b]	23.4 ± .43[bc]	48.2 ± .46[b]	4.1 ± .32[a]	52.3 ± 2.8[d]
Duroc	76	5.50 ± .04[bc]	.045 ± .002[c]	21.65 ± .66[cd]	2.7 ± .11[b]	23.5 ± .48[bc]	48.3 ± .51[b]	4.9 ± .36[bc]	53.1 ± 3.2[d]
Hampshire	44	5.48 ± .04[ab]	.043 ± .002[bc]	20.89 ± .64[bc]	2.8 ± .11[b]	22.1 ± .47[a]	46.9 ± .49[a]	5.2 ± .35[bd]	41.3 ± 3.4[c]
Landrace	50	5.43 ± .04[ab]	.054 ± .003[d]	22.93 ± .72[d]	2.3 ± .12[a]	25.1 ± .52[d]	49.9 ± .55[c]	5.6 ± .39[cdf]	30.9 ± 3.6[ab]
Poland Ch.	13	5.51 ± .07[acd]	.045 ± .004 [ac]	21.08 ± 1.2[bd]	2.6 ± .21[ab]	24.5 ± .88 [bcd]	47.6 ± .92[ab]	5.6 ± .64[bef]	38.6 ± 5.7[ac]
Spots	10	5.36 ± .08[ab]	.050 ± .005[cd]	21.62 ± 1.4[cd]	2.5 ± .24[ab]	26.1 ± 1.0[d]	51.0 ± 1.1[c]	5.4 ± .75[acde]	41.9 ± 6.8[bcd]
Yorkshire	63	5.39 ± .04[a]	.050 ± .002[cd]	21.65 ± .69[cd]	2.2 ± .11[a]	25.8 ± .50[d]	50.7 ± .53[c]	6.1 ± .37[df]	26.7 ± 3.3[a]

*Significant at p≤.05, **Significant at p≤.01, ***Significant at p≤.001

[1]GP= glycolytic potential, pHu = ultimate loin pH, WHC = water holding capacity (mg exudate), CL = cooking loss, MIN = Minolta color reflectance, HUNT = Hunter color reflectance, OFF FL = off flavor subjective score (1-10), Q Index = quality index (Instron, Minolta color and loin pH)

[2]RN positive ≥ 160 µmole/g (High GP), RN negative < 160 µmole/g (Low GP); Napole class LS means with common superscripts are not different (p≥.001)

[3]Breed LS means with common superscripts are not different (p≥.05)

Table 5. PPARγ genotypic[1] and allelic frequencies.

Breed	n	11	12	22	1	2
Berkshire	168	.44	.49	.07	.69	.31
Duroc	76	.67	.32	.01	.83	.17
Hampshire	152	.80	.20		.90	.10
Landrace	50	.80	.20		.90	.10
Total Pop.	**446**	**.64**	**.33**	**.03**	**.81**	**.19**

[1]PPARγ genotypes: 11 = homozygote for allele 1, 12 = heterozygote of allele 1 & 2, 22= homozygote for allele 2

Table 6. HFABP1 genotypic[1] and allelic frequencies.

Breed	n	11	12	22	1	2
Berkshire	170	.68	.30	.02	.83	.17
Duroc	70	.36	.40	.24	.56	.44
Hampshire	148	.23	.76	.01	.61	.39
Landrace	49	.02	.24	.74	.14	.86
Total Pop.	**437**	**.40**	**.46**	**.14**	**.63**	**.37**

[1]HFABP1 genotypes: 11 = homozygote for allele 1, 12 = heterozygote of allele 1 & 2, 22= homozygote for allele 2

Table 7. Least squares means[1] for associations between pork quality characteristics[2] and PPARγ genotypes[3] for the within breed analysis.

Berkshire	**n**	**TEND (p=.06)**		
11	75	6.7 ± .26		
12	82	7.5 ± .25		
22	11	7.7 ± .65		
Duroc	**n**	**LMA***	**OFF FL***	**LRBF (p=.08)**
11	51	39.14 ± .77[b]	4.4 ± .40[b]	1.91 ± .10
12	24	35.94 ± 1.0[a]	3.6 ± .46[a]	2.26 ± .13
Hampshire	**n**	**ADG***	**CL (p=.09)**	
11	121	.77 ± .02[a]	22.13 ± .64	
12	31	.81 ± .02[b]	20.64 ± .85	
Landrace	**n**	**LRBF****	**INSTR****	
11	40	2.44 ± .23[b]	4.63 ± .42[a]	
12	10	1.88 ± .28[a]	5.95 ± .51[b]	

*Significant at p≤.05, **Significant at p≤.01

[1]LS means with common subscripts are not different (p≥.05)

[2]TEND = tenderness score (1-10), LMA = loin muscle area (cm^2), OFF FL = off flavor subjective score, LRBF = last rib backfat (cm), ADG = average daily gain (kg), CL = cooking loss (%), INSTR = Instron tenderness (kg)

[3]PPARγ genotypes: 11 = homozygote for allele 1, 12 = heterozygote of allele 1 & 2, 22= homozygote for allele 2

Table 8. Least squares means for association between pork quality characteristics[1] and PPARγ genotypes[2] for the total population analysis.

