ABSTRACT

FREDERICK, BRENT RAY. Magnesium supplementation through drinking water to improve pork quality. (Under the direction of Eric van Heugten and Jerry Spears.)

Four experiments were conducted to investigate the effect of short term magnesium supplementation through drinking water prior to harvest on pork quality of pigs. Two days of magnesium supplementation (experiment 1) appeared optimal to improved pork quality. Older, slower growing pigs produced higher pork quality than younger faster growing pigs; however, magnesium supplementation had no effect (experiment 2). The presence of the Halothane and to a greater extent the Rendement Napole mutation reduced the quality of pork (experiment 3). Furthermore, pigs with both mutations had poorer pork quality than pigs with one mutation. Magnesium supplementation reduced pork quality when supplemented to pigs with the Halothane mutation. Pork quality was not affected when dose of magnesium supplemented in the water was altered (experiment 4). Although plasma magnesium was increased by magnesium supplementation, muscle magnesium was not affected. Overall, magnesium supplementation through drinking water for 2 days prior to harvest does not appear to be a method to consistently improve pork quality.

MAGNESIUM SUPPLEMENTATION THROUGH DRINKING WATER TO IMPROVE PORK QUALITY

by

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BIOGRAPHY

Brent Frederick grew up on a farrow-to-finish pig farm in Cairo, Georgia. He actively exhibited livestock including pigs, cattle, and sheep at 4-H and Future Farmers of America shows. After graduating from Cairo High School, Brent received a scholarship to pursue a Bachelor of Science in Animal Science at Texas A&M University. While at Texas A&M, Brent had the opportunity to work at the university swine farm and assist Dr. Knabe with his nutritional research. After he completed his BS degree, Brent started graduate studies in Animal Nutrition at Iowa State University. While working on his Master of Science degree at Iowa State, his research focused on phosphorus metabolism and phosphorus requirements of growing pigs under the direction of Dr. Tim Stahly. Additionally, Brent met his best friend and future wife while at Iowa State. From there, he was granted the opportunity to investigate potential nutritional strategies to improve pork quality under the direction of Dr. Eric van Heugten. Brent is now looking forward to entering the work force as a swine nutritionist.

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LITERATURE REVIEW

Introduction

Inconsistent product quality within the pork industry has become a reason for concern over the past few decades. Estimates indicate that the economic loss by pork producers due to pale, soft, and exudative pork is approximately $0.79 per pig, resulting in an estimated $70 million loss of revenue for the pork industry (Sonka et al., 1994). This defect in quality can be a the result of a multitude of factors including but not limited to genotype, nutrition, stress prior to harvest, and processing conditions.

Genetic potential for pork quality is a primary determinate of final quality. Two know mutations, namely Halothane and Rendement Napole, negatively influence quality. Few strategies have been successful to diminish the inherent quality problems of pigs with these mutations. Selective breeding to reduce the incidence of the mutations is an option. However, this strategy is lengthy and may be costly to some producers.

Additionally, pig age at harvest may influence pork quality. Pigs are harvested at a constant bodyweight. The variation of growth of pigs within the same farrowing group can result in multiple marketings from a single farrowing group. Therefore, the difference in age between the first and last pig marketed within the same group can be in excess of 21 days. The effect of marketing group within farrowing group has not been investigated extensively.

Therefore, the focus of this literature review is on the basic principles of meat science and the effect genotype and pig age might have on the quality of pork. Furthermore, this review will introduce a nutritional strategy, magnesium supplementation, as a possible method to improve pork quality.

Conversion of muscle to meat

Knowledge of the events and physiological changes associated with the conversion from muscle to meat is critical to the understanding of pork quality. The subject has been reviewed in detail elsewhere (Gregory, 1998; Berg, 2001). This section summarizes the primary processes of importance.

Upon exsanguination, respiration and blood circulation cease to exist. The absence of respiration and oxygen transport shifts the biological environment from aerobic to anaerobic. Secondly, the absence of substrate transport from the body tissues inhibits the export of metabolites due to the loss of blood flow throughout the body. Both the change in biological environment and the inhibition of transport of metabolites are the basis for post-mortem biological changes.

Immediately after exsanguination, post-mortem tissues attempt to maintain consistent energy homeostasis in order to maintain cell function and integrity. The primary source of energy associated with post-mortem muscle is high-energy phosphate groups. The most prevalent high-energy phosphate compound, adenosine triphosphate (ATP), has the greatest biological activity within the body, both pre and post-mortem. ATP's are hydrolyzed to ADP and inorganic phosphorus by multiple ATPases to provide the energy needed. In pigs, ATP concentrations rapidly decline from approximately 6.7 μmol/g of muscle in the live, resting state (Bendall, 1973) to 1 μmol/g or less 3 hours post-mortem (Kastenschmidt, 1970).

In an effort to maintain a constant concentration of ATP, another high-energy compound, creatine phosphate, is incorporated to replenish the ATP pool by

phosphoralytion of Mg-ADP by creatine kinase. A relatively constant ATP concentration is maintained until approximately 70 % of tissue creatine phosphate has been metabolized (Bendall, 1951). Creatine phosphate utilization continues post-mortem until concentrations reach near zero. Muscles with a high number of white, Type II, muscle fibers have higher concentrations of creatine phosphate than muscle with a greater number of red, Type I, muscle fibers (Bendall, 1975). Thus, white muscle has a greater potential for ATP regeneration by creatine phosphate compared to red muscle.

In vivo, ATP is resynthesized by mitochondrial, oxidative metabolism in red muscle fibers or by anaerobic glycolysis in white muscle fibers. However, in the post-mortem state, the lack of blood flow inhibits oxygen transport and thus oxidative ATP synthesis. Glucose transport to the cell is also blocked. Therefore, previously stored glycogen is degraded and utilized as a source of glucose to fuel ATP synthesis via anaerobic glycolysis. Many enzymes are required throughout glycolysis. Some enzymes are rate limiting, thus allowing for points of control.

The endpoint of glycolysis is pyruvate. In the post-mortem anaerobic environment pyruvate is converted to lactate. In vivo, lactate is transported via blood to the liver, undergoes gluconeogenesis, and is transported back to the muscle to maintain energy balance in a futile cycle referred to as the Cori Cycle. However, the absence of blood flow and lack of lactic acid transport results in the accumulation of lactic acid in the muscle. Thus, accumulation of lactic acid leads to a decrease of pH within the carcass.

The rate of post-mortem pH decline is negatively correlated to meat quality. Under normal circumstances, the initial pH of pig muscle immediately after exsanguination is

approximately 7.2 (Bendall, 1973) and decreases to 6.5 at 45 minutes post-mortem continuing to decrease resulting in an ultimate pH of 5.6 to 5.8. However, animals exposed to stress pre-harvest or genetically predisposed to stress have a faster rate of pH decline having a 45 minute pH of least than 6.0 (Brown et al., 1998; Fisher et al., 2000). Thus, the result is a less desirable pork product characterized as pale, soft and exudative (PSE).

Many biological and environmental factors contribute to the rate of glycolysis and thus, lactate accumulation and pH decline post-mortem. The rate of pH decline is dependent on muscle temperature (Pearson and Young, 1989). Heat can accumulate in the carcass and increase the rate of pH decline as a result of metabolic processes or via processing practices such as scalding. Colder storage temperatures immediately post-mortem decreases the rate of pH decline (Marsh, 1954). Thus, it is speculated that if the rate of glycolysis declines, meat quality would be improved.

Rigor mortis is the end result of the structural transformations occurring in post-mortem muscle. The time at which muscle enters rigor mortis is most closely correlated to the absence of ATP. With an insufficient supply of ATP, actin and myosin filaments combine forming a rigid bond. The loss of extensibility associated with muscle in the rigor mortis state is directly related to insufficient ATP concentrations allowing the release of myosin from the actin filaments (Bate-Smith and Bendall, 1974; Bendall, 1951).

Defining and measuring pork quality

Definition

Pork quality is a phrase that encompasses three main areas, which includes appearance, physical structure, and palatability. These areas are not independent and any effect on one is likely to affect the other areas.

Appearance

Consumer preference for meats is most strongly affected by changes in color and appearance (Risvik, 1994). Consumers relate the color of lean to freshness of the meat (Adams and Huffman, 1972). Pork color ranges from dark red to a pale gray color. The National Pork Producer Council coined the phase "the other white meat" in an effort to promote pork as a healthy alternative to other red meats. However, recent efforts have been made to increase the intensity of red color in pork to capitalize on international demands and increase international pork exports.

The color of meat is determined by the pigments. The majority of pigments in meat are derived from the heme group, although other pigments such as cytochromes, catalase, and flavins are present. Myoglobin accounts for 50 to 80% of the total pigment concentration in meat while hemoglobin may comprise 20 to 50% of total pigment (Price and Schweigert, 1987). Therefore, the color of meat is predominately determined by the concentration and form of the heme groups, mainly myoglobin. Muscles having a red color contain a majority of type I muscle fibers with high concentrations of myoglobin. Conversely, muscles appearing white in color have an abundance of type II muscle fibers that are lower in myoglobin content. The reason for the different concentration of

myoglobin is associated with the functions of the muscle fiber. Red, type I, muscle fibers are oxidative in nature and thus require a higher store of oxygen. Therefore, the higher concentration of myoglobin serves as reserve oxygen storage. Conversely, white, type II muscle fibers are glycolytic and therefore do not require high concentrations of oxygen stores and subsequently have a lower myoglobin concentration (Gregory, 1998b).

Furthermore, the retention of heme groups is important in the determination of meat color. The bleeding of the pig can also affect the heme content of meat due to the presence of hemoglobin. Once exanguination and processing are complete, the amount of water loss occurring post-mortem and the retention of sarcoplasmic proteins influence color (Offer and Knight, 1988). The red color of fluid loss, commonly named purge, is associated with myoglobin lost from the sarcomere during storage of meat.

Also important in the determination of color of meat is the oxidative state of the iron in both myoglobin and hemoglobin which changes over time and is affected by storage environment and conditions. As time post-mortem continues, oxygen is allowed to interact with the meat when exposed to air. Oxygenation of myoglobin proceeds forming oxymyoglobin, which is characterized by a bright red color. The intensity of red color increases to a maximum level, at which time oxidation of both unoxygenated, myoglobin and oxymyoglobin occur producing metmyoglobin, which is characterized by a brown color. Changes in color continue during storage due to the oxidative state of the heme pigments (Warriss, 2000).

Objective and subjective measurements are available for quality determinations. For example, L*, a*, and b* values measured by spectrophotometers indicate the lightness,

redness, and yellowness, respectively. Furthermore, the National Pork Producer Council has color standards to assist in subjectively evaluating pork. Perhaps appearance is the simplest to measure because of subjective standards for color and marbling. Marbling or intramuscular fat can be assessed by a subjective score or extraction of lipid from lean meat. However, the distribution of the lipid present is not represented by the latter method. Furthermore, the visual appearance of fluid loss of pork is undesirable and is dependent on the physical structure of pork.

Physical structure

Lean meat contains approximately 75% water immediately post-mortem. However, the water content of meat is not constant over time due to losses including: 1) drip loss, 2) evaporation, and 3) cooking.

The water concentration of meat decreases to 60 to 80 % as post-mortem pH decreases and onset of rigor mortis proceeds (Price and Schweigert, 1987). This is caused by the decreased inter-myofibril space associated with repulsion of muscle proteins. Alternating charges of proteins create repulsion between the proteins thus providing an intracellular space that water may occupy. As the pH of tissue decreases, proteins approach their isoelectric points causing neutral charges, resulting in decreased repulsion and less space for water. Thus, water is forced out of the meat.

A faster rate of decline of pH post-mortem has been correlated with a decrease in the ability of meat tissue to maintain water concentrations. During rapid pH decline, sacroplasmic proteins are denatured causing a decrease in water holding capacity. Pale, soft, and exudative (PSE) pork is extremely watery as a result of an accelerated rate of pH

decline caused by a high rate of glycolysis. Therefore, if glycolysis could be controlled or postponed then water-holding capacity could be improved resulting in a decreased amount of water loss.

Decreasing water loss by increasing water holding capacity would be beneficial to both packer and consumer. The packer would not lose valuable weight due to water loss and would be able to sell a higher percentage of products. Furthermore, the consumer would be more satisfied with juiciness of the product resulting from higher water content.

Tenderness is a non-visual characteristic related to physical structure and contributes to overall consumer satisfaction. However, pork is usually not associated with tenderness problems because of the young age at which pigs are marketed. Most pigs are marketed at or before puberty. The pig has a high collagen content at harvest. However, the covalent bonds of collagen are labile and are not as stable as in the animal marketed later in maturity, such as cattle. Increased stability of collagen bonds is associated with decreased tenderness (Cross et al., 1973).

Post-mortem tenderization is a complex process affected by rate of pH decline, osmolarity of muscle cells, temperature, and inherent genetic factors (Goll et al., 1997). A majority of tenderization occurs within 3 days post-mortem (Calkins and Seideman, 1988). Although minor inherent differences in meat tenderness are present immediately after harvest, most of the difference occurs in the first 24 hours and maximum tenderness occurs 5 days post mortem (Koohmaraie et al., 1995). Post-mortem proteolysis of myofibrillar proteins is involved in the tenderization process. These myofibrillar proteins are important in functionality and structural integrity of the myofibrils. Thus, degradation and weakening

of the myofibril proteins would result in tenderization. However, upon critical analysis of research and knowledge, Goll et al. (1997) suggest that proteolysis has a relatively small role in the majority of changes in tenderization during the first 72 hours post-mortem, but is responsible for tenderization after such time. Changes in the actin/myosin cross-bridge during postmortem storage need further study to determine the effects on tenderization.

The calcium dependent, neutral calpain enzymes have been suggested to have the greatest potential for inducing post-mortem tenderization via proteolysis because they are endogenous to the muscle cell, are able to reproduce post-mortem changes in vitro, and have access to the myofibril (Koohmaraie, 1992, 1994). At one time, cathepsins were considered important in tenderization. However, cathepsins have little or no effect on the myofibrils during storage of meat at 2 to 4ºC (Goll, 1991). Although cathepsins are highly active, they degrade myosin and actin (Goll et al., 1989) which result in a "mushy' texture (Goll, 1991). The calpains are unique in that they do not degrade actin, myosin, or alpha-actinin (Goll et al., 1991). Thus, calpains are important proteases responsible for post-mortem tenderization.

Fat content, both intramuscular and subcutaneous, has been reported to be associated with aspects of meat quality. Over the past several years, controversy has surrounded the issue of intramuscular fat content and tenderness. Early studies had conflicting results on the relationships between fat and tenderness (Murphy and Carlin, 1961; Rhodes, 1970; and Skelly et al., 1973). Savell and Shackelford (1986) introduced the concept of the window of acceptability for fat in beef. Overall palatability is acceptable between a minimum and maximum percent fat within the meat. The threshold value of 2.5

to 3.0% lipid in pork is required for acceptability of tenderness (DeVol et al., 1988). Barton-Grade et al. (1987) concluded maximum tenderization occurred at the threshold value. Thus, intramuscular fat content is only responsible for problems of decreased tenderness when concentrations are below the minimum threshold.

In addition to intramuscular fat concentrations, subcutaneous fat may also play, to a lesser extent, a role in the absolute toughness of pork. Meat stored at cold temperatures below 11ºC undergoes shortening of the myofibrils called cold shortening. Hence, the shortening of the muscle fiber decreases tenderness. Cold shortening occurs mostly in muscles containing a high number of red fibers and thus is more prevalent in beef. However, cold shortening can occur in pork at 2ºC (Hendricks et al., 1971). Cold shortening is most notably attributed to inadequate external fat covering of the carcass, thus inadequately protecting the muscle from the cold. Over the years producers have responded to consumer demand for a leaner product by producing pigs with low subcutaneous fat levels. Furthermore, pork processors have incorporated the use of rapid chill systems to increase some quality attributes such as water-holding. The combination of these two factors increases the chances of cold shortening. Dikeman (1996) suggested marketing pigs with at least 15 mm of backfat (subcutaneous fat) to prevent cold shortening in ultra-chill processing systems.

Research in the area of tenderness continues to be plentiful in beef. Muscle biologists are continually discovering new interactions of muscle proteins, enzymes and interactions between them. Meat scientists continue to improve processing aspects affecting meat tenderness. However, tenderness improvement and advancements in beef are more

applicable because of the high variation across cattle breeds. Pork tenderness is important but is not as high on the priority list of the industry.

Tenderness can be measured subjectively by a sensory panel or objectively by the shear force required to cut a group of muscle fibers in a latitudinal orientation. Nevertheless, tenderness of pork has not been a major concern in the past if cooked properly, while fluid loss has been a major concern.

Palatability

Organoleptic properties include stimulation of sensory perception and eating quality of meat including flavor, aroma, juiciness, and texture. Various compounds including fat within meat determine the flavor and aroma, while water and fat content affect juiciness and texture. The rate of pH decline and ultimate pH can also affect organoleptic properties. An accelerated rate of pH decline results in the softness of texture associated with PSE pork. Furthermore, ultimate pH concentrations above 5.6-5.8, classified as dark, firm, and dry, have been associated with a "mushy" texture and a "bloody" flavor when consumed.

Flavor of meat is a result of compounds affecting both the olfactory and taste receptors. Concentrations of these compounds are altered by initial concentrations, oxidation of the compounds, and damage caused by heat during cooking. The majority of flavor precursors are water-soluble (Hornstein and Wasserman, 1987) and are found within the muscle portion of meat. The water soluble compounds were identified as sugars, salts, amino acids, and low weight polypeptides.

Lipids also play a role in final meat flavor. Hence, lipid oxidation of double bonds and auto-oxidation can result in off-flavors. Oxidation of meat during storage determines

the length of time the product is considered acceptable, commonly referred to as shelf life. The most common method to determine the extent of oxidation of meat is one that measures the amount of malondialdehyde and is called the 2-thiobarbituric acid (TBA) or thiobarbituric acid reactive substances (TBARS) test (Witte et al., 1970). Malondialdehyde is a primary product of lipid oxidation. Therefore, the greater the amounts of unsaturated fatty acids that are present the more oxidation will occur. Oxidation can affect color, texture, and palatability of pork and should be monitored.

Each of these areas of pork quality is not independent and is very important to the overall acceptability of pork by the consumer. Many factors including but not limited to environment, genetics, nutrition, pre-and post-handling are involved in determining pork quality. The following sections address the most applicable factors to this dissertation.

Genotypic effects

Two genetic mutations have been identified that negatively influence pork quality. Although the Halothane and Rendement Napole (RN) mutations on chromosomes 6 and 15 (Mariani et al., 1996), respectively, have similar effects on ultimate quality, the physiological alterations realized by these mutations differ. The Halothane mutation was discovered first and is associated with improper function of the calcium channels that regulate Ca^{2+} concentration and muscle contraction. This mutation results in a rapid decline in post-mortem pH. Conversely, the RN mutation affects the AMP activated protein kinase and is associated with a higher resting glycogen concentration than animals without the mutation. Therefore, more substrate is available for post-mortem glycolysis. The end result

of the RN mutation is a lower ultimate post-mortem pH. Thus, the Halothane mutations increase the rate and the RN mutations increase the extent of post-mortem glycolysis.

Halothane mutation

The Halothane mutation was the first mutation of the two to be identified. As genetic selection towards higher rate of bodyweight gain and protein deposition and reduced fat deposition increased, the incidence of pale, soft, and exudative (PSE) pork increased. Indeed, pigs with the Halothane mutation have a greater percent of bodyweight as lean tissue, a higher rate of bodyweight gain, and greater efficiency of feed utilization (Rundgren et al., 1990). The increased incidence of PSE was linked to the intolerance to halothane gas resulting in malignant hyperthermia (Mickelson et al., 1989). This condition was later found to be caused by a mutation of the calcium release channel (Ryanodine receptor, RYR1) of the sacroplasmic reticulum in muscle (Fujii et al., 1991). The mutation is located on chromosome 6 and is often referred to as HAL-1843. Genetic designation for homozygous normal, heterozygous carrier and homozygous positive are NN, Nn, and nn, respectively.

The effects of the Halothane mutation on pH and pork quality characteristics are presented in Table 1. Di-mutants (nn) have a faster rate of pH decline as measured at 45 to 60 min post-mortem. However, the ultimate pH usually measured at 24 h post-mortem may not differ from non-mutants. This rapid decline of pH occurs when the carcass has not been cooled sufficiently resulting in pale, soft, and exudative pork. The effect of the mono-mutant genotype is not consistent. However, the effects on pork quality are usually

Table 1. Effect of the Halothane genotype on pH and pork quality[ab]

Genotypes Compared[b]	Effect of mutations on pH	Effect of mutation on quality characteristics	Reference
NN Nn nn	Longissimus pH at 60 min post-mortem decreased with each additional mutation.	Minolta reflectance, Hunter color, and drip loss increased with each additional mutation.	Lahucky et al., 1997
NN Nn nn	Longissimus pH at 60 min post-mortem decreased with each additional mutation. Di-mutants had lower pH at 24 h post-mortem than non-mutants.	Fluid loss during thawing, cooking loss, and mechanical resistance were higher for di-mutants than non-mutants. Additionally, di-mutants had lower sensory scores than non-mutants. The mono-mutants were intermediate or closer to non-mutants for many characteristics.	Monin et al., 1999
NN Nn nn	Longissimus pH at 45 min post-mortem decreased with each additional mutation.	Cooking loss was greater from di-mutants than non- or mono-mutants. Shear force was greater for mono-mutants than other genotypes.	Fisher et al., 2000
NN Nn	Semimembranosus pH was lower for mono-mutants than non-mutants at 1, 2, 3, and 4 h post-mortem.	Interactions with dietary treatments prohibited main effect conclusions.	Kerth et al., 2001
NN Nn	Lower pH of Longissimus at 45 min post-mortem	Mono-mutants had lower color scores, and marbling and higher shear force and drip loss from Longissimus and biceps femoris, respectively, than non-mutants.	Maddock et al., 2002

[a]This table is provided as a representation of previous work conducted in this area and is not exhaustive.
[b]The genotype notation includes NN (non-mutant), Nn (mono-mutant), and nn (di-mutant).

intermediate between the non- and di-mutant, but are more similar to the non-mutant genotype. The most significant effects of the mono-mutant may be related to older and/or heavier pigs (Sather et al., 1991; Monin et al., 1999)

Napole mutation

Quantification of reduced pork quality present in the Hampshire breed of pigs was first demonstrated by decreased processing yield (Naveau, 1986). The observation was commonly termed Rendement Napole (RN^-). The word "Rendement" is from French decent meaning "yield." The word Napole was derived from the first two letters of each of the three authors, Naveau, Pommeret, and Lechaux, who developed a technique for measuring processing yield (Naveau et al., 1985). Some Hampshires were observed to have higher glycogen content, lower ultimate pH, and a lower Napole yield than other breeds (Monin and Sellier, 1985; Naveau et al., 1985). Glycogen is degraded post-mortem to yield glucose, the primary substrate for glycolysis. As post-mortem glycolysis proceeds the pH declines as lactate accumulates. A lower pH at 24 hours post-mortem is associated with poor pork quality.

A majority of the negative effects on pork quality associated with the Hampshire breed was determined to be caused by a single locus with two alleles, RN^- and rn^+ (Le Roy et al., 1990). The reduction in yield and ultimate pH was largely explained by the presence of the RN^- allele that results in an increase of muscle glycogen by as much as 70% compared to nonmutants (Estrade et al., 1993a, 1993b). The locus was determined to be located on chromosome 15 (Mariani et al., 1996). Elevated muscle glycogen was identified

to be caused by a mutation in the AMP-activated protein kinase (Milan et al., 2000). Further information on the biological effect of the RN mutation is presented in Appendix I. Although not all Hampshires have the RN^- mutation, pigs with the dominant RN^- mutation have elevated muscle glycogen concentrations and the subsequent reduction in pork quality. The genotypic frequency of rn^+rn^+ (normal), RN^-rn^+ (carrier), and RN^-RN^- (positive) were estimated to be 0.137, 0.446, and 0.397, respectively and the allelic frequency of rn^+ and RN^- were 0.37 and 0.63, respectively in American Hampshire pigs (Miller et al., 2000).

Pigs that have one or two RN mutations (rn^+RN^- or RN^-RN^-) are usually identified by one of two methods, DNA marker (de Vries et al. 1997) or determination of glycogen potential (Monin and Sellier, 1985). The presence of the RN mutation does not always cause elevated glycogen concentration. Conversely, pigs with abnormally high glycolytic potential, greater than 150 to 200 μmol /g of tissue, may not have the RN mutation (Bertram et al., 2000; Moeller et al., 2003). This maximum glycolytic potential is not constant and depends on method of collection and physiological state at collection time. A bimodal distribution of glycolytic potential is usually observed and genotypic determinations are assigned by the peak and relative values (Lundström et al., 1996; Bertram et al., 2000). However, recent research indicates RN determination by glycolytic potential is not sufficient and can account for as much as 37% of animals being misclassified (Moeller et al., 2003). Therefore, DNA analysis is required to accurately identify RN carriers for breeding purposes.

