Glycolysis and pH Decline Terminate Prematurely in Oxidative Muscles despite the Presence of Excess Glycogen

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Abstract: Meat from oxidative skeletal muscle has a higher postmortem ultimate pH, which was originally thought to be a result from decreased antemortem glycogen stores. Therefore, we hypothesized that excess glycogen may not resolve the high ultimate pH of meat from oxidative muscles in ruminants and poultry. To test this hypothesis, an in vitro muscle glycolytic buffer system containing excess glycogen was used to compare glycolysis and pH decline of glycolytic and oxidative muscle from beef, lamb, chicken, and turkey. Glycogen concentration of both glycolytic and oxidative muscle homogenates was similar at 0 min and decreased significantly with time in all species tested. All homogenates contained residual glycogen at 1440 min, indicating glycogen was provided in excess. The ultimate pH of the oxidative muscle homogenates was significantly increased compared to the glycolytic muscle. The oxidative muscle also contained decreased lactate and decreased glucose 6-phosphate in all the species tested at 1440 min. Combined these data suggest that glycolysis and pH decline of oxidative muscles terminate prematurely at higher ultimate pH even in the presence of excess glycogen across livestock species. Additionally, the data indicated that the in vitro glycolytic buffer system can be used to study species specific meat quality problems in beef, lamb, chicken, and turkey.

Keywords: meat quality, poultry, ruminants, ultimate pH

Introduction

During postmortem glycolysis, skeletal muscle mobilizes stored carbohydrate known as glycogen into ATP, lactate, and ultimately H+ ions. The formation of H+ ions result in a drop in the muscle pH from 7.2 in living muscle to a pH near 5.5 in meat under normal conditions (Scheffler et al., 2011). The acidification of the tissue results in the characteristic tastes and textures associated with fresh meat. If this process is altered, meat quality issues can arise. For instance, if glycogen is limited at the time of death, aberrant meat quality attributes like high ultimate pH (pHu) can occur. Meat with a high pHu is known as dark cutting in ruminants (Terrant, 1981), or dark, firm, and dry (DFD) in pigs and poultry. The resulting meat is dark in color, firm in texture, exhibits a dry surface, possesses a shorter shelf life, and exhibits deteriorated flavor. Classification of dark cutting or DFD meat is usually a combination of visual color assessment and pHu. Classically, this meat was downgraded when pHu was at or above pH 6.0 (Briskey, 1964), but in more recent reports carcasses are being classified as dark cutting beef if pHu is above 5.8 (Page et al., 2001). Because glycogen plays an important role in dictating the extent of pH decline above pH 5.5 (Henckel et al., 2002; Immonen and Puolanne, 2000), further investigation into its role in dictating pHu and meat quality is necessary.

The glycogen concentration stored in muscle at the time of slaughter plays an important role in incidence of dark cutter/DFD, and may vary depending on the muscle, species, nutritional status, or stress level prior to slaughter. To achieve the normal pHu (5.5 to 5.6) in ruminants and non-ruminants, the critical threshold levels of glycogen needed to drop pHu...
to 5.5 to 5.6 are between 45 and 57 mmol glycogen/kg muscle (Pethick et al., 1995; Tarrant, 1989; Warriss, 1990). Thus, many muscles contain excess glycogen. For instance, muscle glycogen concentration of sheep and cattle longissimus normally ranges from 75 to 120 mM/kg (Immonen et al., 2000; Monin, 1981; Pethick et al., 1995). Further, bovine longissimus residual glycogen concentration varied from 10 to 80 mM/kg at a pH value below 5.75 (Immonen and Puolanne, 2000). However, some muscles contain glycogen below this critical threshold resulting in elevated pHu above 5.8 and contain little to no residual glycogen (Pethick et al., 1995). These muscle tend to favor oxidative metabolism and contain a greater proportion of type I fibers (oxidative type; Monin, 1981; Pethick et al., 1995). Compared to glycolytic muscles, oxidative muscles contain lower glycogen levels antemortem which may contribute to the higher pHu (Gardner et al., 1999, 2014; Knee et al., 2004; Jacob et al., 2005). This difference led to the hypothesis that excess glycogen could overcome the high pHu problem in oxidative muscle. However, in a previous study, we determined that pH decline in porcine muscle glycogen levels during anoxia which may contribute to glycolytic muscles (Zerouala and Stickland, 1991), the underlying mechanisms responsible for these differences remain elusive and need to be investigated to better understand the meat quality development. Therefore, the aim of this study was to understand the role of glycogen levels in oxidative muscles and the ultimate pH. The objective was to test the hypothesis that excess glycogen does not resolve high pHu of oxidative muscles in ruminants and poultry.