Class[3]	n	OFF FL*	ADG (p=.07)
11	287	4.9 ± .21[b]	.81 ± .01
12	147	4.4 ± .25[a]	.83 ± .01
22	12	4.7 ± .57[ab]	.85 ± .02
Berkshire	168	4.3 ± .32[a]	.82 ± .02
Duroc	76	4.4 ± .40[ab]	.86 ± .02
Hampshire	152	5.1 ± .34[b]	.79 ± .02
Landrace	50	5.1 ± .44[b]	.86 ± .03
Napole positive	52	5.1 ± .32[b]	
Napole negative	394	4.4 ± .30[a]	
Barrows	217		.85 ± .01
Gilts	229		.80 ± .01

*Significant at p≤.05

[1]OFF FL = off flavor score (1-10), ADG = average daily gain (kg)

[2]PPARγ genotypes: 11 = homozygote for allele 1, 12 = heterozygote of allele 1 & 2, 22= homozygote for allele 2

[3]Class LS means with common superscripts are not different (p≥.05): Only fixed effects significant at p≤.05 in the mixed model are included in the table

Table 9. LS means[1] for associations between pork quality characteristics[2] and HFABP1 genotypes[3] for within breed analysis.

Berkshire	**n**	**IMF***	**INSTR***	**LMA****	**GLYC***	**Q Index****
11	116	2.73 ± .09^{b}	4.52 ± .09^{a}	33.5 ± .50^{a}	7.16 ± .71^{a}	59.64 ± 1.8^{b}
12	50	2.33 ± .13^{a}	4.87 ± .13^{b}	35.3 ± .64^{b}	9.39 ± .88^{b}	50.97 ± 2.4^{a}
22	4	2.66 ± .43^{ab}	5.29 ± .42^{ab}	36.5 ± 1.8^{b}	5.52 ± 2.5^{ab}	56.58 ± 7.3^{ab}

Duroc	**n**	**GLYC***	**LRBF (p=.09)**	**JUICE (p=.07)**	**CL (p=.06)**
11	25	13.00 ± 1.5^{b}	2.37 ± .16	5.65 ± .39	18.9 ± 1.1
12	28	9.90 ± 1.4^{a}	2.41 ± .15	4.69 ± .37	21.5 ± 1.0
22	17	8.82 ± 1.7^{a}	2.74 ± .17	5.23 ± .46	20.8 ± 1.3

Hampshire	**n**	**LRBF****	**AVBF***	**WHC***	**MIN (p=.06)**	**IMF (p=.09)**
11	34	2.66 ± .10^{b}	1.98 ± .06^{b}	.036 ± .004^{a}	21.23 ± .51	2.52 ± .17
12	112	2.42 ± .06^{a}	1.84 ± .04^{a}	.048 ± .004^{b}	22.65 ± .54	2.21± .11

Landrace	**n**	**IMF***	**LRBF***	**AVBF (p=.07)**
12	12	2.58 ± .30^{b}	2.55 ± .26^{b}	1.86 ± .18
22	36	2.07 ± .26^{a}	2.16 ± .23^{a}	1.64 ± .17

*Significant at p≤.05, **Significant at p≤.01

[1]LS means with common subscripts are not different (p≥.05)

[2]IMF = intramuscular fat (%), INSTR = Instron tenderness (kg), LMA = Loin muscle area (cm^2), GLYC = loin muscle glycogen concentration (μmoles/g), Q Index = quality index (Instron, Minolta color, loin pH), LRBF = last rib backfat (cm), JUIC = juiciness subjective score (1-10), CL = cooking loss (%), MIN = Minolta color reflectance, WHC = water holding capacity (mg exudate), IMF = intramuscular fat (%), AVBF = average backfat (cm)

[3]HFABP1 genotypes: 11 = homozygote for allele 1, 12 = heterozygote of allele 1 & 2, 22= homozygote for allele 2

Table 10. LS Means for association between pork quality characteristics[1] and HFABP1 genotypes[2] for total population analysis.

Class[3]	n	Loin pH*	Q Index*	IMF (p=.06)	FLAV (p=.09)
11	176	5.57 ± .02[b]	49.97 ± 2.2[b]	2.69 ± .11	1.22 ± .06
12	202	5.53 ± .02[a]	45.07 ± 1.9[a]	2.33 ± .13	1.09 ± .05
22	59	5.60 ± .03[b]	48.25 ± 2.5[ab]	2.66 ± .43	1.10 ± .08
Berkshire	170	5.68 ± .03[c]	54.25 ± 2.6[b]	2.48 ± .12	1.20 ± .06
Duroc	70	5.58 ± .03[b]	57.58 ± 3.2[b]	3.41 ± .16	1.96 ± .08
Hampshire	148	5.52 ± .03[ab]	43.90 ± 2.7[a]	2.31 ± .11	1.03 ± .06
Landrace	49	5.48 ± .04[a]	35.32 ± 3.7[a]	1.95 ± .18	1.12 ± .09
Napole positive	52	5.53 ± .03[a]	43.70 ± 2.6[a]		1.05 ± .08
Napole negative	385	5.61 ± .02[b]	51.82 ± 1.3[b]		1.22 ± .03
Barrows	212		51.84 ± 1.8[b]	2.87 ± .10	
Gilts	225		43.69 ± 1.8[a]	2.21 ± .09	