Growth rate, backfat, and loin muscle area are not affected by the RN mutation unlike the Halothane mutation (Moeller et al., 2003). However, the RN effect on ultimate

pork quality is similar to the Halothane effect. Pork from pigs with the RN mutation has a lower ultimate pH, higher drip loss, higher cooking loss, paler color, and lower shear force than pork from pigs without the RN mutation (Lundström et al., 1996; Moeller et al., 2003). The effects of the RN mutation on pH and pork quality are presented in Table 2. Although a significant amount of the negative effects associated with the Hampshire breed can be explained by the RN mutation, Hampshires that do not have the RN mutation have been shown repeatedly to have less desirable color than Yorkshires (Lundström et al., 1998; Miller et al., 2000) and may not represent the only explanation for poor quality within the Hampshire breed (Bertram et al., 2000).

The Halothane and Napole mutations are located on different chromosomes. Therefore, pigs with both mutations do exist. Given that the Napole mutation provides more glucose from glycogen, the substrate for glycolysis, and the Halothane mutation accelerates glycolysis post-mortem, the assumption that an animal with both mutations would have lower pork quality than one with a single mutation is plausible. Indeed, these mutations have been reported to be additive resulting in lower ultimate pH, objective color, and water-holding capacity (Hamilton et al., 2000).

Often researchers refer to genetic potential for lean gain or growth rate. However, the same term can be applied to pork quality. Some pigs are predetermined to have lower pork quality than others as observed by the Halothane and Napole mutations. Therefore, genotype can account for a majority of pork quality attributes of pigs managed similarly.

Table 2. Effect of the Rendement Napole genotype on pH and pork quality[a]

Genotypes[b] Compared	Method of genotypic determination	Effect of mutations on pH	Effect of mutation on quality characteristics	Reference
Normal and carriers or positive[c]	Glycolytic potential (GP) >180 μmole/g = carrier	Reduction of 0.12 and 0.24 pH units at 24 h post-mortem in the Longissimus and biceps femoris, respectively.	Carrier pigs exhibited higher surface and internal reflectance, drip loss, cooking loss, taste and smell intensities than non-carriers. Carrier pigs had lower shear force values than non-carriers. Tenderness was not affected by the RN mutation. Sixteen percent of pigs with high GP did not exhibit lower Napole yield., hence, were misclassified.	Lundström et al., 1996
Normal and carriers or positive	GP >180 μmole/g = carrier	Reduction of 0.09 and 0.08 pH units at 24 h post-mortem in the Longissimus and Semimembranosus, respectively.	Carrier pigs exhibited higher internal reflectance, drip loss, cooking loss, and filter paper wetness from the Longissimus than non-carriers. Processing yield was lower and internal reflectance was higher from the Semimembranosus of carriers than normal pigs. Normal Hampshire pigs had lower surface reflectance and shear force than normal Yorkshire pigs.	Lundström et al., 1998

Table 2. Cont.

Normal and carriers or positive	GP >185 μmole/g = carrier	Reduction of 0.10 pH units from the Longissimus at 24 h post-mortem	Carrier pigs exhibited greater drip and cooking loss than normal pigs. Carriers had less marbling than normal pigs. Hunter color, visual color scores, and shear force were not affected by the RN mutation. Hampshires without the RN mutation had lower Hunter L* and higher Hunter a* than normal Yorkshires.	Miller et al., 2000
Normal and carriers or positive	GP >225 μmole/g = carrier	Reduction of 0.24 pH units from the Longissimus at 24 h post-mortem.	Carrier pigs exhibited higher Minolta L*, drip and cooking loss, and Minolta b* than normal pigs.	Hamilton et al., 2000
Normal and Carriers	GP >150 μmole/g = carrier and DNA	Reduction of 0.16 pH units from the Longissimus at 24 h post-mortem.	Carrier pigs had greater drip loss, cooking loss, purge, and off-flavor than non-carriers. Carriers exhibited lower Minolta L*, visual color and firmness score, intramuscular fat, and flavor than non-carriers.	Moeller et al., 2003

[a]This table is provided as a representation of previous work conducted in this area and is not exhaustive.
[b]The genotype notation includes normal (no mutation), carrier (one mutation), and positive (two mutations).
[c]Studies solely relying on glycolytic potential can not conclusively state for a fact that the positive genotype was or was not present.

Effects of pig age at harvest

Genetic selection over the past decades has yielded pigs with improved growth rates and efficiency of feed utilization. However, selecting animals for increased performance has resulted in a reduction of pork quality (Cameron, 1990). Some of these effects on pork quality can be explained by discovered mutations including the Halothane and Napole alleles. However, these mutations do not explain all of the reductions of quality associated with selection over time (Lee and Choi, 1999). Therefore, a question is whether age has an effect on ultimate pork quality.

Pigs in the United States are harvested from 140 to 200 days of age. The effect of age of pig at harvest on pork quality has not been researched extensively. One of the difficulties associated with determining the effect of age at harvest on pork quality is that age is often confounded by bodyweight and inherently related to bodyweight gain. The following section will summarize the research available on the topic and attempt to conclude the current knowledge of this integrated topic.

The research investigating the effect of age and/or growth rate is limited (Table 3). Pork from older, slower growing pigs is less pale and may be more or less red than that of younger pigs (Goerl et al., 1995; Oksbjerg et al., 2000). Given that faster growing pigs have more Type IIB (fast-twitch, glycolytic) and less Type I (slow-twitch, oxidative) muscle fibers, pork would be expected to be redder and darker than pork from slower growing animals (Oksbjerg, et al., 2000).

Morrow et al. (2002) demonstrated a reduction in pork quality characteristics associated with increased age and/or feed withdrawals. Single feed withdrawal prior to

Table 3. Effect of age and/or growth rate on pork quality characteristics[a]

Genotypic Description	pH	Color	Fluid loss	Other observations	Reference
Low and high lean growth potential	No effect	Low lean pigs had lower Hunter L*, a*, and b* than high lean pigs.	Low lean pigs had greater water holding capacity than high lean pigs.	Low lean pigs had higher shear force than the high lean pigs.	Goerl et al., 1995
Slow growing (670 g/d) Fast growing (958 g/d)	No effect of growth on post-mortem pH.	Slow growing pigs had less pale (L*) pork that was redder (higher a*) than fast growing pigs. No effect on b*.	Drip loss was not affected. However, faster growing pigs had more cooking loss than slower growing pigs.	Fast growing pigs had less Type I and more Type IIb muscle fibers and less myoglobin and pigments than slow growing pigs.	Oksbjerg et al., 2000
Duroc Control 218 g lean gain/d Select 292 g lean gain/d	The Longissimus of controls had a greater pH at 15, 30, and 45 min and Semitendinosus of controls had greater pH at 15 min and 24 h post-mortem than the select line.	No effect on Hunter L*, a*, or b*.	Control line had less protein drip loss than the select line. Drip loss was lower from the Longissimus, Semitendinosus, and Semimembranosus of control pigs compared to the select pigs.	Control Longissimus had lower shear force than the select line.	Longeran et al., 2001

[a]This table is provided as a representation of previous work conducted in this area and is not exhaustive.

harvest is the most widely used nutritional method of improving pork quality (Bidner et al., 1999). However, multiple feed withdrawals are common in the swine industry because of multiple marketings within a pen. Multiple marketings are necessary because of the variation associated with a populations of grow-finish pigs and because pigs are marketed by weight. Therefore, further research is needed to identify the effect of pig age on pork quality independent of the number of feed withdrawals.

Magnesium

Introduction

Two-thirds of body Mg is located in bone. The remaining third is disbursed in soft tissue and extracelluar fluid. Plasma Mg values usually range from 17 to 25 ppm and muscle concentrations are approximately 950 ppm (Apple et al, 2001). Only 1 to 3% of the total intracellular Mg concentration (5 to 20 mmol) is free ionized Mg. Therefore, a majority of the intracellular Mg is bound to organic compounds including ATP, DNA, RNA, and proteins.

A great deal of information about Mg is available for ruminants because of the occurrence of grass tetany associated with Mg deficiency, especially when fed on lush green pasture. However, much less research has focused on Mg metabolism in nonruminants. Mg is present in adequate concentrations (0.14 to 0.18%) in typical corn-soybean meal diets consumed by pigs (Svajgr et al., 1969; Krider et al., 1975). Deficiency signs include hyperirritability, muscle twitching, reluctance to stand, loss of equilibrium, tetany, and eventually death (Mayo et al., 1959; Miller et al., 1965). Although toxicity is rare, the estimated tolerable concentration is 0.3% (National

Research Council, 1980).

Absorption and Regulation

Magnesium can be absorbed in the small intestine by carrier-mediated and simple diffusion at low and high dietary concentrations, respectively, in certain species (Schweigel and Martens, 2000). However, in the pig net secretion in the small intestine and absorption from the ileum and colon has been reported (Partridge, 1978). Efficiency of absorption is influenced by Mg status of the pig, Mg content of the diet, and presence of other dietary factors including, calcium, phosphorus, fatty acids, and phytate, which can impair Mg absorption (Patience and Zijlstra, 2001). Apparent absorption of Mg is relativity low, typically 20 to 30% (Patience et al., 1987). The kidney is a major site of Mg regulation and is able to excrete Mg at high dietary concentrations and reabsorb Mg with greater efficiency at low dietary concentrations (Shils, 1988).

Magnesium Status and Biological Role

Magnesium is involved in more than 300 enzyme systems. Perhaps one of the more important functions is to stabilize ATP. Approximately 90% of ATP is bound to Mg (Godt and Maughan, 1988). The free Mg concentration is estimated to be ~1 mM (Westerblad and Allen, 1992; Konishi et al., 1993). It is this small intracellular ionized fraction of Mg that is thought to activate enzymes and influence many biological functions.

The determination of cytosolic free Mg is rather difficult and has been evaluated using sublingual cells through x-ray dispersion (EXA) and tissues using ^{31}P nuclear magnetic resonance spectrometry (NMR), and fluorescent probes (Saris et al., 2000). Debate concerning use of sublingual cells to assess tissue intracellular Mg is extensive

because of the inherant differences between cells. Therefore, if possible, the NMR approach is preferred because of its specificity. However it is expensive and availability is often limited.

Magnesium supplementation has been reported to increase intracellular Mg using sublingual cells as an indicator (Shechter et al., 2000, 2003). However, intracellular free Mg concentrations of skeletal muscle and brain tissues were not affected by Mg supplementation of humans as determined by ^{31}P-NMR (Wary et al., 1999). In that study only urine Mg was increased suggesting the presence of Mg regulation by the kidney and Mg intake was not high enough to increase plasma Mg (Wary et al., 1999). Whether or not intracellular Mg is increased as a result of increased plasma Mg remains to be proven.

Dietary Mg supplementation has been identified to reduce signs of stress. Undoubtedly, the degree of stress an animal experiences is difficult to determine. However, plasma cortisol, epinephrine, and norepinephrine have been used to attempt to objectively determine differences in stress. These compounds can stimulate cAMP that ultimately results in glycogenolysis leading to a more rapid post-mortem pH decline and a reduction in pork quality. Kietzmann (1989) reported a significant reduction in the increase in catecholamine and corticosterone concentration in serum caused by stress in rats provided supplemental Mg gluconate. Dietary Mg reduced the stress response of pigs determined by cortisol and catecholamine concentrations of plasma (Kietzmann and Jablonski, 1985). Furthermore, pigs provided Mg 5 d prior to harvest had reduced plasma epinephrine at harvest (D'Souza et al., 1998). Indeed, pigs receiving Mg supplementation were visually calmer upon arrival to the lairage facility (Kuhn, 1981). However, a significant response to

neither epinephrine nor norepinephrine is required to improve pork quality of pigs supplemented Mg (D'Souza et al., 1999).

Another possible action of Mg to influence pork quality is to antagonize calcium (Lopez et al., 1990). The channel opening of the Ryanodine receptor that is involved in intracellular Ca^{2+} regulation is inhibited by physiological concentrations of Mg^{2+} (Endo, 1977; Meissner, 1994). Calcium release into the intracellular area of the muscle fiber causes muscle contraction through Ca^{2+} binding to troponin. Inhibiting rapid Ca^{2+} release from the sacroplasmic reticulum into the intracellular space by Mg^{2+} ante-mortem could prevent excess muscle contraction and potentially improve pork quality. However, the inhibition effect of Mg seems to be diminished in pigs with the Halothane mutation (Laver et al., 1997; Balog et al., 2001).

Magnesium deficiency has been reported to increase intracellular oxidation compared to the non-deficient state (Dickens et al., 1992). Furthermore, free radical induced membrane damage as a result of Mg deficiency may be caused by abnormal intracellular calcium regulation (Astier et al., 1996). Supplemental Mg well above the Mg requirement has shown some success in reducing lipid oxidation of pork (Apple et al., 2001) but has not been studied extensively.

Pork Quality

Dietary Mg supplementation has been implemented to improve pork quality, specifically color and fluid loss (D'Souza et al., 1998, 1999, and 2000). Furthermore, Mg supplementation has been reported to reduce oxidation of meat during storage (Apple et al., 2001). However, the presence or consistency of the positive effect has been questioned

(Apple et al., 2000; Hamilton et al., 2002) even when plasma Mg was increased 10% (van Laack, 2000). The following section will summarize the most relevant research.

The literature pertaining to Mg supplementation can be divided into two main sections dependent on the physiological status of pigs at harvest and the manner in which the pigs are harvested. Pigs harvested in Australia are boars or gilts weighing 70 to 90 kg of bodyweight and are stunned by carbon dioxide administration (D'Souza et al., 1998). However in the United States and Canada, boars are not harvested and pigs are usually heavier (110 to 125 kg BW) when harvested primarily by electrical stunning. The presence and consistency of a Mg response to improve pork quality have been different across conditions.

The first three experiments presented in Tables 4 and 5 summarize the research conducted by D'Souza in Australia. These studies have shown the most consistent, positive effect of Mg on pork quality characteristics including reduced drip loss (D'Souza et al., 1998, 1999, and 2000) and paleness (L*, D'Souza et al., 1998 and 2000). Although a consistent effect of pork quality was present, the effect of Mg on glycogen, lactate, and pH post-mortem were not consistent. Nevertheless, these three experiments to demonstrated a positive effect of Mg on drip loss and to a lesser extent paleness regardless of amount (1.6 or 3.2 g/d), source (aspartate, sulfate, or chloride), or duration (2 or 5 d prior to harvest) of Mg supplementation.

The data collected in North America have been less consistent within and across experiments than those collected in Australia (Tables 4 and 5). Drip loss was reduced in one experiment (Hamilton et al., 2002) conducted in the United States. Furthermore, the

reduction of drip loss within the experiment was not present across all durations, 2 and 5 d, but not 3 d of supplementation. The effect of Mg on color was more consistent than that on drip loss, indicating that Mg may increase redness (Apple et al., 2000 and 2001) and reduce paleness (Hamilton et al., 2002). Thus, effects of Mg supplementation in North America have not been as consistent and positive as those in experiments conducted with lighter weight pigs stunned with carbon dioxide.

Magnesium supplementation has shown positive results to improve pork quality. However, practical application of Mg supplementation through feed for brief periods of time prior to harvest is difficult especially with multiple marketing within a pen of pigs. Therefore, Mg supplementation through drinking water is a novel and practical approach to attempt to improve pork quality prior to harvest. Research addressing delivery of Mg through drinking water is not present in the literature and therefore is an area of research that is needed.

Table 4. Effect of dietary magnesium on post-mortem glycolysis and pH

Mg addition	Duration of Mg	Initial pH	Ultimate pH	Glycolysis	Reference
3.2 g/d Aspartate	5 d	Magnesium supplemented pigs had greater pH at 40 min in white and red muscles.	The pH at 24 h was greater in the white muscle, but not the red muscle, when supplemented with Mg.	Magnesium reduced lactate concentrations at 5 and 40 min, but not 24 h post-mortem. No effect on glycogen.	D'Souza et al., 1998
3.2 g/d Aspartate, Sulfate, or chloride	5 d	No effect	No effect	No effect of Mg on the rate of glycogenolysis. However, glycogen was higher and lactate was lower 5 min post-mortem than non-Mg supplemented pigs.	D'Souza et al., 1999
1.6 or 3.2 g/d Aspartate or Sulfate	2 or 5 d	No effect	No effect	Magnesium reduced lactate concentration at 24 h but not 5 or 40 min post-mortem. No effect on glycogen.	D'Souza et al., 2000

Table 4. Continued.

Mg addition	Duration of Mg	Initial pH	Ultimate pH	Glycolysis	Reference
5 or 40 mg/kg BW Aspartate	43 d (5 mg/kg) 7 d (40 mg/kg)	Halothane carriers with Mg had lower initial pH than non-supplemented carriers.	Short-term (7 d) Mg increased pH compared to 0 d.	Glycogen was lower and lactate was higher at 20 min post-mortem for Halothane carriers supplemented with Mg.	Caine et al., 2000
3.2 g of Mg/d Sulfate	0, 2, 3, or 5 d	N/A	No effect	N/A	Hamilton et al., 2002

Table 5. Effect of magnesium supplementation on pork quality characteristics

Mg addition	Duration of Mg	Drip loss	Color	Incidence of PSE	Reference
3.2 g/d Aspartate	5 d	Reduced drip loss at P<0.06 in both muscles.	Pigs fed Mg had lower L* of the white muscle, but not the red muscle, compared to pigs not receiving Mg.	Pigs fed Mg had no PSE compared to pigs not fed additional Mg and subjected to normal and negatively handling methods, 8 and 33%, respectively.	D'Souza et al., 1998
3.2 g/d Aspartate, Sulfate, or chloride	5 d	Drip loss was lower from pigs supplemented Mg compared to non-supplemented pigs. However, Mg did not have an effect on exudate.	No effect	No PSE carcass for the Mg supplemented pigs and 17% of non-supplemented pigs were considered PSE.	D'Souza et al., 1999
1.6 or 3.2 g/d Aspartate or Sulfate	2 or 5 d	Magnesium reduced drip loss.	Mg aspartate reduced L*.	Pigs supplemented with Mg had a lower incidence of PSE than non-supplemented pigs.	D'Souza et al., 2000

Table 5. Continued.

Mg addition	Duration of Mg	Drip loss	Color	Incidence of PSE	Reference
0% 1.25% 2.50% Mg mica	27 to 107 kg BW	No effect	Exp. 1 Magnesium supplementation increased redness compared to controls Exp. 2 - Lower Mg (1.25% Mg mica) reduced redness and yellowness compared to 0 or 2.5% Mg mica.	N/A	Apple et al., 2000
5 mg/kg BW 40 mg/kg BW Aspartate	43 d (5 mg/kg BW) 7 d (40 mg/kg BW)	No effect	No effect	N/A	Caine et al., 2000
00% 1.25% 2.50% Mg mica	27 to 107 kg BW	N/A	Lower Mg (1.25% Mg mica) reduced chroma, redness and yellowness compared to 0 or 2.5% Mg mica	N/A	Apple et al., 2001
3.2 g of Mg/d Sulfate	0, 2, 3, 5 d	Supplementation for 2 and 5 d reduced drip loss.	Two days of Mg reduced Hunter L*.	N/A	Hamilton et al., 2002

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CHAPTER 2

Timing of magnesium supplementation through drinking water to improve fresh and stored pork quality[1,2]

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[2]Funded in part by North Carolina Pork Council, the North Carolina Agricultural Research Service, and the Institute of Nutrition of the University of North Carolina System.

ABSTRACT

Thirty-two pigs were used to determine the timing effect of Mg supplementation through drinking water on pork quality. Pigs (16 castrated males, 16 females) were individually penned, provided 2.7 kg of feed (0.12% Mg) daily and allowed free access to water via a nipple waterer for the duration of the study. After 5 d of adjustment, pigs (120 ± 0.8 kg BW) were randomly allotted by weight and sex to 900 mg/L supplemental Mg from Mg sulfate heptahydrate in drinking water for -6, -4, -2, or 0 d relative to harvest. The Longissimus dorsi (LD) and Semimembranosus (SM) muscles were removed 24 h post-mortem. Retail display storage was simulated for 8 d and LD was vacuum-packed for 25 or 50 d at 4°C. Magnesium did not affect pH of the LD at 45 min or 24 h post-mortem ($P > 0.10$). However, pH of the SM at 24 post-mortem tended ($P = 0.08$) to be greater from pigs consuming Mg supplementation for 2 d than those not supplemented. The LD display fluid loss after 8 d of storage was lower ($P < 0.05$) from pigs supplemented with Mg for 6 d than those without supplementation. Furthermore, SM display fluid loss from pigs provided supplemental Mg for 2 d, but not 4 or 6 d, was lower during each storage period than pigs without Mg supplementation. Minolta L*, a*, and b* color measurements of the LD during display storage were not affected by Mg supplementation. However, Mg supplementation for 2 or 4 d reduced lightness (Minolta L*) after 25 d ($P < 0.05$), but not 50 d, of vacuum-packed storage. Magnesium addition for 2 d reduced the extent of oxidation (thiobarbituric acid reactive substances) of the LD after 4 d of display storage compared to 0 d of Mg ($P < 0.05$). Oxidation of the SM during 8 d of display storage increased incrementally ($P < 0.05$) as duration of supplementation increased from 2 to 6 d but did not differ from 0 d of Mg

supplementation ($P > 0.05$). Oxidation of the LD after 50 d of storage increased incrementally ($P < 0.05$) as duration of supplementation increased from 2 to 6 d but did not differ from 0 d of Mg supplementation ($P > 0.05$). Although the response to Mg supplementation was variable, supplementation for 2 d prior to harvest was considered most efficacious because of reduced fluid loss of the SM during display storage, decreased lightness of LD during vacuum-packed storage, and lower oxidation and cost compared to longer durations.

Keywords: Pork quality, magnesium sulfate, drinking water

Introduction

Magnesium is an important divalent cation involved with over 300 enzymes essential for metabolism including protein and energy metabolism. Furthermore, Mg has been shown to decrease acute stress response resulting from handling prior to harvesting (Kietzmann, and Jablonski, 1985), control intracellular calcium (Laver et al., 1997), and delay the initiation of glycolysis by maintaining high energy phosphates post-mortem (Moesgaard et al., 1993). For these reasons Mg has been evaluated as a nutritional means of improving pork quality (D'Souza et al, 1998; Apple et al., 2001; Hamilton et al., 2002).

Indeed, short-term supplemental dietary Mg has been reported to decrease fluid loss (D'Souza et al., 1998, 1999, 2000) and improve color (D'Souza et al., 1998, 2000) of pork. Furthermore, dietary Mg may affect lipid oxidation of stored pork (Apple et al., 2001). The effect of Mg on pork quality does not seem to be dependent on Mg source between Mg aspartate, sulfate, or chloride (D'Souza et al., 1999). However, the response may or may not be dependent on timing of supplementation (D'Souza et al., 2000; Hamilton et al., 2002). However, others have not been able to demonstrate a positive or consistent effect of Mg on pork quality (van Laack, 2000; Hamilton et al., 2002). Although many studies have concentrated on supplemental dietary Mg in feed, supplementation through drinking water is a novel, practical approach that has not been reported previously. Therefore, the objective of this study was to determine the optimal duration of Mg supplementation through drinking water to improve pork quality.

Materials and Methods

Animals and Treatments

All animal procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Sixteen barrows and sixteen gilts weighing 120 ± 0.8 kg from the North Carolina State University Swine Education Unit were placed into 2.03 m by 0.74 m individual pens and provided free access to water via a nipple waterer. Pigs were fed 2.7 kg of feed containing 0.12% Mg from feedstuffs per day (Table 1) during a 5 d adjustment and 6 d treatment period. After the adjustment period, pigs were allotted by weight within sex to water supplemented with 900 mg/L of elemental Mg from Mg sulfate heptahydrate (9.8% Mg, Giles Chemical Corp., Waynesville, NC) for -6, -4, -2, and 0 d relative to harvest. Plastic water containers (23 L capacity) were filled daily with 15 L of water containing the appropriate Mg concentration. These containers were suspended from the ceiling and gravimetrically emptied into a galvanized pipe leading to nipple waterer regulated to dispense 600 ml of water/min. Daily water disappearance volumes were determined by weight loss of the water containers assuming that 1 kg equaled 1 L of water. Feed was removed 12 h prior to transport. However, pigs had free access to experimental water treatments until transport to the abattoir**.**

Harvest Data Collection

On d 7 (0800) all pigs were loaded and transported 110 km (1 h 45 min) to a commercial abattoir. Pigs were unloaded by abattoir personal. After 45 min of lairage, pigs were moved by replicate 15 m to the stunning area. Pigs were electrically stunned and blood was collected during exsanguination for plasma Mg determination. Hot carcass

weights were collected prior to refrigeration to determine dressing percent. The temperature and pH of the loin were measured between the 10th and 11th rib at 45 min post-mortem using an Argus Sentron (Gig Harbor, WA) pH meter.

