THE EFFECT OF DNA MARKER ASSISTED SELECTION FOR THE RENDEMENT NAPOLE GENE ON GROWTH PERFORMANCE AND CARCASS COMPOSITION, WHILE IN CONJUNCTION WITH ENHANCEMENT TREATMENT FOR LEAN QUALITY, SENSORY, AND SHELF LIFE CHARACTERISTICS

By

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Bachelor of Science

Oklahoma State University

Stillwater, OK

2001

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2003

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ACKNOWLEDGEMENTS

One can not successfully complete a graduate program without the assistance of many others. Hence, when recognizing my many shortcomings, that circle of appreciation, expands exponentially. I would first like to thank my family who offered constant encouragement, and instilled within me the value of hard work. I would like to extend my most cordial thank you to my major professor, Dr. Brad Morgan, whose guidance, scholarly advice, and personal friendship I value immensely. I would like to extend my most gracious appreciation to Dr. Fred Ray, who helped to secure funding for this research, and whose extension programs helped to further garner teaching and leadership skills, which will be invaluable to my future endeavors. I would like to thank Dr. Scott Carter for serving upon my graduate committee and who offered extensive advice during the initial experimental design of the project.

This research initiative would have been impossible without the countless work hours by Mr. Kim Brock and Mr. Cecil Hooper, as well as all of their work staff. These men granted me the opportunity to conduct this research as well as taking care of the daily husbandry of the livestock from conception to harvest. To Ms. Linda Gunther, Mr. Jake Nelson, Mrs. Betty Rothermel, and Mrs. Kris Novotny, without their patience, hard work, and steady guidance, this project, as well as the daily operations of the meat science section would be impossible. I would like to extend appreciation to Dr Carla Goad and Dr. Dave Buchanan who guided me through the statistical analysis of the project. Additionally, I deeply value the guidance and friendship of Dr. Glen Dolezal, Dr. Mark Johnson, and Dr. Gordon Jones, all who encouraged me and helped me recognize the value of an advanced degree. Finally, I would then like to extend my most sincere appreciation to all of my fellow graduate students and my entire extended family within the Department of Animal Science at Oklahoma State University. The camaraderie and relationships I acquired during my stay in Stillwater I will cherish for a lifetime.

PREFACE

This thesis is submitted in accordance to the style guide of the Journal of Animal Science for subsequent manuscript publication.

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CHAPTER I

INTRODUCTION

The changing phenotype of the porcine specie within the United States has been very dramatic over the last one hundred years. These overwhelming, yet relatively rapid phenotypic changes are primarily explained via the idea that swine are a litter bearing specie with a relatively short production cycle. Until the conclusion of World War II, U.S. pork production was not actually driven by the need for consumable protein, but rather by the need for high caloric density lard. Following the conclusion of the war, the necessity of "fat-type" hog production lessened due to the lower energetic requirement for a peacetime population, as well as a decreasing need for nitroglycerine. Additionally, it was in this timeframe that university researchers recognized the lack of productive and reproductive efficiency associated with "fat-type" animals. Hence, the next thirty-five years were devoted to the production of "meat-type" hogs.

Over time, intensive selection for percent lean yield led to problems relative to productive efficiency. Additionally, single-trait selectiveness for percent lean yield diverted attention from parameters such as carcass quality, reproductive efficiency, and progeny livability, all which suffered during this selection period. It was also during this production era that first significant genetic mutation was identified which affected carcass quality. Topel et al. (1968) and Christian (1972) at Iowa State University first identified Porcine Stress Syndrome (PSS), or the halothane gene. Since its initial characterization, PSS or its genetic descriptor (HAL 1843) as described by Fuji et al. (1991), has been the most

significant genetic hurdle in reference to maximizing lean efficiency, while maintaining adequate carcass quality. Breeders and producers recognized the lack of overall productiveness in their swine and the increased stress susceptibility of pigs with a high percentage of lean, which lead to the selection of "production-type" hogs during the 1980's.

The "production type" hogs of the 1980's were in theory, evaluated and selected for a proportional balance of carcass and production traits. During the decade, breeders overcompensated for a lack of productiveness, and consequently composition was compromised excessively. Consumer groups began complaining about pork that was excessively fat at the retail level, which had negative feedback to breeders and producers. These problems led to the last and most recent type change within the domestic swine industry, to that of the "modern meat hog", individuals of desirable composition that maximize growth and lean efficiency.

During the domestic era of "production hogs", Europeans were already selecting individuals to maximize lean efficiency. It was during this time frame that European researchers identified the second major genetic cause of quality variation, the Rendement Napole gene (RN). The RN gene has been found to be primarily associated with lines of Hampshire breeding. Considering that Hampshires have a compositional advantage as compared to all other domestic purebred lines (NPPC, 1995), it stands to reason why they would be an integral part of breeding schemes for the "modern meat hog".

In order to strengthen the marketability of pork, the industry has been forced to recognize the qualitative problems associated with its product. According to the Pork Chain Quality Audit, (NPPC, 1994; Cannon et al., 1996) it was estimated that $10.10 was lost per harvested market hog at the packer level due to overall qualitative nonconformities. Furthermore, $1.11 of these economic losses was specifically attributed to problems with pork lean quality. These parameters were recently evaluated again, finding $ 1.83 was lost per harvested market hog due to inadequate lean quality (NPB, 2003). Pork lean quality can be defined as the physiochemical properties of pork that potentially effect palatability and/or processing characteristics. The increasing concern and awareness involving pork quality has forced the pork industry to make a concerted effort to increase the consistency and palatability of its product. In part it is this enhanced awareness of pork quality accompanied by the suspicions regarding the effects of Hampshires on quality that have decreased this breed's utilization in many modern commercial breeding schemes. In order for the Hampshire breed to again be appreciated and fully utilized for its advantages in cutability in reference to the other breeds commonly utilized, the industry must fully understand the problems involving the RN- gene and how it effects pork quality.

The present series of studies was conducted to investigate the growth performance, carcass composition, lean quality, shelf life, and sensory characteristics of individuals who were heterozygous carriers (RN-/rn+), and homozygous normal/recessive (rn+/rn+) for the Rendement Napole gene (RN gene or Napole gene) according to the DNA marker assisted test. Additionally,

the validity of the DNA test for the Napole gene was evaluated as compared to fresh lean quality parameters, such as ultimate pH, drip loss, and glycolytic potential. The results of this research initiative can be used in combination with previous research to determine the role of DNA genotyping for the Napole gene in accordance to pork quality endpoints.

CHAPTER II

REVIEW OF LITERATURE

The Identification of a Qualitative Defect

Researchers at the University of Wisconsin-Madison made the first documentation of a potential "Hampshire effect" relative to post-mortem muscle metabolism. Sayre et al. (1963) reported that the glycogen content of Hampshire longissimus dorsi (LD) immediately postmortem was two and three times higher than for LD muscle originating from Chester White and Poland China market hogs, respectively, at 0 hrs post-mortem. Additionally, residual 24 hr glycogen levels of LD were at 4% for Hampshires as compared to .3% for Chester Whites and .02% for Poland Chinas. Even so, Hampshires were not significantly different than the other two represented breeds relative to muscle lactic acid percentage or pH at 24 hr post-mortem. Longissimus dorsi from Hampshire individuals also possessed significantly higher phosphorylase and phosphofructokinase enzyme activities than LD muscle from pigs of the other represented breeds.

Jensen et al. (1967) reported that LD muscle originating from Hampshire market hogs had significantly lower 48 h pH values, and greater expressible juice area than LD samples from carcasses of Yorkshire and Duroc market hogs. Hedrick et al. (1968) reported that carcasses from Hampshire barrows had significantly lower loin and ham subjective firmness and color scores than carcasses from Duroc barrows. Additionally carcasses from Hampshire barrows exhibited significantly lower LD, gluteus medius (GM), and gluteus accessorius

pH, and greater LD cooking loss as compared to traits from the same muscles for Duroc barrows.

The Quantification of an Abnormality- "The Plight of the Hampshire Breed"

The initial groundwork investigations by American researchers stimulated interest within European pork production circles that were heavily utilizing Hampshire sire lines in terminal crossbreeding systems (Lundstrom et al., 1996). This was contusive to the development of the first groundbreaking research specifically quantifying the "Hampshire effect" and its negative influence on pork carcass quality (Monin and Sellier, 1985). Muscle samples attained from carcasses of Hampshire progeny exhibited significantly higher levels of glucose-6-phospahate, significantly lower ham cooking yields, and significantly more exudative LD wetness scores as compared to muscle samples from Large White (LW), Halothane normal Pietrains (HNP), and Halothane positive Pietrains (HPP) animal lines. Longissimus dorsi samples of carcasses derived from Hampshire individuals possessed numerically higher, less desirable average fiber optic values when compared to LD samples from carcasses generated from HNP and LW individuals. Loin samples from carcasses of Hampshire individuals exhibited poorer lean firmness scores as compared to LD muscle from carcasses of HPP and LW animals. Additionally, Hampshire progeny displayed carcasses with significantly lower 48 hr pH values for the LD, adductor femoris, biceps femoris (BF), gluteus profundus, and gluteus superficialis as compared to the same specific muscles from carcasses of the three other tested genetic lines. However, carcasses from Hampshire progeny did not differ as compared to the

carcasses derived from Halothane normal genetic lines in reference to 1 hr pH, yet were significantly higher than the Halothane carrier genetic line for 1 hr pH, implying that carcasses from individuals of Hampshire lineage had a normal rate of post-mortem pH decline.

The most appreciable development of this specific research initiative was the establishment of the protocol to calculate an individuals glycolytic potential (GP). Glycolytic potential is determined by analysis of glucose-6-phosphate, glycogen, glucose, and lactate levels/g. These values are then put into the formula: (2 x (glycogen + glucose + glucose-6-phosphate)+ lactate), to derive the total GP for a muscle sample. The unit measurement for this final GP is micromoles of lactate equivalent per gram-wet weight. The GP of muscle tissue from Hampshire progeny were significantly different, as compared to muscle samples from the other three genetic lines (229 Hampshire vs. 150 others). The authors concluded that the poor meat quality and processing yield of pork associated with Hampshire genetics was due to the "very low ultimate pH" of the product. The authors further hypothesized that the lower ultimate pH could be explained via a dramatically higher GP. Additionally it was noted that lower pH values of carcasses from Hampshire individuals were not due to an elevated lactate value, which was not different compared to carcasses from LW or HNP individuals, but possibly by drastically higher levels of glucose-6-phosphate ($P < 0.05$), compared to carcasses of LW or HNP individuals (Monin and Sellier, 1985).

To appreciate the value of quantifying an individual's GP, one must understand the role of glycogen in muscle metabolism. The primary source of muscle carbohydrate is glycogen. Glycogen is a polymer of glucose units linked with α 1-4 and α 1-6 linkages formed around a protein, P-glycogenin. When energy is required, glycogen is hydrolyzed to glucose by the action of glycogen phosphorylase, which cleaves α 1-4 bonds, and glycogen debranching enzyme, which cleaves α 1-6 bonds. These processes are referred to as glycogenolysis, and produce glucose, which is metabolized via glycolysis. During glycolysis, two adenosine triphosphate (ATP) molecules are produced per glucose molecule. Upon the cellular utilization of ATP, lactic acid is produced as an end product. In living muscle, lactate is transported in the blood to the liver where it encounters the Cori cycle and ultimately returns to the muscle cell as glucose. Yet in post-mortem muscle metabolism, the biological system is inactivated. It is this cellular increase in lactic acid that results in the pH decline of muscle tissue post-mortem (Brooks et al., 1996). Hence, it could be readily hypothesized as to the impact of an elevated initial glycogen level on ultimate muscle pH.

Naveau et al. (1985) derived a testing methodology titled Napole yield (NY), which reflects the percentage of product lost during a short-term, intensive curing process. This test procedure is a laboratory prediction of a meat block's ultimate yield of the popular "Paris-style" ham. The procedure is conducted by cutting 100 grams of post-rigor muscle into 1-centimeter cubes. The cubes are then immersed in 20 grams of brine solution containing sodium nitrite for 24 hours. The cubes are cooked in a boiling water bath for 10 minutes, and then

cooled for 2.5 hours before being reweighed. A higher percentage of NY reflects less product loss during processing. The authors alluded to the possibility that these individuals could presumably be associated with the "Hampshire effect", discussed by Monin and Sellier (1985). Scientists involved with this research initiative found that Hampshire individuals had significantly lower NY as compared to other represented genetics, though they could not definitively correlate these results to those of Hampshire individuals having an increased amount of glycogen or a lower ultimate muscle pH.

The development of this test lead to the deriving of the name "Rendement Napole", (RN) for the syndrome associated with individuals who had low percentages of NY. The French word "Rendement" has an English translation equivalent to "yield". The history behind the word "Napole" came from the three researchers involved in this particular study, **Na**veau, **Po**mmeret, and **Le**chaux.

Naveau (1986) then followed the initial study to raise the hypothesis that individuals of Hampshire lineage that exhibited poor NY percentages and low ultimate pH were potentially affected by the previously alluded to Rendement Napole abnormality. The author went on to suggest that individuals with these characteristics could possibly be carriers of a dominant gene that affects processing yields and fresh lean quality. To complement this, the author gave the prospective genetic disorder the representative (RN-) allele denoting a potential dominant affect for low NY as opposed to the (rn+) allele designating a potential recessive affect for normal NY. Ultimately, the writer suggested that the presence of the (RN-) allele caused "acid meat".

Genetic Causes and Biochemical Effects of the Rendement Napole Gene

In a research initiative that was focused toward muscle composition analysis and biological activity, Monin et al. (1986) found carcasses from Hampshire individuals to possess a unique combination of qualitative properties. Carcasses derived from Hampshire individuals exhibited significantly lower nitrogen contents and a significantly higher water to nitrogen ratio when compared to muscle from LW, HNP, and HPP carcasses. Little discernable differences were found for any of the represented genetic lines in reference to myosin isozyme 1, 2, and 3, or phosphorylase a + b activity, yet Hampshire progeny had significantly greater myosin isozyme 4 activity when evaluated versus Halothane normal individuals (Monin et al., 1986). Furthermore, Hampshire progeny generated carcass lean with significantly greater levels of glycogen synthase I + D, involved in glycogen anabolism, and citrate synthase, a mitochondrial enzyme, when compared to the muscle from other represented genetic lines. These authors alluded that a higher water to nitrogen ratio could be responsible for a lower yield of cured cooked ham in carcasses from Hampshire individuals. Additionally, the enhanced glycogen synsthase activity observed in Hampshires presumably explains the high level of glycogen characterizing these pigs.

Monin et al. (1987) helped to solidify these initial hypotheses by the evaluation of terminal hybrid market hogs that were 50% Hampshire, 35% Duroc, and 15% LW, versus purebred Landrace, Pietrain, and LW individuals. Citrate synthase activity was significantly higher in the LD and semimembranosus (SM)

of carcasses from Hampshire hybrids as compared to the same muscles attained from carcasses of purebred LW, Pietrain, and Landrace individuals. Carcasses derived from Hampshire hybrids also possessed significantly greater glycogen synthase I + D activity in the LD in relation to LD muscle from carcasses of the other three represented breeds. Additionally, the GP of Hampshire influenced individuals for LD and SM samples were dramatically higher and significantly different, as compared to LD and SM samples from the other three genetic lines. This could indicate that the high GP associated with LD and SM samples acquired from carcasses involving Hampshire genetics exhibits a dominant mode of inheritance in the Hampshire crossbred individuals.

LeRoy et al. (1990) confirmed that the unfavorable (RN-) allele was the dominant allele in reference to phenotype, in comparison to the (rn+) allele. Implying that individuals presumably displaying one RN- allele, regardless of the other allele (RN-/--) would be phenotypically quantified as carriers of the Napole gene, or heterozygous dominant in reference to Napole genotype. Conversely, individuals presumably displaying two rn+ alleles (rn+/rn+) would be phenotypically classified as being normal relative to the Napole gene, or homozygous normal or recessive. The study included NY analysis of Hampshire crossbred market hogs (n=6509) representing two separate hybrid lines. The means of the two supposed homozygotes differed by approximately three phenotypic standard deviations relative to NY, thus implying complete dominance in accordance to this particular trait.

Marinova et al. (1992) found there were not differences in reference to the percentage of α-red, α-white, and β-red muscle fiber types between LD samples of Hampshire hybrid and purebred LW individuals. Yet, the circumferences of β-red and α-white fibers tended to be larger in LD samples from Hampshire hybrids than in LD samples attained from LW carcasses. Furthermore, α-red fibers of LD samples from Hampshire hybrids were significantly larger in circumference compared to LD samples attained from LW carcasses. Lebret et al. (1999) found similar results for LD samples from individuals determined to be carriers for the Napole gene by GP assessment, as compared to LD samples from Napole normal individuals. Additionally, Marinova et al. (1992) reported the GP of LD samples from Hampshire hybrids were significantly higher than LD samples from LW individuals. Beyond this, the glycogen levels within α-white fibers taken from Hampshire and LW LD samples at harvest were higher for samples from Hampshire individuals than LW animals. Even though glycogen levels were higher in samples from Hampshire LD, these glycogen levels remained proportional for LD muscle derived from both Hampshire hybrid and LW individuals until 24 hr post-mortem, which further substantiates the hypothesis that carcasses derived from Hampshire influenced individuals, have a normal rate of post-mortem glycolysis. Furthermore, the authors' observations help to further confirm the hypothesis that the excess muscle glycogen exhibited by Hampshire influenced individuals is more evident in fibers with fast glycolytic and contractile capabilities.

Fernandez et al. (1992) evaluated the GP of two independent populations of Hampshire lineage. The samples were taken from live animal muscle utilizing a shot biopsy technique. In both populations, the distributions of the GP were bimodal in nature. This suggested that a dominant gene affected GP within Hampshire influenced populations. Comparatively, GP within LW populations had been documented as being normally distributed (Talmant et al., 1989). Additionally, NY was obtained for one of the represented populations. Within this particular population, individuals were segregated into Napole genotypes based on a bimodal distribution of NY. Individuals with a NY lower than 91% were considered RN- carriers (RN-/rn+). Individuals within this population classified as (RN-/rn+) using this criterion showed significantly higher GP than individuals classified as normal (rn+/rn+). The authors' suggested that the gene affecting muscle GP, and the dominant gene RN-, known to influence meat quality and processing characteristics, is the same gene. Furthermore, the authors implied that individuals of Hampshire lineage could be effectively phenotypically classified based on the location of the bimodal separation or threshold value regarding the GPs, with those below the separation being classified as genetically normal, or (rn+/rn+), and those above being genetically dominant, or (RN-/rn+), relative to the Napole gene.

