PE1.63 Glycogen and lactate content do not fully explain differences in pork ultimate pH 393.00

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Abstract— Pork quality development is largely governed by postmortem energy metabolism. It is widely believed that extent of postmortem glycolysis is substrate driven, and thus greater degradation of glycogen results in increased lactic acid accumulation and inferior pork quality. Our objective was to generate differences in ultimate pH by manipulating muscle metabolism. The beta-adrenergic agonist Ractopamine was fed for 0, 1, or 8 d to generate differences in antemortem metabolism, whereas one side of the split carcass was exposed to electrical stimulation (ES) to accelerate postmortem glycolysis. Initial glycogen content and ultimate pH were strongly associated (correlation = -0.69, P<0.0001). The relationship was curvilinear, and it appeared that higher values of glycogen (>40 µmol/g) did not have an additional effect on ultimate pH. ES resulted in a small (-0.06) but significant decrease in ultimate pH, but was not associated with differences in lactate. Lactate at 24h was strongly related to ultimate pH; yet when extreme values were removed, this association disappeared. Altogether, these results agree with previous studies that demonstrated glycogen and lactate are related to ultimate pH over a broad range of pH values; however, our results suggest that other biochemical mechanisms in postmortem muscle influence ultimate pH and hence pork quality development.

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Index Terms—glycolysis, glycolytic potential, muscle metabolism, pork quality.

I. INTRODUCTION

Pale, soft, and exudative (PSE) is a major industry concern due to its undesirable appearance and reduced functionality. The rate and extent of postmortem glycolysis are the main factors controlling pork quality development [1] . Hastened postmortem glycolysis generates heat and causes a rapid accumulation of lactate and hydrogen ions. This combination of high carcass temperature at relatively low muscle pH results in the denaturation of sarcoplasmic and myofibrillar proteins . Conversely, 'acid meat' or meat with a low ultimate pH (pHu), is generally considered to occur from abnormal glycogen metabolism [2]. Pigs with the Rendement Napole (RN) mutation possess elevated muscle glycogen. This 'extra' glycogen is suggested to allow greater glycolysis and a low ultimate pH. Certainly, there is a significant relationship between initial muscle glycogen and ultimate pH. In the case of dark, firm, and dry (DFD) meat, the muscle exhibits little postmortem metabolism because substrate is limiting. In this case, there is a direct cause and effect relationship between muscle glycogen content, glycolysis, and pH decline. Although muscle glycogen content accounts for 40-60% of the variation in ultimate pH [3, 4], the relationship between glycogen content and pHu is not linear. Residual glycogen is highly variable at low pH and pH values may plateau in the presence of residual glycogen [5] . Variation in glycogen content alone cannot fully explain differences in ultimate pH. Many have also suggested that lactic acid drives pH decline. Scientists have erroneously used 'lactic acidosis' and 'lactic acid' to explain the pH decline observed in exercising muscle as well as in postmortem muscle. However, because lactic acid is a strong acid, it dissociates into lactate and hydrogen ions (H+), and thus lactic acid is present at very low levels. It seems more appropriate to consider that pH decline is a function of H+ accumulation. Undoubtedly, lactate increases postmortem due to degradation of glycogen, but other biochemical aspects of muscle metabolism are likely involved in H+ accumulation and pH. Therefore, our objective was to generate differences in pHu by manipulating antemortem muscle metabolism as well as postmortem glycolysis. Beta-adrenergic agonists have a major effect on muscle metabolism, and thus ractopamine (RAC) was employed to alter antemortem muscle metabolism. Additionally, electrical stimulation simulates PSE development by accelerating

postmortem glycolysis and may also lower pHu. We hypothesized that variability in pHu may not be directly related to initial glycogen or 24h lactate.