Fabrication and Storage

After 20 h of chilling at 2˚C the entire right side loin and ham were removed and transported 60 km (45 minutes) at 4˚C to a commercial meat fabrication facility for further processing. A total of 4 chops (2.54 cm thick) of the Longissimus dorsi (LD) muscle were obtained beginning at the 7th and 8th rib interface and extending posterior. The first LD chop was placed in a plastic bag and stored at -20˚C for dry matter and tissue Mg determination. The second chop (located posterior to the first chop) was used for drip loss determination on the same day of collection. The third chop posterior to the first was placed on an absorbent pad (Cryovac Sealed Air Corp., Saddle Brook, NY) within a Styrofoam tray (Cryovac Sealed Air Corp.), wrapped with a polyvinyl chloride film (Cryovac Sealed Air Corp.), and stored at 4˚C in the presence of fluorescent lighting to simulate retail display for 4 d. At the end of the 4 d display storage period the chop was analyzed for extent of oxidation. The fourth and final chop was taken immediately posterior to the third chop and stored in a similar environment as the third chop for 8 d of displayed storage. The remaining posterior portion of the LD muscle was split into equal sections, weighed, vacuum-packed in B2651T Cryovac bags with a Mulitvac machine (Cryovac, Ducan, SC), and stored at 4˚C in the absence of light for 25 or 50 d. The Semimembranosus (SM) was removed from the ham. Four SM chops (2.54 cm thick) were obtained and processes similarly to the LD chops.

Plasma and muscle Mg

Plasma and muscle Mg concentration was determined by atomic absorption. Briefly, each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). After two grams of sample were dried at 103˚C overnight, the dried sample was quantitatively transferred to a polypropylene tube (Corning). Ten ml of nitric acid (Fisher Scientific, Fair Lawn, NJ) were added to the tube and allowed to predigest overnight. The next day the samples were placed in a microwave oven (MARS 5, CEM, Matthews, NC), ramped for 10 min to 110˚C, maintained at 110˚C by thermowell, and cooled for 20 min before adding 2 ml of hydrogen peroxide (Sigma, St. Louis, MO) to terminate digestion. Tubes were brought up to 25 ml with deionized water. Fifty microliters of digested sample were combined with 5 ml of lanthium chloride (0.5%, Fisher) and read by atomic absorption.

Pork Quality Measurements

Fluid loss of the LD and SM was evaluated by two separate methods. Drip loss was determined by a method developed by Honikel et al. (1986). Briefly, a 70 g core sample of each muscle was manually removed and suspended by a fish hook (barb removed) in a plastic, covered container, and stored at 4˚C for 48 h. Drip loss was reported as the weight loss of the sample after 48 h of storage divided by the initial weight of the muscle prior to storage multiplied by 100. Display fluid loss was determined on chops designated for 8 d of display storage. The muscle was removed from the tray on d-2, 4, 6, and 8, placed on a paper towel for 5 seconds, and reweighed to determine display fluid loss. Display fluid loss was reported as the weight loss of displayed chops divided by the initial weight of the chop

prior to storage multiplied by 100. Each chop was returned to its original tray, rewrapped, and returned to display storage for subsequent measurements.

Purge loss was identified as the amount of fluid lost from the LD muscle after 25 or 50 d of vacuum-packed storage. After the appropriate storage period, each muscle was removed from the vacuum-packed bag, blotted with a paper towel, and reweighed. Purge loss was reported as the weight loss during storage divided by the initial muscle weight multiplied by 100.

Color in the LD and SM was objectively evaluated by Minolta L*, a*, and b* measurements using a Minolta Chroma Meter (CR-200, Ramsey, New Jersey) calibrated with a standard white plate. Minolta values were reported as the average color values collected at 4 positions in a diamond pattern on the surface of each chop. All color measurements were conducted on chops designated for 8 d of display storage. The initial measurement of color was performed after 45 minutes of the initial cut. Additionally, color was determined every 2 d for 8 d of display storage. Color of vacuum-packed LD was determined on an interior chop after a 45 min bloom period.

Longissimus and SM chops displayed for 4 or 8 d and the LD chops from vacuum-packed loin sections for 25 or 50 d were vacuum-packed in Cryovac bags (B2651T) and stored at -20°C until oxidation was determined by thiobarbituric acid reactive substances (TBARS). At time of analysis, samples were removed from -20˚C and allowed to thaw overnight at 6˚C. Each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). Four grams of ground muscle were homogenized, in duplicate, with 16 ml of ice cold phosphate buffer (pH = 7.0)

prepared to contain 50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 0.1% EDTA (Fisher Scientific) and 0.1% propylgallate (Sigma) in a stainless steel cup for 20 s using an Omni-Mixer (Model 17105, Sorvall Corp., Newtown, CT) at 16,000 rpm. Then, 4 ml of 30% trichloroacetic acid (Sigma) were added and samples were homogenized for an additional 10 s at 16,000 rpm. After the homogenate was filtered (P8, Fisher Scientific), 2 ml of the clear filtrate and 2 ml of 2-thiobarbituric acid (0.02 M, Sigma) were transferred to 16x125 screw-cap glass test tubes, vortexed, and heated in a 100˚C water bath for 30 min. Test tubes were allowed to cool in an ice cold water bath for at least 15 min and vortexed prior to measuring absorbance on a spectrophotometer at 533 nm (Model DU 640, Beckman, Fullerton, CA). The absorbance of samples was compared to tetraethoxypropane (Sigma) standard concentrations of 2, 4, 8, 10, 20, 40, and $80x10^{-7}$ M. Reported TBARS values reflect a correction of percent recovery ranging from 92 to 102%.

Statistical Analyses

Data were analyzed as a randomized complete block design using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). Pigs within individual pens were considered the experimental unit and were blocked by bodyweight within sex. Means were compared using the PDIFF and STDERR options of SAS.

Results and Discussion

Feed and water disappearance

Limiting feed intake was implemented to limit the variation of dietary Mg intake. No feed refusal was observed during the adjustment or treatment period. Therefore, feed intake was the same across treatments and Mg intake from feed was considered to be a

constant 3.24 g of elemental Mg per day. Thus, any difference of Mg intake was a result of Mg intake provided through the drinking water.

Magnesium concentration of water used to dilute the stock solution was 3 mg/L. Therefore, the total Mg concentration of water was 3 and 903 mg/L for pigs receiving water without and with Mg supplementation, respectively. Assuming that water disappearance equaled water consumption, the maximum Mg intake from drinking water was 0.03, 10.47, 8.53, and 7.10 g of elemental Mg/d for 0, 2, 4, and 6 days of supplementation, respectively. Water requirements have been estimated to be 2 L of water per 1 kg of feed (Cumby, 1986). Assuming this water to feed ratio, Mg intake from drinking water would have been approximately 4 to 5 g/d. However, the exact Mg intake from water is not known.

Harvest Data

Plasma Mg tended to increase linearly as duration of Mg supplementation increased ($P < 0.11$, Table 3) and was highest (12% increase) when Mg was supplemented for 6 d. Similarly, van Laack (2000) reported a 10% increase of plasma Mg when pigs were supplemented 2 g of Mg /kg of feed as Mg sulfate for 5 d. Furthermore, D'Souza et al. (1999) reported a 10 % increase of plasma Mg with dietary supplementation of 3.2 g of Mg/d from Mg sulfate, aspartate, or chloride for 5 d prior to harvest. Magnesium concentration of neither LD nor SM in the present study was affected by Mg supplementation and is in agreement with Schaefer et al. (1993) and Apple et al. (2001). Dressing percentage was greater ($P < 0.05$) for pigs receiving Mg supplementation for 6 d compared to 2 d (Table 3). The laxative effect of Mg sulfate may decrease the amount of digesta in the gastrointestinal tract resulting in a greater dressing percentage. Given the

short period of time of Mg supplementation of 2 to 6 d, it is unlikely that Mg significantly increased protein or lipid deposition and thereby affect carcass yield, although this was not directly determined.

Magnesium supplementation did not affect temperature ($P = 0.35$) or pH of the LD at 45 min ($P = 0.26$) or 24 h post-mortem ($P = 0.54$), which is consistent with the results of D'Souza et al. (1999; 2000) and Hamilton et al. (2002). However, the pH of the SM from pigs supplemented with Mg for 2 d tended to be greater (P = 0.08) at 24 h post-mortem than those not receiving Mg supplementation. Apple et al. (2000) reported that ultimate pH of the loin was greater when pigs were fed 1.25% Mg mica, although 2.5% did not affect ultimate pH. Even though pH values are often used as an indicator of pork quality, a difference of initial pH or ultimate pH is not required to effectively alter pork quality (D'Souza et al., 1999 and 2000).

Fluid loss

Drip loss of the LD and SM was not affected by Mg supplementation (Table 4, $P = 0.34$ and $P = 0.51$, respectively), which is consistent with other studies using dietary Mg mica, Mg aspartate, or Mg sulfate prior to harvest (Apple et al., 2000; Caine et al., 2000; van Laack, 2000). However, these results differed from research in which a negative handling strategy was used to evaluate the effects of supplemental dietary Mg (D'Souza et al., 1998, 1999, and 2000). Fluid loss of displayed LD chops was not affected during the first 6 d of storage. However, after 8 d of display storage fluid loss from the LD chops from pigs supplemented for 6 d was lower ($P < 0.05$) than from pigs that did not receive Mg supplementation. Fluid loss of displayed SM was lower ($P < 0.05$) for pigs consuming

supplemental Mg for 2 d prior to harvest compared to no Mg supplementation during all storage periods measured, resulting in a 36% and 24% reduction after 2 and 8 d of display storage, respectively. A reduction of fluid loss from the SM contradicts the observations by van Laack (2000) who found no effects when pigs were fed 2 g of supplemental Mg/kg of feed as Mg sulfate for 5 d.

These results indicate that effects of Mg supplementation on fluid loss differed across muscles. This difference in response may be a result of the physiological differences between muscles. The LD is considered to function primarily for structural support while the SM muscle is primarily used for work. Furthermore, the LD has higher percentage of Type II and lower percentage of Type I muscle fibers than the SM muscle (Gentry et al., 2002).

Color

Magnesium supplementation did not affect Minolta L*, a* or b* of the LD during display storage (Table 5), which is consistent with results reported by D'Souza (1999) and Caine et al.(2000). However, D'Souza et al. (1998, 2000) reported a reduction of L* of the LD when Mg was supplemented through the feed. Hamilton et al. (2002) also reported a reduction of L* of the LD with 2 d but not 3 or 5 d of Mg supplementation. In the present experiment Mg supplementation for 2 d decreased ($P < 0.05$) initial yellowness (Minolta b*) of the SM compared to the control and lowered ($P < 0.05$) lightness (Minolta L*) after 2 d of display storage compared to 6 d of Mg supplementation. However, this effect was not apparent at any other measurement period during display storage, indicating that there was only a slight and transient effect of Mg supplementation on color of displayed pork.

Magnesium supplementation for 2 or 4 d reduced ($P < 0.05$) the lightness of chops from vacuum-packed LD sections stored for 25 d compared to control pigs (Table 6). However, Mg supplementation did not affect lightness after 50 d of vacuum-packed storage. Furthermore, Mg addition did not influence redness or yellowness at 25 or 50 d of vacuum packed storage. Apple et al. (2001) found that supplementation of 1.25% Mg mica reduced redness and yellowness of boneless loins but supplementation of 2.5% Mg mica did not result in differences from non-supplemented pigs. In that same study neither concentration of Mg mica affected loin lightness. The results from the present experiment indicate Mg supplementation for 2 or 4 d prior to harvest may reduce paleness of vacuum-packed loins without negatively affecting redness or yellowness.

Purge Loss

Purge loss of vacuum-packed LD was not affected by Mg supplementation (Table 6), which is in agreement with previous research (Apple et al., 2001; Hamilton et al., 2002). This result may indicate that any effect of Mg on fluid loss is expected to occur during relatively short periods of displayed storage and the effect is less noticeable during longer periods of vacuum-packed storage.

Oxidation

Oxidation of the LD after 25 d of vacuum-packed storage or SM after 4 d of displayed storage was not affected by Mg supplementation (Table 7). However, LD chops from pigs receiving Mg supplementation for 2 d had less oxidation after 4 d of display storage than pigs supplemented with Mg for 0 or 6 d. The extent of oxidation of the LD after 4 d and LD and SM after 8 d of display storage increased incrementally with duration

of Mg supplementation from 2 to 6 d ($P < 0.05$). A similar response was observed in the vacuum packed LD after 50 d of storage. However, neither 4 nor 6 d of Mg supplementation affected oxidation of either muscle compared to 0 d of Mg at any time period. Apple et al. (2001) reported greater oxidation of the loin when pigs were fed 1.25% compared to 2.5% Mg mica after 4 w of vacuum packed storage. Over an additional 4 w, oxidation of loins from pigs provided with 1.25% Mg mica remained constant and the oxidation of loins from pigs provided with 2.5% Mg mica increased to a level higher than that of the 1.25% Mg mica group. Data presented in the current experiment indicate that Mg supplementation can potentially reduce oxidation when supplemented for a brief period of time. However, longer durations of Mg supplementation may increase oxidation relative to brief duration (2 d prior to harvest). Thus, briefer periods of Mg supplementation may be more desirable when the length of shelf life is considered.

Implications

Product quality is important in any industry. The pork industry is no exception. Magnesium supplementation through drinking water is a practical, efficient method of providing supplemental Mg to pigs prior to harvest. The present study suggest Mg supplementation through drinking water for as brief as 2 d prior to harvest may improve pork quality. However, these results are inconsistent across supplementation durations and muscles. Further research is required in this area to evaluate the efficacy of Mg supplementation through drinking water on pork quality.

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Table 1. Composition of experimental diets (%, as is)[ab]

Ingredient	Percent of total
Corn, yellow dent	79.07
Soybean meal, 47.5% CP	15.80
Poultry Fat	2.50
Dicalcium phosphate	1.03
Limestone	0.76
Salt	0.35
Vitamin/Mineral Premix [c]	0.25
L-Lys HCl[d]	0.15
Tylan[e]	0.10
Analyzed Composition	
Total Mg	0.12%

[a]Diet was fed for the 5 d adjustment and 6 d experimental periods.

[b]Formulated to contain 14.2% crude protein, 0.80% lysine, 0.52% P, and 0.60% Ca.

[c]Supplied per kg of complete diet: 5,540 IU of vitamin A as retinyl acetate, 1,108 IU of vitamin D_3, 22 IU of vitamin E as dl-α-tocopherol acetate, 1.98 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 165 mg of choline as choline chloride, 22 mg/kg of niacin as niacinamide, 17.6 mg of d-pantothenic acid as dl-calcium pantothenate, 4.4 mg of riboflavin, 1.1 mg of pyridoxine as pyridoxine·HCl, 0.55 mg thiamine as thiamine mononitrate, 0.022 mg of vitamin B_{12}, 0.33 mg of folic acid, 0.04 mg of d-biotin, 110 mg Zn as $ZnSO_4$, 110 mg Fe as $FeSO_4$, 22 mg Cu as $CuSO_4$, 55 mg Mn as MnO, 0.28 mg I as ethylenediamine dihydriodide, and 0.30 mg Se as $NaSeO_3$.

[d]Contained 78.8% lysine.

[e]Provided 22 mg of tylosin/kg of feed.

Table 2. Least squares means of average water disappearance (L/d) for each 2 day period of magnesium supplementation[a]

	Days of Mg supplementation relative to harvest				
Experimental period	0	6	4	2	SEM
d -4 to -6	11.2			8.4	1.0
d -2 to -4	10.9[b]		9.5[bc]	7.4[c]	1.0
d 0 to -2	11.0	11.6	9.4	8.8	1.8

[a]Water disappearance was measured for pigs receiving 0 or 900 mg/L of elemental magnesium supplementation during a given treatment period.
[bc]Means without a common superscript within rows differ ($P < 0.05$).

Table 3. Least squares means for the effect of magnesium supplementation on harvest data[a]

	Days of Mg supplementation				
Criteria	0	2	4	6	SEM
Live bodyweight, kg	119.9	119.6	120.1	120.0	0.8
Dressing Percent	72.3[bc]	72.0[b]	73.4[bc]	73.5[c]	0.5
Temperature ºC, 45 min	38.4	38.5	38.9	38.9	0.2
Longissimus pH					
45 min	6.31	6.41	6.35	6.48	0.06
24 h	5.60	5.66	5.60	5.60	0.03
Semimembranosus pH					
24 h	5.62	5.71	5.65	5.66	0.03
Plasma Mg, ppm[d]	19.9	21.7	21.8	22.2	0.9
Longissimus Mg, ppm[e]	979	975	1002	975	23
Semimembranosus Mg, ppm[e]	1064	1046	1056	1043	16

[a]Values represent the mean of 8 observations/treatment.
[bc]Means with differing superscripts within a row are different ($P < 0.05$).
[d]Linear effect of duration of magnesium supplementation ($P = 0.11$).
[e]Dry matter basis.

Table 4. Least squares means of the effect of magnesium supplementation on fluid loss of fresh pork[a]

	Days of Mg supplementation				
Criteria	0	2	4	6	SEM
Drip Loss, %[b]					
Longissimus	3.29	2.46	3.16	3.55	0.42
Semimembranosus	3.33	3.26	3.83	3.36	0.30
Display fluid loss, %[c]					
Longissimus					
2d	2.73	2.82	2.84	2.18	0.37
4d	3.85	3.79	3.81	3.10	0.39
6d	4.78	4.59	4.68	3.75	0.45
8d	5.73[d]	5.27[de]	5.39[de]	4.33[e]	0.47
Semimembranosus					
2d	4.30[d]	2.75[e]	3.95[de]	3.78[de]	0.42
4d	6.08[d]	4.15[e]	6.04[d]	5.63[de]	0.52
6d	7.23[d]	5.37[e]	7.31[d]	6.85[de]	0.60
8d	8.22[d]	6.25[e]	8.25[d]	7.76[de]	0.62

[a]Values represent the mean of 8 observations/treatment.

[b]Drip loss was determined by the method of Honikel et al. (1986).

[c]Display fluid loss represents weight loss of muscle chops during simulated retail storage.

[de]Means with differing superscripts within a row are different ($P < 0.05$).

Table 5. Least square means of the effect of magnesium supplementation on Longissimus pork color[a]

	Days of Mg supplementation				
Criteria	0	2	4	6	SEM
Minolta L*					
Longissimus					
0d	57.8	57.0	56.9	55.6	1.7
2d	58.8	57.3	57.6	57.0	1.7
4d	59.6	56.8	57.6	57.1	1.7
6d	59.2	57.8	58.3	57.3	1.5
8d	58.8	57.3	58.5	56.5	1.7
Semimembranosus					
0d	52.3	49.9	50.8	52.8	1.3
2d	52.2[bc]	48.7[b]	49.5[bc]	52.6[c]	1.2
4d	51.4	48.8	49.1	52.3	1.3
6d	52.5	49.6	50.0	52.7	1.1
8d	52.2	50.2	49.0	52.6	1.2
Minolta a*					
Longissimus					
0d	9.33	9.44	9.24	7.71	0.59
2d	9.37	9.54	8.89	8.41	0.48
4d	8.70	8.86	8.10	7.77	0.48
6d	8.04	8.29	7.22	7.22	0.48
8d	7.59	7.67	6.52	6.60	0.50
Semimembranosus					
0d	11.83	10.98	11.48	11.18	0.68
2d	11.78	11.52	12.23	11.09	0.77
4d	11.72	10.37	11.03	10.17	0.73
6d	11.00	9.85	10.20	9.72	0.70
8d	9.95	8.51	9.42	8.90	0.55

Table 5. (cont.)

Criteria	Days of Mg supplementation 0	2	4	6	SEM
Minolta b*					
Longissimus					
0d	7.61	7.88	7.55	6.41	0.70
2d	8.98	8.92	8.51	8.14	0.52
4d	8.96	8.55	8.29	7.93	0.48
6d	8.66	8.57	8.25	7.81	0.45
8d	8.60	8.48	8.10	7.72	0.47
Semimembranosus					
0d	8.95[b]	6.85[c]	7.54[b]	7.94[b]	0.59
2d	10.60	9.40	9.81	9.99	0.54
4d	10.06	8.98	9.27	9.62	0.40
6d	10.02	9.04	9.33	9.82	0.38
8d	9.70	9.06	9.01	9.60	0.39

[a]Values represent the mean of 8 observations/treatment.
[bc]Means with differing superscripts within a row are different ($P < 0.05$).

Table 6. Least square means of the effect of magnesium supplementation on vacuum packed Longissimus quality[a]

	Days of Mg supplementation				
Criteria	0	2	4	6	SEM
			Minolta Color		
25 d					
L*	56.4[b]	51.6[c]	51.9[c]	54.3[bc]	1.1
a*	10.8	10.4	10.4	10.1	0.5
b*	9.0	7.9	8.0	8.2	0.4
50 d					
L*	55.2	53.5	54.1	54.0	1.2
a*	10.5	10.0	10.0	9.7	0.5
b*	8.6	7.9	8.0	8.1	.3
			Purge loss, %		
Days of storage					
25 d	7.8	9.6	8.7	8.7	0.9
50 d	9.4	8.3	9.0	9.7	1.0

[a]Values represent the mean of 8 observations/treatment.
[bc]Means with differing superscripts within a row are different ($P < 0.05$).

Table 7. Least square means of the effect of magnesium supplementation on oxidation (TBARS)[ab]

	Days of Mg supplementation				
Criteria	0	2	4	6	SEM
	TBARS, μg/kg of muscle				
Longissimus					
4d	192[c]	145[d]	171[cd]	187[c]	11
8d	192[cd]	154[c]	198[cd]	216[d]	16
25d	132	115	123	128	9
50d	183[cd]	166[c]	178[cd]	210[d]	12
Semimembranosus					
4d	181	169	176	201	14
8d	219[cd]	194[c]	235[cd]	250[d]	17

[a]Values represent the mean of 8 observations/treatment.
[b]Thiobarbituric acid reactive substances (TBARS).
[cd] Means with differing superscripts within a row are different ($P < 0.05$).

CHAPTER 3

Effect of pig age at market weight and magnesium supplementation through drinking water on pork quality[1,2]

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[2]Funded in part by North Carolina Pork Council and the North Carolina Agricultural Research Service.

ABSTRACT

Thirty-two halothane negative pigs (109±0.6 kg BW) were used to determine the effect of pig age and magnesium supplementation through drinking water on pork quality. Two initial groups of 50 pigs that differed by 30±2 d of age were fed diets to meet or exceed nutrient requirements from 28 kg BW. Sixteen pigs were selected from each group, individually penned, provided 2.7 kg of feed (0.12% Mg) daily, and allowed free access to water for the duration of the study. After 7 d of adjustment, pigs were randomly allotted by sex and weight to 0 or 900 mg/L of supplemental Mg as $MgSO_4$ in drinking water 2 d prior to harvest. All 32 pigs were then transported (110 km) to a commercial abattoir on the same day and harvested 2.5 h after arrival. Longissimus dorsi (LD) and Semimembranosus (SM) chops were packaged and stored to simulate display storage for fluid loss and Minolta color determination at 0, 2, 4, 6, and 8 d. Two remaining sections of the LD were vacuum-packed and stored at 4°C for 25 or 50 d. Fast (younger) and slow (older) growing pigs differed by 27±0.3 d of age, 153 and 180±0.3 d ($P < 0.001$) at similar BW 108 and 110±0.6 kg BW ($P = 0.13$), respectively. Purge loss, color, nor oxidation of vacuum-packed LD or SM nor oxidation or fluid loss of displayed LD or SM were affected by age of pig or Mg supplementation ($P > 0.10$). Surface exudate, measured by filter paper, of the SM from older pigs was less than younger pigs, 61 vs. 74±6 mg ($P < 0.05$). Surface exudate of the LD was not affected by age of pig ($P = 0.22$). The LD from older pigs displayed for 4 and 8 d had lower L* than younger pigs, 51.9 vs. 53.7±0.7 ($P < 0.05$) and 54.4 vs. 55.6±0.6 ($P < 0.06$), respectively. The SM from older pigs had lower L* after 8 d, 54.4 vs. 56.0±0.4 ($P <$

0.05), and tended to have higher a* after 4 and 8 d of display storage, 9.66 vs. 9.22±0.40 and 9.43 vs. 8.84±0.37 (P = 0.09), respectively. Although Mg supplementation increased plasma Mg more than 10%, Mg did not affect pork quality of either older, slower growing or younger, faster growing pigs. However, the SM from older pigs had less exudate, tended to be redder, and the LD and SM from older pigs were darker than younger pigs.

Keywords: Pig age, pork quality, magnesium

Introduction

Pigs are often harvested at a constant bodyweight to maintain uniformity of pork products and maximize profits. However, the variation of bodyweight within pens is often too great to market the entire pen of pigs at one time. Therefore, multiple marketings from a particular group or pen is required to accomplish these goals. Morrow et al. (2002) reported that increased frequency of feed withdrawal associated with increased age at marketing within a pen had a negative effect on several pork quality characteristics. However, the results did not indicate whether the effect on pork quality was caused by frequency of feed withdrawal or age of the pigs when marketed because these factors were confounded by one another. Short-term supplemental dietary Mg has been reported to decrease water loss (D'Souza et al. 1998, 1999, 2000) and improve color of pork (D'Souza et al., 1998, 2000). Furthermore, dietary Mg potentially decreases lipid oxidation of stored pork (Apple et al., 2001). Most of the recent nutritional approaches to improve pork quality have focused on supplementation through feed delivery. This practice is difficult to implement for brief supplementation periods (2 days) and is further complicated by multiple marketings within pens. Developing an approach of supplementing Mg through the water to improve pork quality would simplify delivery by ensuring proper timing of supplementation.