**Materials and Methods**

**Sample collection**

Market weight lambs (n = 6) and 6 market weight steers (n = 6) were raised at The Ohio State University under The Institutional Animal Care and Use Committee (IACUC) project #2012A0000091-R2. Animals were harvested at The Ohio State University Meat Science Laboratory abattoir under USDA-FSIS inspection using accepted commercial processing procedures. Cutaneous trunci (glycolytic) and masseter (oxidative) muscle were selected as representative examples of ruminant muscles containing predominantly glycolytic or oxidative characteristics based on previous classification in the scientific literature (Devine et al., 1984; Egelandsdal et al., 1995; Muroya et al., 2002; Muroya et al., 2006; Ringkob et al., 2004; Suzuki 1977; Tuxen and Kirkeby, 19903-hydroxybutyrate: NAD+ oxidoreductase (HBOX; Fretheim et al., 1986; Young and Davey 1981; Xiong 1994). Samples were collected from each animal immediately following exsanguination. Muscle samples were trimmed for fat, snap frozen in liquid nitrogen, and stored at –80°C until further analysis. Similarly, broiler chickens (n = 6) were raised at The Ohio State University under IACUC project #2012A0000091-R2 and harvested at The Ohio State University Meat Science Laboratory abattoir under USDA-FSIS inspection. Pectoralis major (glycolytic) and sartorius (oxidative) muscles were selected as representative examples of poultry muscles containing predominantly glycolytic or oxidative characteristics based on previous classification in the scientific literature (Asghar et al., 1984; Ashmore and Doerr, 1971a, 1971b; Suzuki et al., 1985; Ueda et al., 2005; Xiong 1994). Commercial weight turkeys (n = 6) were slaughtered at Poultry Research Center, OARDC Wooster, and all procedures were approved by IACUC #2016A00000109 at The Ohio State University. Pectoralis major and sarto-
Bovine muscle samples were collected immediately after exsanguination before defeathering and evisceration. Muscle samples were snap frozen in liquid nitrogen and stored at −80°C until further analysis.

**Muscle fiber typing/myosin heavy chain isoform analysis**

Because there was less scientific literature to demonstrate the muscle fiber typing in lamb cutaneous trunci and masseter muscles, we confirmed the classification of cutaneous trunci (glycolytic) and masseter (oxidative) muscle in lambs by utilizing myosin heavy chain isoforms separation as per the previously reported methods (Park et al., 2009; Picard et al., 2011). Briefly, samples from cutaneous trunci and masseter muscles were powdered in liquid nitrogen and stored on dry ice. Muscle was solubilized in a buffer containing 8 M urea, 75 mM ATP, 0.5 mM ADP, 0.5 mM NAD⁺/w/v ratio (wt/vol; England et al., 2014; Matarneh et al., 2017; Scheffler et al., 2015). Muscle homogenization for the separation of bovine myosin heavy chain isoforms was performed in liquid nitrogen and stored on dry ice. Muscle and buffer homogenates were maintained at 25°C for 10 min followed by centrifugation for 5 min at 13,000 × g at room temperature. The resulting supernatant was neutralized with 1.25 M KOH (Bergmeyer 1984) and stored at −20°C until further analysis. Similarly, for lactate, glucose, and glucose 6-phosphate determination, samples were withdrawn from the muscle homogenate and an equal volume of ice-cold 1 M perchloric acid was added. The muscle homogenate and perchloric acid mixture was vortexed and centrifuged for 5 min at 13,000 × g at room temperature. The supernatant was neutralized with 2 M KOH and processed as per the previously reported methods (Hammelman et al., 2003). The enzymatic reactions were conducted in borosilicate Fisherbrand disposable glass tubes (Thermo Scientific, Pittsburg, PA) and metabolites were quantified spectrophotometrically at 340 nm in triplicate using 96-well microplates with the Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA).

