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## Determinants of ultimate pH of pork longissimus muscle

Jun Yang

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To the Graduate Council:

I am submitting herewith a thesis written by Jun Yang entitled "Determinants of ultimate pH of pork longissimus muscle." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Riëtte van Laack, Major Professor

We have read this thesis and recommend its acceptance:

Sharon L. Melton, Clark J. Brekke

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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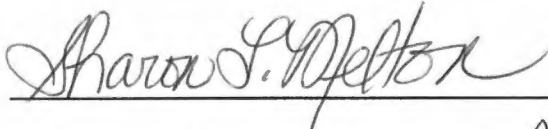
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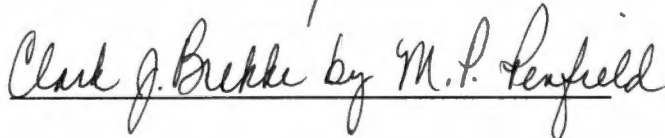
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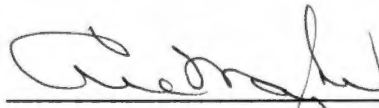


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Accepted for the Council:



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Associate Vice Chancellor and  
Dean of The Graduate School

DETERMINANTS OF ULTIMATE pH OF  
PORK LONGISSIMUS MUSCLE

A thesis

Presented for the Master of Science Degree  
The University of Tennessee, Knoxville

Jun Yang  
December 2000

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## ABSTRACT

Annually, millions of dollars are lost due to the variation in meat quality. Ultimate pH (pHu), one of the most important quality traits, has a great influence on water holding capacity and meat color. The National Pork Producers Council (NPPC) quality solutions team suggested an ultimate pH within the range of 5.6-5.9 as optimum.

The project of the study was to evaluate the possible determinants of ultimate pH. The effects of glycolytic potential (GP), creatine phosphate (CP), buffering capacity (BFC) and titratable acidity (TA), and the enzyme activities of phosphorylase (GPa) and AMP deaminase (AMPD) were investigated. Sixty pigs of 6 different genetic lines were used. At 5 min post mortem, the longissimus muscle was sampled for analysis of various metabolites (CP, GP, lactate) and enzyme activities. The meat quality characteristics (pHu, color (L\*-value), drip loss and filter paper wetness), BFC and TA were measured at 18-24 hr post mortem

The pigs were classified into PSE (Pale, Soft and Exudative) and 'normal' based on their physical and biochemical characteristics. Seven pigs were identified as PSE meat (L\*-value > 58 and drip loss > 5%). The remaining 53 were normal. No dark, firm and dry meat (DFD; pHu > 6.0) was found. The correlations among GP, GPa, BFC and AMPD were significant. Glycolytic potential, where all

components potentially can be converted to lactate, explained 42% of the variation in pHu; increased glycolytic potential was associated with a lower pHu. The GPa explained 28% of the difference in pHu; a high activity of GPa was associated with a lower pHu. A high BFC decreased pHu ( $r = -0.43$ ). The activity of AMP deaminase explained about 10% of the difference in pHu; pHu increased with an increased AMPD.

The best statistic model for predicting pHu was developed as:  $\text{pHu} = 5.75 - 1.21 \times 10^{-3} * \text{GP} - 3.14 \times 10^{-5} * \text{GPa} * \text{GPa}$ . This model explained 47% of variation of pHu. Only when we know what determines ultimate pH, can we produce animals with a specific pHu and, thus, with a consistent quality.

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## CHAPTER 1

### INTRODUCTION

Annually, millions of dollars are lost due to pork quality defects (Carr et al., 1997). The ultimate pH (pHu) is one of the important characteristics of pork. It influences the water holding capacity (Warner et al., 1997; Joo et al., 1999), meat color (Cross et al., 1986) and keepability (Kraft, 1986). The National Pork Producers Council (NPPC) quality solutions team suggested a pHu of 5.6-5.9 as a target for acceptable pork quality (NPPC, 1998).

Post mortem, muscle undergoes many biochemical changes. The role of the high-energy phosphates (ATP, ADP, creatine phosphate) and their metabolites is central to post-mortem muscle conversion (Pearson and Young, 1989). After slaughter, the ATP in muscle is broken down rapidly by ATPase. Post mortem, ATP can be resynthesized through creatine phosphate ( $ADP + CP \rightleftharpoons ATP + Cr$ ), anaerobic glycolysis and through ADP by the action of myokinase (Greaser, 1986).  $2ADP \rightleftharpoons ATP + AMP$

Through post-mortem anaerobic glycolysis, glycogen is converted to lactic acid. The accumulation of lactic acid accounts for pH decline. Glycogen has received most of the attention in explaining ultimate pH (Bendall, 1973; Warriss et al., 1989). Because cutting or touching the muscle stimulates the degradation of glycogen, it is impossible to accurately measure muscle glycogen content (Van

Laack, 2000). Glycolytic potential (GP) has been used as an approximation of muscle glycogen content (Fernandez et al., 1992). Post mortem, all components that can potentially be converted to lactate are represented by glycolytic potential (Monin and Sellier, 1985; Maribo et al., 1999). In a study on the relationship between GP and pHu of pork loin, Van Laack and Kauffman (1999) found a trend towards higher pH with lower GP. Glycolytic potential explained approximately 40% of the variation in ultimate pH. Similar results have been reported by Maribo et al. (1999).

Different glycogen levels may result in the same pHu in meat. In comparison with beef, horse has a higher glycogen level but the same pHu as beef (Van Laack, 2000). The Rendement Napole (RN) gene, a dominant gene first identified in the Hampshire breed (Naveau, 1986), results in a high glycolytic potential and a low ultimate pH (Lundström et al., 1996). According to Monin and Sellier (1985) and Lundström et al. (1998), there is a 30-70% higher GP in muscle of RN-gene pigs than muscle without RN gene, but the pHu difference is only about 0.1 units. It appears that the lactate production ceases before all glycogen in the muscle is used.

Glycogen phosphorylase is a key enzyme in glycolytic control. It regulates the reaction from glycogen to glucose-1-phosphate. Most of the phosphorylase is in the inactive b form at 10 min post mortem (Sayre et al., 1963). Scopes (1974)

found that, when glycogen content is not the limiting factor, the pHu mostly depends on the amount of phosphorylase a. He indicated that higher levels of phosphorylase a gave a lower pHu.

Post mortem, AMP is produced via the myokinase reaction ( $2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$ ). Through AMP deaminase, AMP is deaminated into inosine monophosphate (IMP):  $\text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3$  (Greaser, 1986). The IMP is further degraded to inosine and finally to hypoxanthine (Bendall, 1973).

According to Chapman and Atkinson (1973), AMP deaminase may be implicated in the energy regulation through controlling the relative concentrations of adenine nucleotides. Scopes (1974) found that higher amounts of AMP deaminase resulted in a higher pHu.

Post mortem, CP rapidly degrades into creatine through the Lohman reaction (Greaser, 1986). Since creatine is an alkaline substance, its presence may limit pH decline.

The ultimate pH is not only determined by the presence of acids and bases. Differences in muscle buffering capacity and the concentration of strong ions such as  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  will also affect ultimate pH (Stewart, 1981).

Glycolytic potential cannot explain more than 50% of the variation in pHu. The enzyme systems such as phosphorylase and AMP deaminase may also be important, but how important has not yet been established. The objective of the

present study was to evaluate the possible determinants of ultimate pH. These determinants included levels of substrates (glycogen and creatine phosphate), buffering capacity, titratable acidity, and activities of glycogen phosphorylase and AMP deaminase.