Estrade et al. (1993b) were the first researchers to involve phenotypic segregation of Hampshire influenced individuals via GP analysis as a predictor of true Napole genotype, as an integral part of an alternative research initiative. The location of the bimodal separation utilized for segregation was approximately

200 µmol lactate equivalent /g loin sample. Samples of LD from carrier individuals exhibited significantly higher levels of glycogen as compared to LD from normal individuals at four different levels of cellular extraction. An ultrastructural evaluation was made on samples from the two respective genotypes utilizing electron microscopy. Samples of LD from carrier individuals possessed approximately 70% more glycogen in the sarcoplasm of the white fibers as compared to LD from normal individuals. More specifically, individuals deemed to be carriers expressed enlarged and irregular sarcoplasmic compartments as compared to individuals deemed to be Napole normal, as well as ill-structured mitochondria with abnormally shaped cristae. No histological differences were discerned in red fibers for carriers compared to normals. These results help to explain the findings of Marinova et al. (1992), and implied these authors to theorize that the defective Napole carrier genotype was associated with alterations in glycogen metabolism, or a malformation in protein accretion within white fibers.

Estrade et al. (1993a) further evaluated the last hypothesis. Researchers found that LD muscle from Napole carrier individuals, which had a GP threshold value > 201 µmol/g, had approximately 10% less protein as compared to LD muscle derived from carcasses of Napole normal animals, thus entertaining the hypothesis of a deformity in the accretion of protein. Yet the proportions of myofibrillar, stromal, and sarcoplasmic proteins, as well as non-protein nitrogen, within LD muscle were not different when compared over the two genotypes, implying no abnormality in the formation of the proportions of the different protein

compartments. Thus, leaving the alternative hypothesis that the RN gene is associated with an aberration of glycogen metabolism.

Another biochemical evaluation of the mode of action regarding the RN- gene focused on the levels of anabolic and catabolic enzymes involved in glycogen metabolism. In reference to anabolic activity, Estrade et al. (1994) reported significantly higher levels of glycogen branching enzyme activity, as well as a trend to have higher glycogen synthase I and I + D activity in LD muscle from Napole carrier animals as compared to LD muscle from normal animals. However, there were no significant differences for catabolic glycogen enzyme activity -- glycogen phosphorylase or debranching enzyme -- when compared over genotypes. This helped to solidify the hypothesis that not only do carrier individuals multiply a greater quantity of glycogen, but fail to metabolize more glycogen than a non carrier, thus resulting in a surplus of sarcoplasmic glycogen. The production of extra glycogen normally has a negative feedback on glycogen synthase and branching enzyme, and consequently has a positive feedback for the catabolic glycogen enzymes (Danforth, 1965; Munger et al., 1993); however, this does not appear to be the case with carriers of the Napole gene. Additionally, the authors suggested that cellular metabolism somewhat attempts to compromise for this abnormality by over exerting the mitochondria. This is supported by the findings of significantly higher levels of citrate synthase activity and mitochondrial respiration in muscle tissue of carrier individuals.

Lebret and coworkers (1999) were the first authors to attempt to identify individual animals that were homozygous dominant for the gene RN-/RN-, from

individuals that exhibited heterozygous dominance RN-/rn+, or from those of a homozygous recessive rn+/rn+ nature. The sample population was accomplished by backcrossing individuals of known NY via live animal muscle biopsy. The great-grandparent stock utilized in the development of this backcross had an estimated probability close to 1.0 of being homozygous normal or dominant, rn+/rn+ or RN-/RN-. The GP for dominant genotypes, RN-/RN- 222 µmol/g and RN-/rn+ 195 µmol/g, were significantly different compared to the recessive genotype, rn+/rn+ at 108 µmol/g. In regard to enzymatic evaluations, enzyme activity levels in LD muscle for lactate dehydrogenase were significantly lower in individuals exhibiting at least one dominant allele, as compared to individuals determined to be normal for the Napole gene. Additionally, β-hydroxy-acyl-coenzyme A dehydrogenase, and citrate synthase activities were significantly higher from LD muscle derived from carcasses of individuals with at least one dominant allele compared to Napole normal individuals. Yet, no differences were distinguished between carrier and homozygous dominant animals. These findings led to the calculation that carrier individuals significantly exhibited a 40% lower ratio of lactate dehydrogenase to citrate synthase. Thus, the authors concluded that these findings suggested a shift toward a more oxidative and less glycolytic muscle metabolism within LD tissue from carrier individuals. No significant differences were observed relative to Napole genotype for any previously alluded to enzymatic activity in the semispinalis capitis, a muscle thought to be more oxidative in nature.

Mariani et al. (1996) and Milan et al. (1996) discovered the chromosomal location (chromosome 15) and the region along that chromosome responsible for the quantitative traits associated with the Napole gene. However, these researchers were not able to identify one specific genetic locus, nor a group of multigenic loci deemed to be responsible for the characteristics associated with the RN- allele. It was not until Milan et al. (2000) that the specific quantitative trait locus as well as the true biochemical reaction that causes the genetic RN- mutation was identified.

Several quantitative trait loci that were believed to have an effect on muscle glycogen were mapped to chromosome 15 of the pig genome. Utilizing presumably Napole carrier tissue, three convincing matches were identified, two of which were identified within the human genome and one within the yeast genome (Milan et al., 2000). The two human sequences were associated with traits completely unrelated with muscle glycogen metabolism. Yet, the other codon showed significant sequence similarity to a gene identified in yeast affecting adenosine monophosphate-activated protein kinase (AMPK) subunits. Within yeast, AMPK has three subunits a catabolic α chain and two regulatory chains, γ and β (Milan et al., 2000). All three enzymes were evaluated for activity within all bodily tissues. The γ AMPK subunit was found specifically only within skeletal muscle, confirming its significance to the Napole gene. The coding sequence representing γ AMPK within the porcine genome was determined to be the PRKAG3 gene, and was deemed the primary gene responsible for the RN-

genotype. The actual mutation occurs via an arganine to glutamine substitution around the R 200Q residue of the gene (Milan et al., 2000).

The enzyme AMPK has a key role in regulating energy metabolism within animal tissues. It is biochemically activated by an increase in the ratio of AMP to adenosine triphosphate (ATP). The enzyme AMPK is released when the cytosol of the cellular system is in need of energy. A build up of AMP within the cytosol activates AMPK, which turns on anabolic ATP producing pathways, such as glycolysis, and inhibits catabolic ATP consuming pathways such as the citric acid cycle. Hence in a system with normal AMPK function, glycogen synthesis is decreased; glycogen degradation is increased, as well as an increase in glucose transport for the production of ATP. The authors found that AMPK activity was approximately three times higher in pigs deemed normal for the Napole gene than in individuals with a dominant Napole allele. This suggested that the mutation doesn't directly effect the chemical formation of AMP or AMPK, but rather the amount of AMPK that is synthesized and or released. This led to the authors' conclusion that carrier individuals released an abnormally low amount of AMPK, which leads to greater glycogen synthesis and lower glycogen degradation. A commercial diagnostic test for the PRKAG3 was developed by the sequencing of the reverse strand of the affected locus between nucleotide 595 to 599 (Milan et al., 2000). The DNA marker assisted test was made available to the domestic swine industry via licensing acquired by Geneseek, Lincoln, NE, in the spring of 2001.

Applied Effects of the Rendement Napole Gene

Ultimate pH

The findings by Sayre et al. (1963) and Monin and Sellier (1985) relative to carcasses from Hampshire animals having lower LD ultimate pH values were later substantiated by Enfalt et al. (1994) and the National Pork Producers Council Terminal Line Program Results (1994), who also found carcasses from Hampshire individuals with lower ultimate pH values within the LD. These findings are rationalized when considering the Napole gene was presumably segregating within these populations. Several authors have utilized GP from LD muscle as a phenotypic predictor of Napole genotype within Hampshire or Hampshire-influenced populations. Individuals with an interpreted GP, greater than the threshold value, as determined by the location of the bimodal valley of the particular population, were deemed to be carriers for the Napole gene (Fernandez et al., 1992). Multiple authors who used this methodology to phenotypically classify individuals found LD samples from carrier carcasses exhibited significantly lower ultimate pH values as compared to LD samples from carcasses of Napole normal individuals (LeRoy et al., 1996; Lundstrom et al., 1996; Enfalt et al., 1997a, b; Miller, 1998; Lundstrom et al., 1998; Lebret et al., 1999; Bidner et al., 1999b; Hamilton et al., 2000; Miller et al., 2000). Contrastingly, only one study suggested no difference between LD samples from Napole carrier and Napole normal individuals (Enfalt et al., 1997a). When segregated for the Napole gene by DNA assessment (Milan et al., 2000), ultimate pH of LD muscle from carcasses of Hampshire-influenced individuals of

carrier genotype were significantly lower than the ultimate pH of LD muscle from carcasses derived from animals of Hampshire genetics deemed normal according to DNA testing (Moeller et al., 2003 and Josell et al., 2003b). Additionally, Josell et al. (2003b) reported carcasses derived from carrier individuals produced semimembranosus, biceps femoris, quadriceps femoris, and gluteus medius muscles all displaying lower ultimate pH values than the same muscles evaluated within carcasses from normal individuals. All of these findings are hypothesized to have been caused by the greater initial glycogen substrate present within the white muscle fibers of carrier individuals (Marinova et al., 1992).

Technological Lean Quality

Samples of LD from carcasses attained from animals characterized by GP as Napole carriers depicted significantly greater drip loss percentages compared to LD samples derived from normal individuals (LeRoy et al., 1996; Lundstrom et al., 1996; Sutton, 1997; Enfalt et al., 1997b; Lundstrom et al., 1998; Bidner et al., 1999b; Hamilton et al., 2000; Miller et al., 2000). Samples of LD from carcasses attained from animals characterized by DNA testing as Napole carriers depicted significantly greater drip loss percentages compared to LD samples derived from Napole normal animals (Moeller et al., 2003 and Josell et al., 2003b). Additionally, Bidner et al. (1999a) and Moeller et al. (2003) reported greater purge loss percentages for vacuum packaged, wholesale, boneless center loin sections from carcasses of Napole carrier individuals than loins sections treated the same from carcasses of Napole normal individuals. Regardless of method of

characterization, no reports suggested a contrary relationship between LD from individuals of carrier Napole genotype in relation to drip loss percentages compared to lean samples from individuals deemed normal for the Napole gene. The phenomena of high drip loss percentages within populations where the RN gene is present is hypothesized to be the result of a low ultimate pH (McKeith et al., 1998) closer to the pH associated with the isoelectric point of postmortem muscle tissues, estimated to be approximately 5.2 (Forrest et al., 1975). If muscle proteins have a balance of molecular charges (i.e. isoelectric), proteins are molecularly bound to each other as opposed to immobilized or free water molecules, hence increasing drip loss (Forrest et al., 1975).

Irrelevant of ultimate muscle pH, another factor that potentially affects drip losses are the percentages of protein and water within muscle tissue. Upon proximate analysis of LD tissue attained from carcasses of both RN genotypes, Lundstrom et al. (1996), Lundstrom et al. (1998), Lebret et al. (1999), Bidner et al. (1999a), Miller et al. (2000) and Josell et al. (2003b), all found samples from carrier individuals to exhibit significantly lower protein percentages compared to samples from normal individuals. Additionally, Bidner et al. (1999a) and Josell et al. (2003b) displayed significantly higher moisture percentages from LD muscle from carrier individuals as compared to LD muscle from Napole normal individuals.

According to McKeith et al. (1998) water in muscle is bound to both glycogen and protein (2-4 g of water per 1 g of glycogen or protein). Hypothetically, more water is bound to glycogen in tissues from Napole carrier

individuals, due to the approximately 70% elevated levels of the substrate within white muscle fibers of individuals of this genotype (Estrade et al. 1993a). In turn, glycogen is more likely to be hydrolyzed postmortem as compared to protein, hence potentially increasing the drip loss percentages of individuals of carrier genotypes. Marinova et al. (1992) and Lebret et al. (1999) stated that LD muscle from Napole carrier individuals have larger diameters of fast, red, muscle fibers compared to LD muscle from Napole normal individuals. If muscle samples from Napole carrier individuals have lower protein percentages compared to normal individuals, as stated previously, the combination of these two ideologies help to validate the hypothesis by McKeith et al. (1998). Yet another assumption is that muscle tissues with low percentages of protein with a normal percentage of water could simply have fewer sites for water to bind than muscle tissue with normal percentages of protein and water.

Results similar to those from drip loss assessment were found for measurements of cooking loss and NY. Cooked loin chops from carcasses attained from animals characterized by GP as Napole carriers depicted significantly greater cooking loss percentages compared to cooked loin chops samples derived from normal individuals (Lundstrom et al., 1996; LeRoy et al., 1996; Lundstrom et al., 1998; Miller 1998; Bidner et al., 1999a; Hamilton et al., 2000; Miller et al., 2000). Only Enfalt et al. (1997b) reported levels of cooking loss indifferent relative to loin samples derived from carcasses of both represented Napole genotypes. In the only published assessment of cooking loss percentages for loin samples from carcasses derived from a population

genotyped by DNA technology, samples from Napole carrier carcasses displayed significantly higher levels of cooking loss compared to samples from Napole normal carcasses (Moeller et al., 2003).

Utilizing methodology modified from the procedure described by Naveau et al. (1985), NY assessment was conducted by numerous authors: LeRoy et al. (1996), Lundstrom et al. (1996), Sutton (1997), Enfalt et al. (1997b), and Lundstrom et al. (1998), all of whom found LD samples from carcasses of individuals deemed carriers by GP to have significantly lower NY percentages compared to LD samples from carcasses of individuals phenotypically determined to be normal for the Napole gene. These occurrences of poor cooking and curing yields of LD samples from Napole carrier individuals was potentially explained by McKeith et al. (1998) by similar modes of action by which the interaction for percentage protein and residual glycogen with drip loss was explained. It was hypothesized that as the excess residual glycogen in fresh lean tissue of Napole carriers was hydrolyzed during the cooking process, additional water was released, and greater cook losses ensued as compared to cooked lean tissue of Napole normal individuals.

Lean Color

Samples of LD obtained from carcasses of individuals phenotypically determined to be carriers of the RN gene via GP assessment, expressed significantly higher, more reflective, objective lean surface reflectance values as compared to LD samples from carcasses derived from LD muscle from carcasses of animals deemed normal for the RN gene (Lundstrom et al., 1996;

LeRoy et al., 1996; and Enfalt et al., 1997b). Furthermore, LD samples obtained from carcasses of Napole carrier individuals by GP assessment were determined by Lundstrom et al. (1998) as having significantly higher internal reflectance values, by Lebret et al. (1999) as having higher Hunter L* values ($P < 0.05$), and by Hamilton et al. (2000) as displaying higher Hunter L* and b* values, and lower subjective color and firmness scores (NPPC, 1991) ($P < 0.05$), compared to LD muscle from carcasses of Napole normal individuals. Samples of LD from carcasses of individuals determined as carriers for the Napole gene according to DNA assessment were determined to have significantly higher Hunter L* as well as significantly lighter and softer scores for subjective lean color and lean firmness appraisal (NPPC, 1991) compared to LD muscle from animals assessed as Napole normal by DNA procedures. In LD samples fabricated from carcasses of both carrier and normal individuals as determined via GP, no distinguishable differences were identified between Napole genotypes by Enfalt et al. (1997a) for surface reflectance values nor by Miller et al. (2000) for Hunter L*, a*, b* values or subjective firmness and color values. Only one study (Bidner et al., 1999b) reported significantly more desirable 'ean color parameters for individuals of carrier genotype in that LD samples obtained from carcasses deemed to be carriers by GP assessment exhibited significantly higher Japanese lean color score values, and in turn more desirable, lower Hunter L* and higher a* values when evaluated versus LD from carcasses derived from Napole normal individuals. Though exceptions remain, the RN- allele seems to be of at least partial dominance relative to lean color.

When considering the rationale associated with the tendency for pork from Napole carriers to be more reflective, one should remember the rather well documented and non-conflicting tendency for Napole carrier individuals to exhibit greater drip and purge loss. When combining this characteristic of pork from Napole carriers with the assumption that the primary protein substrate within meat purge is the sarcoplasmic protein, myoglobin, suggest that muscle samples from the Napole carrier genotype might have more initial myoglobin, that ultimately leaches out when compared to samples from individuals of normal Napole genotype, resulting in more reflective color (Monin and Sellier, 1985). Greater reflectance of pork from carrier individuals could also be attributed to the higher percentage of water within the lean tissue (Bidner et al., 1999a) and (Josell et al., 2003b). Tissues exhibiting high volumes of free, extracellular water have many reflecting surfaces that totally reflect light, but only a limited light absorption capacity (Forrest et al., 1975), potentially resulting in softer, more reflective lean tissue from carrier individuals as compared to Napole normal animals.

Sensory and Textural Characteristics

Though many lean quality parameters are less than desirable from individuals determined to be Napole carriers, several authors have suggested advantages for carrier individuals when compared to normal individuals relative to several palatability traits. The first suggestion of a possible "Hampshire effect" was made by Enfalt et al. (1994) and NPPC (1994) who found that LD samples from carcasses derived from purebred Hampshire carcasses depicted

significantly lower Warner-Bratzler shear force (WBS) values compared to LD samples from carcasses attained from purebred Yorkshires. Conflicting results have been generated relative to the RN gene and it's impact on palatability traits. When Hampshire influenced individuals were phenotypically classified by GP assessment, LD samples derived from carcasses of carrier individuals produced significantly lower WBS measurements compared to LD samples attained from carcasses from normal individuals (Lundstrom et al., 1996; Sutton, 1997; Enfalt et al., 1997b; Miller, 1998). Additionally, LD samples generated from carcasses of animals determined by GP to be carriers for the Napole and by DNA assessment to be normal for the Halothane gene had significantly WBS values compared to LD samples from carcasses of animals determined to be normal for both genes. However, these reports were contrasted by Lundstrom et al. (1998) and Miller et al. (2000), who reported no differences in reference to Napole genotype for LD samples relative to WBS assessment attained from animals determined by GP segregation for Napole status. For animals segregated for the Napole gene by way of DNA analysis, conflicting results were again evident relative to instrumentative tenderness assessment. Moeller et al. (2003) reported no differences for WBS values between LD samples from carcasses of either Napole genotype. Even so, Josell et al. (2003a) reported LD from carrier individuals exhibited significantly lower WBS values than normal individuals. Furthermore, Josell et al. (2003a) found WBS values for LD samples from carcasses of carrier individuals were significantly more tender at 1 and 4 days postmortem aging than LD samples from carcasses of normal individuals of the

same aging period. Additionally, LD samples from carcasses of normal individuals needed 7 days of age to reach the same WBS values attained form LD samples from the carcasses of carrier individuals at 4 days postmortem age.

Sensory characteristics from lean tissues of animals affected by the RN- allele generally followed the trends reported for textural analysis. Bidner et al. (1999a) and Miller (1998) found that LD samples from individuals determined by GP assessment to be Napole carriers expressed higher trained sensory panel values for tenderness and juiciness compared to individuals determined to be Napole normal. Conflicting results were reported by Lundstrom et al. (1996), LeRoy et al. (1996), Sutton (1997), and Lundstrom et al. (1998) who found no differences for sensory panel values of tenderness and juiciness from individuals phenotypically classified for either Napole genotype by GP assessment. Yet, Sutton (1997) and Lundstrom et al. (1998) reported trained sensory scores significantly higher for acidity from LD samples derived from carcasses of Napole carrier genotype as compared to cooked LD samples from carcasses of normal individuals. Furthermore, Lundstrom et al. (1996) reported higher sensory values for taste and smell intensity, along with higher acidity values from LD samples generated from carcasses of Napole carrier individuals as opposed to LD samples attained from carcasses of normal individuals.