Therefore, the objective of this study was to determine if the age of pig at harvest affects pork quality and if Mg supplementation through drinking water could affect pork quality in pigs of different ages.

Materials and Methods

Animals and Treatments

All animal procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. A total of 32 pigs (109 ± 0.6 kg BW) were used to determine the effect of age of pigs and Mg supplementation through drinking water on pork quality. The goal of the experiment was to select two groups of pigs of similar market weight, but the groups were intended to differ by approximately 30 days of age. Two initial groups of 50 pigs, approximately 28 kg BW, were selected from two groups of sows farrowed 30 d apart. The initial groups of pigs were fed the same grower and finishing diet during the appropriate weight ranges to meet or exceed nutrient requirements of each phase of growth. Specifically, pigs from each group were moved from a nursery to a finishing barn at 60 d of age, penned by bodyweight (4-5 pigs/pen), and were fed the grower diet ad libitum until the average pen BW was 68 kg. Pigs were then fed the finisher diet (Table 1) ad libitum from 68 kg BW until harvest. Sixteen pigs were selected from each of the two initial groups of 50. Thus, 16 pigs were selected from the older initial group, representing slow growing pigs that reached market weight at 180 d of age and 16 pigs were selected from the younger initial group (fast growing pigs), which reached market weight at 150 d of age.

The 32 pigs selected for this study were placed into 2.03 m by 0.74 m individual pens and provided with free access to water via a nipple waterer. Pigs were fed 2.7 kg of feed (Table 1, 0.13% Mg) per day for a 7 d adjustment period. After the adjustment period,

pigs were allotted by sex and weight to water supplemented with 900 mg of Mg/L as Mg sulfate heptahydrate (9.8% Mg, 12.9% S, Giles Chemical Corp., Waynesville, NC) of drinking water for 0 or 2 d prior to harvest. Plastic water containers (23 L capacity) were filled daily with 15 L of water containing appropriate Mg concentrations. Water containers were suspended from the ceiling and gravimetrically (approximately 600 ml/min) emptied into a galvanized pipe leading to a nipple waterer. Daily water disappearance volumes were determined by weight.

Harvest Data Collection

On d 3 (0800) of the experimental period all pigs were loaded and transported 110 km (1 h 50 min) to a commercial abattoir. Pigs were unloaded by abattoir personal. After 2 h 30 min of lairage, pigs were moved by replicate, 15 m to the stunning area. Pigs were electrically stunned and blood was collected during exsanguination for plasma Mg. Hot carcass weights were collected prior to refrigeration to determine dressing percent. The temperature and pH of the loin was measured between the 10^{th} and 11^{th} rib at 45 min post-mortem using an Argus Sentron (Gig Harbor, WA) pH meter.

Fabrication and Storage

After 20 h of chilling at 2˚C the entire right side loin and ham were removed and transported 60 km (45 minutes) at 4˚C to a commercial meat fabrication facility for further processing. A total of 3 chops (2.54 cm thick) of the Longissimus (LD) muscle were obtained beginning at the 7^{th} and 8^{th} rib interface and extending posterior. The first LD chop was placed in a Ziploc bag and stored at -20˚C for dry matter and tissue Mg determination. The second chop posterior to the first was place on an absorbent pad (Cryovac Sealed Air

Corp., Saddle Brook, NY) within a Styrofoam tray (Cryovac Sealed Air Corp.), wrapped with an oxygen permeable film (Cryovac Sealed Air Corp.), and stored at 4˚C in the presence of fluorescent lighting to simulate retail display for 4 d. At the end of the 4 d display storage period the chop was analyzed for extent of oxidation. The third and final chop was taken immediately posterior to the second chop and stored in a similar environment as the chop for 8 d of displayed storage. The remaining posterior portion of the LD muscle was split into equal sections, vacuum packed in B2651T Cryovac bags with a Mulitvac machine (Cryovac, Ducan, SC), and stored at 4˚C in the absence of light for 25 or 50 d. The Semimembranosus (SM) muscle was removed from the ham. Three SM chops (2.54 cm) were obtained and processed similarly to the LD chops with the exception that SM was not vacuum-packed for storage for 25 or 50 d.

Plasma and muscle Mg

Plasma and muscle Mg concentration was determined by atomic absorption. Briefly, each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). After two grams of sample were dried at 103˚C overnight, the dried sample was quantitatively transferred to a polypropylene tube (Corning). Ten ml of nitric acid (Fisher Scientific, Fair Lawn, NJ) were added to the tube and allowed to predigest overnight. The next day the samples were placed in a microwave oven (MARS 5, CEM, Matthews, NC), ramped for 10 min to 110˚C, maintained at 110˚C by thermowell, and cooled for 20 min before adding 2 ml of hydrogen peroxide (Sigma, St. Louis, MO) to terminate digestion. Tubes were brought up to 25 ml with deionized water.

Fifty microliters of digested sample were combined with 5 ml of lanthium chloride (0.5%, Fisher) and read by atomic absorption.

Pork Quality Measurements

Fluid loss in the LD and SM was evaluated by two separate methods. Surface exudate was determined by a method developed by Kauffman et al. (1986). Briefly, a preweighed filter paper, 4.5 cm (#589, Schleicher and Schuell, Inc., Keene, NH) was placed on the surface of each muscle for 2 s, 20 min after the initial cut. The filter paper was reweighed to determine weight gain of the filter paper associated with extent of surface exudate accumulation.

Display fluid loss was determined on chops designated for 8 d of display storage. The chops from each muscle were removed from the tray on d 2, 4, 6, and 8, placed on a paper towel for 5 seconds, and reweighed to determine display fluid loss. Display fluid loss was reported as the weight loss of displayed chops divided by the initial weight of the chop prior to storage multiplied by 100. Each chop was returned to its original tray, rewrapped, and returned to display storage for subsequent measurements.

Purge loss was identified as the amount of fluid lost from the LD muscle after 25 or 50 d of vacuum-packed storage. After the appropriate storage period, each muscle was removed from the vacuum-packed bag, blotted with a paper towel, and reweighed. Purge loss was reported as the weight loss during storage divided by the initial muscle weight multiplied by 100.

Color of the LD and SM was objectively evaluated by Minolta L*, a*, and b* measurements using a Minolta Chroma Meter (CR-200, Ramsey, New Jersey) calibrated

with a standard white plate. Minolta values were reported as the average color values collected at 4 positions in a diamond pattern on the surface of each chop. The initial measurement of color was performed after 45 min of the initial cut. Additionally, color was determined every 2 d for 8 d of display storage. Color of vacuum-packed LD was determined on an interior chop after a 45 min bloom period for the 25 and 50 d vacuum-packed storage periods.

Longissimus and SM chops that had been displayed for 4 or 8 d and the LD vacuum-packed loin sections for 25 or 50 d were vacuum-packed in Cryovac bags (B2651T) and stored at -20°C until oxidation was determined by thiobarbituric acid reactive substances (TBARS) as described by Witte et al. (1970). Briefly, samples were removed from a -20˚C freezer and allowed to thaw overnight at 6˚C. Each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). Four grams of ground muscle were homogenized, in duplicate, with 16 ml of ice cold phosphate buffer (pH = 7.0) prepared to contain 50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 0.1% EDTA (Fisher Scientific, Fair Lawn, NJ), and 0.1% propylgallate (Sigma, St. Louis, MO) in a stainless steel cup for 20 s using an Omni-Mixer (Model 17105, Sorvall Corp., Newtown, CT) at 16,000 rpm. Then, 4 ml of 30% trichloroacetic acid (Sigma) were added and samples were homogenized for an additional 10 s at 16,000 rpm. After the homogenate was filtered (#P8, Fisher Scientific), 2 ml of the clear filtrate and 2 ml of 2-thiobarbituric acid (0.02 M, Sigma) were transferred to 16x125 screw-cap glass test tubes, vortexed, and heated in a 100˚C water bath for 30 min. Test tubes were allowed to cool in an ice cold water bath for at least 15 min and vortexed prior to measuring absorbance on a

spectrophotometer at 533 nm (Model DU 640, Beckman, Fullerton, CA). The absorbance of samples was compared to tetraethoxypropane (Sigma) standard concentrations of 2, 4, 8, 10, 20, 40, and $80x10^{-7}$ M. Reported TBARS values reflect a correction of percent recovery ranging from 92 to 102%.

Statistical Analyses

Data were analyzed by split-plot design with age as the main plot and Mg supplementation as the subplot using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC).

Halothane and Napole genotypes were identified by DNA tests (GeneSeek Inc., Lincoln, NE) were performed on LD samples. All pigs tested negative for the Halothane mutation. However, the presence of the Napole gene was used as a covariate (0 = normal, 1 = heterozygous, 2 = homozygous). Pigs were blocked by weight within sex and pig was the experimental unit.

Results and Discussion

Feed and Water Disappearance

Access to feed was limited in an attempt to limit the variation of Mg intake from feed. Older, slower growing pigs tended to refuse more feed ($P = 0.07$) than the younger, faster growing pigs (Table 2). Lower feed intake of older pigs likely explains the difference of water disappearance between age groups because water disappearance relative to feed intake did not differ ($P = 0.93$).

Magnesium concentration of water used to dilute the stock solution was 3 mg/L. The final Mg concentration of drinking water was 3 and 903 mg/L for control pigs and pigs

supplemented with 900 mg/L, respectively. Therefore, the maximum mean intake of Mg from drinking water was 0.03 and 8.7 g of Mg/d for pigs receiving water without and with Mg supplementation, respectively. The water requirement of pigs has been estimated to be 2 L of water per 1 kg of feed (Cumby, 1986) compared to our reported 4.2 L of water disappearance per kg of feed. Assuming this water to feed ratio, mean Mg intake from drinking water would have been approximately 0.01 and 4.4 g of Mg/d for non-supplemented and supplemented, respectively. Water Mg intakes of supplemented pigs were likely within the range of 4 to 8.7 g/d.

Age by Magnesium Interactions

Older pigs supplemented with Mg had a lower pH of the LD ($P < 0.05$) at 24 h post-mortem than younger pigs supplemented with Mg. Similarly, older pigs consuming Mg had a lower pH of the SM ($P < 0.06$) at 24 h post-mortem than older pigs not consuming supplemental Mg. There is no clear explanation for this interaction. However, no other interactions between Mg and age were found and, therefore, this observation may not be biologically significant.

Effect of Age at Harvest

Pigs classified as 150 or 180 d of age differed in age ($P < 0.0001$) by 27±0.4 d, 153 vs. 180 days of age, respectively (Table 3). However, live BW did not differ on the day of harvest ($P = 0.13$). Therefore, younger pigs grew 96±5 g of BW/d faster than older pigs ($P < 0.0001$). Dressing percent was not affected by age ($P = 0.99$). Therefore, our goal of obtaining pigs that were raised in similar conditions and differ by age at a similar bodyweight was accomplished.

The pH of the LD at 45 min post-mortem was greater ($P < 0.05$) for older, slower growing pigs than younger, faster growing pigs indicating a slower rate of pH decline during early post-mortem processes. However, the extent of pH decline in the LD ($P = 0.48$) or SM ($P = 0.62$) was not affected by age because the pH at 24 h post-mortem was not different between age groups. These data are in agreement with results reported by Lonergan et al. (2001) who observed Duroc pigs not selected for rate of lean gain had greater pH of the LD at 15, 30, and 45 min but not 24 h post-mortem than Duroc pigs selected for a greater rate of lean gain. However, in the same study a muscle similar to SM, the Semitendinosus, had a greater pH at both 15 min and 24 h post-mortem when rate of lean gain of Duroc pigs was lower. Huff-Lonergan et al. (1997) found similar results in the Semitendinosus from the previous 2 generations of selection of Durocs in the same line. Oksbjerg et al. (2000) reported no difference in pH of the LD at 45 min or 24 h post-mortem when slow growing pigs were compared to fast growing Landrace pigs. Furthermore, Goerl et al. (1995) reported no difference in pH when a fast growing Hampshire line was compared to a 14 breed composition line selected for reproductive traits.

Initial exudate of the LD was not affected ($P = 0.22$) by age of pig at harvest (Table 4). However, initial exudate of the SM was reduced ($P \leq 0.05$) by 18% as age increased from 153 to 180. Display fluid loss of the LD and SM was not affected on any days measured ($P > 0.10$) by age of the pig (Table 5). The reduction of surface exudate of the SM contradicts the absence of an effect on display fluid loss. These estimates of fluid loss were determined by different methods. Kauffman et al. (1986) demonstrated a strong,

positive correlation between surface exudate determined with filter paper and fluid loss during storage. However, in the present study correlations between surface exudate and fluid loss after 8 d of display storage of the LD and SM were consistent across muscles but low, $r^2 = 0.41$ and $r^2 = 0.37$, respectively. Nevertheless, the fluid loss during display has more practical significance than surface exudate. The coefficient of variation associated with display fluid loss was higher than surface exudates measurements, 13 vs. 10%, respectively. Therefore, the measurement of surface exudates was able to detect a smaller difference than the display fluid loss method.

Water-holding capacity of the LD has been reported to be lower for pigs selected for growth performance and lean gain efficiency (Goerl et al. 1995; Huff-Lonergan et al., 1997). Furthermore, drip loss was higher in the LD, SM, and Semitendinosus from Duroc pigs with a faster rate of lean gain, which was most noticeable storage of more than 2 d (Lonergan et al., 2001). Conversely, faster growing Landrace pigs did not have higher drip loss from the LD than slower growing Landrace pigs (Oksbjerg et al., 2000), which is in agreement with results from the present study.

Initial lightness (Minolta L*) of the LD was not affected by pig age ($P = 0.30$, Table 6). However, the LD from older pigs was darker after 4 ($P < 0.01$) and 8 d ($P < 0.06$) of storage than younger pigs. Neither redness nor yellowness of the LD was affected by age at any time period measured. Although age did not affect yellowness of the SM, the SM from older pigs were darker ($P < 0.05$) after 2, 6, and 8 d and tended ($P < 0.10$) to be darker initially and after 4 d of display storage than younger pigs (Table 6). Additionally, the SM from older pigs tended to be redder ($P < 0.10$), as measured by Minolta a*, after 4 and 8 d

of storage than younger pigs (Table 7). Yellowness was not affected by age of pig (Table 8, $P > 0.10$).

The reduction in lightness associated with increased age observed in the present study was also reported by Oksbjerg et al. (2000). Huff-Lonergan et al. (1997) reported lower lightness (Hunter L), but not redness or yellowness (Hunter a or b), of the LD from pigs selected for high rates of lean gain and efficiency of gain. Lonergan et al. (2001) reported no difference in Hunter L, a, or b when the fifth generation of the same Duroc line was compared to previous generations. However, Goerl et al. (1995) reported higher Hunter L, a, and b when a fast growing Hampshire line was compared to a slow growing 14 breed composite line. In that study the incidence of the Napole gene was not identified and may have been higher in the Hampshire line than the composite line. The meat from Hampshire pigs in that study could be referred to as red, soft, and exudative (RSE) if the color and fluid loss mentioned above are considered.

A possible explanation for the observed difference, primarily in color, is a difference in muscle fiber type and/or size. Type I and II muscle fibers are considered slow and fast-twitch, respectively (Brooke and Kaiser, 1970). Type II fibers can be further designated as IIA and IIB. Type I and IIA fibers are red, have a high myoglobin content and function aerobically, whereas type IIB fibers are white and function anaerobically. A negative correlation between daily BW gain and percentage of type I fibers has been observed (Dietl et al., 1993; Larzul et al., 1997). Conversely, a positive correlation between daily BW gain and percentage of type IIB fibers has been reported (Dietl et al., 1993; Oksbjerg et al., 2000). Indeed, type IIB fibers have been shown to grow at a rate two-fold

higher than Type I or Type IIA fibers (Oksbjerg et al., 1994). Taken together these observations support the theory that pigs with a higher proportion of Type IIB fibers grow faster (Sosnicki, 1987), although, the relationship remains controversial (Larzul et al., 1997).

Genetic correlations indicate an increased percentage of Type IIB (fast-twitch nonoxidative) muscle fibers would likely decrease pH at 30 min and 24 h post-mortem, glycolytic potential, and lightness (Larzul et al., 1997). Furthermore, percentage of type IIB fibers and percentage and cross-section area of Type I (slow-twitch) fibers have been estimated to be highly heritable (Larzul et al., 1997). Oksbjerg et al. (2000) reported a greater proportion of type IIB (fast-twitch) and lower proportion of type I (slow-twitch) fibers in faster growing pigs than slower growing Landrace pigs. These faster growing pigs had lower total pigment concentration, haematin, and myoglobin, which could explain the higher lightness and lower redness that was observed in that study. However, there was no difference in drip or thawing fluid loss between growth rates. Conversely, Lonergan et al. (2001) observed no difference in muscle fiber distribution when comparing Duroc pigs selected for increased rates of lean gain and efficiency for 5 generations compared to previous generations. In that study, color was not affected when the percentage of muscle fiber types within specific muscles were distributed evenly across selection lines, which supports the theory that no change in muscle fiber type results in no subsequent change of muscle pigments and myoglobin concentration or no effect on color. The absence of an effect of selection on muscle type distribution was also observed after 7 generations of

selection for daily food intake, daily BW gain, or efficiency of lean tissue growth (Cameron et al., 1997).

Purge loss and color of the LD stored for 25 or 50 d in vacuum-packed bags were not affected by age of pigs (Table 9, $P > 0.10$). Furthermore, oxidation of the LD or SM during display or LD during vacuum-packed storage was not affected by age (Table 10, $P > 0.10$). Limited information is available in the literature regarding the effects of growth rate on these measurements of pork quality. However, these results indicate that the effect of age and growth rate on pork quality may be limited to fresh and displayed pork.

Effect of Magnesium

Feed intake, water disappearance, nor water disappearance relative to feed intake were affected by Mg supplementation (Table 2). Plasma Mg increased 10.6% with Mg supplementation ($P < 0.06$, Table 3). However, Mg concentration was not affected by Mg supplementation in either the LD or SM (Table 4). This observation is consistent with those reported by Schaefer et al. (1993) and Apple et al. (2001). In the present study, Mg supplementation through drinking water for 2 d prior harvest did not affect pork quality (Tables 3-10).

Data from the present experiment are similar to those of van Laack (2000) who reported a 10% increase in plasma Mg without an effect on pork quality of the LD or SM with Mg supplementation in feed. These data are also in agreement with Apple et al. (2000) that reported no effect on drip loss when pigs were supplemented with Mg mica. Caine et al. (2000) also reported no effect on drip loss or color when pigs were supplemented with Mg aspartate. However, these data contrast those reported by D'Souza et al., (1998, 1999)

who demonstrated a 6.5 and 8.3% increase of plasma Mg with Mg supplementation, which improved color and drip loss. Schaefer et al. (1993) reported halothane carrier pigs supplemented with a reported 2.52 mg /d of Mg as Mg aspartate for 5 d had 14% higher plasma Mg, lower initial loin temperature and increased redness of the LD with no effect on drip loss. However, a second experiment within the same paper comparing pigs supplemented with a reported 5.04 mg/d of Mg from Mg aspartate for 5 d indicated no effect on color or temperature of the loin, but lower drip loss than pigs not supplemented with Mg. Although plasma Mg is a response indicator to validate that administration of dietary or water Mg resulted in ingestion and absorption, it may not be a good predictor of the potential effect of Mg on pork quality in the present study.

Implications

Maximizing growth through genetic selection may negatively impact pork quality, irregardless of genetic mutations known to reduce quality. Furthermore, the quality of pork can be greater from older pigs than younger pigs. Although supplementation through drinking water is a convenient means of providing Mg to market pigs and has shown modest success to improve pork quality in the past, the response to Mg is not consistent. More research is required to establish a consistent response to Mg.

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Table 1. Composition of experimental diets (%, as is)[ab]

Ingredient	Percent of total
Corn, yellow dent	79.07
Soybean meal, 47.5% CP	15.80
Poultry Fat	2.50
Dicalcium phosphate	1.03
Limestone	0.76
Salt	0.35
Vitamin/Mineral Premix[c]	0.25
L-Lys HCl[d]	0.15
Tylan[e]	0.10
Analyzed Composition	
Total Mg	0.13%

[a]Diet was fed for the 7 d adjustment and 2 d experimental periods.

[b]Formulated to contain 14.2% crude protein, 0.80% lysine, 0.52% P, and 0.60% Ca.

[c]Supplied per kg of complete diet: 5,540 IU of vitamin A as retinyl acetate, 1,108 IU of vitamin D_3, 22 IU of vitamin E as dl-α-tocopherol acetate, 1.98 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 165 mg of choline as choline chloride, 22 mg/kg of niacin as niacinamide, 17.6 mg of d-pantothenic acid as dl-calcium pantothenate, 4.4 mg of riboflavin, 1.1 mg of pyridoxine as pyridoxine·HCl, 0.55 mg thiamine as thiamine mononitrate, 0.022 mg of vitamin B_{12}, 0.33 mg of folic acid, 0.04 mg of d-biotin, 110 mg Zn as $ZnSO_4$, 110 mg Fe as $FeSO_4$, 22 mg Cu as $CuSO_4$, 55 mg Mn as MnO, 0.28 mg I as ethylenediamine dihydriodide, and 0.30 mg Se as $NaSeO_3$.

[d]Contained 78.8% lysine.

[e]Provided 22 mg of tylosin/kg of feed.

Table 2. Effect of pig age at harvest and magnesium supplementation through drinking water on feed and water disappearance[a]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Feed intake, kg/d	2.65	2.30	2.51	2.44	0.06	0.07	0.48	0.29
Water disapperance, L/d	11.3	9.4	11.1	9.6	1.0	0.05	0.32	0.96
Water per feed, L/kg	4.2	4.3	4.4	4.1	0.5	0.93	0.68	0.66

[a]Least squares means are reported.

Table 3. Effect of pig age at harvest and magnesium supplementation through drinking water on measurements obtained on the day of harvest and pH measurements at 24 h post-mortem[a]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Age at harvest, d	153	180	167	166	0.3	<0.0001	0.32	0.09
Live bodyweight, kg	108	110	109	108	0.6	0.13	0.44	0.40
Growth rate, g/d	698	602	651	649	5	<0.0001	0.85	0.26
Dressing percent, %	72.7	72.7	72.6	72.9	0.3	0.99	0.55	0.35
Plasma Mg, ppm	23.1	22.8	21.8	24.1	0.8	0.79	0.06	0.95
Longissimus pH, 45 min	6.23	6.37	6.27	6.33	0.05	0.04	0.40	0.80
Longissimus pH, 24h					0.02	0.48	0.79	0.04
153 d of age			5.72[bc]	5.78[b]				
180 d of age			5.75[bc]	5.70[c]				
Semimembranosus pH, 24h					0.02	0.62	0.68	0.04
153 d of age			5.62[bc]	5.69 [bc]				
180 d of age			5.71[b]	5.62 [c]				

[a]Least squares means are reported.
[bc]Means with differing superscripts within a row are different ($P < 0.05$).

Table 4. Effect of pig age at harvest and magnesium supplementation through drinking water on surface exudate of muscles[ab]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Longissimus Mg, ppm[c]	1000	1025	1021	1004	11	0.08	0.29	0.20
Semimebranosus Mg, ppm[c]	1080	1099	1082	1097	11	0.44	0.27	0.28
Exudate, mg								
Longissimus	73	58	61	70	7	0.22	0.38	0.80
Semimembranosus	74	61	68	67	6	0.05	0.96	0.36

[a]Least squares means are reported.
[b]Surface exudate was determined by the filter paper method (Kauffman et al., 1986).
[c]Dry matter basis.

Table 5. Effect of pig age at harvest and magnesium supplementation through drinking water on fluid loss of the Longissimus and Semimembranosus during simulated retail display storage [a]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Agex Mg
Longissimus display fluid loss, %								
Days of storage								
2 d	2.92	2.44	2.47	2.89	0.46	0.49	0.56	0.61
4 d	4.16	3.65	3.64	4.17	0.52	0.51	0.49	0.83
6 d	4.85	4.41	4.45	4.82	0.59	0.60	0.68	0.86
8 d	5.36	4.95	4.94	5.37	0.59	0.64	0.63	0.97
Semimembranosus display fluid loss, %								
Days of storage								
2 d	4.18	3.56	3.78	3.96	0.36	0.36	0.74	0.45
4 d	5.94	5.02	5.4	5.56	0.41	0.20	0.80	0.48
6 d	7.13	6.06	6.62	6.56	0.46	0.15	0.93	0.39
8 d	7.96	6.88	7.50	7.34	0.49	0.17	0.83	0.37

[a]Least squares means are reported.