## CHAPTER 2

### LITERATURE REVIEW

The pork chain quality audit reported that the two primary pork quality problems were inadequate meat color and water holding capacity (Cannon et al., 1996). The US swine industry loses about \$ 100 million dollars per year due to the variation in pork quality (Cannon et al., 1996; Carr et al., 1997). The ultimate pH has a great influence on meat color (Cross et al., 1986) and water holding capacity (Warner et al., 1997). The NPPC quality solutions team suggested a pHu of 5.6 to 5.9 as the target for acceptable pork quality (NPPC, 1998).

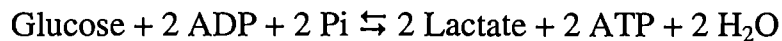
### CONVERSION OF MUSCLE TO MEAT

The conversion of muscle to meat undergoes many complex processes, which involve metabolic, physical and structural alterations (Greaser, 1986). Post-mortem changes-- conversion of muscle to meat—greatly influence the chemical and physical properties of meat. After slaughter, the blood circulation is blocked and oxygen is no longer available. These changes result in limiting the synthesis of high-energy compounds and removing the breakdown products from the system (Pearson and Young, 1989).



## Glycolysis and pH decline

After death, the supply of blood ceases, resulting in the lost of oxygen and glucose supply to muscle. The way to restore ATP through glycolysis of glycogen stored in muscle is particularly important (Greaser, 1986). The sum reaction of glycolysis is:



The anaerobic breakdown of one molecule of glucose yields a net gain of two molecules of ATP and two molecules of lactate. The accumulation of lactate during anaerobic glycolysis accounts for the pH decline (Bendall, 1973). In resting muscle of the pig, the concentration of lactic acid is 6.0 to 11.2  $\mu\text{mol}$  per gram of muscle (Greaser, 1986). Within 3 hr post mortem, Kastenschmidt (1970) observed a lactate concentration higher than 80  $\mu\text{mol/g}$ . Ultimate lactate levels may be as high as 130  $\mu\text{mol/g}$  (Bendall et al., 1963).

The pH value is a reliable and sensitive indicator of the rate and extent of post-mortem glycolysis (Greaser, 1986). Meat or muscle pH can be measured with a portable pH meter (Sayre et al., 1963; Van Laack and Kauffman, 1999) and in iodoacetate solution (Bendall et al., 1963) The rate of pH decline is extremely variable (Cassens, 1966) and dependent on species (Pearson and Young, 1989). In pig muscle, the pH may change 0.64 units/hour at 37 °C (Hallund and Bendall,

1965). At the same temperature, pH decline in beef, sheep and rabbit muscle is considerably slower (about 0.27-0.40 units/hour) (Greaser, 1986).

Early post mortem, the pH declines very fast in pork. As the temperature and pH continually go down, enzymatic activity and metabolic rate decrease (Bendall, 1973; Maribo et al., 1998). According to Bendall (1973), the initial pH value of resting pig muscle is 7.18-7.30. Post mortem, it declines to an ultimate pH of 5.4 -5.7 for longissimus muscle. The different extents of pH decline result in variation in pork quality. A low glycogen reserve in the pig before slaughter or stress will result in DFD (Dark, Firm, Dry) meat. DFD meat has a high ultimate pH (>6.0) because of limited lactate production. Pale, soft and exudative (PSE) pork is the result of a fast glycolytic rate combined with a high temperature (Bendall and Wismer-Pedersen, 1962). According to Van Laack (2000), the ultimate pH in PSE and normal pork are at a similar level (5.4-5.8). A rapid glycolysis will not cause PSE if the ultimate pH is 5.7 or higher (Fernandez et al., 1994). Pork from RN carriers has a lower ultimate pH than pork from non-RN carriers. The lower pH results in a reduction of water holding capacity and a paler color (Fernandez et al., 1992; Lundström et al., 1996).

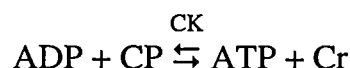
### **High-energy phosphates and their metabolites**

The major high-energy compound in muscle is adenosine triphosphate (ATP),

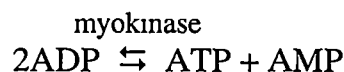
which is required for many processes in cells (Greaser, 1986). The hydrolysis of ATP, by means of ATPase, produces one molecule of adenosine diphosphate (ADP):



The ATP stored in skeletal muscle degrades very fast after slaughter (Greaser, 1986). ATP must therefore be recovered for sustaining muscle work. Post mortem, ATP can be regenerated through the breakdown of creatine phosphate, or through anaerobic glycolysis with formation of lactate. Through the Lohman reaction, ADP is immediately reconverted to ATP with the presence of creatine kinase (CK) (Greaser, 1986):



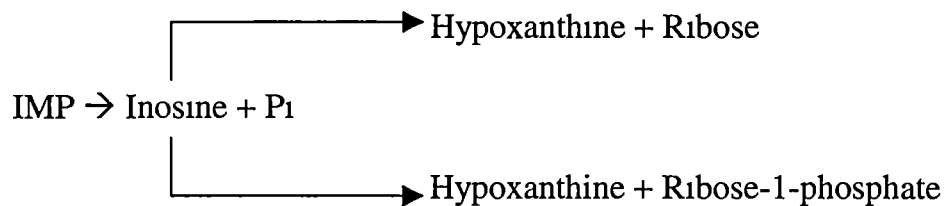
After slaughter, the creatine phosphate and pH in pig decrease very fast. With the continual depletion of CP, glycolysis cannot keep up with ATP resynthesis. ADP plays a role in recovering ATP. This reaction is catalyzed by myokinase (Greaser, 1986):



In pig longissimus muscle, the ADP levels start to decline at about 1 h post mortem (Kastenschmidt, 1970). The produced AMP is deaminated into inosine monophosphate (IMP) by AMP deaminase (Greaser, 1986):



IMP is further degraded into inosine and finally to hypoxanthine (Bendall, 1973):



### **ULTIMATE pH AFFECTS MEAT QUALITY**

The major concerns for economic output and desirable pork quality are reduction of drip loss and improvement of color (Maribo et al., 1998). Ultimate pH strongly influences meat color and water holding capacity (Cross et al., 1986; Warner et al., 1997; Joo et al., 1999, Van Laack, 2000).

#### **pHu affects water holding capacity (WHC)**

Weight loss is economically important to the meat industry. Because drip holds about two-thirds the protein concentration of meat, there is a great loss due to this animal protein waste (Van Laack and Solomon, 1994). Water holding capacity strongly affects juiciness, yield and palatability of the cooked meat product (Kauffman et al., 1994). According to Hamm (1986), the huge economic loss due to storage, freezing and thawing, or cooking of meat is related to the

binding of water within the muscle. Therefore, the power of binding water is of particular importance for meat quality. The ability of meat to retain its own water despite the application of force is defined as water holding capacity (Van Laack and Solomon, 1994).

Meat contains about 75-80% water. A very small part of water (4 to 10g/100 g protein) is bound tightly. A portion of tissue water (20 to 60 g/100 g protein) is electrostatically bound and located between the protein molecules (Van Laack and Solomon, 1994). The major part of water is present as free water, which can migrate throughout the muscle tissue. According to Offer and Knight (1988), most tissue water is located in myofibrils trapped in the three-dimensional network of the filaments.