II. MATERIALS AND METHODS

A. Animals

Sixteen pigs were reared under uniform conditions at the Purdue University Swine Research Unit and all animal procedures were carried out in accordance with the guidelines and prior approval of the Purdue University Animal Care and Use Committee. Pigs were blocked by initial weight and assigned to either 0, 1, or 8 d of RAC (20 ppm) feeding prior to slaughter. All pigs had ad libitum access to feed and water. At the end of the treatment period, pigs (~117 kg) were transported to the Purdue University Meat Science Research and Education Center and slaughtered. Pigs were rendered unconscious by electrical stunning and exsanguinated following standard industry procedures. Exsanguination was considered time 0.

B. Muscle sampling

At 0 min, muscle samples (~5 g) were collected from the lumbar region of the longissimus muscle using a coring device. Samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

C. Electrical stimulation

All carcasses were scalded at 5 min postmortem, followed by dehairing, evisceration, and splitting. After carcass splitting, the left side was designated as control and the right side was subjected to electrical stimulation (ES, 25 pulses, 1s on and 1s off). Carcasses were held at room temperature until 60 min postmortem, at which time they were placed in a chill cooler (4°C).

D. Meat quality

At 24h postmortem, additional muscle samples were collected from the lumbar region of the longissimus muscle on the right and left sides of the carcass. Each side was ribbed between the 10th and 11th costae. Two 2.54-cm thick chops were removed anterior to the cut surface and trimmed to remove excess fat and connective tissue. Objective color measurements were determined on one chop using a Hunter Lab D25-PCΔ2 colorimeter (Hunter and Associates Laboratory Inc., Reston, VA, USA). Mean L* (lightness), a* (redness), and b*(yellowness) were collected from two separate locations on the surface of each chop. Water-holding capacity was determined on the adjacent chop using the drip loss method. Drip loss analysis was evaluated in triplicate.

E. Muscle pH determination

Approximately 1g of frozen muscle was powdered in liquid nitrogen and 10ml of 5mM sodium iodoacetate was added. Samples were homogenized and pH was measured using an Orion 3-star pH meter and probe (ThermoScientific, Waltham, MA, USA).

F. Muscle metabolite analysis

Muscle glucose, glucose-6-phosphate, glycogen and lactate concentrations were determined using enzyme analytical methods modified to a 96-well plate. Metabolite concentrations were used to calculate glycolytic potential (GP) using the formula: GP (μmoles/g wet tissue, lactate equivalents) = 2(glucose + glucose-6-phosphate + glycogen) + lactate.

G. Statistics

To evaluate the effect of duration of RAC feeding and electrical stimulation on ultimate pH, data were analyzed using the proc mixed procedure of SAS. Model included the fixed effects of RAC, period, and electrical stimulation. Least square means were generated using the lsmeans statement. Pearson correlations were generated using the proc corr procedure. Effects were considered significant at $P<0.05$.

III. RESULTS AND DISCUSSION

The β-agonist RAC was fed for 1 and 8d in an attempt to induce changes in antemortem muscle metabolism. Subsequent analysis of glycogen revealed that 2 pigs (both 1d RAC treatment) exhibited much higher levels of glycogen than anticipated. Genotyping tests revealed that these pigs were RN carriers. Additionally, another pig (8d RAC) possessed very low levels of glycogen at 0min and high pHu. Due to these unexpected and somewhat atypical results, it was difficult to make conclusions regarding the effect of 1 and 8d RAC feeding on metabolism and pH. Nonetheless, the variation in metabolite and pH data was useful for examining the relationship between glycogen, lactate, and pH. Glycolytic potential represents the muscle's capacity for postmortem glycolysis. An