Table 6. Effect of pig age at harvest and magnesium supplementation through drinking water on Minolta L*(lightness) values of muscles during retail storage [a]

	Age at harvest, d		Magnesium			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Days of storage								
Longissimus								
0 d	50.5	49.6	49.8	50.3	0.8	0.30	0.68	0.48
2 d	52.2	51.6	51.5	52.3	0.6	0.45	0.40	0.17
4 d	53.7	51.9	52.7	53.0	0.7	0.01	0.77	0.92
6 d	54.8	53.5	53.8	54.5	0.8	0.11	0.52	0.88
8 d	55.6	54.4	54.9	55.1	0.6	0.06	0. 86	0.87
Semimembranosus								
0 d	49.5	48.0	48.3	49.2	0.5	0.09	0.25	0.13
2 d	51.1	49.7	50.32	50.5	0.4	0.04	0.76	0.74
4 d	51.6	50.5	50.83	51.4	0.4	0.09	0.38	0.72
6 d	54.8	53.5	53.8	54.5	0.7	0.02	0.30	0.05
8 d	56.0	54.4	55.1	55.2	0.4	0.02	0.78	0.29

[a]Least squares means are reported.

Table 7. Effect of pig age at harvest and magnesium supplementation through drinking water on Minolta a*(redness) values of muscles during retail storage [a]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Days of storage								
Longissimus								
0 d	7.56	7.25	7.20	7.61	0.28	0.40	0.34	0.72
2 d	8.68	8.44	8.36	8.76	0.30	0.43	0.39	0.96
4 d	8.48	8.44	8.13	8.79	0.32	0.89	0.18	0.83
6 d	8.19	8.03	7.74	8.48	0.22	0.58	0.04	0.78
8 d	7.99	7.65	7.60	8.05	0.24	0.36	0.23	0.84
Semimembranosus								
0 d	8.31	8.52	8.33	8.50	0.32	0.61	0.73	0.30
2 d	9.68	10.01	9.69	10.00	0.40	0.46	0.60	0.52
4 d	9.22	9.66	9.26	9.63	0.34	0.09	0.47	0.59
6 d	8.77	9.00	8.85	8.92	0.37	0.24	0.90	0.55
8 d	8.84	9.43	8.94	9.32	0.37	0.09	0.50	0.61

[a]Least squares means are reported.

Table 8. Effect of pig age at harvest and magnesium supplementation through drinking water on Minolta b*(yellowness) values of muscles during retail storage [a]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Days of storage								
Longissimus								
0 d	8.16	4.62	4.71	5.07	0.27	0.22	0.39	0.82
2 d	7.94	7.58	7.58	7.94	0.21	0.15	0.27	0.68
4 d	8.30	7.76	7.79	8.27	0.26	0.08	0.23	0.97
6 d	8.52	7.99	7.93	8.59	0.22	0.18	0.06	0.58
8 d	8.83	8.20	8.37	8.67	0.24	0.11	0.42	0.52
Semimembranosus								
0 d	5.42	5.16	5.16	5.42	0.26	0.57	0.52	0.16
2 d	9.17	8.97	9.02	9.13	0.28	0.53	0.79	0.45
4 d	8.30	7.76	7.79	8.27	0.26	0.91	0.66	0.32
6 d	8.52	7.99	7.93	8.59	0.22	0.15	0.68	0.10
8 d	10.84	10.71	10.80	10.75	0.29	0.68	0.90	0.24

[a]Least squares means are reported.

Table 9. Effect of pig age at harvest and magnesium supplementation through drinking water on purge loss and color values of vacuum-packed Longissimus muscles [a]

	Age at harvest, d		Magnesium, mg/L			P values		
Criteria	153	180	0	900	SEM	Age	Mg	Age x Mg
Longissimus purge loss, %								
Days of storage								
25 d	8.71	8.88	8.48	9.12	0.70	0.89	0.55	0.71
50 d	9.08	8.44	8.55	8.98	0.73	0.58	0.52	0.69
Minolta color								
25 d of storage								
L*	53.5	53.3	52.8	54.0	0.60	0.87	0.24	0.73
a*	9.41	9.45	9.28	9.58	0.29	0.92	0.50	0.84
b*	8.43	8.37	8.22	8.58	0.21	0.88	0.26	0.89
50 d of storage								
L*	54.4	53.7	53.6	54.4	0.43	0.34	0.23	0.23
a*	9.49	9.67	9.34	9.82	0.22	0.57	0.16	0.71
b*	8.58	8.26	8.29	8.55	0.12	0.10	0.14	0.51

[a]Least squares means are reported.

Table 10. Effect of pig age at harvest and magnesium supplementation through drinking water on lipid oxidation of retail and vacuum-packed storage [a]

	Age at harvest, d		Magnesium, mg/L			P values		
Days of Storage	153	180	0	900	SEM	Growth	Mg	Growth x Mg
Longissimus	**TBARS[b], μg maldonaldehyde/kg of muscle**							
0 d	137	133	133	137	3	0.46	0.43	0.61
4 d	239	219	227	231	11	0.20	0.80	0.21
8 d	304	282	290	296	11	0.12	0.72	0.20
25 d	213	226	220	219	13	0.35	0.97	0.69
50 d	299	255	277	278	14	0.10	0.97	0.54
Semimembranosus								
0 d	150	147	146	151	4	0.63	0.42	0.66
4 d	219	197	198	218	14	0.15	0.37	0.38
8 d	374	356	355	375	27	0.42	0.61	0.29

[a]Least squares means are reported.
[b]Thiobarbituric acid reactive substances (TBARS) is a measurement of the extent of oxidation.

CHAPTER 4

Pork quality of pigs with the Halothane and/or Rendement Napole mutation supplemented with magnesium through drinking water[1,2]

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[2]Funded in part by the North Carolina Pork Council and the North Carolina Agricultural Research Service, and the North Carolina Agricultural Foundation.

ABSTRACT

Sixty-four pigs (117±0.7 kg BW) representing 1) normal (NN/rn^+rn^+), 2) Rendement Napole (RN) carriers (NN/rn^+RN^-), 3) Halothane carriers (Nn/rn^+rn^+), and 4) carriers of both mutations (Nn/rn^+RN^-) in a factorial arrangement were individually penned and provided ad libitum access to feed (0.13% Mg) and water. Pigs were allotted according to genotype, sex and weight to receive 900 mg of Mg/L of drinking water from $MgSO_4$ for 0 or 2 d prior to harvest. Longissimus dorsi (LD) and Semimembranosus (SM) chops were placed on trays, wrapped, and stored at 4ºC to simulate display storage for 8 d. The posterior LD was split, vacuum packed, and stored at 4°C for 25 or 45 d. Pigs with the RN mutation ($N\_\_/rn^+RN^-$) had lower ($P < 0.05$) pH of the LD and SM at 24 h post-mortem, lower ($P < 0.05$) color scores, and shear force and higher surface exudate, display fluid loss, purge loss, paleness, yellowness, shear force of the LD, drip loss and oxidation of the LD and SM, and initial redness of the SM compared to pigs without the RN mutation ($N\_\_/rn^+rn^+$). Pigs with the Halothane mutation ($Nn/rn^+\_\_$) had higher ($P < 0.05$) paleness, yellowness, display fluid loss, cooking loss, and shear force of the LD than pigs without the Halothane mutation ($NN/rn^+\_\_$). Interactions ($P < 0.05$) between genetic mutations included display fluid loss, yellowness, and redness of the SM. In most instances, pigs with both mutations negatively influenced quality characteristics to a greater extent than either mutation alone. Although Mg supplementation reduced ($P < 0.05$) drip loss of the LD, display fluid loss of the LD was not significantly affected by Mg supplementation. Pigs with the Halothane mutation (Nn/rn^+rn^+) supplemented with Mg had greater ($P < 0.05$)

display fluid loss of the SM than the same genotype not supplemented with Mg. Furthermore, Mg supplementation decreased ($P < 0.05$) paleness of the SM in normal pigs ($NN/rn^{+}rn^{+}$) but increased ($P < 0.05$) paleness of the SM of pigs with the Halothane in the absence of the RN mutation ($Nn/rn^{+}rn^{+}$). The RN mutation had the most significant negative effect on pork quality. The Halothane mutation reduced pork quality to a lesser extent, which supports the theory of N allele dominance. The presence of both mutations reduced pork quality. Magnesium did not negate the negative effects of either genetic mutation and exacerbated the effects of pigs with the Halothane mutation.

Keywords: Rendement Napole, Halothane, Magnesium

Introduction

Inconsistent product quality within the pork industry has become a reason for concern over the past few decades. Estimates indicate that the economic loss by pork producers due to pale, soft, and exudative pork is approximately $0.79 per pig, resulting in an estimated $70 million loss of revenue for the pork industry (Sonka et al., 1994).

Genetic potential for pork quality is a primary determinate of final quality. Two know mutations, namely Halothane and Rendement Napole, have been reported to negatively influence pork quality (Hamilton et al., 2000). Few strategies have been successful to diminish the inherit quality problems of pigs with these mutations. Selective breeding to reduce the incidence of the mutations is an option. However, this strategy is lengthy and may be costly.

Short-term supplemental dietary magnesium has been reported to decrease fluid loss (D'Souza et al., 1998, 1999, 2000) and improve color (D'Souza et al., 1998, 2000) of pork. Furthermore, dietary Mg may affect lipid oxidation of stored pork (Apple et al., 2001). The effect of Mg on pork quality does not seem to be dependent on Mg source between Mg aspartate, sulfate, or chloride (D'Souza et al., 1999). However, the response may or may not be dependent on timing of supplementation (D'Souza et al., 2000; Hamilton et al., 2002). However, others have not been able to demonstrate a positive or consistent effect of Mg on pork quality (van Laack, 2000; Hamilton et al., 2002). The reason for the discrepancy in results between studies are not clear, but may in part be

attributed to differences in genotypes used. Thus, Mg supplementation could potentially provide a strategy to improve pork quality of pigs with low genetic potential for pork quality.

Therefore, the objective of this study was to determine the effect of two genetic mutations and Mg supplementation through drinking water on pork quality.

Materials and Methods

Animals and Treatments

All animal procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Twenty-four sows from the North Carolina State University (NCSU) Swine Educational Unit were identified for the Halothane and Rendement Napole (RN) mutation by DNA tests (GeneSeek, Inc., Lincoln, NE). After genetic typing, sows were artificially inseminated with semen from boars previously tested for each gene. The breeding scheme was designed to obtain pigs with the following genotypes: 1) homozygous normal for both genes ($NN/rn^{+}rn^{+}$), 2) heterozygous carrier for Halothane mutation, homozygous normal for the RN mutation ($Nn/rn^{+}rn^{+}$) 3) homozygous normal for Halothane, heterozygous carrier for the RN mutation ($NN/rn^{+}RN^{-}$), and 4) heterozygous carrier for both mutations ($Nn/rn^{+}RN^{-}$). Briefly, sows were identified as normal for both genes or carriers for the RN mutation. Sows normal for both genes ($NN/rn^{+}rn^{+}$) were bred to either a Hampshire boar homozygous normal for both genes ($NN/rn^{+}rn^{+}$), a Pietrain boar homozygous positive for the Halothane mutation ($NN/RN^{-}RN^{-}$

), Hampshire boar heterozygous for the RN mutation ($NN/rn^{+}RN^{-}$), or a Hampshire boar homozygous positive for both genetic mutations ($nn/RN^{-}RN^{-}$) to produce genotypes 1, 2, 3, and 4, respectively. Sows heterozygous for the RN mutation (NN/rn^{+} RN^{-}) were bred to a Hampshire boar heterozygous for the RN mutation and their offspring was DNA tested to obtain the proper genotypes.

All pigs were born within a 12 d period and raised under the same conditions and management scheme from birth to selection for experimental evaluation of pork quality.

A total of 64 pigs (40 barrows and 24 gilts) were selected based on genotype, sex, and BW for pork quality evaluation, placed into 2.03 m by 0.74 m individual pens and provided with free access to water via a nipple waterer. Two replications were performed (32 pigs/replicate study). Pigs were provided feed (Table 1, 0.13% Mg) ad libitum for the 2 d experimental period prior to harvest. Pigs were allotted by BW and sex within genotype to water supplemented with 900 mg of Mg/L as Mg sulfate heptahydrate (9.8% Mg, Giles Chemical Corp., Waynesville, NC) of drinking water for 0 or 2 d prior to harvest. Plastic water containers (23 L capacity) were filled daily with 15 L of water containing appropriate Mg concentrations. Water containers were suspended from the ceiling and gravimetrically (approximately 600 ml/min) emptied into a galvanized pipe leading to a nipple waterer. Daily water disappearance volumes were determined by weight.

Harvest Data Collection

On the day of harvest, pigs were loaded (0900) and transported 110 km (1 h 50 min) to a commercial abattoir. Pigs were unloaded by abattoir personal. After 6 or 8 h of lairage (replicate study 1 and 2, respectively), pigs were moved by block 40 m to the stunning area.

Pigs were electrically stunned and blood was collected during exsanguination for serum Mg by atomic absorption and cortisol concentration. Hot carcass weights were collected prior to refrigeration to determine dressing percent. The temperature and pH of the loin were measured between the 10th and 11th rib at 60 min post-mortem using an Argus Sentron (Gig Harbor, WA) pH meter.

Fabrication and Storage

After 20 h of chilling at 2˚C the entire right side loin and ham were removed and transported 110 km to a NCSU meat fabrication facility for further processing. A total of 3 chops (2.54 cm thick) of the Longissimus (LD) muscle were obtained beginning at the 7th and 8th rib interface and extending posterior. The first Longissimus chop was placed in a plastic bag and stored at -20˚C for dry matter and tissue Mg determination. The second chop posterior to the first was placed on an absorbent pad (Cryovac Sealed Air Corp., Saddle Brook, NY) within a Styrofoam tray (Cryovac Sealed Air Corp.), wrapped with an oxygen permeable film (Cryovac Sealed Air Corp.), and stored at 4˚C in the presence on fluorescent lighting to simulate retail display for 4 d. At the end of the 4 d display storage period the chop were frozen at -20˚C until cooking loss and shear force could be evaluated. The third and final chop were taken immediately posterior to the second chop and stored in a similar environment as the third chop for 8 d of displayed storage. The remaining posterior portion of the LD muscle was split into equal sections, vacuum packed in B2651T Cryovac bags with a Mulitvac machine (Cryovac, Ducan, SC), and stored at 4˚C in the absence of light for 25 or 50 d. The Semimembranosus (SM) muscle was removed from the

ham. Three SM chops (2.54 cm) were obtained and processed similarly to the Longissimus chops.

Plasma and muscle Mg

Plasma and muscle Mg concentration was determined by atomic absorption. Briefly, each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). After two grams of sample were dried at 103˚C overnight, the dried sample was quantitatively transferred to a polypropylene tube (Corning). Ten ml of nitric acid (Fisher Scientific, Fair Lawn, NJ) were added to the tube and allowed to predigest overnight. The next day the samples were placed in a microwave oven (MARS 5, CEM, Matthews, NC), ramped for 10 min to 110˚C, maintained at 110˚C by thermowell, and cooled for 20 min before adding 2 ml of hydrogen peroxide (Sigma, St. Louis, MO) to terminate digestion. Tubes were brought up to 25 ml with deionized water. Fifty microliters of digested sample were combined with 5 ml of lanthium chloride (0.5%, Fisher) and read by atomic absorption.

Pork Quality Measurements

Fluid loss in the LD and SM was evaluated by two separate methods. Surface exudate was determined in chops designated for 8 d of display storage by a method developed by Kauffman et al. (1986). Briefly, a preweighed 4.5 cm filter paper (#589, Schleicher and Schuell, Inc., Keene, NH) was placed on the surface of each muscle for 2 s twenty min after the initial cut. The filter paper was reweighed to determine weight gain of the filter paper associated with extent of surface exudate accumulation.

Display fluid loss was determined on chops designated for 8 d of display storage. The chops from each muscle were removed from the tray on d 2, 4, 6, and 8, placed on a paper towel for 5 seconds, and reweighed to determine display fluid loss. Display fluid loss was reported as the weight loss of displayed chops divided by the initial weight of the chop prior to storage multiplied by 100. Each chop was returned to its original tray, rewrapped, and returned to display storage for subsequent measurements.

Purge loss was identified as the amount of fluid lost from the Longissimus muscle after 25 or 50 d of vacuum-packed storage. After the appropriate storage period, each muscle was removed from the vacuum-packed bag, blotted with a paper towel, and reweighed. Purge loss was reported as the weight loss during storage divided by the initial muscle weight multiplied by 100.

Subjective color scores (NPPC, 1999) were evaluated on the LD chop designated for 8 d of display storage. Color of the LD and SM was objectively evaluated by Minolta L*, a*, and b* measurements using a Minolta Chroma Meter (CR-200, Osaka, Japan) calibrated with a standard white plate. Minolta values were reported as the average color values collected at 4 positions in a diamond pattern on the surface of each chop. All color measurements were conducted on chops designated for 8 d of display storage. The initial measurement of color was performed after 45 min of the initial cut. Additionally, color was determined every 2 d for a total of 8 d of display storage. Color of vacuum-packed LD was determined on an interior chop after a 45 min bloom period.

Longissimus and SM chops displayed for 0 or 8 d and the LD sections vacuum-packed for 25 or 45 d were analyzed for oxidation by the thiobarbituric acid reactive

substances (TBARS) method as described by Witte et al. (1970). Briefly, samples were removed from -20˚C and allowed to thaw overnight at 6˚C. Each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). Four grams of ground muscle were homogenized, in duplicate, with 16 ml of ice cold phosphate buffer (pH = 7.0) prepared to contain 50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 0.1% EDTA (Fisher Scientific, Fair Lawn, NJ) and 0.1% propylgallate (Sigma, St. Louis, MO) in a stainless steel cup for 20 s using an Omni-Mixer (Model 17105, Sorvall Corp., Newtown, CT) at 16,000 rpm. Then, 4 ml of 30% trichloroacetic acid (Sigma) was added and samples were homogenized for an additional 10 s at 16,000 rpm. After the homogenate was filtered (#P8, Fisher Scientific), 2 ml of the clear filtrate and 2 ml of 2-thiobarbituric acid (0.02 M, Sigma) was transferred to a 16x125 screw-cap glass test tubes, vortexed, and heated in a 100˚C water bath for 30 min. Test tubes were allowed to cool in an ice cold water bath for at least 15 min and vortexed prior to measuring absorbance on a spectrophotometer at 533 nm (Model DU 640, Beckman, Fullerton, CA). The absorbance of samples was compared to tetraethoxypropane (Sigma) standard concentrations of 2, 4, 8, 10, 20, 40, and $80x10^{-7}$ M. Reported TBARS values reflect a correction of percent recovery.

Shear force was performed on LD chops displayed for 4 d. Chops were allowed to thaw for 24 h 35˚C. Once thawed, chops were placed on a Faberware open hearth grill (Walter Kidde Co., Bronx, NY) until the internal temperature reached 35˚C at which time they were flipped once and allowed to reach an internal temperature of 70˚C. After cooking chops were placed in ziploc bags and chilled to 4˚C. Chop weight prior to and after cooking

was recorded to calculate cooking loss. Core samples measuring 1.3 cm in diameter were obtained parallel to the muscle fibers. Peak shear force was measured on core sample using an Instron Universal Testing Machine (Instron, Canton, MA) with a Warner-Bratzler attachment with a crosshead speed of 200 mm/min. The shear force was reported as the average of 5-8 cores per chop.

Statistical Analyses

Data were analyzed by split-plot design with genotype as the main plot in a factorial arrangement and Mg supplementation as the subplot using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). Pigs were blocked by bodyweight within sex and the pig was the experimental unit. Significance and tendencies were determined at $P < 0.05$ and $P < 0.10$, respectively.

Results

Live and Harvest Data

Pigs with the RN and Halothane mutation (rn^+RN^-/Nn) consumed more feed ($P < 0.05$) than any other genotype (Table 2). Water disappearance was not significantly affected by genotype or Mg supplementation. Bodyweight at harvest was lower ($P < 0.05$) for pigs without the Halothane mutation ($NN/rn^+\_\_$) supplemented with Mg than with the Halothane mutation ($Nn/rn^+\_\_$) or pigs without the Halothane mutation not supplemented with Mg. Serum cortisol at harvest was higher ($P < 0.05$) in pigs with the RN mutation ($N\_\_/rn^+RN^-$) than pigs without the RN mutation ($N\_\_/rn^+rn^+$) but was not significantly affected by the Halothane mutation or Mg supplementation. Serum Mg at harvest was higher ($P < 0.05$) for normal pigs (NN/rn^+rn^+) supplemented with Mg and pigs with the Halothane but not the

RN mutation (Nn/rn$^+$rn$^+$) not supplemented with Mg than pigs with the RN but not the Halothane mutation (NN/rn$^+$RN$^-$) not supplemented with Mg and pigs with the RN and Halothane mutation (Nn/rn$^+$RN$^-$) not supplemented with Mg (Table 3). Furthermore, normal pigs and pigs with both mutations tended to have higher ($P < 0.10$) serum Mg when supplemented with Mg than the same genotypes not supplemented with Mg.

Post-mortem pH

The pH of the LD at 60 min post-mortem was not significantly affected by Mg supplementation (Table 4). However, pigs with the RN but not the Halothane mutation (NN/rn$^+$RN$^-$) had greater ($P < 0.05$) pH of the LD at 60 min post-mortem than any other genotype. Furthermore, pigs with both mutations (Nn/rn$^+$RN$^-$) had a lower ($P < 0.05$) pH of the LD at 60 min post-mortem than normal pigs (NN/rn$^+$rn$^+$). Neither the Halothane mutation nor Mg supplementation significantly affected the pH of either muscle at 24 h post-mortem. However, the pH of the LD and SM was lower ($P < 0.0001$) at 24 h post-mortem for pigs with the RN mutation (N__/rn$^+$RN$^-$) than pigs without the RN mutation (N__/rn$^+$rn$^+$).

Fluid Loss

Surface exudate (Table 5) of the LD was higher ($P < 0.05$) for pigs with the RN mutation (N__/rn$^+$RN$^-$) than pigs without the RN mutation (N__/rn$^+$rn$^+$). Pigs with both mutations (Nn/rn$^+$RN$^-$) had more surface exudate ($P < 0.05$) of the SM than pigs of any other genotype. Drip loss of the LD was reduced by Mg supplementation ($P < 0.05$) and the absence of the RN mutation ($P < 0.0001$). The presence of the Halothane mutation tended ($P < 0.10$) to increase drip loss from the LD. Magnesium nor the Halothane mutation

significantly affected drip loss of the SM. However, pigs with the RN mutation ($N\_\_/rn^{+}RN^{-}$) had substantially higher drip loss ($P < 0.0001$) from the SM than pigs without the RN mutation ($N\_\_/rn^{+}rn^{+}$).

Display fluid loss of the LD was not significantly affected by Mg supplementation (Table 6). Pigs with the RN ($N\_\_/rn^{+}RN^{-}$) or Halothane ($Nn/rn^{+}\_\_$) mutation had greater ($P < 0.05$) display fluid loss of the LD than pigs without the RN ($N\_\_/rn^{+}rn^{+}$) or Halothane ($NN/rn^{+}\_\_$) mutation, respectively, after each storage period with the exception of 4 d of storage. After 4 d of display storage, pigs with the RN mutation in the absence of the Halothane mutation ($NN/rn^{+}RN^{-}$) had greater display fluid loss of the LD than pigs without the RN mutation ($N\_\_/rn^{+}rn^{+}$) and pigs with both mutations had a higher fluid loss of the LD than any other genotype.

Fluid loss of the SM measured after 2 d of displayed storage was higher ($P < 0.05$) for pigs with the RN ($N\_\_/rn^{+}RN^{-}$) or Halothane ($Nn/rn^{+}\_\_$) mutation than pigs without the RN ($N\_\_/rn^{+}rn^{+}$) or Halothane ($NN/rn^{+}\_\_$) mutation, respectively (Table 7). Pigs with the RN but not the Halothane mutation ($NN/rn^{+}RN^{-}$) had a higher ($P < 0.05$) fluid loss of the SM than pigs without the RN mutation ($N\_\_/rn^{+}rn^{+}$) and pigs with both mutations ($Nn/rn^{+}RN^{-}$) had a higher ($P < 0.05$) fluid loss of the SM than any other genotype after 4, 6, and 8 d of display storage. However, Mg supplementation increased ($P < 0.05$) display fluid loss of the SM from pigs with the Halothane mutation ($Nn/rn^{+}\_\_$) but not from pigs without the Halothane mutation after 2, 6, and 8 d of display storage (Table 8).

Purge loss of the LD was not significantly affected by the Halothane mutation or Mg supplementation after 25 d of vacuum-packed storage (Table 9). However, pigs with

the RN mutation (N__/rn$^+$RN$^-$) had higher (P < 0.001) purge loss than pigs without the RN mutation (N__/rn$^+$rn$^+$). Pigs with both mutations (Nn/rn$^+$RN$^-$) had higher ($P < 0.05$) purge loss than any other genotype after 45 d of vacuum-packed storage.