The influence of ultimate pH on WHC, in either whole or ground meat, is obvious. A pH of 5.0, which corresponds to the isoelectric point (I.E.P) of the myofibrillar proteins, results in a minimum net charge of protein (Hamm, 1986). When the pH is higher or lower than the I.E.P, proteins have a surplus of negative or positive charges, respectively. Repulsion between filaments caused by the surplus of charges increases the interfilament space, and therefore increases the WHC. In the range of pH 5.0-6.5, the range of practical interest in meat, any change of pH has a great influence on the WHC (Hamm, 1986).

The amount of drip loss in fresh uncooked meat is commonly used to

describe WHC (Van Laack and Solomon, 1994). Many studies on ultimate meat quality have reported a relationship between pHu and drip loss. They all found low drip loss values (higher WHC) accompanied with higher pHu in pigs (Warriss and Brown, 1987; Kauffman et al., 1993; Lundström et al., 1996; Maribo et al. 1998; Gariépy et al., 1999).

### **pHu affects color of pork**

For a long time, the meat industry has been challenged to seek ways to produce meat products with acceptable physical appearance. For the consumers, meat color is one of the most important quality factors affecting their purchase decision (Risvik, 1994).

Meat color is mainly determined by the amounts of muscle pigments (myoglobin, hemoglobin and cytochrome) and their chemical state. Within the heme portion of myoglobin, two chemical reactions are involved in meat color changes. The interchange of myoglobin (purple color) and oxymyoglobin (red color) corresponds to a different molecule bound at the free binding site of heme. When oxygen occupies the sixth binding site of ferrous heme iron, myoglobin is converted to bright-red oxymyoglobin. The reduced forms of myoglobin (myoglobin and oxymyoglobin) readily oxidize to the undesirable brownish-red color of metmyoglobin, where the heme iron is oxidized from the ferrous ( $\text{Fe}^{2+}$ ) to

the ferric ( $\text{Fe}^{3+}$ ) state (Cross et al., 1986).

The ultimate pH of “normal” meat is within the range of 5.4-5.8. According to Cross et al. (1986), low pH favors the oxidation of myoglobin by denaturing the globin protein moiety. The globin protein moiety functions to protect the heme and causes the subsequent disassociation of oxygen as well as oxidation of the iron molecule

Another example of ultimate pH affecting meat color is DFD meat. DFD meat is characterized by a dark color, firm texture, and dry appearance. It is associated with low muscle glycogen reserves at the time of slaughter and has an ultimate pH of higher than 6.0. The high pHu in DFD is responsible for a higher WHC than normal meat (Hamm, 1986). A tighter structure in high-WHC meat retards the rate of oxygen diffusion and subsequent pigment oxygenation. Consequently, the light absorption and reflection-characteristics-from-the meat surface are decreased.

Dransfield et al. (1985) reported that the lightness ( $L^*$ ), hue angle (H) and saturation (S) of pork decreased with increasing pHu. According to Warriss and Brown (1987), low ultimate pH is associated with high reflectance values.

Based on the  $L^*$  values and drip loss, pork can be classified as follows (Kauffman et al. 1993):

- PSE: pale, soft and exudative;  $L^* > 58$ ; drip loss  $> 5.0\%$
- RSE: reddish, soft and exudative;  $L^* = 52-58$ ; drip loss  $> 5.0\%$
- PFN: pale, firm and non-exudative;  $L^* > 58$ ; drip loss  $< 5.0\%$
- RFN: reddish, firm and non-exudative;  $L^* = 52-58$ ; drip loss  $< 5.0\%$
- DFD: dark, firm and dry;  $L^* < 52$ ; drip loss  $< 5.0\%$

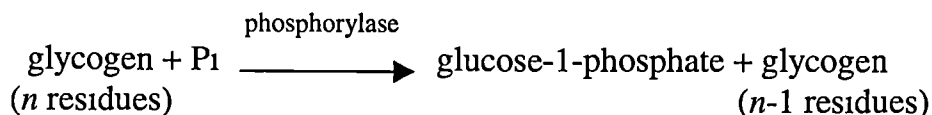
Ultimate pH in PSE, RSE, PFN, RFN are all in the same range (pH 5.4-5.8). DFD has an ultimate pH higher than 6.0.

### **FACTORS THAT MAY AFFECT ULTIMATE pH**

Currently, the determinants of ultimate pH are not clear. Before we can consistently produce pork with a pH of 5.6-5.9, we need to know what factors determine ultimate pH of pork.

### **Glycogen**

Glycogen is a branched polysaccharide made from  $\alpha$ -D-glucose units linked through  $\alpha$ -1,6-glycosidic and  $\alpha$ -1,4-glycosidic bonds (Stryer, 1998). It is the main storage carbohydrate present in animal cells. Glycogen is hydrolyzed to glucose-1-phosphate by the action of glycogen phosphorylase.





After slaughter, the muscle converts glycogen via glucose-1-phosphate into lactate and energy. The degradation of glycogen helps to replenish the high-energy phosphate compound ATP, and results in the formation of lactic acid accounting for the pH decline in post-mortem muscle (Pearson and Young, 1989). Ultimate pH in meat depends primarily on muscle glycogen levels at slaughter (Bendall, 1973; Warriss et al., 1989).

According to Van Laack (2000), it is impossible to accurately measure the glycogen content in muscle. The great variability of breed, type of stresses and different muscle types all affect the glycogen content (Pearson and Young, 1989). Glycolytic potential (GP) is a good approximation of muscle glycogen content (Fernandez et al., 1992). Post mortem, all components that can potentially be converted into lactate are represented by glycolytic potential (Monin and Sellier, 1985; Maribo et al., 1999). Recently, studies on the relationship between ultimate pH and GP have been reported. Van Laack and Kauffman (1999) found that there was a trend towards higher pH with lower GP. Forty percent of the variation in ultimate pH could be explained by the variation in GP. They concluded that there are other determinants of ultimate pH. Similar results have been reported by Maribo et al. (1999).

In comparison with other breeds, the RN-gene pig has a lower pH<sub>u</sub> and higher glycolytic potential (Monin and Sellier, 1985; Lundström et al., 1996).

There is a lack of a correlation between GP and pHu in RN-gene muscle (Lundström et al., 1996; Gariepy et al., 1999). Identification of RN-carrier pigs is judged by glycolytic potential. The pigs with GP > 180  $\mu\text{mol/g}$  are considered RN carriers (Lundström and Enfalt, 1997). According to the results of Monin and Sellier (1985) and Lundström et al. (1998), the GP in muscle of RN-gene pigs is 30-70% higher than the muscle of pigs without the RN gene. The difference in pHu is only about 0.1 units. It seems that the glycolysis ceases at a 'limit value' (around pH 5.4-5.5), even when a large amount of residual glycogen remains (Monin et al., 1987; Van Laack, 2000).