animal with elevated glycogen, such as a pig that is a carrier for the RN mutation, will possess a higher GP. The high glycogen values (>60 μmol/g) in Figure 1 represent RN carriers pigs (each side is represented). In contrast, the points above pH 6.0 represent a pig with extremely low glycogen at 0 min, thereby illustrating that very low levels of initial glycogen largely limit the extent of postmortem pH decline. Higher levels of initial glycogen are associated with lower ultimate pH (correlation= -0.69, P<0.0001), but this effect may diminish as glycogen increases above ~40 μmol/g. ES can be used to mimic rapid pH decline postmortem. In our hands, ES also had a small (-0.06) but significant (P=0.0083) effect on pHu (Figure 2), which is consistent with a previous report that ES induces a small decrease in pHu [6] . However, ES was not associated with altered lactate content. When all data were examined, increasing lactate concentration was associated with pHu (Figure 3, correlation= -0.67, P<0.0001). Interestingly, when extreme values were removed (leaving points within box in Figure 3), the relationship between lactate and pH was lost (correlation= -0.10, P=0.63). The remaining points, however, still represent a large variation in pH (~0.35 units) and lactate (~30 μmol/g). Interestingly, the connection between lactate and ultimate pH in pork has always been rather inconsistent. For example, Hampshire and Large White pigs had different ultimate pH despite similar lactate levels in the longissimus [2]. Undoubtedly, lactate increases during the postmortem period due to degradation of glycogen, but other biochemical aspects of muscle metabolism appear to alter H+ accumulation and pH. In agreement with previous studies, low ultimate pH was associated with lighter pork color (higher L* values) and decreased water holding capacity, evidenced by higher drip loss.

IV. CONCLUSION

Inferior pork quality has been an important industry concern for several decades, yet this phenomenon still is not well understood. Although initial glycogen and lactate at 24h are, in general, related to pHu, there are several inconsistencies between these metabolites and pHu. First, residual glycogen is highly variable at low pH, and pH decline may plateau in the presence of residual glycogen. Additionally, the relationship between lactate and pHu is variable, and low pHu does not always correspond with elevated lactate. Our results support that in addition to glycogen and lactate, other biochemical mechanisms have a significant influence on development of ultimate pH.

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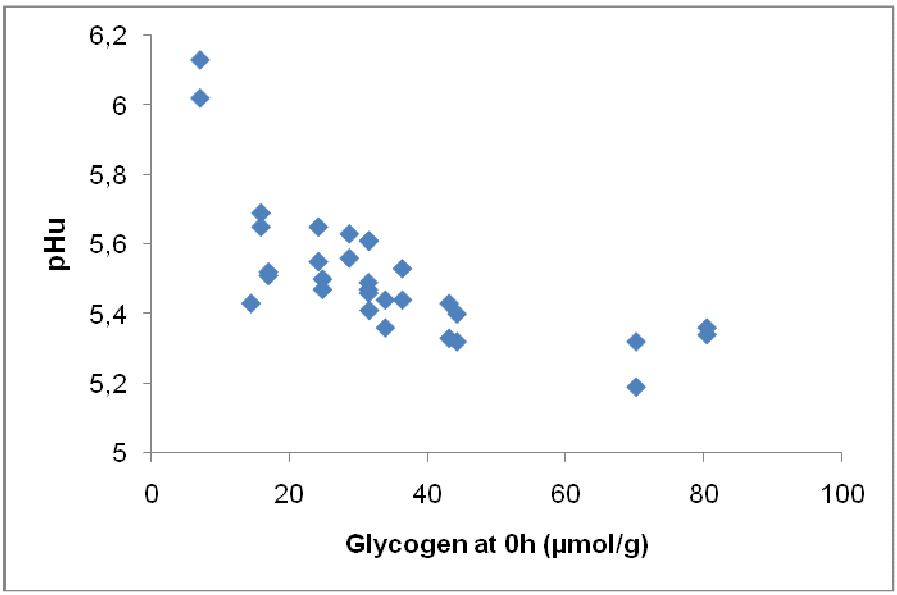

Figure 1. Relationship between glycogen (0h) and ultimate pH.