**In vitro buffer system**

Muscle samples collected for different species (cutaneous trunci and masseter in beef and lamb, and pectoralis major and sartorius in chicken and turkey) were powdered in liquid nitrogen. Powdered muscle tissue was homogenized into the glycolytic buffer containing 5 mM MgCl₂, 10 mM Na₂HPO₄, 30 mM creatine, 60 mM KCl, 10 mM sodium acetate, 5 mM ATP, 0.5 mM ADP, 0.5 mM NAD⁺, 25 mM carnosine, and 30 mM glycogen (glucose equivalents) at a 10% w/v ratio (wt/vol; England et al., 2014; Matarneh et al., 2017; Scheffler et al., 2015). Muscle homogenization conditions were 3000 rpm for 15 s and was consistent between muscle types. The initial pH of the glycolysis buffer was adjusted to 7.4 and 7.2 for glycolytic and oxidative muscle homogenates, respectively, to set similar starting pH for both muscles at time zero (England et al., 2016). The muscle and buffer homogenates were maintained at 25°C in a digital cooling dry bath (Thermo Scientific, Pittsburg, PA) for 24 h during further metabolite and pH analysis.

**Metabolite analysis**

For determining the pH decline and determination of glycolytic metabolite concentration in the muscle homogenates, aliquots were collected at 0, 60, 120, 240, and 1440 min and processed as per previously reported methods (England et al., 2016). Briefly, for glycogen analysis samples were collected from the muscle homogenate and mixed with an equal volume of 2.5 M HCl, followed by heating at 90°C for 2 h. After heating, the homogenate and HCl mixture was centrifuged for 5 min at 13,000 × g at room temperature and the resulting supernatant was neutralized with 1.25 M KOH (Bergmeyer 1984) and stored at −20°C until further analysis. Similarly, for lactate, glucose, and glucose 6-phosphate determination, samples were withdrawn from the muscle homogenate and an equal volume of ice-cold 1 M perchloric acid was added. The muscle homogenate and perchloric acid mixture was vortexed and centrifuged for 5 min at 13,000 × g at room temperature. The supernatant was neutralized with 2 M KOH as per the previously reported method (Bergmeyer 1984).

Glycogen, glucose 6-phosphate, glucose, and lactate were measured as per the aforementioned described acidification and neutralization preparations using previous enzymatic methods (Bergmeyer 1984) with slight modification for a microplate method (Hammelman et al., 2003). The enzymatic reactions were conducted in borosilicate Fisherbrand disposable glass tubes (Thermo Scientific, Pittsburg, PA) and metabolites were quantified spectrophotometrically at 340 nm in triplicate using 96-well microplates with the Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA).

**pH analysis**

The pH of the muscle homogenates was measured as per the previously reported method (Bendall 1973) with a slight modification (England et al., 2016). Briefly, 4 volumes of the muscle homogenate were collected and mixed with 1 volume of a solution containing 25 mM sodium iodoacetate, and 750 mM KCl (pH 7.0). The mixture was vortexed, equilibrated to 25°C for 10 min followed by centrifugation for 5 min at 13,000 × g. Immediately after centrifugation, pH was measured using a calibrated Orion ROSS Ultra pH Electrode (Thermo Scientific, Pittsburg, PA).
Statistical analysis

Data were analyzed with a mixed model in JMP and were analyzed separately for all the species reported in this study. In the present investigation, multiple time point data were analyzed with a split-plot design and individual animals were recognized as an experimental unit. Main effects of muscle and time, and the interactions between the two were considered. All the data in this manuscript have been presented as means ± SE. Evaluation of the least squares means was undertaken with a Student’s *t* test and considered significant at *P* ≤ 0.05.

Results

Muscle fiber-typing

Like the same bovine muscle, lamb masseter contained predominantly type I myosin heavy chain where lamb cutaneous trunci contained type IIA, and IIX/IIB myosin heavy chain (Maier et al., 1992; Sayd et al., 1998; Sazili et al., 2005). Combined, the lamb cutaneous trunci and masseter muscles exhibited divergent muscle fiber-types (Fig. 1).

Glycogen concentration

Glycogen concentration of cutaneous trunci and masseter muscle homogenate in lambs and beef cattle, and pectoralis and sartorius muscle homogenate in chicken and turkey was similar at 0 min within each species and decreased significantly (*P* < 0.05) with time from 0 to 1440 min (Fig. 2). There was significant (*P* < 0.05) interaction between muscle and time in all the species. Residual glycogen concentrations were increased (*P* < 0.05) in the masseter compared to the cutaneous trunci at all time points from 30 to 1440 min in lamb and 60 to 1440 min in beef (Fig. 2). Similarly, residual glycogen concentrations were increased (*P* < 0.05) in the sartorius compared to the pectoralis major at all time-points from 60 to 1440 min in chicken and 30 to 1440 min in turkey. Additionally, residual glycogen was present in all animal and muscle combinations at 1440 min indicating that glycogen was provided in excess.