Color

Subjective color scores (Table 10) were negatively affected ($P < 0.05$) by the presence of the RN or Halothane mutations compared to pigs without the RN or Halothane mutation, respectively. Likewise, lightness (Minolta L*) was higher ($P < 0.01$) from pigs with the RN (N__/rn$^+$RN$^-$) or Halothane (Nn/rn$^+$__) mutation compared to pigs without the RN (N__/rn$^+$rn$^+$) or Halothane (NN/rn$^+$__) mutation, respectively, at each time period of display storage. Lightness of the LD after vacuum-packed storage showed similar results on d 25 ($P < 0.01$) and d 45 ($P < 0.07$)

Pigs with the Halothane mutation (Nn/rn$^+$__) supplemented with Mg had lower ($P < 0.05$) LD color scores and higher lightness values of the LD after 2 d of display storage than pigs without the Halothane mutation (NN/rn$^+$__) regardless of Mg supplementation (Table 11). Magnesium reduced lightness ($P < 0.05$) of the LD of pigs without the Halothane mutation (NN/rn$^+$__) after 4 d of display and after 25 d of vacuum-packed storage.

Lightness of the SM during display storage was consistently higher ($P < 0.05$) for pigs with both mutations (Nn/rn$^+$RN$^-$) than any other genotype (Table 12). Magnesium supplementation reduced lightness of the SM for each storage period after the initial measurement at d-0 for normal pigs (NN/rn$^+$rn$^+$). However, Mg supplementation increased lightness of the SM for pigs with the Halothane but not the RN mutation (Nn/rn$^+$rn$^+$) at each measurement period during display storage.

Initial redness (Minolta a*, Table 13) of the LD was not significantly affected by the Halothane mutation within pigs that did not have the RN mutation (N__/rn$^+$RN$^-$). The LD from pigs with the Halothane and RN mutation (Nn/rn$^+$RN$^-$) were initially more red ($P <$ 0.05) than the LD from pigs with the Halothane but not the RN mutation (Nn/rn$^+$rn$^+$). However, by the 8th d of display storage the LD from pigs with the RN mutation (N__/rn$^+$RN$^-$) had become less red ($P < 0.05$) than the LD from pigs without the RN mutation (N__/rn$^+$rn$^+$). Redness of the LD after 25 d of vacuum-packed storage was higher ($P < 0.05$) from pigs with the RN mutation (N__/rn$^+$RN$^-$) than pigs without the RN mutation (N__/rn$^+$rn$^+$). Furthermore, redness of the LD after 45 d of vacuum-packed storage was higher ($P < 0.05$) for pigs with both mutations (Nn/ rn$^+$RN$^-$) than pigs with the RN but not the Halothane mutation (NN/rn$^+$RN$^-$) or pigs with the Halothane but not the RN mutation (Nn/rn$^+$rn$^+$) and was lowest ($P < 0.05$) for pigs with the Halothane but not the RN mutation (Nn/rn$^+$rn$^+$).

Initial redness of the SM was lowest ($P < 0.05$) for pigs with the Halothane but not the RN mutation (Nn/rn$^+$rn$^+$) and highest for pigs with both mutations (Nn/rn$^+$RN$^-$) (Table 14). The SM of pigs with the Halothane but not the RN mutation (Nn/rn$^+$rn$^+$) was the least red ($P < 0.05$) after 2 d of display storage. Magnesium supplementation had no significant effect on the SM redness.

Initial yellowness of the LD was higher ($P < 0.05$) for pigs with either mutation and was highest ($P < 0.05$) for pigs with both mutations (Table 15). Each mutation alone increased ($P < 0.01$) yellowness of the LD after d-2, 4, and 6 of display storage. The RN mutation increased ($P < 0.001$) yellowness of the LD after 8 d of display and 25 d of

vacuum-packed storage. Yellowness of the LD vacuum-packed for 45 d was lower ($P < 0.05$) for pigs with the Halothane but not the RN mutation ($Nn/rn^{+}rn^{+}$) than pigs without the Halothane mutation ($NN/rn^{+}\_\_$) and pigs with both mutations ($Nn/rn^{+}RN^{-}$) had a higher ($P < 0.05$) yellowness value than any other genotype. Pigs with the Halothane but not the RN mutation ($Nn/rn^{+}rn^{+}$) consistently had a lower ($P < 0.05$) and pigs with both mutations ($Nn/rn^{+}RN^{-}$) had a higher ($P < 0.05$) yellowness values of the SM than any other genotype (Table 16). Furthermore, pigs with the RN but not the Halothane mutation ($NN/rn^{+}RN^{-}$) had higher yellowness values than normal pigs ($NN/rn^{+}rn^{+}$). Pigs with the Halothane mutation supplemented with Mg had higher yellowness values than pigs without the Halothane mutation supplemented with Mg (Table 17).

Cooking Loss, Shear Force, and Oxidation

Cooking loss was not significantly affected by the RN mutation or Mg supplementation (Table 18). However, pigs with the Halothane mutation ($Nn/rn^{+}\_\_$) had a higher ($P < 0.05$) percentage of cooking loss than pigs without the Halothane mutation ($NN/rn^{+}\_\_$). The shear force was higher ($P < 0.05$) for pigs with the Halothane mutation and lower ($P < 0.05$) for pigs with the RN mutation than pigs without the Halothane or RN mutation, respectively. Oxidation of the LD after 8 d the SM after 0 and 8 d of displayed storage and the LD after 25 and 45 d of vacuum-packed storage was higher ($P < 0.05$) for pigs with the RN mutation ($N\_\_/rn^{+}RN^{-}$) than pigs without the RN mutation ($N\_\_/rn^{+}rn^{+}$) (Table 19).

Discussion

Halothane Effect

Although the genetic mutations of interest for this study are associated with different physiological events, their negative effects on pork quality are similar. The Halothane mutation reduces the ability of the sacroplamic reticulum to sequester calcium leading to increased muscle contractions and has been reported to increase the rate of post-mortem pH decline of the LD (Lahucky et al., 1997; Fisher et al., 2000; Maddock et al., 2002). In the present study the Halothane mutation alone did not affect pH of the LD at 60 min post-mortem suggesting the rate of pH decline did not differ from that of pigs without the Halothane mutation. This result contrasts those reported by Lahucky et al. (1997) and Monin et al. (1999) who indicated an increased rate of pH decline. The ultimate pH (24 h post-mortem) was not affected by the Halothane mutation in the present study agreeing with results from Pommier et al. (1998) and Tam et al. (1998) who reported no effect on ultimate pH. Furthermore, the latter paper indicated a high correlation between the Halothane mutation and pH at 45 min but not ultimate pH. However, these results contrast those reported by Leach et al. (1996) and Hamilton et al. (2000), which reported a lower ultimate pH for pigs with the Halothane mutation.

Although the pH of the SM during the early post-mortem period was not determined in the present study, Kerth et al. (2001) reported the pH of the SM of pigs with the Halothane mutation was lower than non-carriers from 1 to 4 h post-mortem. Furthermore, Le Roy et al. (1999) reported that pigs with a single Halothane mutation had a lower pH of the SM at 35 min post-mortem compared to pigs without the mutation. However, the pH of

the Semispinalis capitis, a muscle similar to the SM, at 35 min was not affected by a single Halothane mutation in that study. Given that the pH of the SM tended to be higher at 24 h post-mortem for pigs with the halothane mutation it is unlikely that the rate of pH decline was affected in the present study.

A single Halothane mutation alone did not affect initial fluid loss measurements including drip loss or surface exudate of the LD or SM. In the past, research addressing the Halothane mutation has used the drip loss method to evaluate fluid loss and some have reported an increase in drip loss from the Longissimus (Lahucky et al., 1997; Hamilton et al., 2000) but others did not observed any affect of drip loss (Tam et al., 1998) or thaw loss (Monin et al., 1999) of pigs with a single Halothane mutation. Results obtained from the LD and SM during display storage in the current study indicates a Halothane mutation alone may not have a significant effect on fluid loss stored in retail conditions.

Perhaps the most dramatic effect of the Halothane mutation was on subjective color scores and lightness of the LD. Color scores were negatively affected supporting results of Leach et al. (1996), Hamilton et al. (2000), and Maddock et al. (2002) who reported a reduction in subjective color scores. Furthermore, the LD from pigs with the Halothane mutation were less dark than pigs without the Halothane mutation after each display period and 25 d of vacuum-packed storage, which is supported by previous reports (Leach et al., 1996; Fisher et al., 2000; Hamilton et al., 2000). However, lightness of the SM was not affected by the Halothane mutation and is supported by Tam et al (1998) who evaluated light and heavy weight pigs separately and Kerth et al. (2001) both indicating no difference

in SM lightness but contradicts Maddock et al. (2002) who reported a reduction in lightness of the SM.

Cooking loss was higher for pigs with the Halothane mutation than pigs without the mutation. This observation contrasts reports by Leach et al. (1996), Monin et al. (1999), and Moelich et al. (2003). Most of these reports reported reduced drip loss that could result in more fluid present in the meat at time of cooking. In the present study no such fluid loss occurred prior to cooking indicating more fluid was present and could account for more fluid loss during the cooking process.

Shear force was higher in the present study indicating that tenderness may be lower in pigs with the Halothane mutation compared to pigs without the mutation. These results contrast those reported by previous reports (Leach et al., 1996; Moelich et al. 2003) that indicated no effect of a single Halothane mutation on shear force and Hamilton et al. (2000) who indicated a reduction in shear force with the Halothane mutation. However, results from the present study agree with Murray and Jones (1994), McPhee and Trout (1995), and Brewer et al. (2002).

Results from the present study support the dominance of the N allele for fluid loss of the LD and SM. However, the dominance of the N allele may be trait dependent as suggested by Monin et al. (1999) because color of the LD was affected by a single Halothane mutation. Indeed, pork color has been found to be the pork quality characteristic most highly correlated with the Halothane mutation (Tam et al., 1998) which is supported by results of the present study.

Napole Genotype Effect

The RN mutation of AMP-activated protein kinase results in pigs that have approximately 70% more glycogen in muscle than pigs without the mutation (Milan et al., 2000). Therefore, more substrate is available to fuel post-mortem glycolysis allowing greater accumulation of lactate in muscle and subsequently a greater extent of pH decline (Lundström et al., 1996; Hamilton et al., 2000; Moeller et al., 2003). Indeed in the present study, pigs heterozygous for the RN mutation had a 0.2 and 0.6 reduction of pH at 24 h post-mortem in the LD and SM, respectively. Unexpectedly, pigs with only the RN mutation had a higher pH of the LD at 60 min post-mortem than any other genotype. This observation is difficult to explain. However, Le Roy et al. (1999) found no difference in pH of the LD at 35 min post-mortem between RN genotypes without the Halothane mutation. The rate of pH decline post-mortem has been reported to increase with the RN mutation between 45 min to 5 h post-mortem (Josell et al., 2003). The early post-mortem pH measurement in this study was conducted at 60 min and could have been prior to the increased rate of pH decline post-mortem observed by Jossel et al. (2003).

Initial fluid loss and drip loss of the LD was lower for pigs with the RN mutation and is supported by the majority of previous research (Hamilton et al., 2000; Miller et al., 2000; Moeller et al., 2003). The present results also indicate that fluid loss is higher for pigs with the RN mutation during 8 d of display storage and 25 d of vacuum-packed storage. Furthermore, results indicate the LD from pigs with the RN mutation is paler than that of pigs without the RN mutation agreeing with many previous reports (Hamilton et al., 2000; Moeller et al., 2003). The reduction in color scores associated with the RN mutation is likely a result of the increased paleness and yellowness because redness of the LD was not

affected by a single RN mutation alone. This theory is supported by Hamilton et al. (2000) but not by others (Miller et al., 2000) who reported conflicting results of objective color measurements.

Purge loss after 25 d of vacuum-packed storage was consistently reduced by a single RN mutation alone and is in agreement with results reported by Moeller et al. (2003). Cooking loss was not affected by the RN mutation in the present study, which is consistent with Brewer et al. (2002) and Moeller et al. (2003) but different than others (Lundström et al., 1996; Miller et al., 2000) who reported an increase in cooking loss for pigs with the RN mutation. The RN mutation alone reduced shear force of the LD as reported by many others (Lundström et al., 1996; Hamilton et al., 2000; Josell et al., 2003) but not by Miller et al. (2000) or Moeller et al. (2003) who reported no differences in shear force between pigs with or without the RN mutation.

The RN mutation increased oxidation of the LD and SM during all storage periods with the single exception of the initial oxidation of the LD. The oxidative effect of the RN mutation has not been studied extensively in the past. However, Nilzén et al. (2001) reported that pigs with a single RN mutation raised outdoors in a pasture system had higher oxidation of pork when compared to pigs without the mutation in the same environmental conditions and was related to leanness especially in gilts. Indeed, pigs with the RN mutation had higher omega-3 fatty acids and C22:5 than pigs without the mutation in that study. It is widely accepted that more unsaturated fatty acids in meat increases the potential for oxidation and could explain the results from the present experiment; however fatty acid composition was not confirmed. Although diet greatly affects the fatty acid profile of meat

in pigs, pigs in the present study were provided the same diet removing any effect or dietary manipulation.

Halothane and RN Interactions

Pigs with both mutations had a lower early post-mortem pH than pigs with only one of the mutations indicating that the rate of pH decline of the LD was increased. However, an interactive effect was not observed at 24 h post-mortem indicating the total extent of pH decline was additive in each muscle. Indeed, Hamilton et al. (2000) reported no interaction between these two mutations on the ultimate pH of the Longissimus. Although pH of the SM was not measured during the early post-mortem period, Le Roy et al. (1999) indicated that the SM but not the LD from pigs with both the Halothane and RN mutation had a lower pH at 35 min post-mortem than any other genotype represented in the present study.

No interactions with Halothane and RN mutations were observed for surface exudate or drip loss of the LD and only one was observed during display storage of the LD. Similarly, Hamilton et al. (2000) reported no Halothane by RN interaction for drip loss of the LD. However, in the present study the interactive affects between mutations on fluid loss was most notable in the SM. Initial surface exudate of the SM was higher with both mutations than any other genotype. Moreover, the RN mutation increased displayed fluid loss of the SM more than 40% an average compared to normal pigs. The addition of the Halothane to the RN mutation increased that effect to over 75% of pigs.

No genotype interactions were observed in the LD with the exception of initial redness and yellowness and color after 45 d of vacuum-packed storage. However, interactions were not observed after 2 d of display storage. No Halothane by RN interaction

on paleness, redness, or yellowness was reported in the Longissimus by Hamilton et al. (2000). However, in the present study interactions were plentiful in regards to color of the SM. Paleness, redness, and yellowness were reduced by the Halothane mutation and increased by the RN mutation during display storage. Furthermore the presence of both mutations produced pork that was paler and more yellow than any other genotype.

No interactions were observed for cooking loss, shear force, or oxidation with the exception of oxidation of the LD after 45 d of vacuum-packed storage. Shear force was lower for the RN mutation but higher for the Halothane mutation. Hamilton et al. (2000) reported that normal pigs had the highest shear force values of any genotype represented in the present study and no difference in shear force between the other three genotypes. However, many other studies have indicated pigs with the Halothane mutation have higher shear force values than pigs without the mutation (Murray and Jones,1994; McPhee and Trout, 1995; and Brewer et al., 2002).

Magnesium Effect and Genotype by Magnesium Interactions

Magnesium supplementation did not affect pH of either muscle at any time period, which is consistent with the work of D'Souza et al. (1999) and Hamilton et al. (2002) but contradicts D'Souza et al. (1998) and Caine et al. (2000) who reported a reduction in pH when pigs were supplemented with Mg. Although, Mg supplementation reduced drip loss of the LD, which is in agreement with (D'Souza et al., 1998, 1999, 2000) but not (Apple et al., 2000; Caine et al., 2000; van Laack et al., 2000) Mg did not affect display fluid loss of the LD. Drip loss is not always highly correlated with display fluid loss (Chapter 2). Of the two measurements, display fluid loss is the more important from a practical standpoint.

Therefore, Mg supplementation in the present study was not able to influence fluid loss of the LD in practical conditions.

No interactions between the RN mutation and Mg supplemention were observed and no interactions have been reported previously by Hamilton et al. (2002). However, several interactions were observed between the Halothane mutation and Mg supplementation. Magnesium supplementation did not affect display fluid loss of the SM in pigs without the Halothane mutation. However, Mg supplementation increased fluid loss of the SM of pigs with the Halothane mutation during display storage. Furthermore, Mg supplementation improved color of the SM from pigs without the Halothane mutation but not the RN mutation and negatively affect color of the SM from pigs with the Halothane mutation but not the RN mutation. In regards to paleness, this effect of SM color was most noticeable in the absence of the RN mutation.

The inhibitory effect of Mg on the ryanodine receptor-Ca^{2+} release channels is reduced by the Halothane mutation (Laver et al., 1997; Balog et al., 2001). Therefore, any effect Mg has on regulating calcium could be diminished with the Halothane mutation and could explain the absence of a positive effect on pork quality. Indeed, Apple et al. (2002) reported no effects of long term Mg supplementation on pork quality of pigs with or without the Halothane mutation. Conversely, Schaefer et al. (1993) reported that supplemental Mg increased redness or reduced drip loss of pigs with a single Halothane mutation, with 20 or 40 g of Mg asparate/d, respectively. Nevertheless, negative effects of Mg on fluid loss and color of pigs with the Halothane mutation in the present study are difficult to explain. However, others have reported similar results. Caine et al. (2000)

reported lower glycogen concentrations early post-mortem when pigs with the Halothane gene were provided Mg supplementation, which resulted in lower structure score of the Longissimus and tended to cause greater drip loss and spectral shift in color toward higher luminosity. Results from that study indicate the possibility of higher paleness, softness, and exudative pork from pigs with the Halothane supplemented with Mg. The mechanism by which Mg exacerbates the Halothane mutation warrants additional research.

Implications

Pigs heterozygous for the RN mutation produced lower quality pork than pigs heterozygous for the Halothane mutation indicating that the N allele of the Halothane mutation may be dominant for most traits. Magnesium supplementation does not seem to be a plausible strategy to reduce the negative effects associated with either gene. In fact, Mg supplementation might exacerbate the effects of the Halothane mutation. Thus, elimination of both mutations, especially the RN mutation, through selective breeding is imperative to obtain the highest quality pork possible.

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Table 1. Composition of finisher diet (% of total)[ab]

Ingredient	Percent of total
Corn, yellow dent	78.95
Soybean meal, 47.5% CP	15.80
Poultry Fat	2.50
Dicalcium phosphate	1.30
Limestone	0.60
Salt	0.35
Vitamin/Mineral Premix[c]	0.25
L-Lys HCl[d]	0.15
Tylan[e]	0.10
Analyzed Composition	
Total Mg	0.13%

[a]Diet was fed from 65 kg BW until harvest.

[b]Formulated to contain 14.2% crude protein, 0.80% lysine, 0.57% P, and 0.60% Ca.

[c]Supplied per kg of complete diet: 5,540 IU of vitamin A as retinyl acetate, 1,108 IU of vitamin D_3, 22 IU of vitamin E as dl-α-tocopherol acetate, 1.98 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 165 mg of choline as choline chloride, 22 mg/kg of niacin, 17.6 mg of d-pantothenic acid as dl-calcium pantothenate, 4.4 mg of riboflavin, 1.1 mg of pyridoxine as pyridoxine·HCl, 0.55 mg thiamine as thiamine mononitrate, 0.022 mg of vitamin B_{12}, 0.33 mg of folic acid, 0.04 mg of d-biotin, 110 mg Zn as $ZnSO_4$, 110 mg Fe as $FeSO_4$, 22 mg Cu as $CuSO_4$, 55 mg Mn as MnO, 0.28 mg I as ethylenediamine dihydriodide, and 0.30 mg Se as $NaSeO_3$.

[d]Contained 78.8% lysine.

[e]Provided 22 mg of tylosin/kg of feed.

Table 2. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on data collected prior to and at harvest[a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Feed intake, kg/d					2.59	2.58	0.04	0.59	0.29	0.05	0.99	0.91	0.27
rn^+, rn^+			2.60[b]	2.54[b]									
RN^-, rn^+			2.50[b]	2.70[c]									
Water disappearance, L/d	9.32	9.27	9.42	9.17	8.92	9.67	0.4	0.94	0.76	0.34	0.22	0.98	0.61
Harvest BW, kg	116.0	117.4					0.7	0.34	0.38	0.95	0.33	0.77	0.04
				NN	117.7[b]	114.5[c]							
				Nn	116.7[b]	117.9[b]							
Serum cortisol	10.4	13.1	10.8	12.7	11.9	11.7	0.7	0.03	0.12	0.95	0.86	0.35	0.42

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bc]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 3. Three way interaction between the Rendement Napole mutation, Halothane mutation, and magnesium supplementation on serum magnesium concentration[a]

Napole	rn^+rn^+				rn^+RN^-					P-value						
Halothane	NN		Nn		NN		Nn					RN x Hal		Mgx RN	Mgx Hal	Mg x RN x Hal
Mg	0	900	0	900	0	900	0	900	SEM	RN	Hal		Mg			
Serum Mg	28.3[bc]	31.0[c]	31.4[c]	29.5[bc]	27.8[b]	28.2[bc]	26.5[b]	29.3[bc]	1.1	0.04	0.73	0.66	0.20	0.49	0.49	0.03

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bc]Means without a common superscript differ ($P < 0.05$).

Table 4. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on post-mortem pH[a]

	Napole		Halothane		Magnesium			P-value					
Criteria	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
LD pH, 60 min					5.86	5.91	0.02	0.4	<0.01	0.03	0.18	0.92	0.30
rn^+rn^+			5.89[b]	5.84[bc]									
rn^+RN^-			6.02[d]	5.78[c]									
LD pH, 24 h	5.81	5.59	5.74	5.70	5.70	5.73	0.02	<0.0001	0.27	0.45	0.41	0.80	0.11
SM pH, 24 h	6.00	5.68	5.79	5.89	5.87	5.81	0.03	<0.0001	0.07	0.06	0.16	0.45	0.10

[a]Napole: rn^+rn^+ = normal and rn^+RN^-=carrier; Halothane: NN= normal and Nn=carrier; Magnesium: 0 or 900 mg/L of water.

[bc]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 5. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on surface exudate and drip loss[a]

	Napole		Halothane		Magnesium			P-value					
Criteria	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Surface exudate, mg													
LD	98	121	104	115	116	103	5	0.01	0.13	0.57	0.07	0.78	0.13
SM					86	87	5	<0.0001	0.03	0.02	0.91	0.50	0.30
rn^+rn^+			73[b]	72[b]									
rn^+RN^-			88[b]	112[c]									
Drip loss, %													
LD	9.24	12.83	10.44	11.63	11.76	10.31	0.5	<0.0001	0.07	0.06	0.04	0.40	0.73
SM	1.99	6.65	4.19	4.45	4.75	3.89	0.5	<0.0001	0.69	0.09	0.22	0.23	0.22

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcd]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 6. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on display fluid loss of the Longissimus dorsi (% of initial weight) [a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Days of storage													
d-2	4.20	6.50	4.87	5.82	5.38	5.31	0.30	<0.0001	0.01	0.14	0.88	0.61	0.06
d-4					7.24	7.22	0.40	<0.0001	0.01	0.04	0.97	0.60	0.08
rn^+rn^+			5.68[b]	5.93[b]									
rn^+RN^-			7.73[c]	9.60[d]									
d-6	6.98	10.03	7.94	9.08	8.56	8.45	0.38	<0.0001	0.01	0.11	0.84	0.59	0.08
d-8	7.61	10.79	8.61	9.79	9.29	9.11	0.39	<0.0001	<0.01	0.07	0.74	0.58	0.06

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcd]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 7. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on display fluid loss of the Semimembranosus (% of initial weight) [a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Days of storage													
d-2	3.00	4.87	3.45	4.41	3.64	4.23	0.24	<0.001	0.05	0.06	0.09	0.20	<0.001
d-4					5.41	6.67	0.44	<0.0001	0.13	0.02	0.05	0.09	0.25
rn^+rn^+			4.56[b]	4.12[b]									
rn^+RN^-			6.77[c]	8.68[d]									
d-6					6.90	7.90	0.30	<0.0001	0.33	0.04	0.02	0.10	0.002
rn^+rn^+			5.87[b]	5.22[b]									
rn^+RN^-			8.39[c]	10.12[d]									
d-8					7.91	8.73	0.32	<0.0001	0.25	0.02	0.08	0.29	0.01
rn^+rn^+			6.68[b]	5.92[b]									
rn^+RN^-			9.31[c]	11.38[d]									

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcd]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 8. Halothane genotype by magnesium interaction on display fluid loss of the Semimembranosus (% of initial weight) [a]

Halothane	NN		Nn		
Magnesium	0	900	0	900	SEM
Display fluid loss, %					
d 2	3.80[b]	3.11[b]	3.47[b]	5.36[c]	0.33
d 6	7.32[b]	6.94[b]	6.47[b]	8.87[c]	0.40
d 8	8.19[b]	7.79[b]	7.62[b]	9.67[c]	0.44

[a]Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bc]Means without a common superscript within rows differ ($P < 0.05$).