### **Creatine phosphate**

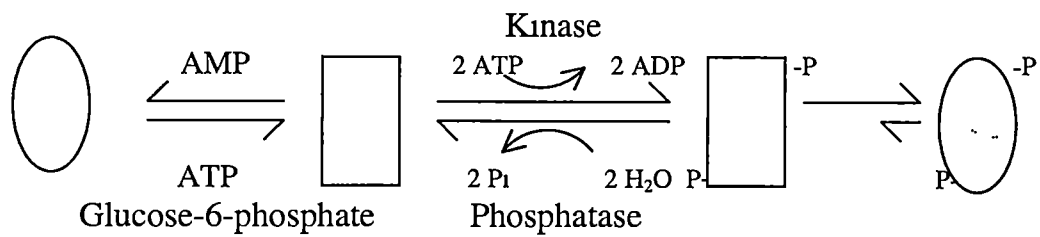
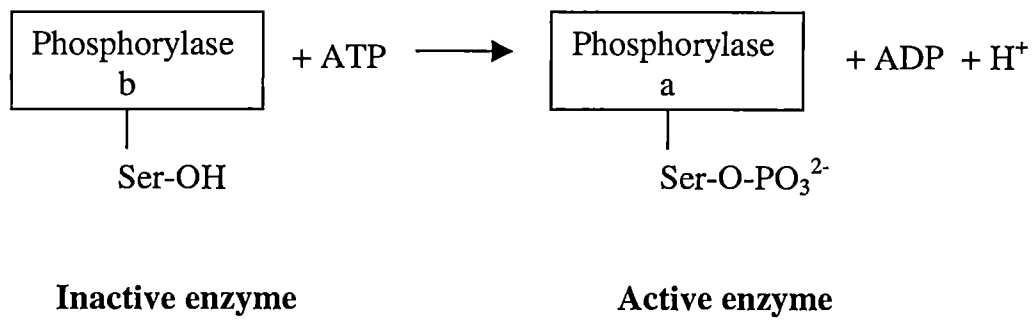
Creatine phosphate is an energy-rich compound with a free energy of hydrolysis ( $\Delta G^0$ ) of -10.2 Kcal/mol. Post mortem, CP plays an important role in the process of conversion of muscle to meat (Pearson and Young, 1989). CP serves to buffer ATP levels through the Lohman reaction ( $\text{ADP} + \text{CP} \rightleftharpoons \text{ATP} + \text{Cr}$ ) (Greaser, 1986). When ATP is broken down, the ADP is immediately reconverted to ATP. The initial levels of CP in muscles vary considerably with species (Bendall, 1973). The produced creatine has an alkaline character, its presence may limit pH decline.

## Enzymes

Various enzymatic processes are involved in post-mortem muscle metabolism.

The important enzymes affecting pHu are glycogen phosphorylase and AMP deaminase (Scopes, 1974).

Glycogen phosphorylase is a key enzyme in glycolytic control. It is specific for  $\alpha$ -D-glucose-1-phosphate and  $\alpha$ -1,4-glucosidic bonds in polysaccharides. Glycogen phosphorylase exists in muscle in two forms—active phosphorylase a and inactive phosphorylase b. Phosphorylase b is activated by the presence of adenosine monophosphate (AMP) and inactivated by ATP (negative allosteric effectors by competing with AMP) and glucose-6-phosphate (primarily through binding AMP site) Phosphorylase a is relatively insensitive to AMP, ATP and glucose-6-phosphate. Glycogen phosphorylase is regulated by allosteric mechanisms and phosphorylation (Fig.1) (Stryer, 1998). The conversion of phosphorylase b to phosphorylase a is under the presence of ATP and  $Mg^{2+}$  by phosphorylase kinase. There are two conformations for phosphorylase a and b—the inactive T form and the active R form (Fig.1). Phosphorylase a is dominated by the active R form. Phosphorylase b is mostly in the inactive T form unless there are high levels of AMP and low levels of ATP and glucose-6-phosphate. Phosphorylase b is converted to phosphorylase a in the presence of ATP and phosphorylase kinase. The reports on glycolytic rate related to phosphorylase



**Phosphorylase b**      **Phosphorylase b**      **Phosphorylase a**      **Phosphorylase a**  
 (Active R form)      (Inactive T form)      (Inactive T form)      (Active R form)

Fig. 1. Control of glycogen phosphorylase in skeletal muscle (Stryer, 1998)

activities and pork quality are contradictory. Ono et al. (1977) found that there was a higher glycogen phosphorylase activity in the muscle of PSE vs. normal pigs. In a study of glycolytic intermediates in “fast-” and “slow-glycolyzing” muscle, Kastenschmidt et al. (1968) supported that the increased phosphorylase activity in “fast-glycolyzing” muscle was the main cause for the accelerated glycolytic rate. However, Schwagele et al. (1996) could not find any differences in structural and kinetic characteristics of phosphorylase from normal and PSE muscles.

In a reconstituted glycolytic system, Scopes (1974) studied the influence of substrate concentration and enzyme activity on ultimate pH. He suggested that if glycogen is not limiting, the ultimate pH is mostly related to the amount of phosphorylase a. His results showed that higher amounts of phosphorylase a gave lower pHu values.

Another enzyme that affects ultimate pH is AMP deaminase (Scopes, 1974). AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) is a sarcoplasmic protein that is associated with the thick filaments in myofibrils (Cooper and Trinick, 1984). It catalyses the irreversible hydrolytic deamination of AMP into IMP and ammonia:  $AMP + H_2O \rightarrow IMP + NH_3$ . Chapman and Atkinson (1973) suggested that AMP deaminase might be implicated in the regulation of energy metabolism through controlling the relative concentrations of adenine nucleotides. Lowenstein (1972) suggested that this regulation is either by displacing the

myokinase reaction ( $2 \text{ ADP} \rightleftharpoons \text{AMP} + \text{ATP}$ ) to the direction of ATP formation by removing AMP, or by stimulating the key glycolytic enzyme phosphofructokinase (PFK) with ammonia. The activity of AMP deaminase is related to the control of pH and PFK, the higher AMP concentration, the faster the AMP is deaminated (Scopes, 1970). Scopes (1974) found that higher level of AMP deaminase resulted in a higher pHu.

### **Other possible determinants of pHu**

The ultimate pH is not only determined by the presence of acids and bases, but also by the presence of strong ions (Stewart, 1981). Differences in muscle buffering capacity and the concentration of strong ions such as  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  will affect ultimate pH (Stewart, 1981). Buffering capacity (BFC) is the ability of weak acids to resist the change of pH when acid or alkali is added. Titratable acidity (TA) is used to test the quantity of acid in meat (Gault, 1985). The change in free amino groups or carboxylic groups will result in an alteration of TA (Madovi, 1980). In order to understand how the ions affect the ultimate pH, buffering capacity and titratable acidity need to be evaluated.

### **Conclusions**

Ultimate pH of pork is one of the most important quality determinants of pork.

The NPPC quality solutions team suggested a pHu of 5.6 to 5.9 as a target for acceptable pork quality. In order to consistently produce pork products with ultimate pH within the range of 5.6 to 5.9, we need to understand what factors are important in determining pHu. Glycolytic potential seems important for pHu, but cannot explain more than 50% of the variation in pHu. The enzyme systems such as the activities of phosphorylase and AMP deaminase may also be important, but how important has not yet been established. In order to evaluate the possible determinants of ultimate pH, the effects of substrate level (glycogen and creatine phosphate), buffering capacity, titratable acidity, activities of glycogen phosphorylase and AMP deaminase have to be evaluated.

## CHAPTER 3

### MATERIALS AND METHODS

#### SLAUGHTERING AND SAMPLING

Sixty pigs, 10 each of six genetic lines, were slaughtered at the University of Tennessee Meat Laboratory in groups of 20, 25 and 15. Within 5 min after stunning, a muscle sample of 25 g longissimus muscle (LM) was collected by coring (diameter of 1.7 cm) and frozen immediately in liquid nitrogen. This sample was stored at -80 °C until the analysis of various metabolites (creatine phosphate, glycolytic potential, ATP) and enzyme activities, such as AMP deaminase and phosphorylase a and a+b. Temperature and pH were measured at 5 min post mortem.