When animals were genotyped via DNA technology, Moeller et al. (2003) found no differences between LD samples from carcasses of either Napole genotype relative to tenderness or chewiness, though samples from carriers tended to be juicier than samples from normal individuals ($P < 0.10$).

Additionally, cooked LD muscle from carrier individuals exhibited significantly lower values for traditional pork flavor and significantly higher values for off flavor compared to cooked LD samples from carcasses of normal individuals (Moeller et al., 2003). Josell et al. (2003a, b) depicted sensory attributes including: tenderness, juiciness, chewing resistance, and chewing time, to be more desirable for LD samples fabricated from carcasses of individuals determined to be carriers for the Napole gene by DNA assessment as compared to LD muscle from carcasses deemed to be Napole normal. Yet, both studies also reported LD samples from carrier individuals to have higher trained sensory values for acidity as weighed against LD samples from carcasses of individuals deemed normal for the Napole gene (2003a,b).

The tenderness and juiciness phenomenon associated with Napole carriers was rationalized by Lundstrom et al. (1996) via the idea that loin samples from carriers have a greater amount of sarcoplasm, i.e. cellular fluid, surrounding the myofibrils (Estrade et al., 1993b), resulting in an improved lubricating or diluting effect (Hedrick et al., 1994). Other hypotheses have been raised as to the mode of action relative to the enhanced tenderness associated with lean tissue from Napole carriers versus lean tissue from normal individuals. Josell et al. (2003a) found that LD from carcasses of Napole carriers, while still having a pH decline deemed normal or non-Halothane type in nature, displayed a significantly faster decrease in pH within the LD during the initial 5 hr rigor onset, than LD samples attained from the carcasses of normal individuals. Additionally, LD samples from the carcasses of carrier individuals exhibited significantly lower

values for isometric tension than LD samples from carcasses of normal individuals. Isometric tension is a mechanical quantification of the force per unit area necessary to contract; suggesting the LD tissue from carriers remained more elastic. Yet, there were no significant differences between genotypes relative to sarcomere length, though LD samples from carriers statistically tended to have longer sarcomeres than samples from normal individuals. Samples of LD from the carcasses of carriers possessed significantly shorter myofibrils after 1 and 4 days postmortem aging as compared to LD samples obtained from carcasses of normal individuals. After 4 days, the length of the myofibrils from the LD of carrier individuals had not changed, whereas the myofibrils from the LD samples of normal individuals continued shortening until 7 days postmortem aging. This implies that LD muscle in the carcass of carrier individuals have greater early postmortem proteolytic activity than LD muscle derived from a Napole normal carcass (Josell et al., 2003a). O'Halloran et al. (1997) suggested that pork muscle exhibiting rapidly glycolysing muscle tissue early postmortem (3 h postmortem) showed enhanced release of cathepsin B and L, higher calpain I activity, and lower calpastatin activity compared with pork of normal to slow postmortem glycolysis.

From the perspective of the flavor profile of muscle from carrier carcasses, the formation of furanthiols and disulphides, which are important to the development of a meaty flavor, are more active at a pH below 5.5 (Farmer and Mottram, 1990). Recognizing the innate biological tendency for carcasses of carrier genotype to display lower ultimate pH values possibly explains the

incidence of the more acidic and intense flavor for the cooked loin. Another possible contributing factor to the differences in the flavor perceptions between lean tissue of carrier and normal individuals involves the presence of excess residual glycogen present in lean tissue of carrier individuals. Inosine monophosphate (IMP) and hypoxanthine are flavor components produced as a by-product of denaturing protein tissue during the cooking process. These compounds have been associated to affect the flavor profile of meat products. Since IMP and hypoxanthine are both breakdown by-products of the metabolizing of ATP, it could be rationalized that muscle tissue with high volumes of energy (i.e. residual glycogen) could have stronger more pronounced flavors (Forrest et al., 1975).

Live Animal Performance and Carcass Composition

Several research initiatives have assessed growth and carcass composition parameters within populations where the RN allele was segregating. Within populations where Napole genotype was phenotypically determined by GP assessment no significant genotype effect was discerned as reported by Miller et al. (2000) in accordance to last rib fat depth, or by Hamilton et al. (2000) in reference to any measure of carcass composition including 1st rib, last rib, and last lumbar vertebra fat thicknesses, 10th rib fat depth, and 10th rib loin eye area or depth. When genotyped by DNA assessment no differences relative to Napole genotype in reference to ADG, or from carcasses derived from the same individuals in reference to carcass length, last rib fat thickness, or 10th rib fat depth, though a statistical trend existed for carcasses from carrier animals to

display larger loin eye areas as compared to carcasses from Napole normal individuals. Animals phenotypically classified by GP testing as Napole carriers showed statistical trends for carcass trait or live animal performance advantages as stated by Enfalt et al. (1997a) in reference to carcass length, 10^{th} rib fat depth, and percent lean yield, by LeRoy et al. (1996) relative to percent lean yield and loin eye area, and by Miller (1998) in terms of 10^{th} rib fat depth, loin eye area, ADG, and feed to gain ratio.

However, some reports do suggest statistically significant carcass composition and growth performance advantages for carrier individuals when both Napole genotypes are evaluated. LeRoy et al. (1996) reported that individuals determined by GP assessment as carriers depicted significantly less 10^{th} rib fat depth (1.3 cm) and significantly greater ADG (50 g/d) compared to Napole normal animals. Additionally, Enfalt et al. (1997a) depicted individuals determined as carriers by GP classification displayed significant advantages in ADG (26 g/d) when compared to normal animals. The carcasses from these faster growing carrier individuals depicted statistical trends for carcass composition advantages via less 10^{th} rib fat depth, and greater percent lean yield, as compared to carcasses from normal individuals. These marginal cutability advantages were further quantified by cutting analysis of the major muscles of the ham. Actual denuded weights and percent of carcass weights were significantly greater in carcasses from heterozygous dominant individuals for the following muscles: (SM), (BF), and gluteus medius (GM), when compared to the same muscles from carcasses of homozygous recessive individuals. Yet, the

percentage of quadriceps femoris (QF) on a dissected ham muscle basis was significantly greater in QF from homozygous recessive carcasses as opposed to heterozygous dominant carcasses. When recognizing that QF muscle is the most oxidative in nature of the compared muscles, Enfalt and coworkers (1997a) concluded that the proportions between the individual muscles of the ham were changed due to the RN- allele. A greater proportion of glycolytic muscles and a smaller proportion of oxidative muscles in the hams from carcasses of individuals with the heterozygous dominant genotype substantiated this suggestion. Yet in a subsequent assessment, no differences were recognized between GP determined Napole genotypes relative to percent lean yield or ham cutting analysis (Enfalt et al., 1997b). Lebret et al. (1999) reported significantly larger loin eye areas in carcasses from individuals deemed Napole carriers and homozygous dominant, RN-/RN-, compared to carcasses from Napole normal individuals. This was theoretically due to an increased diameter of Type IIA and Type IIBr (i.e. fast, red) muscle fibers within carrier individuals as compared to Napole normal individuals. No published hypothesis attempted to explain the trends for advantages of carrier individuals relative to carcass leanness.

Gene Frequency

In reference to gene frequency, LeRoy et al. (1990) utilized phenotypic GP assessment of LD musculature of individuals to estimate the frequency of the segregating RN- allele within the French Hampshire population to be approximately 60%. Enfalt et al (1997b) estimated the RN- allelic frequency within the Swedish Hampshire population via phenotypic assessment of GP from

LD tissue of individuals to determine a threshold value of 183 µmol of lactate/g. Results from this assessment suggested 85 percent of the individuals evaluated were phenotypically classified according to GP as having at least one RN- allele, thus giving an approximate genotypic frequency of 61 % for the RN- allele within the Swedish Hampshire population. Miller et al. (2000) quantified the frequency of the segregating RN- allele within the American Hampshire population. Napole carrier individuals were determined at a GP threshold value of 180 µmol of lactate/g. The percentage of U.S. Hampshires exhibiting the phenotypic characteristics of the Napole mutation was estimated at slightly over 85%, thus the dominant RN- allele exhibited an estimated frequency of approximately 63%. Moeller et al. (2003) were the first researchers to estimate the allelic frequency of the RN- allele based on the DNA PCR-based test. Within their American Hampshire crossbred population, the estimation of the RN- allele was approximately 69%, significantly higher that all previous estimates.

DNA Validation of Phenotypic Classifying by Glycolytic Potential

Utilizing the previously reported findings of Milan et al. (2000), Hamilton et al. (2002) utilized GP tissue samples, which were derived from individuals via either live animal biopsy or postmortem sampling of the LD. These same samples were subjected to the DNA based polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test for the status of the PRKAG3 gene. The GP threshold values regarding phenotypic quantification for the Napole gene were 190 and 158 µmol/g for the live animal biopsy and postmortem samples, respectively. In reference to DNA genotyping, longissimus

GP was significantly higher for LD samples from heterozygous dominant individuals compared to homozygous recessive animals (242.6 vs. 168.2 µmol/g, respectively) for live animal samples and (185.7 vs. 118.6 µmol/g, respectively) for postmortem samples, thus implying the validity of the GP assay. However, predicting genotype on the basis of LD GP resulted in 12.5% (n=32) of the samples (n=251) being misclassified compared to the DNA test. Yet, there was a trend for more effective classification utilizing postmortem samples as compared to live animal samples (8.9 and 18.3% misclassified, respectively). These findings compelled the authors to suggest that genotyping for the Napole gene by GP testing, as done in all previous research, does a relatively acceptable job of effectively discerning individuals possessing a RN- allele.

In a collaborative study between Iowa State University and Ohio State University, the efficacy of GP based classifying for the RN- allele, as well as quantifying qualitative relationships for the DNA based genotyping for the mutation was investigated (Moeller et al., 2003). Hampshire crossbred market hogs (n=118) were segregated at a bimodal threshold level of 150 µmol/g, based on postmortem LD sampling. According to GP segregation, 37 pigs were classified as RN-/rn+ and 81 were classified as rn+/rn+. Ironically, the precise antitheses of these values were found when the same samples were subjected to the DNA test for the Napole mutation, where 81 pigs were classified as RN-/rn+ and 37 as rn+/rn+. All 37 animals classified as RN-/rn+ by GP classification, were also genotyped as RN-/rn+ by the DNA test. Approximately two-thirds were misclassified by GP procedures when levels were between 120 and 150 µmol/g.

These problems were perceived by the authors to have been caused by a lack of a truly bimodal distribution, and more specifically an inability to correctly quantify the best value to declare as the threshold value. Nonetheless, the authors overwhelmingly concluded that DNA testing far supersedes GP analysis for the identification of the RN- allele.

As documented, the genetic influence on pork quality comprises differences among breed as well as differences among animals within the same breed. (Rosenvold and Andersen, 2003). These differences can be derived by way of a large number of genes with small effects, known as polygenic effects, or by large monogenic, major gene effects, such as the case with the Rendement Napole gene (Andersson, 2001). The RN gene is quantified as a major gene, due to the fact that the mean value of individuals homozygous for the gene RN-/RN-, and that of individuals not carrying the gene rn+/rn+, is equal to or greater than one phenotypic standard deviation, relative to the trait of interest (Sellier and Monin, 1994). It could be summarized that the Rendement Napole gene serves as a major gene with complete dominance for glycolytic potential, ultimate pH, protein percentage, NY, cooking and drip loss percentages. Even so, the Napole gene appears inconsistent in dominance relative to subjective color and firmness, Hunter L*, a*, and b* values, ADG, and carcass composition (Lundstrom and Andersson, 2001).

The Hampshire breed has serious issues to address concerning its performance as a breed as it relates to pork quality. One potential step to the improved perception of pork derived from Hampshire genetics would be to

merchandise pork produced from carrier genetics as a guaranteed tender branded pork product to a particular market that desires fresh pork as opposed to a processed product. This process would take advantage of the apparent tenderness advantages that Hampshires have in comparison to other heavily utilized domestic breeds. Although documented trends exist between research reports involving the Napole gene, the development of the DNA test has opened new doors to investigate. Further understanding of these problems and the corrective procedure asserted to them could easily resurrect the Hampshire breed back to its prior dominance as "The Meat Breed".

Pork Quality

Over the past 15 years, pork quality within the United States has become an increasingly important parameter relative to carcass value. Lean color is the primary criterion relative to consumer purchase intent of fresh pork (Brewer and McKeith, 1999). Consumers tend to prefer more reddish-pink, less light reflective lean as compared to pale gray to white lean, with greater light reflectance (Norman et al., 2002). Additionally, some domestic packers value based pricing systems now include a qualitative component. According to the Pig Improvement Company, PIC (2003) carcasses must exhibit an L* value lower than 50 to be eligible for the premiums associated with the Japanese export market. However, lean color is not the only criteria of concern with regards to pork quality. The amount of visible exudate lost during storage affects the value of the product (Joo et al., 1995). A study that attempted to quantify this problem was recently

completed, by way of the NPB funded, Pork Quality Benchmarking Assessment (NPB, 2003). The audit stated that approximately $0.50 per harvested market hog was lost due to inadequacies specifically relative to water holding capacity of pork muscle. It is important to note that this assessment was made purely at the packer level, suggesting that an all inclusive value assessment including retailers and processors would result in a much higher value loss.

One must appreciate the market share stability exhibited by fresh pork products during the past 10 years. At the same time, only 20-35% of pork worldwide is consumed fresh, where as 65-80% is further processed (Andersen, 1999). Thus implying the importance of not only fresh pork quality, but also processing characteristics of raw materials. Recognizing the well-established relationship of Napole carriers and further processing, more through discussion will be given to the association between ultimate pH, light reflectance, and water holding capacity and their impact on pork quality.

The phenomena known as pale, soft, and exudative (PSE) pork was first reviewed and characterized by Briskey (1964), where the author suggested that its development is caused by an extensive protein denaturation due to the combination of a low pH and simultaneously high temperature early postmortem. It is well established that there is a relationship between early postmortem pH and ultimate pH values, along with the association of these two pH estimates to pork quality endpoints such as lean color and water holding capacity. Yet, the true significance of these relationships is highly debatable. Numerous studies have estimated the amount of variability in objective reflectance values explained

by 45 min pH. Yet, only three of the ten published studies reviewed by Bendall and Swatland (1988) exhibited r^2 values higher than .45, and two of these studies evaluated less than 100 individuals. Additionally, Kauffman et al. (1993) clearly displayed that 45 min pH was not appropriate for predicting ultimate pork quality for individual carcasses, and also demonstrated that measurement of ultimate pH alone is not a reliable indicator of the PSE lean condition.

In terms of ultimate pH assessment, Warriss and Brown (1987) reported that the r^2 value of the relationship between ultimate pH and water holding capacity was approximately .15. Van Laack et al. (1994) reported only 24% of the variation in water holding capacity was explained by ultimate pH, as compared to Schafer et al. (2002), which reported a minuscule r^2 of .04 in reference to the proportion of variation in water holding capacity explained by ultimate pH. Yet, a study by Joo et al. (1995) reported that ultimate pH accounted for 67 and 69% of the variability exhibited in L* and drip loss values, respectively. However, these authors recognized that these values were indicative of the populations, which were evaluated. Kauffman et el. (1993), Warriss and Brown (1987), Van Laack et al. (1994), and Schafer et al. (2002) all evaluated loin samples that were considered to be from genetically normal populations. Additionally, all samples were deemed to be of either red, firm, and normal lean quality (RFN) or red, soft, and exudative (RSE) lean quality. However, Joo et al. (1995) investigated samples of unknown genotype that expressed phenotypic lean quality characteristics of not only RFN and RSE, but also dark, firm, and dry (DFD) as well as PSE samples. Thus, when the DFD and PSE samples were segregated

from the population, it resulted in a dramatically lower relationship between ultimate pH, water holding capacity, and colorimetric reflectance scores.

These findings are of substantial significance to animals affected by the Napole gene. Though the relationship involving lean color and RN- genotype is not fully understood, no documentation has been made of carrier individuals producing particularly "pale" lean color. Thus most pork of carrier Napole genotype is probably phenotypically classified as at least mildly RSE as opposed to RFN or PSE. Warner et al. (1997) stated that pork definitively expressing the phenotypic profile of PSE or RSE, did not respond to the endogenous calpain protelytic system responsible for postmortem tenderization. This is in contradiction to published results which suggest that pork from carcasses of Napole carrier individuals have greater drip loss values and generally tend to have higher lean reflectance values, however still exhibit lower Warner-Bratzler shear force values when compared to pork from carcasses of individuals deemed normal for the Napole gene (Hamilton et al., 2000).

In another comparative analysis of PSE, RSE, RFN, and DFD pork, Warner et al. (1997) addressed the basic physiochemical properties of these attributes in that samples were selected with the significantly different mean ultimate pH values of 6.3, 5.6, 5.4, and 5.3 for DFD, RFN, RSE, and PSE phenotypes, respectively. Solubility measurements were significantly lower for all protein types (sarcoplasmic, myofibrillar, and total), and myosin denaturation was higher for PSE samples compared with the other phenotypic characterizations. This supported the hypothesis by Offer in (1991) that the excessive drip loss of

PSE pork was caused by myosin denaturation, resulting in bound water loss. However, RSE and RFN samples were similar for both protein solubility and myosin denaturation, but RSE samples did have a significantly higher proportion of the sarcoplasmic protein, phosphorylase, compared to RFN individuals. RFN samples had significantly less drip loss, though no differences were distinguished in terms of thaw loss, cook loss and total water loss when compared to RSE samples. Still, RSE and RFN were both significantly different and intermediate for all measures of water loss compared to PSE and DFD. PSE and RSE samples exhibited less degradation of the protein titan, and inversely nebulin was more degraded than in RFN or DFD samples.

Warner et al. (1997) suggested that the pre-rigor conditions in RSE muscle caused precipitation of the sarcoplasmic proteins, which are the most sensitive to pH and temperature conditions existing immediately postmortem. Even so, the pre-rigor conditions in RSE muscle did not cause extensive denaturation of myofibrillar of sarcoplasmic proteins, yet RSE product still exhibits unacceptable drip losses. The only truly distinguishable difference between RFN and RSE individuals was found within ultimate pH. Thus implying that the properties expressed by RSE pork – high drip loss, yet acceptable lean color – could be loosely associated to the same phenomena expressed by carriers for the Napole gene. Still, the authors noted that the lean quality problems associated with RSE are to a lesser extent (Warner et al., 1997).