Table 9. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on purge loss of the Longissimus dorsi (% of initial weight) [a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Days of vacuum-packed storage													
d 25	11.73	14.11	12.89	12.95	12.52	13.32	0.39	<0.001	0.91	0.69	0.15	0.59	0.09
d 45					12.09	12.96	0.40	<0.01	0.02	0.03	0.13	0.67	0.22
rn^+rn^+			11.47[b]	11.56[b]									
rn^+RN^-			12.02[b]	15.05[c]									

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bc]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 10. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on color scores and lightness (Minolta L*) of the Longissimus dorsi[a]

	Napole		Halothane		Magnesium			P-value					
Days of storage	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Color score[b]	2.9	2.2	2.9	2.2	2.6	2.5	0.2	<0.01	<0.01	0.47	0.68	0.33	0.02
Display, Minolta L*													
d-0	57.4	60.4	57.1	60.7	59.0	58.9	0.7	<0.01	<0.001	0.83	0.94	0.72	0.07
d-2	57.0	60.6	56.8	60.7	58.9	58.6	0.7	<0.01	<0.001	0.92	0.77	0.77	0.02
d-4	57.0	60.4	56.9	60.6	58.8	58.6	0.7	<0.01	<0.01	0.62	0.81	0.56	0.02
d-6	57.0	60.4	57.5	60.9	59.3	59.2	0.7	< 0.001	<0.01	0.73	0.90	0.70	0.06
d-8	57.8	61.7	58.1	61.4	59.8	59.6	0.7	<0.01	<0.01	0.95	0.89	0.67	0.08
Vacuum-packed, Minolta L*													
d-25	56.3	61.6	57.3	60.6	59.2	58.8	0.8	< 0.001	<0.01	0.22	0.73	0.62	0.05
d-45	56.7	62.3	58.3	60.7	59.6	59.4	0.7	< 0.001	0.06	0.56	0.89	0.41	0.09

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[b]American color score: 1=pale pinkish gray and 6=dark purplish red (NPPC, 1999).

Table 11. Halothane genotype and magnesium interaction on color of the Longissimus dorsi[a]

Halothane	NN		Nn		
Magnesium	0	900	0	900	SEM
Color score	2.69[b]	3.13[b]	2.49[bc]	1.94[c]	0.24
Display lightness					
d 2	56.1[b]	54.1[b]	57.7[bc]	59.7[c]	0.96
d 4	58.2[b]	55.5[c]	59.4[bd]	61.7[d]	0.96
Vacuum-packed lightness					
d 25	58.6b[cd]	56.0[b]	59.7[cd]	61.6[d]	1.07

[a]Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcd]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 12. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on lightness (Minolta L*) of displayed Semimembranosus[a]

Napole	rn^+rn^+				rn^+RN^-					P-value						
Halothane	NN		Nn		NN		Nn									
Mg	0	900	0	900	0	900	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mgx Hal	Mg x RN x Hal
Semimembranosus																
d-0	55.3^{bd}	52.3^{bc}	49.7^{c}	53.6^{bd}	56.1^{de}	55.5^{bd}	59.1^{ef}	61.3^{f}	1.2	<0.0001	0.18	<0.001	0.49	0.49	0.85	0.01
d-2	53.3^{b}	49.4^{c}	49.0^{c}	52.6^{b}	54.8^{b}	55.0^{b}	59.2^{d}	60.1^{d}	1.2	<0.0001	0.02	<0.01	0.81	0.67	0.03	0.07
d-4	53.9^{b}	50.2^{cd}	48.9^{c}	53.3^{bd}	56.1^{b}	55.7^{b}	59.1^{e}	59.9^{e}	1.2	<0.0001	0.13	0.01	0.77	0.91	<0.01	<0.05
d-6	55.1^{b}	51.2^{cd}	49.8^{d}	53.7^{bc}	56.3^{b}	55.8^{b}	60.4^{e}	60.5^{e}	1.3	<0.0001	0.05	<0.001	0.91	0.92	0.03	0.05
d-8	55.9^{be}	51.7^{cd}	49.3^{d}	54.0^{ce}	57.8^{bf}	56.5^{be}	60.5^{f}	61.0^{f}	1.2	<0.0001	0.43	<0.01	0.94	0.75	<0.01	0.06

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcdef]Means without a common superscript within a row differ ($P < 0.05$).

Table 13. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on redness (Minolta a*) of displayed and vacuum-packed Longissimus dorsi[a]

Days of storage	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Displayed													
d-0					9.88	9.76	0.18	<0.001	0.03	0.01	0.31	0.59	0.11
rn^+rn^+			9.77^{bc}	9.32^{b}									
rn^+RN^-			9.95^{bc}	10.25^{c}									
d-2	9.54	10.10	9.86	9.79	9.88	9.76	0.18	0.13	0.84	0.30	0.63	0.19	0.87
d-4	9.18	8.93	9.34	8.77	9.01	9.11	0.18	0.49	0.13	0.43	0.71	0.08	0.60
d-6	8.18	9.85	8.81	8.24	8.50	8.55	0.21	0.07	0.18	0.49	0.88	0.22	0.60
d-8	8.65	7.56	8.44	7.77	8.09	8.12	0.21	0.01	0.10	0.46	0.92	0.29	0.54
Vacuum-packed													
d-25	10.06	11.28	10.69	10.64	10.80	10.54	0.15	0.01	0.90	0.14	0.21	0.68	0.20
d-45					11.01	10.93	0.16	0.01	0.08	<0.001	0.73	0.46	0.46
rn^+rn^+			11.48^{bc}	9.40^{d}									
rn^+RN^-			11.14^{b}	11.86^{c}									

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcd]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 14. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on redness (Minolta a*) of displayed Semimembranosus[a]

	Napole		Halothane		Magnesium			P-value					
	rn^+rn^+	rn^+RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Days of storage													
d-0					11.50	11.61	0.24	<0.0001	0.03	0.01	0.31	0.59	0.11
rn^+rn^+			10.91[b]	9.49[c]									
rn^+RN^-			12.12[d]	13.72[e]									
d-2					10.34	10.59	0.25	<0.01	0.04	<0.01	0.51	0.61	0.66
rn^+rn^+			10.77[b]	8.95[c]									
rn^+RN^-			10.94[b]	11.19[b]									
d-4	9.51	9.94	10.00	9.46	9.60	9.86	0.24	0.24	0.14	0.06	0.45	0.92	0.48
d-6	9.14	8.97	9.43	8.67	8.86	9.24	0.25	0.68	0.07	0.27	0.30	0.90	0.72
d-8	9.17	8.50	9.19	8.48	8.64	9.02	0.34	0.13	0.10	0.68	0.43	0.78	0.73

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcde]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 15. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on yellowness (Minolta b*) of the Longissimus dorsi[a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Days of display storage													
d 0					8.09	7.83	0.24	<0.0001	<0.001	0.02	0.44	0.84	0.07
rn^+rn^+			6.64[b]	7.30[c]									
rn^+RN^-			7.68[c]	10.22[d]									
d 2	8.02	9.48	8.34	9.16	8.87	8.63	0.14	<0.0001	<0.001	0.34	0.24	0.60	0.04
d 4	8.05	9.48	8.49	9.04	8.88	8.65	0.15	<0.0001	<0.01	0.44	0.29	0.33	0.09
d 6	8.18	9.85	8.70	9.32	9.09	8.94	0.13	<0.0001	<0.01	0.37	0.44	0.72	0.09
d 8	8.35	10.18	9.04	9.49	9.25	9.28	0.23	<0.0001	0.15	0.98	0.94	0.43	0.79
Vacuum-packed storage													
d 25	8.07	9.40	8.48	8.99	8.37	8.64	0.15	<0.001	0.10	0.23	0.37	0.58	0.03
d 45					9.02	8.77	0.13	<0.01	0.97	0.02	0.19	0.98	0.03
rn^+rn^+			8.76[b]	8.09[c]									
rn^+RN^-			9.04[b]	9.68[d]									

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcd]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 16. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on yellowness (Minolta b*) of the Semimembranosus[a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Days of display storage													
d 0					7.68	7.93		<0.0001	0.14	<0.0001	0.54	0.99	0.04
	rn^+rn^+		6.93[b]	5.50[c]									
	rn^+RN^-		8.13[d]	10.67[e]									
d 2					8.97	9.09	0.19	<0.0001	0.73	<0.001	0.67	0.56	0.03
	rn^+rn^+		8.28[b]	7.21[c]									
	rn^+RN^-		9.69[d]	10.94[e]									
d 4					8.99	9.06	0.16	<0.0001	0.17	<0.001	0.78	0.20	<0.01
	rn^+rn^+		8.33[b]	7.51[c]									
	rn^+RN^-		9.43[d]	10.83[e]									
d 6					9.18	9.27	0.17	<0.0001	0.31	<0.0001	0.72	0.18	0.06
	rn^+rn^+		8.53[b]	7.53[c]									
	rn^+RN^-		9.70[d]	11.13[e]									
d 8					9.55	9.59	0.17	<0.0001	0.22	<0.0001	0.84	0.12	0.01
	rn^+rn^+		8.70[b]	7.84[c]									
	rn^+RN^-		10.16[d]	11.58[e]									

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bcde]Means without a common superscript within a criteria differ ($P < 0.05$).

Table 17. Halothane genotype by magnesium interaction on yellowness (Minolta b*) of the Semimembranosus[a]

Halothane	NN		Nn		
Magnesium	0	900	0	900	SEM
Longissimus					
d 25	8.83[b]	8.13[c]	8.84[b]	9.14[b]	0.22
d 45	9.23[b]	8.57[c]	8.80[bc]	8.97[bc]	0.18
Semimembranosus					
d 0	7.85[bc]	7.22[c]	7.51[bc]	8.65[b]	0.40
d 2	9.24[b]	8.74[b]	8.71[b]	9.44[b]	0.26
d 4	9.20[bd]	8.56[c]	8.78[bc]	9.55[d]	0.22
d 6	9.31[bc]	8.92[c]	9.05[bc]	9.61[b]	0.24
d 8	9.72[bc]	9.15[c]	9.37[bc]	10.04[b]	0.24

[a]Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.
[bc]Means without a common superscript within a row differ ($P < 0.05$).

Table 18. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on cooking loss and shear force[a]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Cooking loss, %	28.0	27.3	25.9	29.3	28.1	27.2	0.7	0.40	<0.001	0.92	0.38	0.31	0.78
Shear force, kg	3.94	3.04	3.28	3.71	3.44	3.55	0.10	<0.0001	0.01	0.82	0.48	0.12	0.39

[a]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.

Table 19. Effect of Rendement Napole and Halothane genotype and supplemental magnesium on oxidation (TBARS)[a] during display and vacuum-packed storage[b]

	Napole		Halothane		Magnesium			P-value					
	rn^+ rn^+	rn^+ RN^-	NN	Nn	0	900	SEM	RN	Hal	RN x Hal	Mg	Mg x RN	Mg x Hal
Display													
LD, d-0	94	109	107	95	96	106	7	0.16	0.25	0.63	0.32	0.13	0.25
LD, d-8	159	322	241	240	247	234	14	<0.0001	0.97	0.26	0.50	0.72	0.68
SM, d-0	98	117	109	106	109	107	3	<0.0001	0.39	0.24	0.67	0.45	0.86
SM, d-8	157	399	271	284	245	310	16	<0.0001	0.77	0.61	<0.01	0.12	0.98
Vacuum-packed													
d-25	126	170	150	146	147	149	4	<0.0001	0.52	0.75	0.67	0.45	0.73
d-45					239	242	8	<0.01	0.28	0.03	0.80	0.99	0.71
rn^+rn^+			231[c]	193[d]									
rn^+RN^-			269[e]	269[e]									

[a]Thiobarbituric acid reactive substances (TBARS, µg/kg of fresh tissue).

[b]Napole: rn^+rn^+ = normal, rn^+RN^-=carrier; Halothane: NN= normal, Nn=carrier; Magnesium: 0 or 900 mg/L of water.

[cde]Means without a common superscript within a criteria differ ($P < 0.05$).

CHAPTER 5

Effect of magnesium concentration of drinking water on pork quality[1,2]

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

[2]Funded in part by North Carolina Pork Council, the North Carolina Agricultural Research Service, and the North Carolina Agricultural Foundation.

ABSTRACT

Thirty-two barrows were used to determine the effect of magnesium concentration in drinking water on pork quality. Pigs were individually penned, provided ad libitum access to feed (0.13% Mg) and allowed free access to water via a nipple waterer for the duration of the study. Pigs were free of the Halothane and Napole mutations as determined by DNA testing. After 7 d of adjustment, pigs (111 ± 1 kg BW) were randomly allotted by bodyweight to 0, 300, 600, or 900 mg/L supplemental Mg from Mg sulfate heptahydrate in drinking water for 2 d prior to harvest. Pigs were not allowed access to feed for 15 h prior to harvest. On d 3 of the experimental period (0900) all pigs were loaded and transported 110 km (1 h 45 min) to a commercial abattoir and remained in lairage for 5 h prior to harvest. The Longissimus dorsi (LD) muscle was removed 24 h post-mortem. Display storage was simulated for 8 d and the remaining LD was vacuum-packed for 25 or 50 d at 4°C. Plasma Mg increased linearly ($P < 0.01$) with Mg supplementation. However, Mg concentration of the LD was not affected (linear, $P = 0.99$) by Mg supplementation. Surface exudate, drip loss, and display fluid loss of the LD were not affected by Mg supplementation. Additionally, paleness and redness of the LD were not affected by Mg supplementation. However, Mg supplementation resulted in a cubic effect ($P < 0.05$) with regard to yellowness of the LD displayed for 0 to 6 d. Specifically, pigs supplemented with 300 or 900 mg/L of Mg had lower yellowness of the LD displayed for 0 to 6 d than pigs supplemented with 0 or 600 mg/L of Mg (cubic, $P < 0.05$). Oxidation of the LD after 4 d of display storage increased linearly ($P < 0.05$) as Mg concentration in drinking water increased. Furthermore, oxidation of the LD after 8 d of display storage tended to increase

linearly ($P < 0.10$) primarily because of the high oxidation of LD from pigs supplemented with 900 mg/L of Mg compared to the control (224 vs 171 ± 19, respectively). Oxidation of the LD was higher for pigs supplemented with 300 or 900 mg/L compared to 0 or 600 mg/L of Mg (cubic, $P < 0.06$) after 25 d of vacuum-packed storage. Magnesium did not improve pork quality characteristics of practical significance in pigs without the Halothane and Rendement Napole mutation.

Keywords: Pork quality, magnesium sulfate, drinking water

Introduction

Magnesium is an important divalent cation involved with over 300 enzymes essential for metabolism including metabolism of protein and energy. Furthermore, Mg has been shown to decrease acute stress response resulting from handling prior to harvesting (Kietzmann, and Jablonski, 1985), control intracellular calcium (Laver et al., 1997), and delay the initiation of glycolysis by maintaining high energy phosphates post-mortem (Moesgaard et al., 1993). For these reason Mg has been evaluated as a nutritional means of improving pork quality.

Indeed, short-term supplemental dietary Mg has been reported to decrease fluid loss (D'Souza et al., 1998, 1999, 2000) and improve color (D'Souza et al., 1998, 2000) of pork. The effect of Mg on pork quality does not seem to be dependent on Mg source when comparing Mg aspartate, sulfate, or chloride (D'Souza et al., 1999) but is dependent on timing of supplementation (Hamilton et al., 2002 and Chapter 2) and the response may be dependent on Mg intake (Schaefer et al., 1993).

However, others have not been able to demonstrate a positive or consistent effect of Mg on pork quality (van Laack, 2000; Hamilton et al., 2002). Although many studies have concentrated on supplemental dietary Mg in feed, supplementation through drinking water is a novel, practical approach that received less attention.

Results previously reported by our laboratory have indicated that 2 d may be the most efficacious timing for Mg supplementation through drinking water (Chapter 2). Schaefer et al. (1993) reported that the response to Mg for certain pork quality characteristics differed between pigs receiving varying dietary Mg intakes. However,

D'Souza et al. (2000) reported that the effects of Mg on pork quality were independent of dietary Mg intake.

Therefore, the objective of this study was to determine the optimal Mg concentration in drinking water to improve pork quality.

Materials and Methods

Animals and Treatments

All animal procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Thirty-two barrows weighing 111.0±1.0 kg from the North Carolina State University Swine Education Unit previous determined to be free of the Halothane and Rendement Napole mutations by DNA testing (GeneSeek, Lincoln, NE) were placed into 2.03 m by 0.74 m individual pens and provided free access to water via a nipple waterer. Pigs were provided ad libitum access to feed containing 0.13% Mg from feedstuffs (Table 1) during a 7 d adjustment and 2 d treatment period. After the adjustment period, pigs were allotted by bodyweight to water supplemented with 0, 300, 600, or 900 mg of elemental Mg/L from Mg sulfate heptahydrate (9.8% Mg, Giles Chemical Corp., Waynesville, NC) for 2 d prior to harvest. Plastic water containers (23 L capacity) were filled daily with 15 L of water containing the appropriate Mg concentration. These containers were suspended from the ceiling and gravimetrically emptied into a galvanized pipe leading to nipple waterer regulated to dispense 600 ml of water/min. Daily water disappearance volumes were determined by weight loss of the water containers assuming that 1 kg equaled 1 L of water. Feed was removed 15 h prior to transport to the abattoir. However, pigs had free access to experimental water treatments until loading time.

Harvest Data Collection

On the d 7 (0900) all pigs were loaded and transported 110 km (1 h 45 min) to a commercial abattoir. Pigs were unloaded by abattoir. After 5 h of lairage, pigs were moved by replicate 50 m to the stunning area. Pigs were electrically stunned and blood was collected during exsanguination for plasma Mg determination. The temperature and pH of the loin were measured between the 10^{th} and 11^{th} rib at 45 min post-mortem using an Argus Sentron (Gig Harbor, WA) pH meter.

Fabrication and Storage

After 20 h of chilling at 2˚C the entire right side loin was removed and transported 60 km (45 minutes) at 4˚C to a commercial meat fabrication facility for further processing. A total of 4 chops (2.54 cm thick) of the Longissimus dorsi (LD) muscle were obtained beginning at the 7^{th} and 8^{th} rib interface and extending posterior. The first chop was used for drip loss determination on the same day of collection. The second chop was used for initial oxidation measurements and determination of LD Mg concentration. The third chop was placed on an absorbent pad (Cryovac Sealed Air Corp., Saddle Brook, NY) within a Styrofoam tray (Cryovac Sealed Air Corp.), wrapped with a polyvinyl chloride film (Cryovac Sealed Air Corp.), and stored at 4˚C in the presence of fluorescent lighting to simulate retail display for 4 d. At the end of the 4 d display storage period the chop was analyzed for extent of oxidation. The fourth and final chop was taken immediately posterior to the third chop and stored in a similar environment as the third chop for 8 d of displayed storage. The remaining posterior portion of the Longissimus muscle was split into two

equal sections, weighed, vacuum-packed in B2651T Cryovac bags with a Mulitvac machine (Cryovac, Ducan, SC), and stored at 4˚C in the absence of light for 25 or 50 d.

Plasma and muscle Mg

Plasma and muscle Mg concentration was determined by atomic absorption. Briefly, each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). After two grams of sample were dried at 103˚C overnight, the dried sample was quantitatively transferred to a polypropylene tube (Corning). Ten ml of nitric acid (Fisher Scientific, Fair Lawn, NJ) were added to the tube and allowed to predigest overnight. The next day the samples were placed in a microwave oven (MARS 5, CEM, Matthews, NC), ramped for 10 min to 110˚C, maintained at 110˚C by thermowell, and cooled for 20 min before adding 2 ml of hydrogen peroxide (Sigma, St. Louis, MO) to terminate digestion. Tubes were brought up to 25 ml with deionized water. Fifty microliters of digested sample were combined with 5 ml of lanthium chloride (0.5%, Fisher) and read by atomic absorption.

Pork Quality Measurements

Fluid loss of the LD was evaluated by two separate methods. Drip loss was determined by a method developed by Honikel et al. (1986). Briefly, a 70 g core sample of each muscle was manually removed and suspended by a fish hook (barb removed) in a plastic, covered container, and stored at 4˚C for 48 h. Drip loss was reported as the weight loss of the sample after 48 h of storage divided by the initial weight of the muscle prior to storage multiplied by 100. Display fluid loss was determined on chops designated for 8 days of display storage. The muscle was removed from the tray on d 2, 4, 6, and 8, placed

on a paper towel for 5 seconds, and reweighed to determine display fluid loss. Display fluid loss was reported as the weight loss of displayed chops divided by the initial weight of the chop prior to storage multiplied by 100. Each chop was returned to its original tray, rewrapped, and returned to display storage for subsequent measurements.

Color in the LD was objectively evaluated by Minolta L*, a*, and b* measurements using a Minolta Chroma Meter (CR-200, Ramsey, New Jersey) calibrated with a standard white plate. Reported Minolta values were reported as the average color values collected at 4 positions in a diamond pattern on the surface of each chop. All color measurements were conducted on chops designated for 8 d of display storage. The initial measurement of color was performed after 45 minutes of the initial cut. Additionally, color was determined every 2 d for 8 d of display storage.

Longissimus chops displayed for 4 or 8 d and loin sections vacuum-packed for 25 or 50 d were vacuum-packed in Cryovac bags (B2651T) and stored at -20°C until oxidation was determined by thiobarbituric acid reactive substances (TBARS) (Witte et al., 1970). At time of determination, samples were removed from -20˚C and allowed to thaw overnight at 6˚C. Each muscle was ground and passed through a 5 mm screen twice (Oster Food Grinder, Sunbeam Corp., Canada, Ltd., Mississaug, Ontario). Four grams of ground muscle were homogenized, in duplicate, with 16 ml of ice cold phosphate buffer (pH = 7.0) prepared to contain 50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 0.1% EDTA (Fisher Scientific, Fair Lawn, NJ) and 0.1% propylgallate (Sigma, St. Louis, MO) in a stainless steel cup for 20 s using an Polytron homogenizer (Model PT10/35, Kinematica, Switzerland). Then, 4 ml of 30% trichloroacetic acid (Sigma) were added and samples were homogenized for an

additional 10 s. After the homogenate was filtered (P8, Fisher Scientific), 2 ml of the clear filtrate and 2 ml of 2-thiobarbituric acid (0.02 M, Sigma) were transferred to 16x125 screw-cap glass test tubes, vortexed, and heated in a 100˚C water bath for 30 min. Test tubes were allowed to cool in an ice cold water bath for at least 15 min and vortexed prior to measuring absorbance on a spectrophotometer at 533 nm (Model DU 640, Beckman, Fullerton, CA). The absorbance of samples was compared to tetraethoxypropane (Sigma) standard concentrations of 2, 4, 8, 10, 20, 40, and $80x10^{-7}$ M.

Statistical Analyses

Data were analyzed as a randomized complete block design using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). Pigs within individual pens were considered the experimental unit and were blocked by bodyweight. Linear, quadratic, and cubic contrasts were used to determine the effect of Mg supplementation. Significance was determined at $P < 0.05$.

Results and Discussion

Feed intake and water disappearance were not affected by Mg supplementation (Table 2). Water used to mix the Mg contained 3 mg/L of Mg. Therefore, the mean maximum intake of Mg from drinking water was 0.04, 3.27, 6.27, and 10.57 g/d for Mg concentrations of 0, 300, 600, and 900 mg/L, respectively. However, the actual intake may have been lower because waste water was not measured. Assuming the actual water intake was between the estimated requirements of 2 L per kg of feed (Cumby, 1986) and the maximum mean Mg intake, the Mg intake in the present study was well within the range of Mg intake shown to improve pork quality by D'Souza et al. (1998, 1999, and 2000) who

fed 1.6 or 3.2 g of elemental Mg from sources including aspartate, chloride, or sulfate for 2 or 5 d.

Although plasma Mg measured at time of harvest increased linearly ($P < 0.01$) as Mg concentration in drinking water increased, Mg concentration of the LD was unchanged. Therefore, the effects of Mg supplementation does not appear to be a result of total Mg concentration in muscle but could be an effect of altered intracellular concentration or other effects not directly associated with muscle Mg concentration. Schaefer et al. (1993) reported that plasma Mg increased 34% when pigs were reported to be supplemented 2.52 mg of Mg (20 g of Mg aspartate product containing 1.3% was Mg aspartate, which contained 9.7% elemental Mg) for 5 d. However, in that study Mg concentration of skeletal muscle, the liver, and heart tissue did not change with Mg supplementation.

The pH of the LD was not affected by Mg supplementation suggesting that the rate of pH decline did not differ during early post-mortem processes. However, the pH of the LD at 24 h decreased linearly ($P < 0.05$) with increased Mg concentration in the drinking water. This suggests that either the rate after 60 min post-mortem or the extent of pH decline was increased by Mg supplementation. These results contradict results reported by D'Souza et al. (1998) who reported a higher pH with Mg supplementation and D'Souza et al. (1999, 2000) and Hamilton et al. (2002) who reported no differences in ultimate pH between Mg supplemented and non-supplemented pigs. The post-mortem pH is often used as a predictor of pork quality (NPPC, 2001) and, therefore, the reduction in ultimate pH with high concentrations of Mg in the present study would be expected to have lower pork quality.