Carcasses were further processed and chilled. At 18-24 h post mortem, ultimate pH, surface color, water holding capacity (drip loss and filter paper wetness), buffering capacity and titratable acidity were assessed.

The protein denaturation will result in enzyme denaturation, PSE pork was not included in our study for evaluation of determinants of pHu. Samples with L\* > 58, drip loss > 5% were considered PSE (Kauffman et al., 1993).



## **MATERIALS AND METHODS**

### **pH**

At 5 min and 24 h post mortem, the pH was assessed in each sample by using an Orion 250 portable pH meter (Orion Research, Beverly, MA) and a Mettler-Toledo combination spear tip glass electrode (Mettler-Toledo Process, Analytical Inc., Wilmington, MA). I placed the glass electrode directly into the longissimus muscle and measured the pH.

### **Surface color (CIE-L\*,a\*,b\*)**

The measurement was taken at 24 h post mortem. Surface color was measured in triplicate on a freshly cut surface of a pork loin chop after 30 min of exposure to air ("blooming"), using a Minolta Spectrophotometer CM-508d (Minolta Camera Co., LTD., Japan). Measurement was run at 2° viewing angle and D65 illuminant.

### **Drip loss**

Drip loss was measured in duplicate as the weight loss during 48 h suspension of a standardized (4-cm diameter, 2.5-cm thickness) sample of approximately 50 g at 4 °C (Kauffman et al., 1986).

### **Filter paper wetness method**

Filter paper wetness was determined in each sample according to the method reported by Kauffman et al. (1986).

### **Glucose, lactate, glycogen and glycolytic potential**

Glucose, glucose-6-phosphate, lactate and glycogen were determined according to Bergmeyer (1974). To determine glycolytic potential, I used the formula described by Monin et al. (1987).

Glycolytic potential ( $\mu\text{mol}$  of lactate/gram of meat) = 2 X (glucose + glycogen + glucose-6-phosphate) + lactate.

Samples with glycolytic potential of more than 180  $\mu\text{mol}$  lactate/g were considered RN carriers.

### **Metabolites**

#### *ATP*

ATP measurement was determined according to Bergmeyer (1974).

#### *CP*

A UV Scanning Spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD) was used for this measurement. A modification of the procedure by Bergmeyer (1974) was used. Creatine kinase was prepared as 10,000 U/mL

with triethanolamine buffer, instead of 1000 U/mL. This high concentration of creatine kinase resulted in a complete conversion of creatine phosphate into creatine within 30 min.

The mixture was incubated (25 °C in water bath) for 50 min instead of 25 min. Measure and record the absorbance at 340 nm as E1. Five µl of creatine kinase was added into the mixture, mixed and extinction E2 (340 nm) read after 30 min incubation instead of 15 min. Doubling the time of incubation made the reactions of ATP to ADP and creatine phosphate to creatine complete.

### **Buffering capacity**

The measurement of buffering capacity was modified according to Monin and Sellier (1985) and Sayre et al. (1963). We used 2.5 g of muscle (in 25 mL of 0.005 M Na-iodoacetate solution) instead of 5 g of muscle (in 50 mL of solution).

### **Titrateable acidity**

Titrateable acidity of each sample was determined by method reported by Madovi (1980).

### **Enzyme activities determination**

#### *1. Phosphorylase a and a+b*

The activity of phosphorylase a was measured as described by Bergmeyer (1974). The activity of phosphorylase a + b was measured by adding AMP (2mM final concentration) in the reaction medium (Monin et al., 1987). The state of activation of phosphorylase was expressed as the ratio between activities of forms a and a+b. One unit (U) of enzyme activity was defined as the production of 1  $\mu$ mol of glucose-1-phosphate per min per gram of muscle at 30 °C.

Before we ran the enzyme test, we prepared  $\text{NH}_4^+$ -free phosphoglucomutase (PgluM). Millipore filter VMWP 047 (Millipore Corporation, Bedford, MA 01730) was used to remove ammonia by dialyzing against acetate buffer (50 mM, pH 4.8). The procedure was as follows: the membrane, shiny side up, was placed on the acetate buffer. Then, I carefully pipetted the enzyme onto the surface of the membrane. After 60 min without disturbing, I withdrew the drop of liquid from the surface of the membrane and diluted it to the appropriate concentration (Greaser, personal communication).

## *2. AMP deaminase*

The procedure for AMP deaminase activity by Purzycka-Preis et al. (1975) was modified. The difference was the extraction medium used. I used K-phosphate buffer (pH 7.4) containing 5 mM DTT and 0.5 mM EDTA instead of 0.6 M KCl containing 1mM mercaptoethanol. The released ammonia was measured according

to Chaney and Marbach (1962) The calibration curve was prepared by using ammonium sulfate One unit (U) of AMP deaminase activity was defined as the production of 1 $\mu$ mol ammonia per min per gram of muscle at 30 °C.

### **Statistics**

The effects of glycogen content, creatine phosphate, buffering capacity, titratable acidity, AMP deaminase and glycogen phosphorylase on ultimate pH of pork with and without RN gene were investigated. The correlation between each factor, multiple linear regression and polynomial regression were used to identify which factors explain the variation in ultimate pH (SAS Institute, Inc., 1999). Variable selection (stepwise) was used to develop a model for predicting ultimate pH based on the effects measured in this study.

## CHAPTER 4

### RESULTS AND DISCUSSION

The 60 samples were classified into two groups based on their different physical and biochemical characteristics (Table 1). The meat quality characteristics used for classification were L\*-value, drip loss, filter paper wetness and pHu. The pH decline within 5 min after death reflects the glycolytic rate, so it serves as an additional criterion for classification (PSE samples are expected to have a fast pH decline). Of the 60 pigs, seven were identified as PSE. The remaining 53 were normal. No DFD meat was found in this study.

The means of L\* value in PSE and normal pork were 61.1 and 52.9, respectively (Table 1). According to Kauffman et al. (1993), meat with L\* value > 58 and drip loss > 5% can be considered PSE. The filter paper wetness and drip loss are often used to describe water holding capacity. The means of drip loss in PSE and normal pork were 7.1% and 2.4% (Table 1). The mean values of filter paper wetness in PSE and normal pork were  $100 \pm 36$  and  $44 \pm 26$  mg. All the results of L\* value, drip loss and filter paper wetness in PSE and normal pork were consistent with earlier observations by Kauffman et al. (1986), Kauffman et al. (1993) and Van Laack and Kauffman (1999).

The mean ultimate pH in PSE and normal pork was 5.47 and 5.56, respectively, which is similar to other observations (Kauffman et al., 1986;

Table 1. Quality characteristics at 24 h post mortem, pH changes within 5 min and temperature at 5 min post mortem and glycolytic potential (GP) of pork longissimus muscle (mean  $\pm$  standard deviation)

<b>Characteristic</b>	<b>PSE <sup>(1)</sup> (n=7)</b>	<b>Normal (n=53)</b>
<b>L* value</b>	61.1 $\pm$ 4.1	52.9 $\pm$ 2.5
<b>Drip loss %</b>	7.1 $\pm$ 2.8	2.4 $\pm$ 2.3
<b>FPW, mg</b>	100 $\pm$ 36	43 $\pm$ 26
<b><math>\Delta</math>pH <sup>(2)</sup></b>	0.95 $\pm$ 0.22	0.60 $\pm$ 0.23
<b>Temp, °C</b>	39.9 $\pm$ 1.7	39.2 $\pm$ 0.5
<b>pHu</b>	5.47 $\pm$ 0.08	5.56 $\pm$ 0.12
<b>GP, <math>\mu</math>mol lactate/g</b>	148 $\pm$ 38	132 $\pm$ 48

<sup>(1)</sup> PSE = pale, soft, exudative.