In a comprehensive assessment of pork quality, Rosenvold and Andersen (2003) suggested that continued selection for improved performance and

composition may have indirectly resulted in poorer meat quality attributes even in selection lines free of the HAL 1843 gene. Oksbjerg et al. (2000) compared two different selection lines of Danish Landrace progeny representing the growth potential and cutability trends comparable to current phenotype, while the other genetic line was more conventional, exhibiting growth and composition parameters comparable to individuals circa 1976. The muscle growth rate and lean gain per day of age of modern individuals was dramatically increased due to increased muscle fiber diameter and satellite cell proliferation during the selection period, as compared to individuals of 1976 type. Yet, selection had resulted in carcasses that exhibited significantly lighter more reflective lean with less myoglobin than the carcasses from the compositionally unimproved genetic line. These findings were further supplemented by Lonergan et al. (2001) which compared a selection line of Duroc pigs continually selected for lean growth efficiency to a conventional, genetically unimproved line of Duroc genetics. Performance and lean tissue accretion were significantly improved in the lean growth selected individuals, yet the more efficient animals exhibited carcasses with significantly lower early postmortem pH and greater drip loss in the LD, when compared to the carcass characteristics of the LD in the conventional line. Additionally, Barton-Gade (1990) concluded that there was significantly less pigment content in the LD and BF muscles from purebred Danish pigs on performance test at the end of the 1980's than represented in the LD and BF of the carcasses in Danish pigs at the beginning of the 1980's. These findings

suggest that single trait selectiveness for lean tissue accretion, inevitably has had negative repercussions in reference to lean quality.

Postmortem Enhancement

Consumers value tenderness in whole muscle products, and are willing to pay a premium for products that are guaranteed tender (Boleman et al, 1997). However, continual selection for lean efficiency has possibly indirectly lessened the acceptability of some qualitative carcass parameters, such as: lean color, marbling, and muscle pH, all which have been correlated to effect shear force values in pork (Davis et al., 1975) and beef (Wulf and Page, 2000). Hence, the U.S. pork industry's solution to less than desirable palatability traits has been postmortem injecting of a brine solution, otherwise known as "enhancement" into aged pork muscle. These pumping solutions may include, but are not limited to, phosphate, salt, flavor enhancers, and antioxidants. The primary purpose of the enhancement of fresh pork is to increase palatability characteristics via the improvement of tenderness, juiciness, and flavor (Banks et al., 1998). Yet, fresh pork enhancement has been suggested to improve shelf life, pork color stability and water holding capacity.

The injection of water, sodium chloride, and polyphosphates within a fresh meat system, is very similar to the brine solutions used in fully-cooked products for years within the processed meat industry, though injection solutions are absent of nitrite. The water serves as the medium for the solid ingredients and contributes to the products' palatability. Sodium chloride, or "table salt", is generally added as a flavoring agent, and helps to carry other ingredients into

tissues via the equilibration of osmotic pressure. The use of phosphate within meat substances has long been accepted and utilized within the processed meat industry (Bendall, 1954). However, the U.S. pork industry did not begin to recognize the applicability of postmortem enhancement until the early to mid-1990's.

Marginal introduction of phosphates to a meat protein system has been shown to improve all facets of palatability. Enhancement, specifically the presence of alkaline phosphate, improves juiciness, tenderness, and Warner-Bratzler shear force assessment (Sutton et al., 1997; Sheard et al., 1999; Prestat et al., 2002; and Jensen et al., 2003). The increase in juiciness and decrease in cook loss is accomplished by phosphates raising the pH of the product, thus improving the amount of bound water in the muscle protein lattice (Ellenger, 1972). Specifically, it is hypothesized that alkaline polyphosphates effects on juiciness has two possible modes of action- promoting the depolymerisation of myosin filaments and/or inducing dissociation of actomyosin bonds which open the protein lattice structure, exposing more positively charged bonding sites for water to attach (Offer and Trinick, 1983). Mixed results have been cited in reference to the water holding capacity of enhanced product. Sheard et al. (1999) found greater weight loss in pork loins enhanced with a 10% solution containing .3% phosphate than in non-enhanced loins. Yet, Sutton et al. (1997) found chops from enhanced pork loins had non-statistically different drip loss compared to non-enhanced loins. The discrepancy can possibly be attributed to the greater volume of free water as well as bound water within the protein

structure. The tenderness advantages exhibited by enhanced samples can be attributed to the weakened muscle structure as well as the greater water content of the cooked samples (Sheard et al., 1999), implying a lubrication effect (Hedrick et al., 1994).

The effect of phosphate on flavor has been attributed to the improved moisture retention of proteins (Ellenger, 1972) and the reduction of oxidative rancidity (Keeton, 1983). However, Smith et al. (1993) suggested that the utilization of phosphates at high levels could provide off flavors (i.e. "soapy"). Thus, it is not surprising that conflicting results have surfaced relative to the utilization of enhancement and the product's flavor profile. Sutton et al. (1997) found that enhancement of pork loins with sodium chloride (NaCl) and sodium tripolyphosphate (STTP), produced greater saltiness, but also decreased pork flavor intensity, compared to control samples. Sheard et al. (1999) stated that samples enhanced with NaCl and .5 % STTP had less intense pork flavor and greater abnormal flavor, though samples at the .3% level were no different than control samples. Vote et al. (2000) suggested that enhancement of beef strip loins with NaCl and STTP improved cooked beef flavor, but tended to impart off-flavors described by panelists as soapy and sour, when compared to control samples. Prestat et al. (2002) indicated that samples injected with .4% STTP and NaCl solution levels possessed more intense pork flavor than non-enhanced samples. Jensen et al. (2003) found that STTP and NaCl enhanced chops performed significantly better than controls for pork flavor saltiness, and still were not different in reference to off flavors classified as soapy or acid-like.

Incorporation of sodium lactate (Vote et al., 2000), and/or sodium acetate (Jensen et al., 2003) into enhancement solutions can help to alleviate off flavors. Yet, considering the well-documented "acid meat" nature of pork from Napole carrier individuals (Monin and Sellier, 1985), as well as some documentation of an acetic flavor profile (Josell et al., 2003a, b) the response to STPP might not be as drastic, and could possibly counteract flavors inherent to Napole carriers.

Irrelevant of endpoint palatability advantages, postmortem solution injection has been attributed to the improving of pork lean color (Krause et al., 1978). Research by Banks et al. (1998) suggested that pork enhanced with solutions containing .4% STTP displayed significantly lower L* values and significantly higher a* values when compared to pork with 0 and .2% STTP levels. These results were repeated by Prestat et al. (2002) which again distinguished enhanced samples more desirable for L* and a* compared to non-enhanced samples. These two studies were in direct contrast to results by Jensen et al. (2003) who found that loins enhanced with STTP were significantly higher for L* and significantly lower for b* as compared to control samples.

These findings may be rationalized by recognizing that the mean L* values of lean from non-enhanced samples were much lower in the last article, approximately (L* 53) than the first two articles, approximately (L* 60). This is in accordance with unpublished results by McKeith et al., who suggested that initial lean color played the most vital role to uniform post-enhanced color of the lean surface. As lean color becomes less reflective, it becomes more susceptible to a common problem within enhanced pork, lean striations, or "tiger striping". The

documented tendency regarding lean samples from Napole carrier individuals and light reflectance is that they tend to be more reflective than lean samples from normal individuals (Miller et al., 2000; Moeller et al., 2003), hence one could speculate as to the potential for lean color improvement of pork derived from carcasses of Napole carriers by way of enhancement.

Shelf life Stability

As consolidation and a depleted community infrastructure have swept across rural America, so has the onset of vertical integration within agricultural commodities. Excluding poultry production, no commodity has experienced such dramatic restructuring, as has pork production. Along with the improved production efficiencies associated with swine production has came dramatically improved proficiencies in reference to the packaging and marketing of fresh retail pork, such as the case-ready marketing of modified atmosphere packaged pork. The term "case-ready" implies that products arrive to retail marketers in an all-inclusive package, needing little to no modification prior to being placed in the retail case for potential sale. The meat industry verbiage of case-ready most often connotes to fresh muscle product displayed within a modified atmosphere package. The marketing of case-ready meats is a relatively new concept, yet retail sales of case-ready product have grown exponentially since the mid-1990's. Experts suggest that the construction of new harvest and initial fabrication facilities for America's largest protein providers is at a standstill, and will be for sometime. Yet, the expansion of present facilities and the remodeling of underutilized live to wholesale establishments into value-added or case-ready

facilities emphasizes the importance of better understanding a case-ready protein system.

Modified atmosphere packaging (MAP) can be defined as the packaging of a perishable product in an atmosphere modified so that its composition is other than air (Hintlian and Hotchkiss, 1986). Though several MAP packaging systems exist, the most extensively utilized methodology within the domestic pork industry is a high oxygen system consisting of 80% O_2 and 20 % CO_2. As with fresh meat packaged in any material where the surface is exposed, lean color is the primary constituent to consumers purchasing a modified atmosphere product. Reddish-pink pork color is primarily the result of the sarcoplasmic protein, myoglobin. The ultimate lean color of muscle tissue is most directly determined by the oxidative state of the iron atom that it contains. As oxymyoglobin, the protein commonly associated with reddish-pink color of fresh pork, is oxidized to metmyoglobin, i.e. "browning", it creates a dark gray to tan pigmentation associated with lean discoloration (Forrest et al., 1975.). As the percentage of surface oxymyoglobin particles that have been oxidized to metmyoglobin approaches 50% of the surface protein population, muscle tissue will begin to exhibit characteristics of lean discoloration (Lawrie, 1985).

Pigment oxidation is not the only constituent concerning lean discoloration, lipid peroxidation is associated with the occurrence as well (Greene, 1969; Faustman et al., 1989). Lipid oxidation is associated with the presence of free-radical oxygen to the surface of the product. As lipid molecules become oxidized, the reaction produces acids, aldehydes, and ketones, all of

which are associated with the odors and off-flavors found in meat that has undergone oxidative rancidity (Hedrick et al., 1994; Shahidi, 1994; St. Angelo, 1996). Tissues with high levels of polyunsaturated fatty acids are especially susceptible to oxidation, as they are the least chemically stable form of fatty acid, containing the most double bonds (Gray, 1978; Allen and Allen, 1981).

Nilzen et al. (2001) and Hogberg et al. (2002) depicted that LD attained from carcasses of individuals of carrier Napole genotype displayed a different fatty acid profile than did LD derived from carcasses of individuals displaying normal genotype. Specifically, Nilzen et al. (2001) stated that muscle from carcasses of Napole carrier individuals expressed significantly higher levels of n-3 polyunsaturated fatty acids compared with muscle from carcasses of Napole normal individuals. This suggests that pork from Napole carrier individuals would be more susceptible to lipid peroxidation during retail storage as compared to pork from Napole normal individuals. Recognizing the hypothesized palatability attributes of Napole carrier pork as a fresh product, (Josell et al., 2003a, b) implies that pork from individuals of carrier genotype could possibly be merchandised as a niche market or branded pork product. A guaranteed tender, branded pork product would be very conducive to a case-ready MAP packaged system. Thus, analysis of the shelf life characteristics of lean tissue from populations where the Napole gene is segregating should be deemed important.

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CHAPTER III

THE EFFECT OF DNA MARKER ASSISTED SELECTION FOR THE RENDEMENT NAPOLE GENE ON GROWTH PERFORMANCE AND CARCASS COMPOSITION, WHILE IN CONJUNCTION WITH ENHANCEMENT TREATMENT FOR LEAN QUALITY, SENSORY, AND SHELF LIFE CHARACTERISTICS

C. C. Carr, J. B. Morgan, S. D. Carter, F. K. Ray

ABSTRACT

Progeny (n= 70) from unrelated DNA tested, Napole carrier Hampshire sires and DNA tested, Napole normal Yorkshire dams were genotyped for the segregating RN- allele via DNA based reaction-restriction fragment length polymorphism sequence methodology. Six harvest groups ensued, with littermates all being represented within the same harvest. All carcass fabrication was initiated at 48 h postmortem. The anterior section of each boneless loin from right carcass sides was not subjected to enhancement treatment, whereas the posterior section of the same loin was enhanced with a solution containing .5% sodium chloride and .5% sodium tripolyphosphate to 110% of initial weight. Carrier individuals exhibited greater adjusted lean gain per day of age, and their carcasses possessed less actual and adjusted 10^{th} rib fat depth, and higher actual and adjusted percent lean yield values ($P < 0.05$), compared to normal individuals and their respective carcasses. Successive postmortem pH assessment during rigor onset resulted in lower pH values from LD within carcasses of carriers at 3, 6, 12, and 24 h ($P < 0.05$), and a tendency to have lower 48 h pH values ($P = 0.062$) compared to LD within carcasses of normal animals. Samples of LD from carcasses of carriers compared to LD samples

attained from carcasses of normal animals exhibited higher GP values and greater drip loss percentages, lower pH and higher a* values at fabrication, as well as more desirable trained sensory scores for tenderness, connective tissue amount, and overall sample acceptability ($P < 0.05$). No differences ($P > 0.05$) were found between LD samples at fabrication relative to genotype for L* value, though LD samples from carrier carcasses exhibited higher L* values following 7 d of retail display than corresponding samples from Napole normal carcasses ($P < 0.05$). Semimembranosus samples from carrier carcasses compared to normal counterparts exhibited higher purge loss percentages, lower pH values and higher L* values ($P < 0.05$). Enhanced LD samples exhibited higher drip loss percentages, higher pH and lower L* values at fabrication, along with higher trained sensory values for overall sample acceptability ($P < 0.05$), regardless of Napole status. Non-enhanced LD samples attained from carcasses of carrier barrows exhibited lower shear force values than all other non-enhanced LD samples ($P < 0.05$). LD samples from carcasses of carrier barrows were the only genotype:gender combination not affected by enhancement treatment relative to shear force values ($P > 0.10$), whereas LD samples from carcasses of carrier gilts exhibited the most dramatic response to enhancement treatment when compared to any other genotype gender combination, as assessed by shear force values ($P < 0.05$). Non-enhanced LD samples from carcasses of normal animals exhibited more desirable lean color subjective scores for overall acceptability than LD samples from all other genotype: enhancement treatment combinations ($P < 0.05$). This coincides with findings that LD samples from

carcasses of normal animals compared to counterpart samples of carcasses from carriers exhibited lower thiobarbituric acid reactive substances (TBARS) after 7 d of retail display ($P < 0.05$). These findings suggest that the Napole gene has positive effects for lean tissue accretion and sensory tenderness, but detrimental affects for numerous assessments of technological lean quality and shelf life characteristics, which were often further compounded when subjected to enhancement treatment.

INTRODUCTION

Over the past fifteen years domestic and foreign fresh meat consumers have emphatically stated the importance of product quality, in reference to both visible appearance and palatability traits, to purchase intent (Boleman et al., 1997; Brewer and McKeith, 1999; Norman et al., 2002). This redirected consumer focus from one of percentage edible lean portion, to that of lean quality, has forced the U.S. pork industry to make a concerted effort to characterize it's problems, thus hopefully improving pork lean quality. All segments of the pork chain have been scrutinized as to their inputs on end product quality. As with all corrective procedures, substantial focus should be asserted to the initial source of deviation, which relative to pork quality would be genetics. The presence of the Rendement Napole gene (RN-) has been depicted to have a derogatory affect for numerous traits affecting lean quality (Lundstrom et al., 1996; Hamilton et al., 2000; Miller et al., 2000; Moeller et al., 2003). However, enhancement injection of whole muscle products has been

illustrated to improve several variables involved in lean color stability and sensory assessment (Smith et al., 1984 and Sutton et al., 1997). The present series of studies was conducted to investigate the growth performance, carcass composition, lean quality, shelf life, and sensory characteristics of individuals who were heterozygous carriers (RN-/rn+), and homozygous normal/recessive (rn+/rn+) for the Rendement Napole gene (RN gene or Napole gene) according to the DNA marker assisted test. Additionally, the impact of enhancement treatment in combination with Napole genotype was evaluated relative to lean quality, shelf life, and sensory characteristics of fresh pork cuts.

METHODS AND MATERIALS

Animals

Test animals were bred and reared at the Oklahoma State University swine teaching and research facility. The individuals were produced by the mating of purebred Hampshire boars via artificial insemination to purebred Yorkshire females. Semen was obtained from a commercial boar stud (Swine Genetics International, Cambridge, IA) from three unrelated sires, which were deemed carriers, (RN-/rn+) for the Napole gene by way of DNA based PCR testing. All females were DNA genotyped for Napole status by means of blood sample extraction; where all represented individuals were discovered to be homozygous normal (rn+/rn+). At piglet processing, three days post-farrowing, ear tissue samples produced as a by-product of conventional ear notching were gathered for each test progeny, then individually sealed and frozen for

subsequent assessment of RN- genotype status. All progeny of the seventeen test litters with greater than eight live pigs weaned were subjected to DNA genotyping for Napole status. All dam and progeny testing was completed by a commercial genetic laboratory (Geneseek, Lincoln, NE), according to protocol described by Milan et al. (2000).

Each litter was weaned at 18 to 22 d of age and allotted to pens in a conventional climate controlled nursery. All test individuals were subjected to a sequential three phase nutritional scheme during nursery growth. Test animals were fed the initial starter ration containing 1.47% lysine and 3,415 kilocalories (kcal) ME for the initial 5-7 d. Individuals were then transferred to nursery rations containing 1.32% lysine, 3,430 kcal ME and 1.12% lysine, 3,432 kcal ME after approximately 7 d on subsequent rations, respectively. At the conclusion of the nursery phase (approximately 35 d), individuals were allotted by litter into pens in a mechanically ventilated grower/finisher building with fully slatted floors. Where gender and genotype litter constraints allowed, pens were randomly arranged with at least one Napole carrier barrow and gilt, and one Napole normal barrow and gilt represented in each pen. Upon arrival, individuals were subjected to a growing ration containing 1.00% lysine and 3,320 kcal ME. As individuals approached 84 kg live weight, they were transferred to a finishing ration on which they remained until harvest containing .70 % lysine and 3,338 kcal ME. All rations throughout production met or exceeded all NRC (1998) requirements. Six harvest groups were represented, with littermates all being represented within the same respective harvest. Ultimately, 33 carrier individuals (RN-/rn+),

barrows (n=16) and gilts (n=17), along with 37 normal individuals (rn+/rn+), barrows (n=18) and gilts (n=19), were harvested at an average weight of 112.3 kg. One individual with an actual live weight two standard deviations lower than the sample mean for live weight was eliminated from further analysis. All adjusted carcasses traits were calculated according to protocol described by NSIF (1997). All animals were humanely harvested at the Oklahoma State University Meat Science Laboratory abattoir located within the Oklahoma Food and Agricultural Product Center. Animals were mixed in one group during transport and abattoir holding. At the harvest facility, animals were rested and fasted for approximately 12 h with free access to water prior to harvest.

Carcass Composition and Quality Assessment

A schematic of fabrication and lean quality assessment is depicted in Appendix I. Postmortem pH and muscle temperature assessment of the longissimus dorsi (LD) muscle was measured on the left carcass side intercostally at 45 min, 3 h, 6 h, 12 h, and 24 h utilizing a portable pH meter (pH Star, SFK Co., Cedar Rapids, IA) and temperature probe (Koch Equipment Co., Kansas City, MO). Evaluations were obtained within the LD at 45 min between the 10^{th} and 11^{th} ribs, with successive evaluations being made between the 11^{th} and 12^{th} ribs (3 h), 9^{th} and 10^{th} ribs (6 h), 12^{th} and 13^{th} ribs (12 h), and 8^{th} and 9^{th} ribs (24 h).

