# IDENTIFICATION OF VARIABLES USEFUL FOR PREDICITION OF ULTIMATE PH AND COLOR IN PORK

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***Abstract*—Accurate prediction of specific pork quality traits will allow processors to sort carcasses based on quality characteristics best suited for particular processing or marketing strategies. For instance, muscle pH is associated with functionality, shelf life and eating quality, whereas CIE L* (lightness) color is associated with consumer acceptance. Identification of variables that contribute to these quality measurements will provide potential avenues for predicting pork quality or applying interventions to improve quality. Although pork quality is largely governed by the rate and extent of pH decline, the typical variation observed in quality cannot be completely explained by these factors. Thus, pigs from Pietrain, Duroc×Pietrain and two separate Duroc sired lines were utilized to generate variation; carcass traits, meat quality, key muscle metabolites, and fiber type were evaluated. From these data, prediction equations were developed to identify potential key contributers to ultimate pH and CIE L* color. The equation for ultimate pH ($R^2$=0.5810; RMSE=0.0742) included 24 h glucose and glycogen as well as initial IMP content and percentage myosin heavy chain type IIB as assessed by SDS-PAGE. The equation for CIE L* ($R^2$ =0.4706; RMSE =2.592) included 24 h glycolytic potential and percentage fat content. These variables may serve as potential targets to further investigate the mechanisms controlling postmortem metabolism and pork quality development.**

***Index Terms*—myosin heavy chain, pork quality, ultimate pH.**

## I. INTRODUCTION

A primary goal of the pork industry is to identify means to predict quality; successful prediction of traits will facilitate sorting carcasses to improve utilization and increase profitability. Understanding the metabolic changes that occur postmortem is requisite for development of prediction technologies and intervention strategies to improve pork quality. Pork quality is governed by the rate and extent of postmortem pH decline (Briskey, et al., 1966, Monin and Sellier, 1985). Extreme examples of pork quality, including pale, soft, and exudative (PSE) meat from halothane or stress-susceptible pigs (Honikel and Kim, 1986); ”acid meat” from pigs with the Rendement Napole mutation (Monin, et al., 1985, Le Roy, et al., 2000); and dark, firm, and dry meat (Warriss, et al., 1998) have provided a foundation for understanding the association between postmortem metabolism and pork quality . However, these extreme conditions are often manifested by unique circumstances and provide a skewed perception of pork quality development. The vast majority of pork falls in the ”middle” of this range, and variation in quality cannot be sufficiently explained by the rate of metabolism or glycolytic potential. Thus, the purpose of this study was to utilize a population of pigs generated from Duroc, Pietrain and Duroc×Pietrain sires to provide variation in carcass traits and meat quality while avoiding these extreme scenerios. A thorough assessment of muscle metabolism and fiber type was conducted. With these data we generated predicition equations to help identify indicators of pork quality.

## II. MATERIALS AND METHODS

Animals

Sixty-five crossbred pigs representing four genetic crosses were transported to the Purdue University Meat Science Research and Education Center for processing over two separate days, according to standard industry procedures. Two lines were Duroc-sired (D1 and D2), one line was Pietrain-sired, and the last was sired by a Duroc-Pietrain F1 cross (D×P). All sires were mated with Large White x Landrace cross dams. *Longissimus* (LM) muscle samples were

collected at 0, 30, and 60 min and 24 h after exanguination, frozen at -80°C, and stored until biochemical analysis. At 24 h postmortem, carcasses were ribbed between the 10th and 11th costae.

Whole bone in loins were transported to the University of Illinois Meat Science Lab where they were fabricated into Canadian back loins (NAMP #414), tenderloin (NAMP #415A), and sirloin end. Initially, Canadian back loins were cut at the area of the 10th rib. CIE objective color measurements (L*, a*, and b*) were collected using a Minolta Chromameter CR-300 (Minolta Camera Co., Osaka, Japan) with a D65 illuminant, 0° observer. Subjective evaluations for color, marbling, and firmness were evaluated by trained University of Illinois personnel according to standards provided by the National Pork Producers Council (NPPC, 1991, 2000). Ultimate pH ($pH_u$) was determined on LD (approximately 3 g) taken at the level of the l0th rib and homogenized in 10 mL of distilled water. The pH of the homogenate was measured using an Orion model 720A pH meter fitted with a Ross Sure Flow 81-72 electrode (Orion Research, Boston, MA). Chops were cut for proximate composition and water holding capacity parameters. A 1.25 cm chop was weighed, suspended from a fish hook for 24 h in a whirl-pak bag, and weighed again. Drip loss is expressed as percent change in initial and final weight. A 2.54 cm thick chop was trimmed of external fat, homogenized and used for proximate composition. A 10 g sample was oven dried at 110° C for 24 h to determine percent moisture. The dried sample was then washed in an azeotropic mixture of chloroform and methanol for fat extraction. The remaining sample was dried and weighed to determine fat percentage.