<sup>(2)</sup>  $\Delta$ pH = the pH change in 5 min after slaughter, pH7.2 was used as initial pH

FPW = filter paper wetness

Temp = the temperature at 5 min after slaughter.

Kauffman et al., 1993; Maribo et al., 1998).

The pH change within 5 min after slaughter was used as an additional indicator for glycolytic rate. I took pH 7.2 as pH at death. PSE is the result of protein denaturation caused by a combination of low pH and high temperature. The pH change within 5 min after slaughter in PSE and normal were  $0.95 \pm 0.22$  and  $0.60 \pm 0.23$ . The temperature was  $39.9 \pm 1.7$  ° C in PSE and  $39.2 \pm 0.5$  ° C in normal pork.

In comparison with a report by Van Laack and Kauffman (1999), I got a similar GP value in PSE (mean value of 148  $\mu\text{mol/g}$ ), but the GP value (132  $\mu\text{mol/g}$ ) in normal meat was higher than the 97  $\mu\text{mol/g}$  found by Van Laack and Kauffman (1999). A similar GP value (mean of 131  $\mu\text{mol/g}$ , min-max: 43-242  $\mu\text{mol/g}$ ) in normal pork was reported by Maribo et al. (1999). The high GP value in my study may be the result of inclusion of RN carriers in the sample population.

The CP and ATP levels I found (Table 2) were comparable to those reported by Bendall et al. (1963). Bendall et al. (1963) reported a lower level of lactate (about 5  $\mu\text{mol/g}$ ) than I found (Table 2). Maribo et al. (1999) found a lactate level of 13.4-51.1  $\mu\text{mol/g}$  at 4 min post mortem.

At 5 min after slaughter, the absolute phosphorylase a activity (Pa) was  $21 \pm 12$  U ( $\mu\text{mol/min per gram of muscle at } 30$  °C) and the total phosphorylase (a+b) was  $84 \pm 13$  U. The relative activity of phosphorylase a (GPa) was 26.8



Table 2. The mean value of various biochemical parameters in “normal” pork longissimus muscle (n=53)

Variables	Mean	Std. Dev.	Range
pHu	5.56	0.12	5.31-5.88
GP, $\mu\text{mol lactate/g}$	143	52	48-252
CP, $\mu\text{mol/g}$	8.9	6.7	0.7-26.8
ATP, $\mu\text{mol/g}$	5.3	0.6	3.6-6.7
Lactate, $\mu\text{mol/g}$	41.2	13.0	2.2-66
GPa, %	26.8	16.2	2.3-60.7
Pa, U*	21	12	3-49
Pa+b, U*	84	13	66-132
AMPD, U*	42.2	13.9	1.9-75.0
BFC	5.4	0.2	5.0-5.8
TA	1.39	0.15	1.04-1.77

pHu = ultimate pH, GP = glycolytic potential, CP = creatine phosphate at 5 min post mortem

GPa = relative phosphorylase a activity  $(a/a+b) \times 100\%$

U\* = unit of enzyme activity,  $\mu\text{mol/min per gram of tissue at } 30^\circ\text{C}$

Pa = phosphorylase a activity at 5 min post mortem.

Pa+b = total phosphorylase activity at 5 min post mortem

AMPD = AMP deaminase at 5 min post mortem.

BFC = buffering capacity,  $10^{-5}$  equivalents per pH unit per gram of fresh tissue

TA= titratable acidity, the amount (mL) of 0.02 N NaOH to titrate 20 mL solution sample (1% w/v) in 3% SDS (end point pH=9.00)

$\pm 16.2\%$ . All these results were consistent with those reported by Monin et al. (1987).

AMP deaminase activity (AMPD) at 5 min after slaughter was  $42.2 \pm 13.9$  U. This value is lower than measurements in pig muscle by Fishbein et al. (1993). They reported a higher AMPD (500 U) in pig muscle, while other animals showed a much lower AMPD (rabbit muscle was 78-122 U and human muscle was 62-120 U). Their spectrophotometric method with an unusual high substrate level (10 mmol/L) made the result inaccurate and may have doubled their value of AMPD in animal muscle (Fishbein et al., 1993). Most reports on animal heart muscle showed AMPD activities less than 5 U (Purzycka-Preis and Zydowo, 1987; Jenkins et al., 1991; Wegelin et al., 1995).

The mean buffering capacity (BFC) was 5.4, which is consistent with 4.97-5.84 by Sayre et al. (1963) and the  $5.7 \pm 0.5$  by Monin and Sellier (1985). Titratable acidity (TA) was  $1.39 \pm 0.15$ . This TA level was in agreement with the value of 1.42 reported by Madovi (1980) in pork.

The main objective of the study was to evaluate which factors determine the ultimate pH. Glycolytic potential (GP) and relative activity of phosphorylase a (GPa) were significantly ( $p < 0.001$ ) correlated with ultimate pH (Table 3). A highly significant negative correlation between pH<sub>u</sub> and GP ( $r = -0.61$ ) indicated that an increased GP results in lower ultimate pH. Similar results on the

Table 3. Pearson correlation coefficients (r) among different biochemical characteristics of normal pork longissimus muscle (n=53)

	GP	CP	GPa	AMPD	BFC	TA	Pa	Pa+b
<b>pHu</b>	-0.65***	-0.19	-0.52***	0.32*	-0.43**	-0.27	-0.47***	0.47***
<b>GP</b>	1.00	0.36**	0.54***	-0.47***	0.46***	0.35*	0.52***	-0.26
<b>CP</b>		1.00	0.65***	-0.27*	0.26	0.10	0.69***	0.23
<b>GPa</b>			1.00	-0.40**	0.41**	0.31*	0.98***	-0.58***
<b>AMPD</b>				1.00	-0.30*	-0.17	-0.42**	0.16
<b>BFC</b>					1.00	0.42**	0.42**	-0.30*
<b>TA</b>						1.00	0.31*	-0.18
<b>Pa</b>							1.00	-0.47***

\*\*\* Significant,  $P < 0.001$ , \*\* Significant,  $P < 0.01$ , \* Significant,  $P < 0.05$ .

pHu = ultimate pH, GP = glycolytic potential,  $\mu\text{mol lactate/g}$

CP = creatine phosphate,  $\mu\text{mol/g}$  at 5 min post mortem.

GPa = activity of glycogen phosphorylase a/a+b (%) at 5 min post mortem

Pa = phosphorylase a activity at 5 min post mortem

Pa+b = total phosphorylase activity at 5 min post mortem

AMPD = activity of AMP deaminase at 5 min post mortem

BFC = buffering capacity,  $10^{-5}$  equivalents per pH unit per gram of fresh tissue

TC = titratable acidity, the amount (mL) of 0.02 N NaOH to titrate 20 mL solution sample

(1% w/v) in 3% SDS (end point pH=9.00)

relationship between GP and pHu were reported by Van Laack and Kauffman (1999) and Maribo et al. (1999). The negative correlation between pHu and GPa ( $r = -0.52$ ) suggests that an increased phosphorylase a activity also decreases the ultimate pH. This is in agreement with Scopes (1974), who used a reconstituted glycolytic system.