At 48 h postmortem, dorsal midline last rib backfat and all subsequent carcass measurements were obtained from the right side of each carcass. Carcasses were divided into wholesale cuts by separations made directly

posterior to the 2nd rib, 5 cm directly anterior to the cranial tip of the aitch bone, and 5 cm from the juncture of the 3rd rib and thoracic vertebrae to a point directly ventral to the psoas major. Following carcass separation into wholesale cuts, the loin was divided between the 10th and 11th ribs. After allowing for a fifteen-minute oxygenation period (i.e., bloom) loin muscle area, 10th rib fat depth, and subjective lean color and marbling scores (NPPC, 2000) were assessed at the 10th /11th rib interface. All adjusted carcass traits were calculated according to protocol described by NSIF (1997). Both loin sections were then skinned, deboned, and trimmed to a commodity fat trim level of 6.35 mm. For the most anterior loin section, four 1.91 cm chops were fabricated placing the chops bloom surface up for ultimate muscle pH and objective lean color (L*, a*, b*) analysis utilizing a Mini Scan XE Plus (Hunter Lab, Reston, VA) with an illuminant setting of D65. Chops were randomly allocated for retail evaluation (n=1), with the three remaining chops being vacuum packaged for subsequent Warner-Bratzler shear force (n=1) and sensory analysis (n=2) following a 7 d postmortem aging period. The remaining loin sections were then individually identified, vacuum packaged and stored for 7 d at 4°C for subsequent assessment of purge loss, muscle pH, and objective lean color assessment.

From the loin section posterior to and including the 11th rib, one 2.54 cm and one 1.91 cm chop was generated. A 2.54 x 2.54 cm section was further fabricated from the center of the lean tissue of the 2.54 cm chop for drip loss analysis. Drip loss assessment was accomplished by a method modified from Honikel (1987). Briefly, the initial sample was weighed, then pierced with a

tagging gun, and suspended in a 4°C cooler while covered by an inflated plastic bag for 24 h prior to the sample being re-weighed. Drip loss percentage was calculated by weight loss divided by initial weight x 100. The remainder of the initial chop was immediately frozen in a Whirl-Pac bag for glycolytic potential analysis. The remaining chop was frozen and ultimately utilized as a day 0, base line value for thiobarbituric acid (TBA) assessment for non-enhanced pork loin.

Product Enhancement

The remaining posterior loin sections were enhanced with a solution containing .5% sodium chloride and .5% sodium tripolyphosphate, administered by means of a commercial mechanical injector (Formac-Reiser, Canton, MA). Samples were injected (10% of green weight) and allowed to equilibrate for approximately 1 h prior to further fabrication. Following equilibration, 1.91 cm (n=5) and 2.54 cm (n=1) chops were fabricated from loin sections. The initial, most anterior, 1.91 cm chop was allowed to bloom, then was subsequently evaluated for post-enhancement pH and objective lean color assessment. The single 2.54 cm chop was further fabricated for drip loss assessment, as described previously. The remaining 1.91 cm thick chops were randomly allocated to 1) base line TBA assessment, 2) retail shelf life, 3) sensory analysis, and 4) Warner-Bratzler shear force determination. These chops and the remaining loin sections were treated according to protocol described previously for samples of the same analyses. Additionally, semimembranosus muscles were segregated from the intact hams of each right carcass side and the adductor muscle was removed. Following the denuding process, the lean surface was

allowed to bloom for approximately 30 min. Objective color evaluations and ultimate pH were obtained on the medial side of the semimembranosus muscle. Each semimembranosus muscle was individually identified and stored in a standard vacuum bag for 7 d at 4°C for subsequent assessment of purge loss, muscle pH, and objective lean color assessment.

At the conclusion of the storage period, purge weighing the initial package, dispersing stored fluids, then reweighing, made analysis. Purge loss percentage was calculated by weight loss divided by initial weight x 100. Day 7 objective color assessment and pH values were taken of all storage and retail samples, after allowing for bloom time (approximately 30 min.) of the vacuum packaged storage samples. Retail evaluation samples were individually vacuum packaged and frozen for endpoint TBA analysis following the completion of the previously mentioned qualitative assessments.

Retail Shelf Life

Chops (n=138) from enhanced and non-enhanced treatment groups, representing all animals, were individually packaged and assigned to retail cases for shelf life analysis. Chops were placed in a 0.6 ethelene-vinyl alcohol (EVOH) modified atmosphere packaging (MAP) tray (ROCK-TENN Co., Norcross, GA) and sealed with Cryovac 1050 lidding film (Cryovac, Duncan, SC) within 1 hour of retail fabrication. Packaging was accomplished utilizing a Mondini MAP machine (Model CV/VG-5, G. Mondini S.P.A. Cologne, Italy), which removed atmospheric air and flushed with a pressurized mix of 80% O_2 and 20% CO_2. Test packages were subjected to an oxygen headspace analyzer (Model HS-

750, MOCON Modern Controls Inc., Minneapolis, MN) to ensure the atmosphere contained 80% O_2 prior to subsequent packaging of research samples. All MAP samples were displayed in commercial retail coffin cases under cool-white fluorescent light (1,600 to 1,900 lux) at 2 to 4°C for 7 d. Panelists were instructed to only evaluate parameters affecting the LD muscle of each sample. Samples were subjectively evaluated daily at mid-day by a trained shelf life panel for lean color (7=pale pinkish gray to white; 1=dark purplish red), worst-point lean color (7=pale pinkish gray to white; 1=dark purplish red), muscle firmness (7=very firm; 1=very soft and watery), percent discoloration (7=none; 1=complete), and overall appearance (7=extremely desirable; 1=extremely undesirable).

Warner-Bratzler Shear Force Analysis

Warner-Bratzler shear force assessment was made for all loin samples on two separate days to measure instrumentative tenderness. Following fabrication, all samples (n=138) were placed in individual vacuum packages, where they initiated a 7 d postmortem aging period and were frozen (-10°C) at its conclusion. Samples were tempered for 24 hours at 4°C prior to cooking. Chops were broiled on an impingement oven (Lincoln Impinger, Model 1132-00-A) at 180°C to an internal temperature of 70°C. Temperatures were monitored with a Digi Sense type T thermocouple (Model 91100-20, Cole-Parmer Instrument Company, Vernon Hills, IL). Individual chop weights were obtained prior to and following cooking for the calculation of cooking loss percentages. Cooking loss percentages were calculated by cook loss divided by initial weight x 100. Upon

cooling to 21°C, a minimum of four cores (1.27 cm diameter) were obtained parallel to the muscle fiber orientation and sheared using a Warner-Bratzler attachment on an Instron Universal Testing Machine (Model 4502, Instron, Canton, MS) at a cross head speed of 200 mm per minute. Instron program software was utilized to record the peak load (kg) of each core, which was logged by a Dell Optiplex GS400. Mean peak load of each sample was calculated and analyzed.

Sensory Analysis

Potential panelists were trained for sensory analysis in accordance with guidelines published by American Meat Science Association (1995). Two LD samples per animal:enhancement treatment combination (n=138) were individually vacuum packaged following fabrication and ultimately frozen (-10°C) following a 7 d postmortem aging period. Samples were randomly selected for cooking day and order, then tempered for 24 hours at 4°C. Chops were broiled on an impingement oven as described for Warner-Bratzler shear analysis. During product testing, each session consisted of six panelists. Fourteen samples were presented to each panelist. Two cubic portions (1.3 cm x 1.3 cm x cooked chop depth) from each sample were served warm to panelist under red light. Panelists were instructed to average sensory perceptions for the two individual portions. Samples were evaluated for tenderness (8=extremely tender; 1=extremely tough), juiciness (8=extremely juicy; 1=extremely dry), connective tissue (8=none; 1=abundant), saltiness (5=not salty; 1=extremely salty), off-flavor (5=none detectible; 1=extremely strong), and overall acceptability (7=extremely

desirable; extremely undesirable). Between samples, panelists cleansed their palate with unsalted crackers and distilled water.

Thiobarbituric Acid Assay

Estimates of lipid oxidative rancidity were made on the surface of samples via thiobarbituric acid (TBA) analysis. Samples (n=276) were distributed randomly relative to harvest date and treatment across the four testing days to ensure all genotypes and treatments were effectively represented. Baseline and final TBA samples were taken from samples with 0 and 7 d retail display, respectively. The procedure was preformed following protocol outlined by Buege and Aust (1978). The following modifications were made to the procedure: surface lean tissue of pork loin samples (10g) were homogenized with deionized water in a Waring Commercial Blender (Model 33BL79 (700), Waring Products Division Dynamics Corporation of America, New Hartford, CT) and centrifuged at 1,850 G for 10 minutes at 4°C (Beckman Induction Drive Centrifuge, Model J-6M, Beckman Instruments, Inc. Houston, TX). Procedures from the initial protein substrate homogenate were then conducted in duplicate. Two mL of homogenate, in duplicate, were subjected to TBA reagent and cooked in a boiling water bath. After cooling, absorbencies of the supernatant were measured using a spectrophotometer (Beckman, Model DU 7500 Beckman Instruments, Inc. Houston, TX), at a frequency of 531nm. Results were recorded as thiobarbituric acid reactive substances (TBARS), which are representative of malondialdehyde (MDA) equivalents per kg pork loin.

Glycogen Potential Analysis

The formula to calculate glycogen potential (GP) was calculated as described by Monin and Sellier (1985) as (2 x (glycogen + glucose + glucose-6-phosphate)+ lactate), where the unit measurement is micromoles of lactate equivalent per gram-wet weight (µmol/g). Glycogen potential was analyzed for non-enhanced LD sample frozen at 48 hours postmortem according to a method modified from Miller et al. (2000), Dalrymple and Hamm (1973) and Keppler and Decker (1974). Sample preparation for the assay was performed by homogenizing 6 g of fresh loin tissue in 30 mL of 0.6 N perchloric acid in a Waring blender. Samples were duplicated by pipetting 200 µl of homogenate into 1.5 mL microcentrifuge tubes (n=2). A mL of amyloglucosidase (AGS) enzyme solution (1 mg AGS protein/1 mL 0.2 M acetate buffer, at pH 4.8) and 20 µl 5.4 N potassium hydroxide were added to samples and immediately vortexed and incubated for 2 h at 37°C, while being agitated periodically. Samples were cooled in an ice bath for 10 min and 0.1 mL of 3 N perchloric acid was added to stop the catabolic reaction. Samples were centrifuged at 7,000 g for 5 min at 4 °C (Fischer Scientific Micro-centrifuge Model 594, Fischer Scientific, Pittsburgh, PA). The resulting supernatant contained the hydrolyzed glycogen as glucose, glucose-6-phosphate (glu-6-p) and lactate.

Glycogen determination was initiated by the mixing of an enzymatic buffer solution. The stock of the solution was triethanolamine buffer (5.6 g triethanolamine hydrochloride, 100 mg magnesium sulfate, 12 mL 1 N potassium hydroxide, 50 mL deionized water). This buffer was used in the manufacturing of

an enzymatic buffer solution containing: (60 mg adenosine triphosphate (ATP), 80 mg nicotinamide adenine dinucleotide phosphate (NADP), 300 µl glu-6-p dehydrogenase, 100 mL triethanolamine buffer). A mL of the enzymatic buffer and 5 µl hexokinase was added to 50 µl of extraction supernatant. The mixture was vortexed and incubated at 37°C for 15 min. Glycogen, glucose, and glu-6-p concentrations were determined simultaneously using the sipping attachment of a spectrophotometer at a frequency of 340 nm. Lactate determination was achieved by adding 2.9 mL lactate buffer solution (.005 g nicotinamide adenine dinucleotide (NAD), .95 mL glycine buffer, 1.9 mL deionized water, 0.047 mL lactate dehydrogenase) to 100 µl of extraction supernatant. The mixture was vortexed and incubated at 37°C for 15 min. Lactate concentrations were determined utilizing a spectrophometer at a frequency of 340 nm.

Statistical Analysis

All assessments of live animal performance, lean quality, sensory characteristics, and shelf life characteristics were analyzed using generalized least squares (PROC MIXED, SAS Inst., Inc., Cary, NC). The design structure was a randomized complete block design, where individuals were blocked by litter. Fixed effects included in the model for all analysis were RN genotype, and gender, with harvest date, litter, and sire included as random effects. Enhancement was utilized as a fixed effect in all models except live animal performance, carcass pH, carcass composition traits, and quality assessment of semimembranosus muscle. Days of display (0-7) were utilized as a fixed effect for shelf life analysis, as was storage day (0 and 7) for Hunter L*, a*, and b* and

pH. Mean separation was accomplished using the P-DIFF option of SAS at an Alpha level of .05. The numerically highest standard error of the respective main effect or interaction mean was reported.

RESULTS AND DISCUSSION

Glycolytic Potential

All research reports published subsequent to Fernandez et al. (1992) until 2002, utilized glycolytic potential (GP) testing as a phenotypic prediction of true Napole genotype. Thus, it stands to reason, that all publications involving the Rendement Napole (RN) gene during this period reported individuals of "Napole carrier genotype" to exhibit significantly higher GP values than individuals of "Napole normal genotype". Table 1 depicts the findings within this study in that, GP values were significantly higher ($P < 0.0001$) for LD samples from carcasses of individuals determined by DNA assessment as Napole carriers (312.6 µmol/g) as compared to LD samples of individuals normal for the Napole gene (213.1 µmol/g). Hamilton et al. (2002) and Moeller et al. (2003) found similar results suggesting the efficacy of the DNA test to distinguish GP values, which prior the 2002, was considered the optimum method to segregate individuals for the RN- allele. Also, LD samples from barrow carcasses exhibited significantly higher ($P = 0.02$) GP values when compared to LD samples from gilt carcasses.

Growth Performance and Carcass Characteristics

The documented effects of the RN gene on live animal growth performance and carcass characteristics have been rather inconclusive. Table 2 depicts the results relative to this study in that, carcasses from Napole carrier individuals had significantly less 10^{th} rib fat depths, higher percent lean yields, less adjusted 10^{th} rib fat depths, and higher adjusted percent lean yields, than carcasses from Napole normal individuals. Additionally, carrier individuals displayed 8.57 g/d greater adjusted lean gain per day of age compared to Napole normal individuals. These findings relative to the Napole gene are the most persuasive as to a compositional advantage for Napole carrier individuals that have been reported. LeRoy et al. (1996) reported carrier individuals exhibiting 50 g/d higher ADG ($P < 0.05$) when compared to Napole normal individuals, and that the carcasses from carriers exhibited 1.3 mm less backfat ($P< 0.05$) when compared to carcasses from normal individuals. Additionally, they reported statistical trends for advantages for carriers as compared to normal individuals relative to percent lean yield. Enfalt et al. (1997a) reported Napole carrier individuals displaying a significant advantage in weight per day of age (26 g/d) compared to normal individuals, but only an advantageous statistical trend for the carcass traits of 10^{th} rib fat depth and percent lean yield. LeBret et al. (1999) found an increase in loin muscle area from carcasses of Napole carriers, as compared to carcasses of normal individuals, but no differences in reference to carcass fatness or red meat yield. These results represent the only published documentation of a possible positive association of the RN- allele to cutability

and lean tissue accretion. It should be noted that all literature published prior to 2002 phenotypically segregated individuals based on glycolytic potential relative to Napole gene status. However, the present research initiative is in direct contrast to Moeller et al. (2003) who segregated the test population for the RN gene by DNA assessment. Even so, the present research suggests that the RN- allele could be of at least partial dominance relative to carcass leanness and lean tissue accretion.

In reference to gender influence on live animal performance, barrows expressed a significantly higher weight per day of age and a lower adjusted lean gain per day of age compared to gilts, resulting in carcasses with lower percent lean yields, smaller adjusted loin eye areas, and lower adjusted percent lean yields (Table 2). No differences were distinguished for either gender or genotype effects for the following carcass traits: loin eye area, hot carcass weight, last rib fat thickness, lean gain per day of age, or subjective lean color and marbling. Also, barrows did tend to have heavier carcasses with more last rib backfat ($P < 0.10$), than carcasses from gilts (Table 2). The aforementioned gender effects are in complementary accord with the findings of the NPPC Terminal Line program results (1995). However, the lack of difference between Napole genotypes for subjective lean color scores is in contrast to the findings of previous reports by Hamilton et al. (2000) and Moeller et al. (2003) who found carcasses from carrier individuals to have significantly lower subjective lean color scores compared to carcasses from normal individuals. Additionally, Miller et al. (2000), Hamilton et al. (2000), and Moeller et al. (2003) reported carcasses from

carrier individuals to have lower subjective marbling scores than carcasses from Napole normal individuals ($P < 0.05$).

Carcass pH and Temperature

Successive postmortem pH assessments were acquired for all test individuals, where results are reported in Tables 3. Carcasses derived from Napole carrier animals had significantly lower LD pH values at 3, 6, 12, and 24 h, and statistically tended to have lower ultimate 48 h values ($P = 0.062$), when evaluated versus measurements from carcasses of carrier individuals. The initial pH assessments are in agreement with Josell et al. (2003a) who reported that LD from Napole carrier individuals, while still having a postmortem pH decline deemed normal or non-Halothane type in nature, possessed a significantly faster decrease in pH during the initial 5 h rigor onset compared to LD from normal individuals. The lower ultimate pH value associated with carrier individuals is documented by numerous authors (LeRoy et al., 1996; Lundstrom et al., 1996; Enfalt et al., 1997b; Lundstrom et al., 1998; Miller, 1998; Lebret et al., 1999; Bidner et al., 1999b; Hamilton et al., 2000; Miller et al., 2000; Moeller et al., 2003). Barrow carcasses exhibited significantly higher LD temperature during rigor onset compared to gilt carcasses (Table 4). This can possibly be attributed to the findings that barrow carcasses tended to have heavier hot carcass weights with more last rib fat thickness compared to gilt carcasses.

Physical Water-Holding Capacity

Numerous assessments of physical water-holding capacity of muscle including drip loss, purge loss, and cooking loss percentages, were made to attempt to further quantify the relationships between Napole genotype, enhancement solution, and gender, which are reported in Table 5. In the analysis of conventional 24 h drip loss percentage, LD samples from carcasses of Napole carriers had significantly higher drip loss percentages than LD samples attained from carcasses of normal individuals (i.e., 6.82 vs. 5.86%, respectively). Furthermore, vacuum packaged semimembranosus from carcasses of carrier individuals exhibited higher purge loss percentages ($P < 0.001$) than semimembranosus muscle from carcasses of normal individuals after 7 d of storage. The findings relative to Napole status were in accordance with the findings of multiple authors (Lundstrom et al., 1996; Enfalt et al., 1997b; Sutton, 1997; Miller et al., 2000; Hamilton et al., 2000; Moeller et al., 2003), who reported higher LD drip loss percentages from carriers, though all authors found greater numeric differences than in the present analysis. Results for purge loss percentage are in agreement with Bidner et al. (1999b) and Moeller et al. (2003), who found that carrier individuals exhibited a higher percentage purge loss; however, these results were found utilizing LD muscle.