*Significant at p≤.05

[1]Q Index = quality index (Instron, Minolta color and loin pH), IMF = intramuscular fat (%), FLAV = flavor subjective score (1-10)

[2]HFABP1 genotypes: 11 = homozygote for allele 1, 12 = heterozygote of allele 1 & 2, 22= homozygote for allele 2

[3]Class LS means with common superscripts are not different (p≥.05): Only fixed effects significant at p≤.05 in the mixed model are included in the table

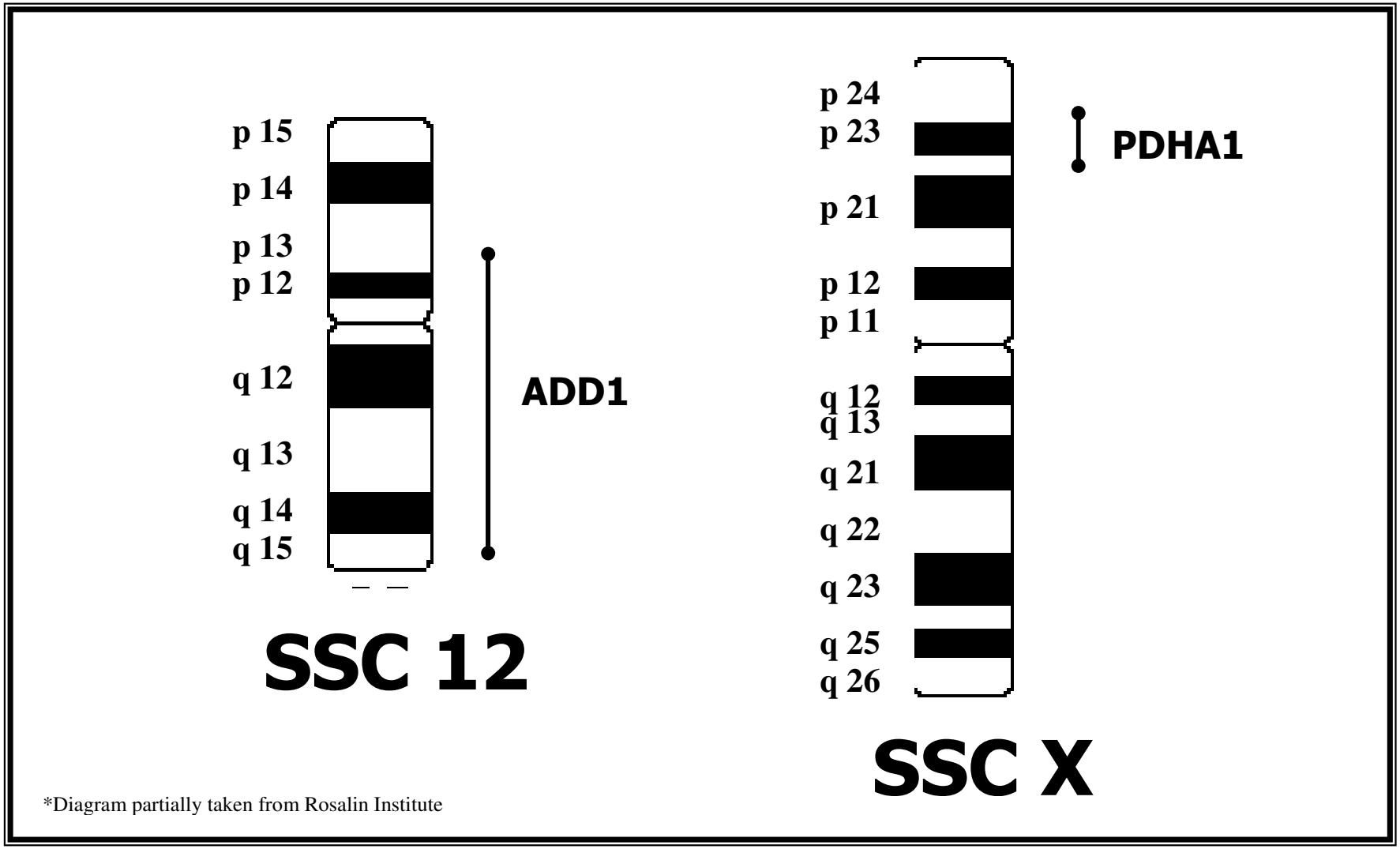

Figure 2. Cytogenetic location of *Adipocyte determination and differentiation factor-1* (*ADD1)* on pig chromosome 12 (SSC 12), and *Pyruvate dehydrogenase E1-alpha* (*PDHA1)* on pig chromosome X (SSC X).