Glycolytic Potential

Muscle glucose, glucose-6-phosphate (G6P), glycogen and lactate concentrations were determined using enzyme analytical methods (Bergmeyer, 1974) modified to a 96-well configuration (Hammelman, Bowker, Grant, Forrest, Schinckel & Gerrard, 2003). These metabolite concentrations were used to calculate glycolytic potential (GP) using the formula described by Monin and Sellier (1985): Glycolytic potential (μmoles/g wet tissue) = 2(glucose+ glucose-6-phosphate + glycogen) + (lactate).

HPLC

HPLC was performed in accordance with procedures by Bernocchi, Ceconi, Cargnoni, Pedersini, Curello & Ferrari (1994) and modified by Williams, Vidt & Rinehart (2008). Briefly, frozen muscle was powdered and homogenized in 0.5M perchloric acid. Samples were neutralized to pH 7.0 with 2.1M $KHCO_3$. Cr and PCr were isocratically separated with a Waters HPLC system equipped with a diode array detector using 100 mM $KH_2PO_4$, 5 mM tetrabutylammonium hydrogen sulfate (TBAHS), and 2.5% acetonitrile (pH 6.0). ATP, ADP, AMP, and IMP were separated on an HP Agilent system with a UV/Vis detector as previously described (Williams, et al., 2008). All injections were 15 μL, flow rate was maintained at 1 mL/min, and all metabolites were separated on a Phenomenex C18 5-μm reversed-phase column (4.6 x 150 mm).

Muscle Fiber Type

Assessment of muscle fiber type by electrophoretic separation of myosin heavy chain (MyHC) was conducted according to the protocols of Talmage and Roy (1993) as modified by Park et al. (2009). Following migration, the gels were silver-stained, images were captured, and the relative amounts of different MyHC isoforms were quantified using ImageJ software (National Institute of Health, USA).

Statistical Analysis.

Data were analyzed using the JMP software version 8.0 (SAS Institute Inc., Cary, NC, USA). LS means were generated and differences were evaluated using Tukey's adjustment for multiple comparisons. Step-wise regression was conducted to generate the best 20 prediction equations for one to four variables. Equations were screened to ensure prediction variables were orthogonal and significant at $P<0.05$.

## III. RESULTS AND DISCUSSION

Carcass performance and meat quality data are reported in Table 1. Live weight and HCW of D1 were greater ($P<0.05$) than D2 and D×P, whereas Pietrain sired pigs were intermediate to D1 and D2. The ultimate pH of D1 gilts was greater that D2 gilts and D×P gilts. Pietrain sired gilts and all barrows were intermediate. Surprisingly, the most divergent groups (live weight, HCW, $pH_u$) were derived from the two Duroc sired lines, while the Pietrain offspring were generally intermediate. Harvest date influenced L*, a*, b* and purge percentage, ($P<0.05$, data not shown) and may reflect different levels of stress over the two days or different chilling rates.

Pooled carcass traits and meat quality data had a SD/range between 17 and 28%, indicating this data set is useful for developing meaningful prediction equations (Qiao, et al., 2007). The prediction equation for $pH_u$ is $pH_u = 6.04+(-0.0175\ \text{glycogen24})+(-0.0574\times\text{IMP0})+(-0.00799\times\text{IIB\%})+(0.899/\text{glucose24})$, where glycogen and glucose measured at 24 h postmortem (μmol/g), IMP measured at T0 (μmol/g) and MyHC IIB as a percentage of total MyHC. The $R^2$ is 0.5810 and RMSE is 0.0742 (Figure 1; left). Glucose accumulates during postmortem metabolism due to the activity of debranching enzyme, whereas glycogen is the remaining substrate once rigor is reached. IMP is formed by the deamination of AMP. Levels of IMP increase during bouts of metabolic stress and high ATPase activity (Sahlin, Gorski

& Edstrom, 1990). Potentially, IMP may be an indicator of metabolic status preslaughter. Muscle fiber type has been used previously to account for variation in $pH_u$ (Eggert, Depreux, Schinckel, Grant & Gerrard, 2002). The inclusion of percentage type IIB fibers is likely a reflection of the metabolism characteristic of these fibers. Indeed, Bowker, Botrel, Swartz, Grant & Gerrard (2004) showed that fast fibers have increased myosin ATPase activity. Further, Ryu and Kim (2006) demonstrated that in fast metabolizing pig muscle, defined by 45 min pH and ratio of inosine to adenosine nucleotides, LM classified as PSE contained a higher percentage of type IIB fibers than LM classified as red, firm, and normal.

The prediction equation for L* is L*= 24.991175+(1.23×Fat%)+(0.190×GP24) where GP24 is the glycolytic potential (μmol/g) at 24 h. The $R^2$ is 0.4706 and the RMSE is 2.592 (Figure 1; right). Increased fat percentage indicates greater marbling, which would contribute to greater whiteness of LM. GP 24 was also positively associated with L*. Reduced GP24 by low carbohydrate feeding has been associated with a reduction in L* values of semitendinosus muscle (Bee, Biolley, Guex, Herzog, Lonergan & Huff-Lonergan, 2006).