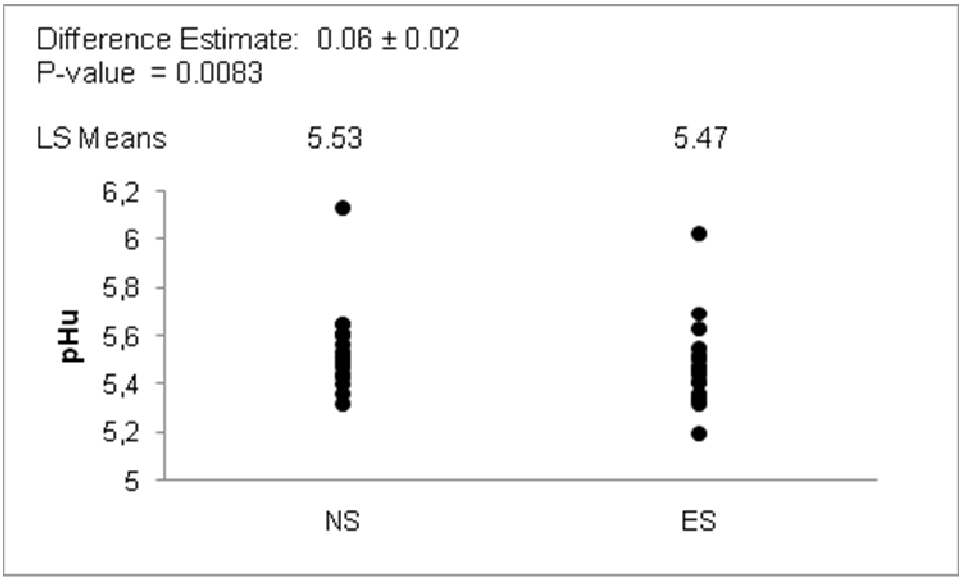

Figure 2. Ultimate pH of carcass sides designated control (non-stimulated, NS) or electrically stimulated (ES).

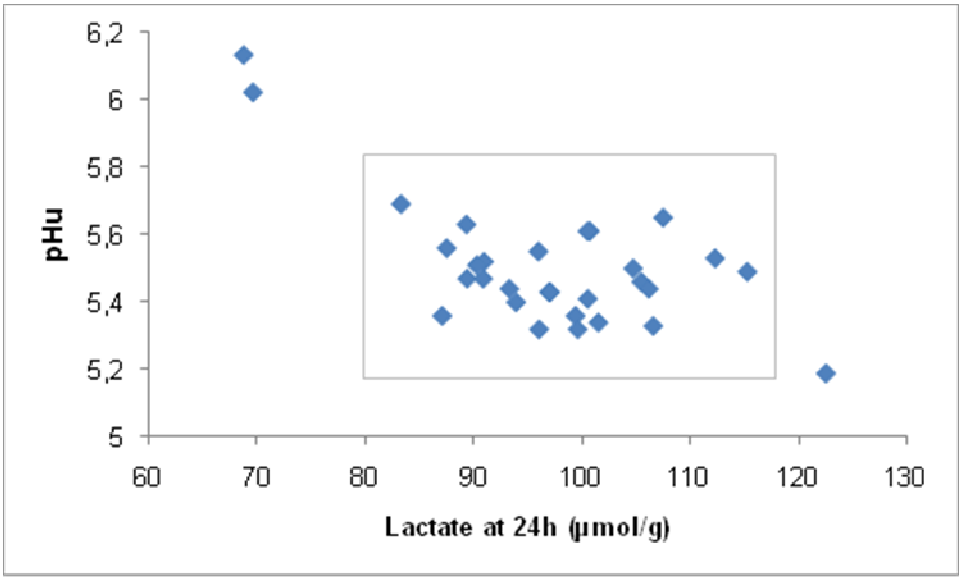

Figure 3. Relationship between lactate at 24h and ultimate pH. When all values are included, lactate is strongly related to pHu (-0.67, P<0.0001). When outliers are removed, the relationship is -0.10 (P=0.63).