Although pH was negatively affected by Mg supplementation, fluid loss measured by surface exudates, drip loss, and display fluid loss was not affected by Mg supplementation (Table 3). These results are in agreement with Caine et al. (2000) and van Laack (2000) but contradict those reported by D'Souza et al. (1998, 1999, 2000). Schaefer et al. (1993) reported that 4.04 mg of Mg from Mg aspartate for 5 d reduced drip loss from pigs with the Halothane mutation. However, a reported 2.52 mg of Mg from Mg aspartate for 5 d did not affect drip loss in that experiment. Apple et al. (2000, 2001) report no effect of feeding 1.25 or 2.5% Mg mica during the starter to finisher phase of production on drip loss.

Paleness (Minolta L*) and redness (Minolta a*) were not affected by Mg supplementation (Table 4). However, pigs supplemented with 300 or 900 mg/L of Mg had lower yellowness (Minolta b*) of the LD displayed for 0 to 6 d than pigs supplemented with 0 or 600 mg/L of Mg (cubic, $P < 0.05$). Furthermore, Mg concentration in drinking water had a quadratic effect ($P < 0.05$) on paleness of the LD after 25 or 50 d of vacuum-packed storage indicating 300 and 600 mg/L of supplemental Mg increased lightness compared to the pigs supplemented with 0 or 900 mg/L of Mg (Table 5). The inconsistent reduction of yellowness associated with different supplemental Mg intakes has been reported previously (Apple et al., 2000, 2001). However, others reported no effects of Mg supplementation on the color of pork (D'Souza et al., 1999; Caine et al., 2000) or only decreased paleness (D'Souza et al., 1998, 2000; Hamilton et al., 2002).

Oxidation of the LD after 4 d of display storage increased linearly ($P < 0.05$) as Mg concentration in drinking water increased (Table 6). Furthermore, oxidation of the LD after

8 d of display storage tended to increase linearly ($P < 0.10$) primarily because of the high oxidation of LD of pigs supplemented with 900 mg/L of Mg compared to the control (224 vs. 171 ± 19, respectively). Oxidation of the LD was higher for pigs supplemented with 300 or 900 mg/L compared to 0 or 600 mg/L of Mg (cubic, $P < 0.06$). These data are in agreement with previous experiments conducted in our laboratory that indicated that 900 mg/L of Mg in the drinking water for 2 d can increase oxidation of pork during display storage (Chapter 4). However, this effect of Mg on oxidation is not consistent between experiments (Chapter 2, 3, and 4) and could be the result of varying Mg intakes. An inconsistent effect was observed by Apple et al. (2001) who reported 1.25% Mg mica in the starter, grower, and finisher diets increased oxidation after 28 d of vacuum-packed storage compared to pigs fed 2.5% Mg mica. Conversely, the oxidation of pork was higher after 56 d of vacuum-packed storage from pigs fed 2.5% Mg mica than pigs fed 1.25% Mg mica in the same study.

Implications

Although Mg supplementation through drinking water has slightly improved pork quality in previous experiments, the effect of Mg is not consistent. Stress associated with handling and transit along with the lairage time prior to harvest may be a reason for variable results across studies. Nevertheless, Mg supplementation at high concentrations may actually reduce quality of pork by increasing oxidation, which could result in a shorter shelf life. Future research is required to establish under which conditions a consistent effect of Mg on pork quality may be expected.

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Table 1. Composition of experimental diets (%, as is)[ab]

Ingredient	Percent of total
Corn, yellow dent	79.07
Soybean meal, 47.5% CP	15.80
Poultry Fat	2.50
Dicalcium phosphate	1.03
Limestone	0.76
Salt	0.35
Vitamin/Mineral Premix[c]	0.25
L-Lys HCl[d]	0.15
Tylan[e]	0.10
Analyzed Composition	
Total Mg	0.13%

[a]Diet was fed for the 7 d adjustment and 2 d experimental periods.

[b]Formulated to contain 14.2% crude protein, 0.80% lysine, 0.52% P, and 0.60% Ca.

[c]Supplied per kg of complete diet: 5,540 IU of vitamin A as retinyl acetate, 1,108 IU of vitamin D_3, 22 IU of vitamin E as dl-α-tocopherol acetate, 1.98 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 165 mg of choline as choline chloride, 22 mg/kg of niacin as niacinamide, 17.6 mg of d-pantothenic acid as dl-calcium pantothenate, 4.4 mg of riboflavin, 1.1 mg of pyridoxine as pyridoxine·HCl, 0.55 mg thiamine as thiamine mononitrate, 0.022 mg of vitamin B_{12}, 0.33 mg of folic acid, 0.04 mg of d-biotin, 110 mg Zn as $ZnSO_4$, 110 mg Fe as $FeSO_4$, 22 mg Cu as $CuSO_4$, 55 mg Mn as MnO, 0.28 mg I as EDDI, and 0.30 mg Se as $NaSeO_3$.

[d]Contained 78.8% lysine.

[e]Provided 22 mg of tylosin/kg of feed.

Table 2. Effect of magnesium concentration of drinking water on criteria prior to and at harvest

	Magnesium, mg/L					P values[b]		
Criteria	0	300	600	900	SEM	L	Q	C
Feed Intake[a], kg	4.31	5.00	4.54	4.51	0.25	0.89	0.16	0.17
Water Disappearance, L/d	12.3	10.8	10.4	11.7	1.1	0.69	0.21	0.92
Harvest BW, kg	110.2	111.8	111.1	110.7	1.0	0.87	0.34	0.56
Dressing percent	74.9	74.3	74.9	74.5	0.43	0.67	0.77	0.39
Plasma Mg, ppm	21.5	25.1	24.4	27.4	1.0	0.001	0.77	0.10
Longissimus Mg, ppm[c]	897	897	891	891	15	0.99	0.82	0.80
Longissimus pH, 60 min	5.91	5.81	5.69	5.95	0.10	0.96	0.09	0.38
Longissimus pH, 24 h	5.96	5.83	5.82	5.79	0.05	0.02	0.27	0.50

[a]Feed intake was cumulative from the beginning of the experimental period (0800, d-1) until feed was withdrawn (1800, d-2).

[b]Linear (L), quadratic (Q), and cubic (C) contrasts.

[c]Dry matter basis.

Table 3. Effect of magnesium concentration of drinking water on Longissimus magnesium concentration and fluid loss

	Magnesium, mg/L					P values[a]		
Criteria	0	300	600	900	SEM	L	Q	C
Surface exudates, mg	139	130	154	133	12	0.90	0.63	0.16
Drip loss, %	3.01	2.91	2.78	3.03	0.45	0.57	0.35	0.64
Display Fluid Loss								
d 2	5.33	5.24	5.09	4.29	0.51	0.16	0.49	0.79
d 4	6.12	6.18	6.34	5.40	0.50	0.38	0.32	0.59
d 6	7.04	7.00	7.33	6.33	0.48	0.41	0.33	0.44
d 8	7.60	7.42	7.80	6.53	0.47	0.18	0.25	0.29

[a]Linear (L), quadratic (Q), and cubic (C) contrasts.

Table 4. Effect of magnesium concentration of drinking water on Longissimus during display storage

	Magnesium, mg/L					P values[a]		
Criteria	0	300	600	900	SEM	L	Q	C
Minolta L*								
d 0	62.3	62.3	64.5	61.0	1.3	0.76	0.21	0.19
d 2	63.1	63.6	65.5	61.8	1.7	0.77	0.22	0.37
d 4	63.8	64.0	66.1	62.5	1.5	0.79	0.22	0.28
d 6	64.3	65.1	67.2	63.3	1.6	0.90	0.16	0.33
d 8	64.6	65.3	67.5	63.6	1.6	0.92	0.16	0.30
Minolta a*								
d 0	10.63	9.42	11.28	9.56	0.72	0.68	0.73	0.05
d 2	9.15	8.42	9.44	8.75	0.44	0.92	0.97	0.10
d 4	8.54	7.85	8.47	8.07	0.44	0.69	0.75	0.25
d 6	7.60	6.56	7.07	6.95	0.54	0.56	0.40	0.38
d 8	6.70	5.97	6.49	6.55	0.40	0.97	0.35	0.35
Minolta b*								
d 0	8.61	8.01	9.94	8.04	0.70	0.95	0.36	0.05
d 2	8.80	8.40	9.60	8.55	0.40	0.80	0.43	0.04
d 4	8.61	8.25	9.36	8.47	0.36	0.68	0.47	0.05
d 6	8.60b	8.26	9.51	8.61	0.40	0.48	0.47	0.04
d 8	8.54	8.55	9.69	8.69	0.46	0.45	0.28	0.13

[a]Linear (L), quadratic (Q), and cubic (C) contrasts.

Table 5. Effect of magnesium concentration of drinking water on Longissimus after vacuum-packed storage

	Magnesium, mg/L					P values[a]		
Criteria	0	300	600	900	SEM	L	Q	C
Day 25								
L*	60.6	64.2	64.7	60.8	1.6	0.86	0.03	0.86
a*	10.86	10.83	10.76	10.72	0.51	0.83	0.99	0.97
b*	9.11	9.55	9.58	9.55	0.40	0.48	0.58	0.85
Day 50								
L*	58.6	64.2	62.7	61.9	1.5	0.23	0.04	0.26
a*	10.57	10.48	10.73	9.35	0.54	0.14	0.28	0.40
b*	8.29	9.25	8.99	8.21	0.47	0.82	0.08	0.74

[a]Linear (L), quadratic (Q), and cubic (C) contrasts.

Table 6. Effect of magnesium concentration of drinking water on Longissimus oxidation (TBARS)[a] during display and vacuum-packed storage

	Magnesium, mg/L					P values[b]		
Criteria	0	300	600	900	SEM	L	Q	C
Display Storage								
d 0	115	123	121	128	5	0.12	0.94	0.47
d 4	142	161	156	183	13	0.05	0.75	0.34
d 8	171	180	167	224	19	0.10	0.22	0.31
Vacuum-packed Storage								
d 25	139	153	136	145	6	0.98	0.64	0.06
d 50	176	171	175	176	11	0.91	0.78	0.78

[a]Thiobarbituric acid reactive substances (TBARS, μg/kg of fresh tissue).
[b]Linear (L), quadratic (Q), and cubic (C) contrasts.

Appendix I

Elevated muscle glycogen: result of the RN^- mutation in AMP-activated protein kinase

Introduction

The phenotypic selection within the Hampshire breed has produced fast growing, muscular pigs with a high percentage of lean tissue. The selection for these growth and carcass characteristics have coincided with a reduction in the quality of pork obtained from many Hampshires. Quantification of reduced pork quality of Hampshires was first demonstrated by decreased processing yield (Naveau, 1986). The observation was commonly termed Rendement Napole (RN^-). The word "Rendement" is from French decent meaning "yield." The word Napole was derived from the first two letters of each of the three authors, Naveau, Pommeret, and Lechaux, who developed a technique for measuring processing yield (Naveau et al., 1985). Some Hampshires were observed to have higher glycogen content, lower ultimate pH, and a lower Napole yield than other breeds (Monin and Sellier, 1985; Naveau et al., 1985). Glycogen is degraded post-mortem to yield glucose, the substrate for glycolysis. As post-mortem glycolysis precedes the pH declines as lactate accumulates. A lower pH at 24 hours post-mortem is associated with poor pork quality.

A majority of the negative effects on pork quality associated with the Hampshire breed was determined to be caused by a single locus with two alleles, RN^- and rn^+ (Le Roy et al., 1990). The reduction in yield and ultimate pH was largely explained by the presence of the RN^- allele that results in muscle glycogen by as much as 70% (Estrade et al., 1993a,

1993b). The locus was determined to be located on chromosome 15 (Mariani et al., 1996). Elevated muscle glycogen was identified to be caused by a mutation in the AMP-activated protein kinase (AMPK) (Milan et al., 2000). Although not all Hampshires have the RN^- mutation, pigs with the dominant RN^- mutation have elevated muscle glycogen concentrations and the subsequent reduction in pork quality. This AMPK is not to be confused with cAMP-activated kinase, whose actions are better established. This appendix will summarize the current knowledge regarding AMPK and the effect of the RN^- mutation on skeletal muscle.

Structure of AMPK

AMPK consists of three subunits, namely α, β, and γ. Multiple isoforms have been identified for each subunit including α_1, α_2, β_1, β_2, γ_1, γ_2, and γ_3 (Beri et al., 1994; Cheung et al., 2000; Hardie et al., 1998). All three subunits are required for full AMPK expression (Cheung et al., 2000; Hardie and Carling, 1997; Hardie et al., 1998).

The α-catalytic subunit contains the kinase activity, while β and γ are the regulatory subunits (Hardie and Carling, 1997; Hardie et al., 1998).

The α-subunit contains the kinase domain at the N terminus and the autoinhibitory region at the C terminus (Crute et al., 1994). Although α_1 and α_2 isoforms have been observed in many tissues, the α_2 isoform is more abundant in skeletal muscle accounting for 80% of the total AMPK activity (Cheung et al., 2000).

The β-subunit is the structural backbone that binds the KIS and ASC domains, which contains the α- and γ-subunits, respectively (Thornton et al., 1998). The β_1 isoform

has been observed in type I fibers (red, oxidative), while β_1 and β_2 have been identified in a muscle with predominately type II fibers (white, glycolytic) (Chen et al., 1999).

The γ-subunit contains 4 tandem repeats of cytathione β-synthase (CBS) domains (Bateman, 1997). Although the exact function of each CBS domains is not known, they are thought to be involved in allosteric activation involving the binding of AMP to the α–subunit (Hardie and Hawley, 2001). The γ subunit is involved in AMPK's sensitivity to AMP. The γ_3 subunit predominates in muscle and is least sensitive to AMP (Hamilton et al., 2001; Cheung et al., 2000).

Experimental techniques applied to AMPK

Before proceeding with a review of AMPK, an introduction to experimental procedures that has lead to the current knowledge is required. Much of the research in this area has been conducted in vitro with conditions that attempt to mimic in vivo situations. Therefore, many of the results address one aspect of regulation that may or may not be true in vivo. Although this is true for most in vivo studies, advancements in AMPK research are complicated by two additional restrictions.

First, the multiple isoforms of each subunit result in at least 12 different structural formations of the enzyme (Hardie and Hawley, 2001). Furthermore, multiple structures of AMPK are present in any given tissue. Thus, developing a mutant for each isoform would be extremely involved. Isolating one structural conformation of AMPK may lead to improper conclusions relating to the actual conditions in vivo and cannot be applied to all tissues.

Secondly, a specific inhibitor of AMPK has not been identified. Therefore, conclusions of AMPK functions are based primarily on studies in which activation of AMPK is stimulated and observations of naturally occurring mutations such as the RN^- mutation. The experimental procedure most widely used is activation of AMPK by 5-aminoimidazole-4-carboxamide riboside (AICAR). AICAR is taken up by muscle cells and phosphorylated to form 5-aminoimidazole-4-carboxamide ribonucleotide (ZMP) that mimics the effects of AMP on AMPK (Sabina et al., 1982). The accumulation of ZMP in skeletal muscle leads to activation of AMPK allosterically and by phosphorylation (Winder and Hardie, 1999). Therefore, the assumption that AICAR activates AMPK in a similar manner as AMP has been accepted and remains the most popular approach to AMPK research.

Thus, the multiple isoforms and lack of a specific inhibitor of AMPK have limited the present knowledge of AMPK's involvement in cellular processes. However, in all likelihood these obstacles will be overcome in the future because of the importance of AMPK in diabetes research.

Regulation of AMPK

AMPK is allosterically activated by AMP and inhibited by ATP and creatine phosphate concentration (Corton et al., 1994; Hawley et al., 1996). As the demand for energy in the cell is increased, phosphate is released from ATP for energy resulting in an increase AMP concentration. The binding of AMP between the γ-subunit and the autoinhibitory region of the α-subunit is thought to activate AMPK (Hardie and Hawley, 2001). AMPK is further activated by phosphorylation of the α-subunit by AMPK kinase

(Hawley et al., 1996). AMPK kinase is an upstream activator of AMPK and is activated by elevated AMP concentration. The binding of AMP to AMPK increases the AMPK kinase's preference for AMPK (Hawley et al., 1996) and makes AMPK a poor substrate for protein phosphatase 2C, which inactivates AMPK by dephosphorylation (Davies et al., 1995). Therefore, AMP increases AMPK activity by activating AMPK directly, activating AMPK kinase, and increasing the affinity of AMPK to AMPK kinase.

The degree of activation is dependent on the isoforms of the α- and γ-subunits (Cheung et al., 2000). The isoform $\alpha_2\beta_1\gamma_1$ complex was observed to have a 13-fold difference in activation compared to other combinations (Stein et al., 2000).
Phosphorylation of AMPK by AMPK kinase can increase AMPK activity 50 to 100 fold (Stein et al., 2000; Hawley et al., 1996).

Inactivation of AMPK is controlled by ATP and phosphocreatine. As a result of AMPK's effects, the AMP:ATP ratio and AMPK activity decreases. ATP binding at the α- and γ-subunits disrupts the stability of the autoinhibitory region inactivating AMPK (Hardie and Hawley, 2001). AMPK is also allosterically inhibited by phosphocreatine (Ponticos et al., 1998). Elevated muscle glycogen concentration reduces AMPK activity (Derave et al., 2000).

Thus, AMPK is allosterically activated by AMP and phosphorylation by AMPK kinase. Conversely, AMPK is inactivated by ATP and phosphocreatine. It seems clear that regulation of AMPK is closely associated with the energy status of muscle.

Effects of AMPK of glycogen metabolism

Glucose transport

Glucose transport is the most researched area involving the activity of AMPK because of its potential role in type 2 diabetes. Insulin and muscle contraction increases muscle glucose uptake. GLUT-4 transporters are translocated from microvesicles beneath the sacrolemma and T-tubule membranes to the surface and are inserted into these membranes increasing the capacity for glucose uptake. The effect of insulin on glucose uptake has been well established. Insulin binds to insulin receptors activating insulin receptor substrate1, subsequently activating phosphatidylinositol 3-kinase, which increases translocation of GLUT-4 to the surface of the membrane. Additionally, chronic AMPK activation by AICAR increases GLUT-4 protein expression (Ojuka et al., 2000). However, studies that inhibited insulin activated GLUT-4 translocation did not inhibit glucose uptake when AMPK was artificially stimulated (Hayashi et al., 1998), simulating muscle contraction, indicating that the insulin and muscle contraction pathways may be independent. Indeed, activation of AMPK increased GLUT-1, which is ubiquitous, transport resulting in a 2- to 3- fold increase in glucose transport (Abbud et al., 2000). Other transporters may be responsible for increased glucose transport. Thus, AMPK may not be essential for glucose transport in skeletal muscle.

The induced contraction increases glucose uptake and AMPK activation is lower in Type II muscles that have elevated glycogen content (Fryer et al., 2000; Hayashi et al., 2000). Indeed, muscle contraction of rats with low muscle glycogen induced 2-and 3-fold lower glucose transport and AMPK activation, respectively, than rats with a 3-fold higher

glycogen concentration (Derave et al., 2000). Thus, glycogen seems to blunt AMPK activity and glucose transport in Type II muscles that is likely a result of higher energy status. Glucose uptake after electrical stimulation was not affected by elevated glycogen concentration in Type I muscles although a complete absence of AMPK activation was reported (Dervae et al., 2000). Mice expressing an inactive α_2-catalytic subunit of AMPK depressed endogenous AMPK activity but hexose transport was only partially blocked (Mu and Birnbaum, 2001). Therefore, alternative, redundant AMPK independent pathway for contraction stimulated glucose uptake of muscle is likely.

Glycogen regulation

Muscle glycogen concentration is regulated by glycogen synthase and phosphorylase. Glycogen synthase is an essential enzyme for the addition of glucose by 1-4 bonds. On the other hand, glycogen phosphorylase degrades glycogen supplying glucose for energy. AMPK can phosphorylate glycogen synthase in vitro (Carling and Hardie, 1989; Wojtaszewski et al., 2002), which would inactivate the synthase and inhibit glycogen accumulation. The ability of AMPK to activate phosphorylase kinase in vitro has been debated (Beyer et al., 2000; Carling and Hardie, 1989). Activation of phosphorylase would increase glycogen degradation. If these phosphorylations are true in skeletal muscle in vivo, then glycogen concentration would decrease, at least short term, with AMPK activation. The inhibition glycogen accumulation by AMPK activation is logical given that glycogen would be degraded to yield glucose that would be used for ATP production through glycolysis instead of glycogen storage.

Although a logical effect of AMPK would be to decrease the activity of glycogen synthase and increased or constant phosphorylase activity, some research has indicated that chronic activation of AMPK actually increases glycogen accumulation (Aschenbach et al., 2002; Holmes et al., 1999). Muscle glucose transport (Kawanaka et al., 2000) and glycogen synthase activity (Nielsen et al., 2001) are inversely related to muscle glycogen concentration. Pigs with the RN^- mutation in the AMPK have 70% higher glycogen concentration and a 2-fold lower AMPK activity (Milan et al., 2000). These observations, along with the observation that glycogen levels do not affect adenine nucleotide concentrations (Wojtaszewski et al., 2002), indicate a possible feedback inhibition on AMPK and/or glycogen synthase in muscles with high glycogen concentration.

Thus, activation of AMPK causes a short-term reduction in glycogen synthase activity decreasing the demand of glucose for glycogen accumulation and increases glucose uptake into muscle, thereby increasing glucose availability for ATP generation through glycolysis. In the case of chronic AMPK activation, either pharmacologically by AICAR or physiological by frequent bounds of exercise, the AMP:ATP ratio is restored and glucose is allocated to glycogen accumulation that leads to reduced AMPK activation.

Effect of RN^- mutation on muscle glycogen

The RN^- mutation that results in a 70% increase in muscle glycogen commonly associated with the Hampshire breed is caused by a nonconservative substitution (R200Q) in the AMP–activated protein kinase gamma-3 subunit (*PRKAG3*) gene, more specifically the first CBS domain (Milan et al., 2000). At first glance, it appears the mutation causes a loss of function because AMPK activity is 2-fold lower in pigs with the mutation opposed

to those without the mutation. However, the regulation of AMPK is much more complex as is apparent by the aforementioned sections. Whether this mutation results in a loss or gain of AMPK function is not currently known. However, some evidence supports both possibilities.

Possible loss of function

The AMPK activity is lower in pigs with the RN^- mutation (Milan et al., 2000). The mutation in the gamma subunit may reduce the ability of AMP to bind the γ-subunit interfering with the interaction between the gamma and alpha subunit. The reduction in AMP binding may inhibit the conformation change that removes the autoinhibitory region from inhibiting the alpha subunit. Reduced AMP binding would also reduce the ability of AMPK kinase to phosphorylate AMPK, further reducing AMPK activity. Thus, AMP would not be able to directly or indirectly activate AMPK. Glycogen synthase inhibition by AMPK would be reduced. Therefore, an increase in glycogen synthase activity would be expected to increase muscle glycogen accumulation. Indeed, RN^- carrier pigs have a higher glycogen branching enzymes and glycogen synthase activity than normal pigs (Estrade et al., 1994). However, if the mutation results in a loss of function, glucose uptake would depend primarily on the response of insulin or muscle contraction because the additional GLUT-4 translocation and expression would likely be reduced. Therefore, glycogen accumulation may be limited by substrate transport into the cell.

Possible gain of function

Conversely, the RN^- mutation in the γ-subunit may cause a gain of function by changing a conformation that makes AMPK less dependent on AMP for activation.

However, activity would only be increased when the elevated glycogen concentration was not maintained. Thus, AMPK activity would be higher with the mutation than without in this situation. This would seem to contradict the observation that AMPK activity is 2-fold lower in pigs with the mutation as opposed to those without (Milan et al., 2000). However, glycogen level may directly affect the activation of AMPK (Wojtaszewski et al., 2002) and cause the reduction in AMPK when the elevated glycogen concentration is maintained.

Support of this hypothesis includes a study conducted to determine the effect of a mutation in the first CBS domain of the γ1 subunit (Hamilton et al., 2001). The R200 residue in the first CBS domain is preserved in all γ subunits. Therefore, the mutation is similar to that found in the RN^- mutation. The γ1 R70Q mutation caused increased phosphorylation of the α-subunit and substantial loss of AMP dependence (Hamilton et al., 2001). However, glucose transport and glycogen synthesis was not affected. Although this study partially agrees with a gain of function, another study more closely related to the RN^- mutation was conducted using variant 199I, which is only three nucleotides from R200Q (Ciobanu et al., 2001). The 199I variant of the first CBS domain of γ3 is associated with lower than normal glycogen concentrations. However, AMPK activity was not reported. Although not known exactly how, it is clear that the CBS domains of the γ-subunit are involved in regulation of AMPK activity.

Conclusion

The RN^- mutation in AMPK elevates muscle glycogen that results in reduced pork quality of carrier pigs. Whether the mutation causes a loss or gain of function is not currently known. Evidence for both possibilities hinders a definitive answer. Future advancements in AMPK research, specifically identifying a specific inhibitor of AMPK, will likely resolve this question.

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