In reference to gender influence, LD samples fabricated from barrow carcasses depicted higher drip loss percentages than LD samples from gilt carcasses. A contributing factor to greater drip loss percentages of LD samples from barrow carcasses could be the significantly higher GP of barrows compared

to gilts. Miller et al. (1998) reported that drip loss was positively correlated with GP ($r = .30$, $P < 0.005$), within non-Hampshire influenced populations with normal GP values (GP < 160). Gender effects for drip loss percentage have not been extensively documented, though numerical values were higher for LD samples from barrows as compared to LD samples from gilts according to NPPC (1995) and Hamilton et al., (2000). Purge loss assessment on d 7 for MAP packaged LD samples depicted no significant differences as affected by gender, enhancement solution, or Napole genotype. This could be speculated to be attributed to product surface dehydration during the latter portion of retail display in the modified atmosphere system, possibly inhibiting the loss of unbound water.

Relative to postmortem injection, enhanced LD samples had higher drip loss percentages compared to non-enhanced LD samples. Additionally, a statistical trend for an interaction between genotype and enhancement treatment was present for d 7 purge loss percentage of vacuum packaged loin sections ($P < 0.10$). Enhanced loins from carcasses of carriers nearly numerically doubled purge loss percentage values compared to enhanced loins (8.03 vs. 4.69%, respectively) fabricated from carcasses of Napole normal individuals. Additionally, enhanced loins from carcasses of Napole normal individuals expressed numerical purge loss values over five times higher than non-enhanced loins from either represented Napole genotype, suggesting that postmortem enhancement overwhelms the biological system relative to water binding capacity. In reference to LD cooking loss percentage, samples from carcasses of carriers statistically tended to possess higher cooking loss percentages ($P =$

0.0754) compared to samples from carcasses of normal animals, while no differences were discerned between enhanced and non-enhanced samples. The results for cooking loss percentage as affected by Napole genotype are complemented by Lundstrom et al. (1996), Miller (1998), Lundstrom et al. (1998), Bidner et al. (1999a), Miller et al. (2000), Hamilton et al. (2000), and Moeller et al. (2003). Only Enfalt et al. (1997b) reported no differences for cook loss percentages from LD samples across Napole genotype.

These trends for purge loss and cooking loss percentages, though not statistically significant at the traditional probability level, suggest that Napole carriers, who theoretically possess a more open protein lattice structure and larger sarcoplasmic compartment (Estrade et al., 1993), tended to demonstrate a lower capacity to bind exogenous water -- regardless of the presence of phosphate in an enhancement solution -- when compared to LD samples from normal individuals.

Results specifically for enhancement treatment and water holding capacity were complementary to the findings of Detienne and Wicker (1999) who found loins enhanced to 110% of initial weight with an enhancement solution containing .5% sodium tripolyphosphate (STPP) to have poorer water-holding capacity than non-injected loins. Yet, the results of this initiative were contrary to the findings of Smith et al. (1984) who reported samples from enhanced loins containing .475% added STPP to exhibit greater water holding capacity than non-enhanced loins. Additionally, Sutton et al. (1997) found pork loins enhanced with a solution containing .4% added STPP to express lower purge and drip loss percentages

compared to non-enhanced loins. However, the present research was in accordance with Sutton et al. (1997) relative to cooking loss percentages, who found no differences between pork loins enhanced with a solution containing .4% added STPP and non-enhanced loins.

Retail and Subprimal pH Assessment

All retail and subprimal pH findings are reported on Table 6. Chops of LD samples from carcasses of carrier individuals exposed to retail evaluation in a MAP system exhibited significantly lower pH values than their counterparts from carcasses of normal individuals, across storage day and enhancement treatment. As well, semimembranosus and boneless vacuum packaged loin subprimal sections from carrier individuals possessed lower ($P < 0.0001$) pH values than the same muscle samples from Napole normal individuals. These findings were similar to findings of numerous authors (LeRoy et al., 1996; Lundstrom et al., 1996; Enfalt et al., 1997a, b; Miller, 1998; Lundstrom et al., 1998; Lebret et al., 1999; Bidner et al., 1999b; Hamilton et al., 2000; Miller et al., 2000; Moeller et al., 2003), though findings from all authors were specifically derived from 48 h pH of LD muscle.

Chops from enhanced LD samples at initiation of retail evaluation, 0 d, exhibited significantly higher pH values when compared to non-enhanced samples at 0 d of retail display within a MAP system. Enhanced boneless vacuum packaged loin subprimal sections at the initiation, 0 d, of vacuum packaged storage exhibited significantly higher pH values compared to their non-

enhanced counterparts at the initiation of vacuum packaged storage. The tendency for pork loin samples enhanced with a solution containing STPP to exhibit higher pH values when compared to non-enhanced pork loins is supported by numerous authors (Smith et al., 1984; Jones et al., 1987; Sutton et al., 1997; Banks et al., 1998; and Detienne and Wicker, 1999). Interestingly, MAP packaged, non-enhanced LD chops within this study exhibited pH values that slightly increased over the display period ($P < 0.05$), whereas their counterpart enhanced MAP LD chops exhibited pH values that significantly decreased while in retail simulation. Enhanced boneless, vacuum packaged loin subprimal sections exhibited no change in pH over storage time. Yet, non-enhanced boneless vacuum packaged loin subprimal sections exhibited significantly higher pH values after the 7 d storage period compared to the pH values of the same samples at storage initiation. Muscle pH should either stay constant or slightly increase during postmortem aging (Forrest et al., 1975) as found in non-enhanced muscle samples. Even so, it could be speculated that as residual metabolites continue to be denatured during postmortem aging, via the endogenous proteases innate to normal musculature, muscle pH could slightly decrease, as found in LD retail samples from enhanced loins. However, no definite explanation exists for the apparent interaction of muscle pH and enhancement treatment with subprimal storage and retail display times.

Retail and Subprimal Objective Lean Color Assessment

All findings for retail and subprimal objective lean color assessment are illustrated in Tables 7, 8, and 9. L^* values from lean samples regardless of

muscle evaluated, packaging type, storage length, or Napole genotype, were higher than ideal, though few, if any, lean samples were visibly classified as "pale". Generally, all muscles evaluated over time at least tended to exhibit higher L* values (more reflective), higher a* values (more reddish colored), and higher b* values (more yellowish colored).

For Hunter L* assessment, no difference ($P > 0.05$) was found relative to Napole genotype from LD chops at the initiation of MAP packaged retail simulation (Table 7). Miller et al. (2000) reported no difference for L* values relative to Napole genotype. However, Hamilton et al. (2000) and Moeller et al (2003) reported significantly higher L* values for LD samples from carcasses of carriers compared to contemporary samples from normal animals. LD chops from carcasses of carriers expressed higher L* values at the conclusion of retail assessment compared to counterparts from normal carcasses ($P < 0.05$). Semimembranosus from carcasses of carriers exhibited significantly higher Hunter L* values than accompanying samples from carcasses of normal animals, as depicted in Table 7 ($P < 0.05$).

Semimembranosus and LD samples from barrow carcasses displayed higher L* values than counterpart samples from gilt carcasses ($P < 0.05$). These findings were complementary to Moeller et al. (2003) who also reported higher L* values from LD samples of barrow carcasses when compared to LD samples attained from gilt carcasses. However, the results of the present research was contrary to reports by NPPC (1995) and Hamilton et al. (2000) who found no difference relative to gender for L* values of LD, though barrow samples

exhibited numerically higher values than gilt samples. Irrelevant of Napole status, significantly lower L* values were observed from enhanced LD samples when compared to non-enhanced LD samples ($P < 0.05$). These results were in complementary accord to findings by Banks et al. (1998) and Sutton et al. (1997) who found pork loins injected with an enhancement solution containing .4% STPP to exhibit significantly lower L* values than non-enhanced pork loins.

Sections of LD from carcasses of Napole carriers exhibited significantly higher Hunter a* values (more reddish lean color), compared to LD sections from carcasses derived from normal individuals ($P < 0.05$), as depicted in Table 8. No difference ($P > 0.05$) for Napole genotype was distinguished in LD chops at the initiation of retail assessment (Table 8). However, LD chops from carcasses of normal individuals, particularly non-enhanced samples, exhibited higher a* values than LD chops from carrier individuals at the conclusion of retail display ($P < 0.05$). Non-enhanced LD samples exhibited higher a* values at the conclusion of retail assessment and subprimal storage than enhanced LD samples ($P < 0.05$).

Subprimal LD sections from carcasses of carriers displayed significantly higher b* values at the conclusion of 7 d of retail display than LD subprimals from carcasses of normal animals (Table 9). Semimembranosus samples from carrier carcasses tended to exhibit higher b* values than accompanying samples from carcasses of normal animals. Enhanced LD samples exhibited higher b* values at the conclusion of retail assessment and subprimal storage than non-enhanced LD samples ($P < 0.05$).

Objective Textural and Subjective Sensory Assessment

In reference to Warner-Bratzler shear force (WBS) assessment, a significant statistical three-way interaction existed between Napole genotype, gender, and enhancement treatment as represented in Table 10. Non-enhanced LD samples attained from carcasses of carrier barrows depicted significantly lower WBS values than all other non-enhanced LD samples. However, LD samples from carcasses of Napole carrier barrows were the only genotype: gender combination not significantly ($P > 0.05$) affected by enhancement treatment relative to their combined influence on WBS. On the other hand, LD samples originating from carcasses of Napole carrier gilts significantly exhibited the most dramatic response ($P < 0.05$) to enhancement treatment when compared to any other genotype gender combination, as assessed by WBS values. The lack of consistency for WBS values of LD samples from carcasses of carrier genotypes theoretically explains the cause of the statistically significant three-way interaction. Furthermore, this lack of consistency presents an anomaly relative to the true mode of action responsible for the lower WBS values of LD samples from carrier individuals when compared to LD samples from Napole normal animals reported by numerous authors (Lundstrom et al., 1996; Enfalt et al., 1997b; Hamilton et al., 2000; Josell et al., 2003a).

If LD samples from the carrier barrows represented in this study, were representative of LD samples of all Napole carriers relative to postmortem enhancement and WBS assessment, it could be hypothesized that the more glycogen filled, larger sarcoplamic compartment present in LD samples from

carrier individuals (Estrade et al., 1993) disenables it's biochemical ability to bind exogenous water, regardless of phosphate. Hence, suggesting that LD samples from carrier individuals are not susceptible to lower WBS values from enhancement application due to an already maximized water-binding threshold, where instrumentative tenderness is already maximized. However, if LD samples from the carrier gilts represented in this study, were representative of LD samples of all Napole carriers relative to postmortem enhancement and WBS assessment; it could be hypothesized that LD muscle from carcasses of Napole carriers is just as susceptible to the biological principals that enhancement solutions utilize to lower WBS in any other normal meat protein.

Both of the previously alluded to hypothesis relative to the potential mode of action affecting the interaction of LD samples from carrier individuals with incorporation of enhancement solution, may be all for not according to the sensory findings summarized in Table 11. LD samples from carcasses of carrier individuals exhibited higher values ($P < 0.0001$) for trained sensory tenderness when compared to LD samples from carcasses of Napole normal individuals (Table 11); yet, least square means for samples from both genotypes still coincided within the written numerical description of "slightly tender" (Appendix II). Nonetheless, these statistical findings are complementary to reports by Miller (1998), Bidner et al. (1999b), and Josell et al. (2003a, b), who found that LD samples from carcasses of carrier individuals exhibited significantly higher values for trained sensory panel tenderness ratings as compared to counterparts from carcasses of normal animals. Additionally, this initiative is also in complement of

the findings of Josell et al. (2003a), who reported that carcasses from Napole carriers exhibited a slightly faster pH decline during the initial postmortem rigor mortis onset stage when compared to carcasses of Napole normal individuals ($P < 0.05$), possibly implying a tenderness advantage for LD samples from carcasses of carrier individuals when compared to counterpart samples from normal individuals due to a more rapid early postmortem glycolysis. However, Moeller et al. (2003) was in contrast of the present research, reporting that carrier individuals as determined by DNA assessment performed no different than Napole normal individuals relative to trained sensory assessment for tenderness.

In reference to trained sensory panel assessment of juiciness, a statistical three-way interaction ($P < 0.01$) existed between Napole genotype, gender, and enhancement treatment (Table 11). Enhanced LD samples from carcasses of Napole carrier barrows exhibited significantly higher juiciness scores than LD samples from all normal individuals, with the exception of enhanced LD samples from carcasses of Napole normal gilts. Non-enhanced LD samples from carcasses of Napole carrier gilts preformed as well as all samples from carcasses of Napole normal individuals, regardless of enhancement treatment. Additionally, non-enhanced LD samples from carcasses of Napole carriers exhibited numerically higher least square means for juiciness than non-enhanced LD samples from carcasses of Napole normal individuals, though these values were not statistically different. The findings of this research were in accordance to reports by Lundstrom et al. (1996), LeRoy et al. (1996), Lundstrom et al. (1998), Sutton (1997), and Moeller et al. (2003), who all found no difference

between LD samples relative to Napole genotype for trained sensory juiciness scores; yet, none of these studies utilized enhancement solution as an affect in the assessment. Furthermore, the results from trained sensory juiciness scores for enhancement treatment and Napole genotype, works against the previously mentioned hypothesis regarding WBS values that implied enhancement had little to no effect for LD samples from Napole carriers.

For trained sensory evaluations of residual tenderness, LD samples fabricated from carcasses of carrier individuals exhibited more desirable values for connective tissue amount than LD samples attained from carcasses of Napole normal individuals ($P < 0.05$). Josell et al. (2003a, b) reported similar results, stating that cooked LD chops from carcasses of carrier animals exhibited less chewing resistance and chewing time than LD chops attained from normal carcasses. The results from this initiative and the previously alluded to research reports were indirectly supportive of findings from Lebret et al. (1999) who found no differences for hydroxyproline concentrations between Napole genotypes. If this is true for cooked LD chops for carriers of the Napole gene, the improved myofibrillar tenderness expressed by LD samples from Napole carriers as compared to LD samples from normal individuals, could have a possible carry over affect with sensory panelists for connective tissue amount.

In terms of enhancement treatment affects, enhanced LD chops depicted significantly poorer values for trained sensory connective tissue amounts when compared versus non-enhanced LD chops ($P < 0.01$). This occurrence is surprising when considering that product enhancement was conducted by using

a commercial mechanized injector. Any physical disruption of the characteristics of the stromal proteins should only help to improve residual tenderness. However, the increased product rubberiness and tooth penetration generally found in enhanced samples could have potentially accentuated the connective tissue present (Morgan et al., 1991), potentially rationalizing the less desirable connective tissue values found from enhanced samples when compared to non-enhanced samples.

Enhanced LD samples exhibited a saltier flavor profile ($P < 0.0001$) compared to LD samples from non-enhanced loins. However, enhanced and non-enhanced samples both exhibited sensory saltiness levels that were within the written description of "slightly salty". Other differences for trained sensory saltiness assessment found cooked LD chops from carcasses of Napole normal gilts to exhibit a significantly saltier flavor profile than cooked LD samples from carcasses of Napole normal barrows. In reference to trained sensory perception for off-flavors, a statistical three-way interaction existed ($P < 0.05$) between enhancement treatment, gender, and Napole genotype. Non-enhanced LD chops from carcasses of Napole carrier individuals exhibited significantly higher values for off-flavors than enhanced LD chops from carcasses of carrier barrows and non-enhanced LD chops from carcasses of normal gilts. Presently, no viable explanation exists for these reported relationships. Though samples from a gender: enhancement treatment: carrier genotype combination depicted the numerically lowest values for off-flavors, samples from a different combination, still possessing carrier genotype exhibited the numerically highest value for off-

flavor. Thus, the sensory results for off-flavors from this particular study does little to validate or contradict the results of Lundstrom et al. (1996), Lundstrom et al. (1998), and Moeller et al. (2003) who all found that LD samples from carcasses of carrier individuals exhibited significantly higher values for trained sensory off-flavor scores compared to LD samples from carcasses of normal individuals.

For overall sample palatability, cooked LD chops fabricated from carcasses of carrier individuals exhibited higher scores ($P < 0.01$) for overall acceptability compared to cooked LD samples attained from carcasses of Napole normal animals (Table 11). This suggests that though relationships for juiciness and flavor profile were inconsistent relative to samples from Napole genotype, panelists perceived cooked LD samples from carcasses of carrier individuals to be more highly palatable overall, when compared to cooked LD samples from carcasses of Napole normal animals. Additionally, enhanced cooked LD samples depicted significantly higher values for overall sample palatability when evaluated versus LD samples from non-enhanced loins ($P < 0.05$).

Shelf life assessment

In terms of subjective lean color evaluation, LD chops from carcasses of normal individuals exhibited higher, more desirable scores ($P < 0.05$) than LD chops from carcasses of Napole carrier individuals (Table 12). Additionally, LD chops attained from carcasses of carrier barrows exhibited significantly lower values than LD chops from carcasses of Napole carrier gilts (Table 12). In reference to the affect of enhancement treatment on subjective lean color

assessment, non-enhanced samples from gilt carcasses exhibited higher lean color values ($P < 0.05$) compared to samples from carcasses of any other gender: enhancement treatment combination (Table 13).

For subjective worst-point lean color assessment (Figure 1), LD chops from enhanced loins decreased, or became lighter colored ($P < 0.05$), for worst-point lean color scores over the 7 d MAP packaged retail assessment period. However, LD chops from non-enhanced loins increased, or became darker colored ($P < 0.05$), for worst-point lean color scores over the 7 d MAP packaged retail assessment period. The darkening of LD chops from non-enhanced loins could be due to continual surface dehydration within the MAP system. Chops from enhanced loins could have became increasingly lighter colored due to exhibiting high volumes of free, extracellular water which has many reflecting surfaces that totally reflect light (Forrest et al., 1975).

In reference to subjective percentage discoloration assessment (Table 14), non-enhanced LD chops from carcasses of Napole normal individuals exhibited less discoloration during the 7 d shelf life assessment than LD chops attained from carcasses of any other genotype and enhancement treatment combination ($P < 0.05$). No differences ($P > 0.05$) were discerned between LD samples from carcasses of carrier individuals for percentage discoloration, irrelevant of enhancement treatment. Collectively, these results imply that lean samples from carcasses of animals of normal genotype are more susceptible to surface lean discoloration, via enhancement treatment, than lean samples attained from carcasses of carrier genotype animals. The results for

enhancement and percentage discoloration were in contrast to findings by Jensen et al. (2003), who reported non-enhanced samples exhibited greater subjective lean discoloration when compared with enhanced samples, during a 96 h shelf life assessment. However, Brewer et al. (2002) reported results that indirectly supported the present research, finding consumers to be more likely to purchase non-enhanced pork loin chops than enhanced pork loin chops, with consumer citing advantages for non-enhanced pork relative to color uniformity and lean firmness.