**Table 1.** Effect of sire line on carcass traits and meat quality

| | Duroc-sired 1 | | Duroc-sired 2 | | Pietrain sired | | Duroc×Pietrain sired | | P Values[A] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Barrow | Gilt | Barrow | Gilt | Barrow | Gilt | Barrow | Gilt | SEM | SL | Sex | SL*Sex |
| Live weight (kg) | 147.5 | 153.7 | 139.6 | 141.7 | 141.4 | 150.4 | 136.3 | 141.7 | 3.3 | * | ** | ns |
| HCW (kg) | 100.6 | 104.6 | 94.9 | 96.3 | 96.3 | 103.3 | 92.4 | 97.1 | 2.6 | ** | * | ns |
| DP (%)[B] | 68.1 | 68.1 | 68.0 | 67.9 | 68.1 | 68.6 | 67.8 | 68.5 | 0.6 | ns | ns | ns |
| LEA ($cm^2$)[C] | 52.7 | 56.4 | 47.9 | 56.1 | 54.2 | 59.5 | 48.6 | 56.1 | 2.1 | ns | *** | ns |
| $pH_u$ | 5.67 | 5.59 | 5.73 | 5.81 | 5.68 | 5.68 | 5.74 | 5.60 | 0.03 | *** | ** | ns |
| L* | 48.59 | 51.06 | 51.20 | 48.95 | 51.79 | 48.50 | 50.06 | 52.32 | 1.14 | ns | ns | ** |
| a* | 8.74 | 7.79 | 10.11 | 8.65 | 8.61 | 8.67 | 9.44 | 8.83 | 0.52 | ns | * | ns |
| b* | 4.55 | 4.61 | 6.61 | 4.69 | 5.53 | 4.77 | 5.48 | 5.79 | 0.53 | ns | ns | ns |
| Color[D] | 3.0 | 2.5 | 2.9 | 2.8 | 2.5 | 2.9 | 2.9 | 2.4 | 0.2 | ns | ns | ns |
| Firmness[E] | 3.0 | 2.9 | 2.9 | 2.9 | 2.4 | 2.7 | 2.7 | 2.4 | 0.2 | * | ns | ns |
| Marbling[F] | 2.6 | 2.6 | 3.1 | 3.0 | 2.0 | 1.9 | 3.2 | 2.2 | 0.3 | ns | ** | ns |
| Purge loss (%) | 1.1 | 2.0 | 0.9 | 1.1 | 1.2 | 1.9 | 1.2 | 2.5 | 0.5 | ns | * | ns |

[A] Significance: * = P < .05, ** = P < .01, *** = P < .001.
[B] DP = Dressing percentage
[C] LEA is adjusted to HCW (LEA/HCW × constant; constant = average HCW of the study)
[D] Subjective color, where 1 = pale, pinkish gray to 6 = dark, purplish red.
[E] Subjective firmness, where 1 = very soft and watery to 5 = very firm and dry.
[F] Subjective marbling, where 1 = 1% intramuscular fat to 10 = 10% intramuscular fat.

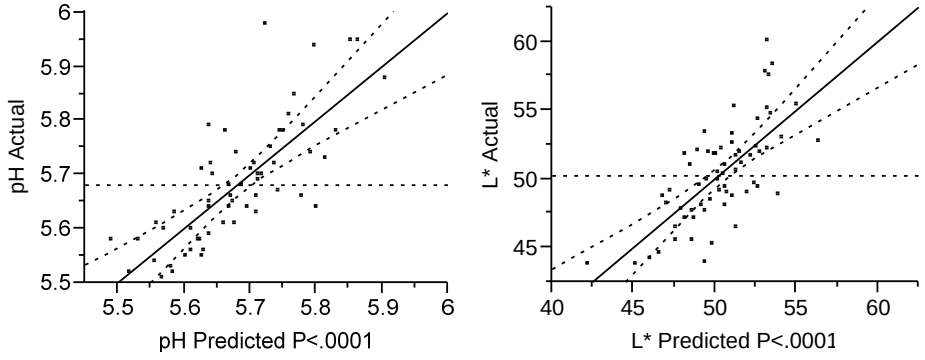


**Figure 1.** Prediction versus actual plots for pH and L* color. The horizontal lines represent the overall means. The diagonal solid line is the line of fit with the 95% confidence interval.

## IV. CONCLUSION

The use of Duroc, Pietrain, and Duroc×Pietrain sired pigs produced sufficient variation in carcass traits and meat quality to allow for development of useful prediction models for ultimate pH and L*. Certainly, we do not propose the use of complicated and expensive metabolic and biochemical analysis to replace the standard pH and color measurements. Rather, use of a prediction model may assist in identifying important variables that contribute to muscle pH decline and meat quality. The prediction model for ultimate pH produced by step-wise regression used components of both postmortem metabolism and preslaughter metabolic status. Specifically, percentage

MyHC IIB and T0 IMP levels may provide an indication of pork quality predisposition. The prediction equation for L*was primarily driven by 24 h glycolytic potential. For both pH and L*, there is still opportunity to account for variation.


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