pH decline

Within a species, the zero-time pH of the cutaneous trunci and masseter muscle homogenates in ruminants, and pectoralis and sartorius muscle homogenate in poultry were not different (Fig. 3). Muscle homogenate pH declined with time (*P* < 0.001). There were significant (*P* < 0.05) differences between muscle types within each species with oxidative muscle homogenates generally having an elevated pH in all the species. Specifically, beef cutaneous trunci homogenates had a significantly lower (*P* < 0.05) pH compared to the masseter at all time-points between 60 and 1440 min. Similarly, lamb cutaneous trunci homogenates had a significantly (*P* < 0.05) lower pH compared to the masseter at all time-points between 30 and 1440 min. In poultry, pectoralis major mean homogenate pH was significantly (*P* < 0.05) lower than sartorius at all time-points from 120 to 1440 min in chicken and 30 to 1440 min in turkey.

Lactate concentration

Lactate concentration increased (*P* < 0.001) with time in all muscles, but the oxidative muscle homogenates generally contained lower lactate concentrations compared to glycolytic muscle homogenates in all species tested (Fig. 4). Lactate concentration was significantly (*P* < 0.05) increased in cutaneous trunci as compared to masseter at all time points from 30 min to 1440 min in both lamb and beef (Fig. 4). In chicken, lactate concentration was increased (*P* < 0.05) in pectoralis major homogenates as compared to sartorius muscle homogenates at 120, 240, and 1440 min time-points. Turkey lactate concentration was significantly (*P* < 0.05) increased in pectoralis major homogenates compared to the sartorius muscle homogenates at all time-points from 30 to 1440 min.
Glucose and glucose 6-phosphate concentration

Glucose concentration in both muscle types increased ($P < 0.05$) with time in each species tested (Fig. 5). Glucose levels in the ruminant cutaneous trunci was elevated at 30, 60, 120, and 240 min compared to the masseter, but no differences were detected at 1440 min. In the poultry, glucose concentration was significantly increased ($P < 0.05$) in the pectoralis major homogenates compared to the sartorius at all time-points from 30 to 1440 min.

Glucose 6-phosphate concentration was not different at 0 min between muscles of all the species tested (Fig. 6). However, G6P increased significantly ($P < 0.05$) from 0 to 30 min in all species and muscle combinations. Glucose 6-phosphate was also significantly ($P < 0.05$) increased in the glycolytic muscle homogenates of all species at all time-points from 30 to 1440 min compared to the oxidative muscles.

Discussion

The primary objective of these experiments was to compare the postmortem muscle glycolysis and pH decline in cutaneous trunci and masseter muscle homogenates of beef and lamb, and pectoralis major and sartorius muscle homogenates of chicken and turkey, by using in vitro muscle glycolyzing buffer system in the presence of excess glycogen. As expected, the glycogen concentration of both glycolytic and oxidative muscle homogenates within a species was similar at 0 min due to the exogenously added glycogen. While the glycogen concentration declined with time from 0 min to 1440 min in both muscle types of all species tested, the amount each muscle type metabolized was different. The oxidative muscles mobilized less glycogen compared to the glycolytic muscles in all the 4 species tested as suggested by the residual glycogen at the later time points. Though, none of the muscles metabolized all the glycogen provided in the system. These glycogen data indicate that oxidative muscles terminated
glycolysis and glycogenolysis prematurely compared to the glycolytic muscles. These findings were corroborated by the higher pHu of the oxidative masseter and sartorius muscles as compared to the glycolytic cutaneous trunci and pectoralis major muscle. The glycogen and pH data align with our previous findings in porcine skeletal muscle where glycolysis, glycogenolysis, and pH decline stopped prematurely in masseter (oxidative) muscle indicating that glycogen was not a limiting factor (England et al., 2016). Further, the starting and ultimate pH in vivo data in the ruminant muscles are similar to ultimate pH of in vivo masseter and cutaneous trunci (Devine et al., 1984). Therefore, these new data suggest that oxidative beef, lamb, chicken, and turkey muscle terminate postmortem metabolism prematurely due to some other factor than limited glycogen.

In all the species tested in the present study, lactate concentration increased with time in all species and muscles. The lactate concentration data also aligns with the glycogen and pHu data as the oxidative muscle homogenates contained lower lactate concentration at 1440 min when compared to glycolytic muscle homogenates for all species. A reduced lactate concentration further suggests reduced total glycolytic flux and a prematurely terminated postmortem glycolysis and glycogenolysis in oxidative muscle. The limited glycolytic flux aligns with previous data in turkey where reduced glycolytic flux was associated with darker, more red turkey with an elevated ultimate pH (Patterson et al., 2016). The limited glycolytic flux is possibly due to pH-mediated inactivation of phosphofructokinase activity (England et al., 2014). Prematurely terminated postmortem glycolysis was also suggested by the glucose and glucose 6-phosphate data due to the similarities between this study and the study using porcine skeletal muscle (England et al., 2016).