Subjective lean firmness scores exhibited a significant decline over the last 6 d of MAP packaged retail simulation (Table 15), after an initial increase from d 0 to d 1, which possibly represented a further equilibration of muscle proteins following fabrication. Chops of LD from carcasses of Napole carrier individuals were softer than LD chops fabricated from carcasses of Napole normal individuals ($P < 0.0001$), in reference to subjective lean firmness scores as shown in Table 16. Also, LD chops from enhanced loins exhibited dramatically lower subjective firmness scores compared to LD chops from non-enhanced loins, 3.96 versus 4.51, respectively ($P < 0.0001$).

Non-enhanced LD samples from carcasses of Napole normal animals exhibited higher values for subjective overall acceptability than their counterpart samples from Napole carrier individuals ($P < 0.05$), as depicted in Table 17. In reference to the affect of enhancement treatment on overall acceptability (Table 17 and 18), non-enhanced LD samples exhibited dramatically higher overall acceptability scores than enhanced LD samples ($P < 0.05$). Additionally,

subjective overall acceptability values from enhanced LD samples attained from barrow carcasses were significantly lower than their accompanying samples from gilt carcasses, as illustrated in Table 16. In accordance to actual days of retail shelf life (Figure 2), LD chops from non-enhanced loins exhibited a 24 h advantage of overall product acceptability by trained panelists compared to LD chops from enhanced loins ($P < 0.05$), 7 d versus 6 d, respectively. Additionally, LD chops attained from non-enhanced loins exhibited a numerical least squares mean for overall acceptability correlating to being "acceptable" after 7 d of retail simulation, where as the least squares mean for LD chops fabricated from enhanced loins after 6 d correlated to being "slightly undesirable"; yet, these differences were not significant ($P > 0.05$). Chops of LD fabricated from carcasses of normal individuals exhibited significantly higher subjective overall acceptability scores after 6 and 7 d of retail simulation, compared to LD chops derived from carcasses of Napole carriers. However, these scores did not differ according to the written description of the values, which were "acceptable" for d 6 assessments and "slightly undesirable" for d 7 assessments.

Generally, LD samples derived from carcasses of carrier individuals preformed poorer for all variables evaluated involving lean color during retail simulation when compared to LD samples fabricated from carcasses of Napole normal individuals. These results for genotype were rationalized via findings for TBA assessment (Table 19), which found higher values for MDA equivalents per kg pork loin from LD chops from carcasses of carrier individuals when compared to LD chops attained from carcasses of normal individuals ($P < 0.05$), after 7 d of

MAP packaged retail storage. When recognizing tissues with high levels of polyunsaturated fatty acids are especially susceptible to oxidation (Gray, 1978; Allen and Allen, 1981), one could hypothesize these previously alluded to differences in lipid peroxidation are a result of differing levels of polyunsaturated fatty acids. Additionally, it could be hypothesized that LD samples from carcasses of Napole carrier individuals have more polyunsaturated fatty acids. This hypothesis is in complement to the findings by Nilzen et al. (2001) who reported lean samples from carcasses of carrier individuals exhibited significantly higher levels of n-3 polyunsaturated fatty acids compared with muscle from carcasses of Napole normal individuals.

Chops of LD from barrow carcasses exhibited significantly lower scores for overall acceptability compared to LD chops from gilt carcasses. This may in part be due to LD chops from barrow carcasses exhibited higher TBAR values than LD chops from gilt carcasses ($P < 0.05$). The findings of the present study imply that LD samples from barrow carcasses exhibited more polyunsaturated fatty acids compared to LD samples from gilt carcasses. These findings are contrary to findings from Hogberg et al. (2002) and Barton-Gade (1987) who found LD samples from gilt carcasses exhibited higher polyunsaturated fat levels compared to LD samples from barrow carcasses.

IMPLICATIONS

The findings of the present research imply the advantages found from carrier individuals compared to normal individuals relative to lean tissue accretion and carcass composition, validate the continued utilization of seedstock of carrier genotype in modern commercial breeding schemes. Though often statistically significant, the lean quality problems associated with muscle samples from carcasses of carrier individuals compared to counterpart samples from normal individuals are of marginal practical significance. Whereas the cutability advantages exhibited by carcasses of carriers compared to carcasses of normal individuals would have a more immediate impact for commercial producers. Even so, the negative lean quality and shelf life parameters exhibited by Napole carriers cannot be ignored. In the eyes of retailers and further processors, lean product from carcasses of Napole carriers would be especially less valuable than lean product from carcasses of Napole normal individuals due to it's genetic predisposition to exhibit a greater product loss, lighter lean color and less desirable shelf life properties. Nonetheless, the positive palatability properties exhibited by chops from carcasses of Napole carriers would be more attractive to food service professionals, compared to loin chops from Napole normal individuals. Thus, if the Napole carrier genotype is not to become obsolete, niche-marketing strategies should be developed, as has been developed with the Berkshire breed via the Berkshire Gold program. These strategies should reward producers for optimizing carcass cutablity as well as producing a product exhibiting excellent potential palatability. Meat marketing should be focused on

consumers in a food service setting or a retail market with extremely rapid product turnover. The marketing scheme should focus upon consumers of adequate social status, so that price is of no consequence, but product satisfaction is of optimum importance.

Another alternative would be to eliminate all individuals deemed by DNA assessment as non-normal (carrier or homozygous dominant) for the Napole gene from parent lines of Hampshire seedstock. U.S. Hampshire breeders with commercial hog producers as their primary clientele are currently utilizing this practice. This would minimize the probability of creating additional lean quality problems throughout the pork chain. However, the elimination of Napole carriers from the U.S. population could potentially have negative repercussions relative to carcass cutability and product palatability. No definitive answers exist to resurrect the utilization of the Hampshire breed in commercial circles. However, if either of the strategies alluded to earlier prove to be successful, the characteristic "belt" exhibited across the shoulders of Hampshires would again be recognized as "The Mark of a Meat Hog".

Table 1. Least squares main effect means for Napole genotype* and gender** of glycolytic potential (GP) values of postmortem longissimus dorsi samples

GP, µmol/g lactate equivalent	***Genotype*[a]**	
	(RN-/rn+)	**(rn+/rn+)**
	312.6	213.1
	Gender	
	Barrow	**Gilt**
	282.0	243.6

*Genotype main effect ($P < 0.0001$) SEM (28.2).
**Gender main effect ($P = 0.02$) SEM (27.9).
[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.

Table 2. Effect of Napole genotype and gender on performance and carcass characteristics

Variable	Gender		Genotype[a]		Level of Significance[b] (SEM)	
	Barrow	Gilt	(RN-/rn+)	(rn+/rn+)	Gender	Genotype
WDA[c], kg	.5933	.6253	.6050	.6135	** (.01)	NS (.01)
HCW[d], kg	85.73	81.65	83.24	84.59	x (3.71)	NS (3.79)
LR FT[e], mm	21.90	20.56	21.17	21.30	x (.70)	NS (.71)
10th FD[f], mm	18.48	15.31	15.73	17.94	NS (1.07)	* (0.96)
Loin Eye Area, cm^2	43.72	45.43	44.91	44.21	NS (2.04)	NS (2.10)
Lean Percentage[g]	53.86	55.73	55.28	53.86	*** (.48)	** (.48)
LGPDA[h], kg	.2565	.2531	.2546	.2550	NS (.0033)	NS (.0033)
Marbling[i]	1.47	1.41	1.36	1.50	NS (.20)	NS (.22)
Color[j]	2.22	2.43	2.31	2.35	NS (.22)	NS (.22)
ADJ 10th FT[k], mm	17.81	15.78	15.91	17.69	NS (.88)	* (.75)
ADJ LEA[k], cm^2	17.04	18.39	17.98	17.45	** (.83)	NS (.83)
ADJ Lean, %[gk]	53.56	55.35	55.03	53.89	*** (.37)	** (.37)
ADJ LGPDA[hk], kg	.2659	.2745	.2731	.2673	*** (.008)	** (.008)

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NS= not significant, x= P < 0.10, *= P < 0.05, **= P < 0.01, ***= P < 0.0001, respectively.
[c]ADG: average daily gain; average age at harvest 184 days.
[d]HCW: hot carcass weight.
[e]LR FT: last rib fat thickness.
[f]10th FD: 10th rib fat depth.
[g]Lean Percentage: calculated according to protocol by NPPC (2000).
[h]LGPDA: lean gain per day of age, calculated according to protocol by NPPC (2000).
[i]Subjective Marbling: 1= 1% intramuscular fat , 10= 10% intramuscular fat; NPPC (2000).
[j]Subjective Color: 1= white to pale pinkish gray, 6= dark purplish red; NPPC (2000).
[k]ADJ: implies adjustments to raw data according to protocol by NSIF (1997).

Table 3. Effect of Napole genotype and gender on postmortem longissimus dorsi pH

	Gender		*Genotype*[a]		*Level of Significance*[b] *(SEM)*	
Variable, Time	**Barrow**	**Gilt**	**(RN+/rn-)**	**(rn-/rn-)**	**Gender x Time**	**Geno x Time**
pH, 45min	6.03	5.98	6.02	5.98	NS (.040)	NS (.046)
pH, 3h	5.47	5.49	5.39	5.57	NS (.043)	** (.044)
pH, 6h	5.46	5.51	5.41	5.57	NS (.033)	*** (.034)
pH, 12 h	5.52	5.55	5.47	5.60	NS (.028)	*** (.030)
pH, 24 h	5.46	5.49	5.42	5.54	NS (.026)	*** (.026)
pH, 48 h	5.45	5.46	5.41	5.50	NS (.039)	x (.039)

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.

[b]Level of significance: NS= not significant, x= P < 0.10, *= P < 0.05, **= P < 0.01, ***= P < 0.0001, respectively.

Table 4. Effect of Napole genotype and gender on postmortem longissimus dorsi temperature

	Gender		*Genotype*[a]		*Level of Significance*[b] *(SEM)*			
Variable, Time	**Barrow**	**Gilt**	**(RN+/rn-)**	**(rn-/rn-)**	**Gender x Time**	**Geno x Time**	**Gender**	**Geno**
Temperature, 45min	38.0	38.0	38.2	37.8	NS (.35)	NS (.36)	NA	NA
Temperature, 3h	22.1	20.9	21.5	21.5	NS (.56)	NS (.58)	NA	NA
Temperature, 6h	12.4	11.5	12.0	11.9	NS (.39)	NS (.42)	NA	NA
Temperature, 12 h	4.1	3.6	3.9	3.8	NS (.28)	NS (.28)	NA	NA
Temperature, 24 h	.60	.53	.61	.51	NS (.22)	NS (.23)	NA	NA
Avg. Temperature	15.4	14.9	15.2	15.1	NA	NA	* (.25)	NS (.26)

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NA= not applicable, NS= not significant, *= $P < 0.05$, respectively.

Table 5. Effect of Napole genotype, gender, and enhancement treatment on physical water holding capacity

	Gender		*Genotype*[a]		*Treatment*		*Level of Significance*[b] *(SEM)*			
Characteristic	**Barrow**	**Gilt**	**(RN-/rn+)**	**(rn+/rn+)**	**Enhanced**	**Non-Enhanced**	**Gender**	**Geno**	**Trt**	**Geno x Trt**
Longissimus										
MAP[c] Purge Loss, %	5.08	5.89	5.32	5.66	5.64	5.33	NS (1.13)	NS (1.15)	NS (1.13)	NS
Loin Section Purge Loss, %	4.89	2.24			6.36	764	NS (2.55)	x	NS (2.68)	x (2.78)
Enhanced			8.03	4.69						
Non-Enhanced			.706	.789						
Drip Loss, %	6.68	6.00	6.82	5.86	6.94	5.74	* (1.20)	** (1.20)	** (1.20)	NS
Cook Loss, %	24.42	25.10	26.27	23.25	25.37	24.15	NS (.78)	X (.92)	NS (.84)	NS
Semimembranosus										
Purge Loss, %	2.26	1.60	2.50	1.36	---	—	NS (.34)	*** (.29)	NA	NA

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NA= not applicable, NS= not significant, x= P < 0.10, *= P < 0.05, **= P < 0.01, ***= P < 0.0001, respectively.
[c]MAP= modified atmosphere packaged.

Table 6. Effect of retail display and vacuum packaging on pH values for longissimimus dorsi and semimembraneous muscles across and within genotype, enhancement treatment, and storage day

	Gender		*Genotype*[a]		*Treatment*		*Level of Significance*[b] *(SEM)*			
Variable	Barrow	Gilt	(RN-/rn+)	(rn+/rn+)	Enhanced	Non-Enhanced	Gender	Geno	Trt	Trt x Day
Longissimus- Chops										
pH	5.45	5.46	5.40	5.51						
Day 0					5.53[c]	5.39[e]	NS	***	***	***
Day 7					5.44[d]	5.46[d]	(.11)	(.11)		(.11)
Longissimus- Section										
pH	5.48	5.52	5.43	5.56						
Day 0					5.53[d]	5.39[e]	X	***	NS	***
Day 7					5.50[d]	5.58[c]	(.12)	(.12)		(.13)
Semimembranosus										
pH	5.42	5.45	5.34	5.52	—	—	NS	***	NA	NA
							(.12)	(.12)		

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NA= not applicable, NS= not significant, ***= P < 0.0001, respectively.
[c, d, e]Values lacking a common superscript within an interaction differ (P < 0.05).

Table 7. Effect of retail display and vacuum packaging on Hunter L* values for longissimimus dorsi and semimembraneous muscles across and within genotype, enhancement treatment, and storage day

	Gender		*Genotype*[a]		*Treatment*		*Level of Significance*[b] *(SEM)*				
Variable	**Barrow**	**Gilt**	**(RN-/rn+)**	**(rn+/rn+)**	**Enhanced**	**Non-Enhanced**	**Gender**	**Geno**	**Trt**	**Trt x Day**	**Geno x Day**
Longissimus- Chops											
Hunter L*	55.90	55.03					**	NS	**	**	*
Day 0			54.11[e]	54.44[e]	53.29[f]	55.26[a]	(.76)			(.79)	(.79)
Day 7			57.27[c]	56.04[d]	56.49[d]	56.82[c]					
Longissimus- Section											
Hunter L*	55.06	54.04	54.76	54.33			**	NS	**	**	NS
Day 0					53.36[d]	55.35[c]	(.62)	(.62)		(.66)	
Day 7					54.78[c]	54.69[c]					
Semimembranosus											
Hunter L*	60.94	59.48	61.25	59.17	---	---	* (1.42)	** (1.41)	NA	NA	NS

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NA= not applicable, NS= not significant, *= $P < 0.05$, **= $P < 0.01$, respectively
[c, d, e, f]Values lacking a common superscript within a main effect or interaction differ ($P < 0.05$).

Table 8. Effect of retail display and vacuum packaging on Hunter a values for longissimimus dorsi and semimembraneous muscles across and within enotype, enhancement treatment, and storage day*

	Gender		Genotype[a]		Treatment		Level of Significance[b] (SEM)					
Variable	**Barrow**	**Gilt**	**(RN-/rn+)**	**(rn+/rn+)**	**Enhanced**	**Non-Enhanced**	**Gender**	**Geno**	**Trt**	**Trt x Day**	**Geno x Day**	**Geno x Trt**
Longissimus- Chops												
Hunter a*	7.66	7.64										
Day 0			6.13[e]	5.65[e]	5.73[e]	6.05[e]						
Day 7			8.94[d]	9.88[c]	8.15[d]	10.31[c]	NS	NS	***	***	***	*
							(.34)			(.36)	(.36)	(.36)
Enhanced			7.19[e]	7.05[e]								
Non-Enhanced			7.88[d]	8.47[c]								
Longissimus- Section												
Hunter a*	7.11	6.25	7.11	6.26								
Day 0					6.09[d]	6.41[d]	NS	***	***	***	NS	NS
Day 7					6.29[d]	7.95[c]	(.29)	(.29)		(.31)		
Semimembranosus							*	**	NA	NA	NS	NA
Hunter a*	5.55	5.78	5.89	5.43	---	---	(1.42)	(1.41)				

Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
Level of significance: NA= not applicable, NS= not significant, x= $P < 0.10$, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.0001$, respectively.
[d, e]Values lacking a common superscript within a main effect or interaction differ ($P < 0.05$).

Table 9. Effect of retail display and vacuum packaging on Hunter b* values for longissimimus dorsi and semimembraneous muscles across and within genotype, enhancement treatment, and storage day

	Gender		*Genotype*[a]		*Treatment*						
Variable	**Barrow**	**Gilt**	**(RN-/rn+)**	**(rn+/rn+)**	**Enhanced**	**Non-Enhanced**	**Gender**	**Geno**	**Trt**	**Trt x Day**	**Geno x Day**
Longissimus- Chops											
Hunter b*	15 16	14.95	15.03	15.07							
Day 0					13.08[e]	13.34[e]	NS	NS	***	*	NS
Day 7					16.46[d]	17.34[c]	(.30)	(.30)		(.31)	
Longissimus- Section											
Hunter b*	14.22	13.95									
Day 0			13.41[e]	13.45[e]	13.30[e]	13.56[e]	*	NS	NS	**	**
Day 7			15.12[c]	14.35[d]	14.29[d]	15.18[c]	(.20)			(.22)	(.22)
Semimembranosus											
Hunter b*	13.89	13.57	13.91	13.54	---	---	NS (.49)	X (.50)	NA	NA	NS

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NA= not applicable, NS= not significant, x= P < 0.10, *= P < 0.05, **= P < 0 01, ***= P < 0.0001, respectively.
[c, d, e]Values lacking a common superscript within a main effect or interaction differ (P < 0.05).

Table 10. Least squares means for interaction* between gender, Napole genotype, and enhancement treatment for Warner-Bratzler Shear Force values (kg) of longissimus dorsi muscle

	Genotype[a]/ Gender			
Treatment	**(RN-/rn+)/ Barrows**	**(RN-/rn+)/ Gilts**	**(rn+/rn+)/ Barrows**	**(rn+/rn+)/ Gilts**
Enhanced	3.32^{de}	2.88^{e}	3.47^{cd}	3.31^{de}
Non-enhanced	3.34^{de}	4.00^{bc}	4.14^{b}	3.81^{bc}

*Gender x genotype x enhancement interaction (P = 0.01).
[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[c, d, e, f]Values lacking a common superscript differ (P < 0.05) SEM (.29).