There was a significant negative correlation ( $p < 0.01$ ) between pHu and buffering capacity (BFC); high buffering capacity in meat increases the ability of meat to resist the pH decrease, and therefore will result in a high ultimate pH ( $r = -0.43$ ). Titratable acidity (TA) is a measure of the quantity of acid in meat. It was significantly correlated ( $p < 0.05$ ) with GP ( $r = 0.35$ ) and GPa ( $r = 0.31$ ); high GP and Gpa were associated with high TA. I did not find a significant correlation between titratable acidity and pHu.

The activity of AMP deaminase (AMPD) had a significant correlation ( $p < 0.05$ ) with ultimate pH. A high AMPD was associated with a high pHu ( $r = 0.32$ ). This is in agreement with the results reported by Scopes (1974).

It was interesting to find a significant correlation ( $p < 0.001$ ) between GP and GPa ( $r = 0.54$ ), and between GP and AMPD ( $r = -0.47$ ). A high glycolytic potential was associated with high activity of phosphorylase a and low activity of AMP deaminase. These correlations may contribute to the explanation of the phenomena (acid meat) occurring in RN-carrier pork. Sayre et al. (1963) found

that Hampshire pork (RN carrier) had a higher total phosphorylase activity than other breeds. Estrade et al. (1994) reported that phosphorylase a activity (GP<sub>a</sub>) was not increased in RN-carrier pork. I found significant correlations between pH<sub>u</sub> and the absolute phosphorylase a (P<sub>a</sub>) ( $r = -0.47$ ,  $p < 0.001$ ), and between pH<sub>u</sub> and total phosphorylase activity (P<sub>a+b</sub>) ( $r = 0.47$ ,  $p < 0.001$ ). I also found a significant correlation between GP and P<sub>a</sub> ( $r = 0.52$ ,  $p < 0.001$ ), but I did not find a significant correlation between GP and total phosphorylase activity ( $p < 0.05$ ). The highly significant correlation ( $r = 0.98$ ,  $p < 0.001$ ) between GP<sub>a</sub> and P<sub>a</sub> suggested that P<sub>a</sub> was sufficient to represent the activity of phosphorylase a in muscle without using relative activity (GP<sub>a</sub>).

The polynomial regression among variables (Table 4) showed that GP is the most important factor in explaining pH<sub>u</sub>; it explained 42% of the pH<sub>u</sub> variability. An increased glycolytic potential would decrease the ultimate pH (Fig. 2). This result was in agreement with Van Laack and Kauffman (1999) and Maribo et al. (1999).

GP could not completely explain the difference of pH<sub>u</sub>. Possibly, the presence of RN carriers influenced results. As previously reported (Monin and Sellier, 1985; Lundström et al., 1998), there is a lack of correlation between GP and pH<sub>u</sub> due to the RN gene. In comparison with normal pork, the RN-gene type pork has a 30-70% higher GP, while the difference in pH<sub>u</sub> between

Table 4. Polynomial regression among biochemical variables that may explain ultimate pH of normal pork longissimus muscle (n=53)

Variables	R-square	Significance	Variables	R-square	Significance
<b>GP (<math>\mu\text{mol/g}</math>)</b>			<b>CP (<math>\mu\text{mol/g}</math>)</b>		
Linear	0.42	***	Linear	0.04	
Quad	0.45		Quad	0.05	
Cubic	0.45		Cubic	0.07	
<b>GPa (U)</b>			<b>AMPD (U)</b>		
Linear	0.28	***	Linear	0.10	*
Quad	0.31		Quad	0.12	
Cubic	0.35		Cubic	0.15	
<b>BFC<sup>(1)</sup></b>			<b>TA<sup>(2)</sup></b>		
Linear	0.19	**	Linear	0.07	
Quad	0.19		Quad	0.16	*
Cubic	0.19		Cubic	0.16	

\*\*\* Significant,  $p < 0.001$ , \*\* Significant,  $p < 0.01$ , \* Significant,  $p < 0.05$

GP = glycolytic potential, CP = creatine phosphate at 5 min post mortem.

GPa = relative phosphorylase a activity ( $a/a+b$ ), % at 5 min post mortem

U = unit of enzyme activity,  $\mu\text{mol/min}$  per gram of tissue at  $30^\circ\text{C}$

AMPD = AMP deaminase at 5 min post mortem

BFC<sup>(1)</sup> = buffering capacity,  $10^{-5}$  equivalents per pH unit per gram of fresh tissue.

TC<sup>(2)</sup> = titratable acidity, the amount (mL) of 0.02 N NaOH to titrate 20 mL solution sample (1% w/v) in 3% SDS (end point pH=9.00)

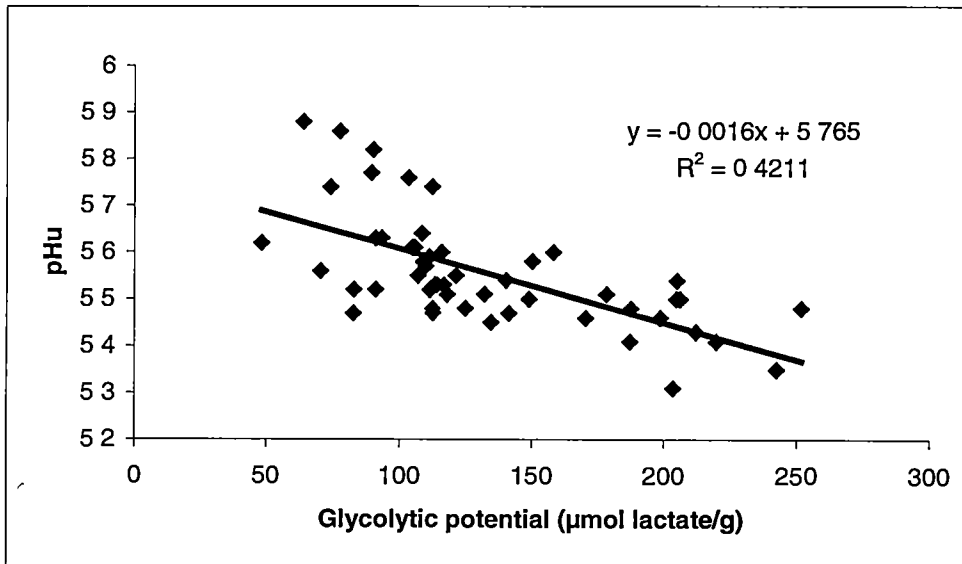


Fig 2 The relationship between glycolytic potential and ultimate pH of pork longissimus muscle (n=53)

normal and RN-gene type pork was only about 0.1 (Monin and Sellier, 1985; Lundström et al., 1998). To test the possible influence of RN carriers on my results, I determined correlations among all factors (SAS, 1999) using only non-RN carriers (n= 42). The Pearson correlation (Table 5) showed a lower correlation ( $r = -0.53$ ,  $p < 0.001$ ) between GP and pHu in non-RN pigs compared to the total samples ( $r = -0.65$ ,  $p < 0.001$ ) (Table 3). There was a significant correlation ( $r = -0.34$ ,  $p < 0.05$ ) between ultimate pH and activity of phosphorylase a (GP<sub>a</sub>) (Table 5).

Glycogen in muscle has been studied for many years. Two different types of glycogen seem to be present in muscle—macroglycogen (MG) and proglycogen (PG). The smaller molecular weight (400 KDa) macroglycogen has 10% protein components, and thus can be extracted by a low concentration of trichloroacetic acid (TCA). Proglycogen has a relatively high molecular weight of  $10^7$  Da and is insoluble in acid solution (Adamo and Graham, 1998). The increase of total glycogen in muscle will mainly increase the MG% (Adamo et al., 1998). Lomako et al. (1991) indicated that PG may be an intermediate in the synthesis and degradation of MG. Adamo and Graham (1998) stated that PG is the precursor of MG. So the content of PG may be more closely related to pHu. To clearly understand the importance of these two forms of glycogen in pork post-mortem glycolysis, further work needs to be done in future studies.