Table 11. Effect of Napole genotype, gender, and enhancement treatment on sensory characteristics

	Genotype[a]		*Treatment*		*Level of Significance*[b] *(SEM)*				
Characteristic[c]	**(RN-/rn+)**	**(rn+/rn+)**	**Enhanced**	**Non-Enhanced**	**Geno**	**Trt**	**Gender x Geno**	**Gender x Trt**	**Gender x Geno x Trt**
Tenderness	5.94	5.50	5.74	5.69	*** (.13)	NS (.13)	NS	NS	NS
Juiciness	5.46	5.08							
(RN-/rn+) Barrows			5.80^{d}	5.21^{e}					
(RN-/rn+) Gilts			5.47^{df}	5.36^{ef}	NS (.28)	**	NS	NS	** (.32)
(rn+/rn+) Barrows			4.96^{e}	5.18^{e}					
(rn+/rn+) Gilts			5.35^{df}	4.85^{e}					
Connective Tissue	6.51	6.30	6.24	6.57	* (.11)	** (.11)	NS	NS	NS
Saltiness			4.67	4.85					
Barrows	4.73^{df}	4.82^{d}			NS	*** (.07)	* (.08)	NS	NS
Gilts	4.78^{df}	4.72^{ef}							
Off-Flavor	4.85	4.88							
(RN-/rn+) Barrows			4.80^{df}	4.93^{e}					
(RN-/rn+) Gilts			4.92^{ef}	4.75^{df}	NS (.03)	NS	NS	*	* (.05)
(rn+/rn+) Barrows			4.86^{ef}	4.89^{ef}					
(rn+/rn+) Gilts			4.88^{ef}	4.87^{ef}					
Overall	5.09	4.77	5.03	4.83	** (.15)	* (.15)	NS	NS	NS

[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b]Level of significance: NS= not significant, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.0001$, respectively.
[c]Characteristic: Tenderness-8 extremely tender, 1 extremely tough; Juiciness-8 extremely juicy, 1 extremely dry; Connective Tissue-8 none, 1 abundant; Saltiness-5 not salty, 1 extremely salty; Off-Flavor-5 none detectible, 1 extremely strong; Overall Acceptability-7 extremely desirable, 1 extremely undesirable.
[d, e, f]Values lacking a common superscript within an interaction differ ($P < 0.05$).

Table 12: Least squares means for interaction* between Napole genotype and gender for subjective lean color scores[a] of modified atmosphere packaged longissimus dorsi chops

	Gender	
Genotype[b]	**Barrow**	**Gilt**
(RN-/rn+)	2.67[c]	2.76[d]
(rn+/rn+)	2.81[c]	2.84[c]

*Genotype x gender interaction (P = 0.04).
[a]Subjective color scores: 1= pale pinkish gray to white; 2= grayish pink; 3= reddish pink.
[b]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[c, d, e]Values lacking a common superscript differ (P < 0.05) SEM (.09).

Table 13. Least squares means for interaction* between enhancement treatment and gender for subjective lean color scores[a] of modified atmosphere packaged longissimus dorsi chops

	Gender	
Enhancement Treatment	**Barrow**	**Gilt**
Enhanced	2.76[c]	2.73[c]
Non-enhanced	2.73[c]	2.86[b]

*Enhancement treatment x gender interaction (P = 0.04).
[a]Subjective color scores: 1= pale pinkish gray to white; 2= grayish pink; 3= reddish pink.
[b, c]Values lacking a common superscript differ (P < 0.05) SEM (.09).

Table 14. Least squares means for interaction* between gender, Napole genotype, and enhancement treatment for subjective percentage discoloration scores[a] of modified atmosphere packaged longissimus dorsi chops

	Genotype[b]/ Gender			
Treatment	**(RN-/rn+)/ Barrows**	**(RN-/rn+)/ Gilts**	**(rn+/rn+)/ Barrows**	**(rn+/rn+)/ Gilts**
Enhanced	5.64[d]	5.72[d]	5.38[e]	5.71[d]
Non-enhanced	5.98[d]	6.00[d]	6.18[c]	6.18[c]

*Gender x genotype x enhancement interaction (P = 0.01).
[a]Subjective percentage discoloration scores: 7= none; 5= 11-25%; 3= 51-75%; 1= complete.
[b]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[c, d, e]Values lacking a common superscript differ (P < 0.05) SEM (.13).

Table 15. Least squares main effect means of subjective lean firmness scores[a] by day of retail display* of modified atmosphere packaged longissimus dorsi chops

Days of Retail Display	Lean Firmness
0 d	4.38[d]
1 d	4.66[b]
2 d	4.49[c]
3 d	4.21[e]
4 d	4.27[e]
5 d	3.96[fh]
6 d	4.02[f]
7 d	3.87[h]

*Days of retail display main effect ($P < 0.0001$).
[a]Subjective lean firmness scores 1= very soft and watery; 3= slightly soft and watery; 4= slightly firm; 6= Very firm.
[b, c, d, e, f, g, h]Values lacking a comon superscript differ ($P < 0.05$) SEM (.11).

Table 16. Least squares main effect means for Napole genotype* and enhancement treatment** of subjective lean firmness scores[a] of modified atmosphere packaged longissimus dorsi chops

Genotype[b]	(RN-/rn+)	(rn+/rn+)
	4.17	4.29
Enhancement Treatment	Enhanced	Non-enhanced
	3.96	4.51

*Genotype main effect ($P < 0.0001$) SEM (.10).
**Enhancement treatment main effect ($P < 0.0001$) SEM (.10).
[a]Subjective lean firmness scores 1= very soft and watery; 3= slightly soft and watery; 4= slightly firm; 6= very firm.
[b]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.

Table 17. Least squares means for interaction* between Napole genotype and enhancement treatment for subjective overall acceptability scores[a] of modified atmosphere packaged longissimus dorsi chops

	Treatment	
Genotype[b]	**Enhanced**	**Non-enhanced**
(RN-/rn+)	5.03[e]	5.54[d]
(rn+/rn+)	5.04[e]	5.82[c]

*Genotype x enhancement treatment interaction ($P < 0.0001$).
[a]Subjective overall acceptability scores: 7= extremely desirable; 6= desirable; 5= slightly desirable; 4=acceptable; 3= slightly undesirable.
[b]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[c, d, e]Values lacking a common superscript differ ($P < 0.05$) SEM (.13).

Table 18. Least squares means for interaction* between enhancement treatment and gender for subjective overall acceptability scores[a] of modified atmosphere packaged longissimus dorsi chops

	Gender	
Enhancement Treatment	**Barrow**	**Gilt**
Enhanced	4.95[d]	5.12[c]
Non-enhanced	5.65[bc]	5.71[b]

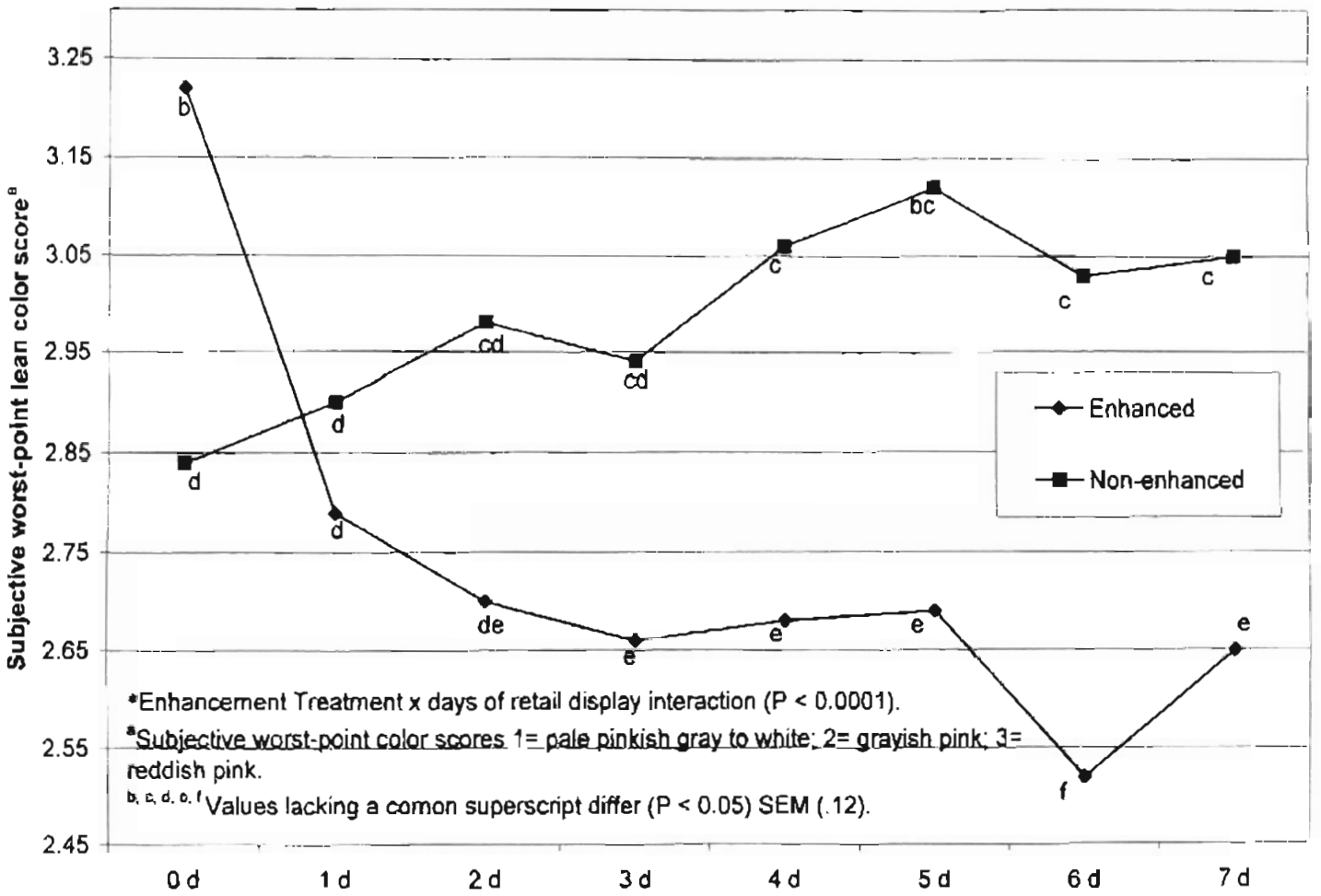
*Enhancement treatment x gender interaction ($P = 0.04$).
[a]Subjective overall acceptability scores: 7= extremely desirable; 6= desirable; 5= slightly desirable;
4= acceptable; 3= slightly undesirable.
[b, c, d]Values lacking a common superscript differ ($P < 0.05$) SEM (.13).

Table 19. Least squares means for interaction* between days of retail display, Napole genotype, and enhancement treatment and main effect means for gender** in reference to thiobarbituric acid (mg of MDA equivalents) values of longissimus dorsi

		Days of Retail Display	
Genotype[a]	**Enhancement Treatment**	**Day 0**	**Day 7**
(RN-/rn+)	Yes	.1594[d]	.4186[b]
(RN-/rn+)	No	.1111[de]	.4536[b]
(rn+/rn+)	Yes	.0892[e]	.2829[c]
(rn+/rn+)	No	.1248[de]	.2664[c]
Gender			
Barrow		**Gilt**	
.2585		.2180	

*Retail display days x genotype x enhancement treatment interaction (P = 0.04) SEM (.032).
**Gender main effect (P = 0.01) SEM (.023).
[a]Genotype: (RN-/rn+), (rn+/rn+)= heterozygous dominant (carrier) and homozygous recessive (normal) for the Napole gene, respectively.
[b, c, d, e]Values lacking a common superscript differ (P < 0.05).

Figure 1. Least squares means for interaction* between enhancement treatment and days of retail display for worst-point lean color assessment of modified atmosphere packaged longissimus dorsi chops

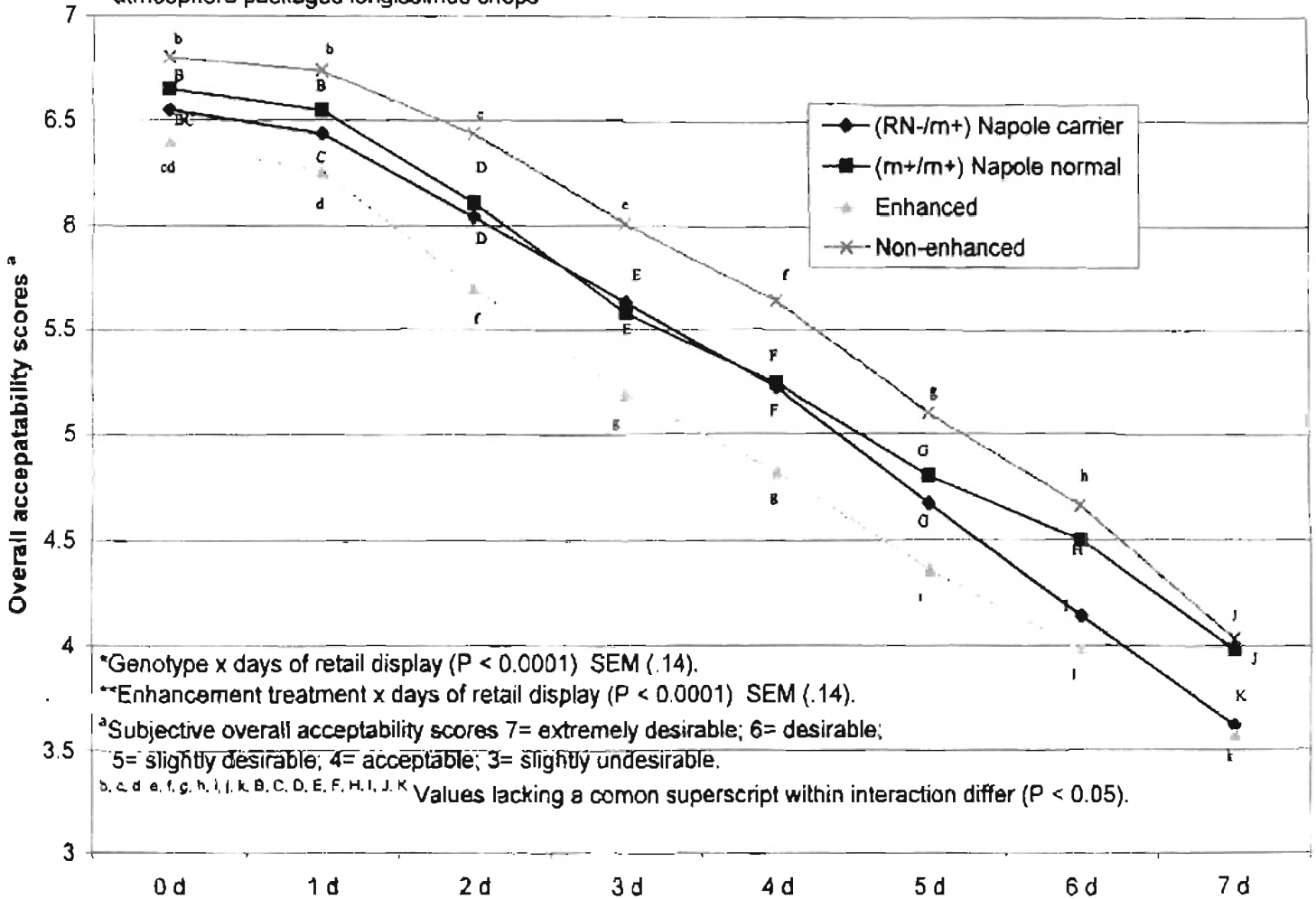


*Enhancement Treatment x days of retail display interaction (P < 0.0001).

[a]Subjective worst-point color scores 1= pale pinkish gray to white; 2= grayish pink; 3= reddish pink.

[b, c, d, e, f]Values lacking a comon superscript differ (P < 0.05) SEM (.12).

Figure 2. Least squares means for interactions between Napole genotype* and days of retail display and enhancement treatment** with days of retail display for subjective overall acceptability assessment of modified atmosphere packaged longissimus chops



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APPENDIX I

Left Carcass Side

1 Carcass

Right Carcass Side

Assessment of loineye pH and temp will be made at 45 min 3,6,12, & 24 and 48 h.

At 48 hr carcass data will be collected, then fabricated

All qualitative assessment, as well as samples cut will come from the $10^{th}/11^{th}$ rib interface

Blade Section (3^{rd}-10^{th} rib)	Sirloin Section (11^{th}-Sirloin)	Semimembranosus
Subjective Color	GP Slice	Minolta
Minolta	Drip Loss	pH
pH	TBA- 1 Chop	Vacuum Pack
Retail - 1 Chop	**Enhancement Injection**	
WBS- 1 Chop	pH	
Sensory- 2 Chop	Minolta	
Vacuum Pack	Retail Chop- 1 Chop	
	TBA 1 Chop	
	Drip Loss Slice- 1 Chop	
	WBS- 1 Chop	
	Sensory- 2 Chop	
	Vacuum Pack	

All chops will be randomly allotted. GP, and initial TBA pieces will be frozen at 48 hr. All three muscle subprimals, both retail packages, and the vacuum packaged shear and sensory chops will be subjected to 7 d postmortem storage. Muscle subprimals and retail packages will be evaluated for purge loss, pH, and Minolta following storage. 7 d retail packages will be analyzed for TBA. Vacuum packaged samples will be frozen following storage.

APPENDIX II

Napole Sensory Ballot

Booth # _____ Date: _____ Time: _____ AM/PM

Sample	Tenderness	Juiciness	Connective Tissue	Saltiness	Off-Flavor	Comments	Overall Acceptability
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Tenderness
8 Extremely Tender
7 Very Tender
6 Moderately Tender
5 Slightly Tender
4 Slightly Tough
3 Moderately Tough
2 Very Tough
1 Extremely Tough

Juiciness
8 Extremely Juicy
7 Very Juicy
6 Moderately Juicy
5 Slightly Juicy
4 Slightly Dry
3 Moderately Dry
2 Very Dry
1 Extremely Dry

Connective Tissue
8 None
7 Practically None
6 Traces
5 Slight
4 Moderate
3 Slightly Abundant
2 Moderately Abundant
1 Abundant

Saltiness
5 Not Salty
4 Slightly Salty
3 Mildly Salty
2 Moderately Salty
1 Extremely Salty

Off-Flavor
5 None Detectable
4 Slightly Detectable
3 Mildly Detectable
2 Moderately Strong
1 Extremely Strong

Overall Acceptability
7 Extremely Desirable
6 Desirable
5 Slightly Desirable
4 Acceptable
3 Slightly Undesirable
2 Undesirable
1 Extremely Undesirable

Appendix III

Napole Visual Appraisal Guidelines

Lean Color

1-Pale Pinkish Gray to White
2-Grayish Pink
3-Reddish Pink
4-Dark Reddish Pink
5-Purplish Red
6-Dark Purplish Red

Worst Point Lean Color

1-Pale Pinkish Gray to White
2-Grayish Pink
3-Reddish Pink
4-Dark Reddish Pink
5-Purplish Red
6-Dark Purplish Red

Firmness

1-Very Firm
2-Firm
3-Slightly Firm
4-Slightly Watery
5-Soft and Watery
6-Very Soft and Watery

Percent Discoloration

7-None
6-1-10
5-11-25
4-26-50
3-51-75
2-76-99
1-Complete

Overall Acceptability

7-Extremely Desirable
6-Desirable
5-Slightly Desirable
4-Acceptable
3-Slightly Undesirable
2-Undesirable
1-Extremely Undesirable

VITA

Charles Chadwick Carr

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF DNA MARKER ASSISTED SELECTION FOR THE RENDEMENT NAPOLE GENE ON GROWTH PERFORMANCE AND CARCASS COMPOSITION, WHILE IN CONJUNCTION WITH ENHANCEMENT TREATMENT FOR LEAN QUALITY, SENSORY, AND SHELF LIFE CHARACTERISTICS

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