Table 5. Pearson correlation coefficients (r) among variables in non-RN pork longissimus muscle (n=42)

	GP	CP	GPa	AMPD	BFC	TA
pHu	-0.53***	0.03	-0.34*	0.14	-0.44**	-0.22
GP	1.00	-0.08	0.27	-0.27	0.58***	0.33*
CP		1.00	0.64***	-0.09	0.09	0.03
GPa			1.00	-0.25	0.39*	0.27
AMPD				1.00	-0.21	-0.04
BFC					1.00	0.38*

\*\*\* Significant,  $P < 0.001$ , \*\* Significant,  $P < 0.01$ ; \* Significant,  $P < 0.05$ .

pHu = ultimate pH GP = glycolytic potential,  $\mu\text{mol/g}$

CP = creatine phosphate,  $\mu\text{mol/g}$  at 5 min post mortem.

GPa = activity of glycogen phosphorylase a/a+b, % at 5 min post mortem.

AMPD = activity of AMP deaminase,  $\mu\text{mol/g}$  at 5 min post mortem

BFC = buffering capacity,  $10^{-5}$  equivalents per pH unit per gram of fresh tissue at 24 hr post mortem

TC= titratable acidity, the mL of 0.02 N NaOH to titrate 20 mL sample solution (1% w/v) in 3% SDS to an end point of pH 9.00.

Enzyme activities are particularly important for post-mortem glycolysis. One key enzyme in metabolism is phosphorylase. At early post mortem, the main form (inactive b form) can be activated by a certain level of AMP. The activity of phosphorylase a (GPa) was expressed as the ratio of phosphorylase a to the total activity of phosphorylase a + b. As shown in Fig.3, phosphorylase a explained 28% of the variation in pHu in normal and RN pork. Higher activity of phosphorylase a is associated with lower ultimate pH. This relationship was in agreement with the results of Scopes (1974).

As shown in Table 3, there was no significant correlation between pHu and the concentration of CP at 5 min after slaughter. Creatine phosphate explained less than 5% of the difference in pHu (Table 4). Creatine phosphate concentration at 5 min after death varied from 0.7 to 26.8  $\mu\text{mol/g}$  in pork longissimus samples (Table 2). Bodwell et al. (1966) found that the initial creatine phosphate in the muscle of all 18 pigs was below detectable amounts ( $<0.3 \mu\text{mol/g}$ ). They suggested that the electric stunning results in the depletion of CP within a few minutes after death. In the Lohman reaction ( $\text{ADP} + \text{CP} \rightleftharpoons \text{ATP} + \text{Cr}$ ), the creatine produced is an alkaline substance. The concentration of CP in muscle may not be high enough to buffer the pH decline.

Buffering capacity (BFC) is the ability of meat to resist pH change caused

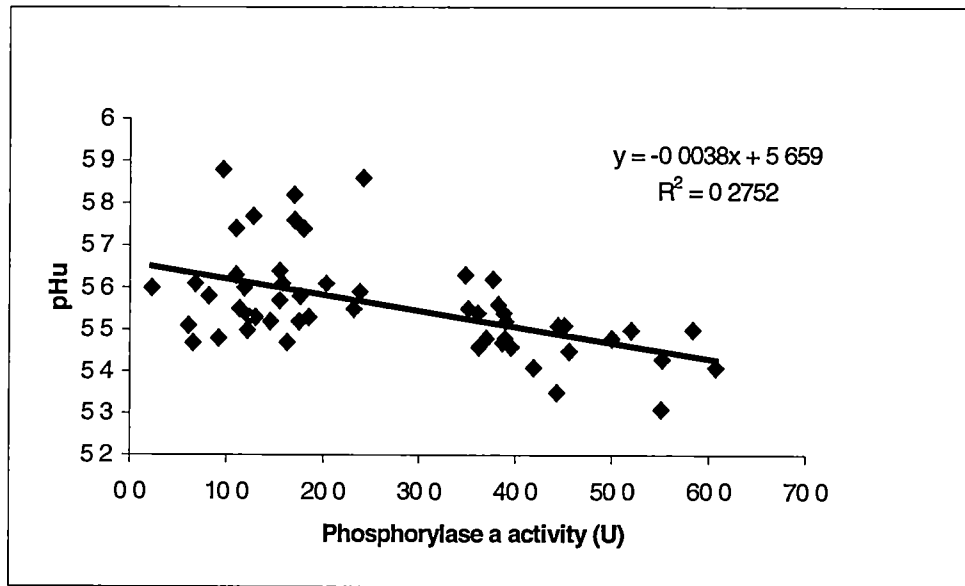


Fig.3. The relationship between ultimate pH (pHu) and phosphorylase a activity of pork longissimus muscle (n=53); U=  $\mu\text{mol}/\text{min}$  per gram of muscle at 30 °C

by the addition of acid or alkali. BFC explained 19% (Table 4) of ultimate pH.

Another enzyme that may affect pHu, is AMP deaminase. It regulates energy by controlling relative concentrations of adenine nucleotides. There was a significant positive correlation between pHu and AMPD (Table 3), but it explained only 10% of the differences in pHu (Table 4). According to Scopes (1974), higher amounts of AMP deaminase accelerate IMP production, thus resulting in an earlier cessation of metabolism. How important of AMPD in affecting pHu has not been reported by Scopes (1974).

Linear regression and polynomial regression with stepwise selection were used to develop a model for predicting pHu based on the effects measured in the study. The best statistic model was:  $\text{pHu} = 5.75 - 1.21 \times 10^{-3} * \text{GP} - 3.14 \times 10^{-5} * \text{GPa} * \text{GPa}$ . This model only can be used when the pHu is lower than 5.75. The combination of GP and GPa\*GPa explained 47% variation of pHu. All other factors were only weakly correlated with pHu. Apparently, there are some other factors affecting ultimate pH.

## **Conclusions**

The most important determinant of ultimate pH in pork was glycolytic potential. It explained 42% of the variation in pHu. High glycolytic potential decreases the ultimate pH. The activity of phosphorylase a (GPa) was also

significantly related to ultimate pH in pork. It explained 28% of the variation in pHu. High activity of phosphorylase a resulted in a low ultimate pH. There was a significant correlation between buffering capacity and pHu ( $r = -0.43$ ,  $p < 0.001$ ), and AMP deaminase activity and pHu ( $r = 0.32$ ,  $p < 0.05$ ). Buffering capacity explained 19% of the variation in pHu. High buffering capacity means high ultimate pH. Activity of AMP deaminase (AMPD) only explains 10% variation in pHu. GP was significantly related to GPa ( $r = 0.54$ ,  $p < 0.001$ ) and AMPD ( $r = -0.47$ ,  $p < 0.001$ ). The correlations of GP and GPa, GP and AMPD may explain why RN carriers have a lower ultimate pH than non-RN carriers.

The combination of GP and GPa explained 47% variation of pHu. All other effects in the study were only weakly correlated with pHu. Apparently, there are some other factors affecting ultimate pH. Proglycogen and macroglycogen seem to be important in the glycolysis processes. Proglycogen is early used for energy supply and may be more closely related to ultimate pH. Further study on proglycogen and macroglycogen is needed.

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