![Figure 3. Mean pH decline of reactions buffer and cutaneous trunci (glycolytic muscle) or masseter (oxidative muscle; n = 6 per muscle) in lamb and beef or pectoralis major (glycolytic muscle) or sartorius (oxidative muscle; n = 6 per muscle) in chicken and turkey. Data are means ± SEM. *P ≤ 0.05 within time.](image-url)
Collectively, the muscle pH and metabolite data suggest that regardless of species, oxidative skeletal muscle postmortem glycolysis is halted by an external factor beyond glycogen concentration. The most likely explanation for this phenomenon is based on results presented in our similar study using porcine skeletal muscle (England et al., 2016). In that study, glycolytic capacity, or a lack thereof, terminated post-mortem pH decline and resulted in an elevated pHu in oxidative muscle. To reiterate, glycolytic capacity is the maximal ability of a cell to convert carbohydrate (i.e., glycogen) to pyruvate or lactate (Mookerjee et al., 2016). This conclusion that limited glycolytic capacity was responsible for terminating postmortem pH was suggested by 3 separate investigations in that study. First, the rate of ATP disappearance between the longissimus lumborum (glycolytic muscle) and masseter (oxidative) was minimal. Second, when glycogen and ATP were added to 24 h (i.e., post-rigor) masseter, glycolysis reinitiated, and pH decline recommenced. Finally, pre-rigor longissimus lumborum and masseter muscle were powdered and mixed at defined percentages to evaluate the ability of skeletal muscle glycolytic capacity to dictate pHu. Ultimate pH decreased linearly as the percent of longissimus lumborum (i.e., glycolytic capacity) was increased when mixed at defined ratios from 0 to 50%. However, no further decline in pHu occurred as glycolytic capacity increased. Thus, the previous data indicated that in porcine skeletal muscle, the rate of ATP hydrolysis outpaced the ability of the oxidative muscle to re-phosphorylate ADP. In doing so, glycolysis and glycogenolysis were halted prior to the inhibition of phosphofructokinase-1 (England et al., 2014). Because the current data aligns with the previous data, a similar effect may exist in the 4 new species tested. Given that skeletal muscle glycolytic capacity is a conserved trait across species (Listrat et al., 2016; Peter et al., 1972; Realini et al., 2013), these data were somewhat anticipated.

Figure 4. Mean lactate concentrations of reactions buffer and cutaneous trunci (glycolytic muscle) or masseter (oxidative muscle; n = 6 per muscle) in lamb and beef or pectoralis major (glycolytic muscle) or sartorius (oxidative muscle; n = 6 per muscle) in chicken and turkey. Data are means ± SEM. *P ≤ 0.05 within time.
However, these data also suggest potential opportunities to remedy species specific problems in the poultry and ruminant meat industries. For instance, the increased glycolytic capacity found in glycolytic skeletal muscle may be reduced if animals were selected for a decreased glycolytic capacity in the pectoralis major. However, selecting against glycolytic capacity in the pectoralis major is likely to be costly and against increased growth potential. Rather, these potential opportunities may be captured through management practices designed to lower glycogen content or slow glycogen metabolism postmortem. For instance, poultry meat routinely develops pale, soft, and exudative attributes typically through accelerated postmortem pH decline (Pietrzak et al., 1997; Rathgeber et al., 1999). The accelerated decline is thought to occur due to accelerated rate of ATP hydrolysis resulting in H⁺ production and muscle acidification (Scopes, 1973). However, when chickens were under-fed in a negative energy balance, ATPase activity was lower compared to ad libitum fed birds (Ashgar et al., 1984). Therefore, in conjunction with their results, there is a possibility that altering dietary management strategies to reduce glycogen may further help reduce the rate of ATP hydrolysis and the incidence of PSE meat. Though, further studies are necessary to corroborate that conclusion.

Conversely and possibly more importantly, these results may provide further insight into management strategies designed to reduce the incidence of dark cutting in beef. Antemortem glycogen concentration in skeletal muscle is central to the extent of postmortem pH decline and incidence of dark cutting. However, glycogen concentrations should not be a limiting factor in healthy animals not stressed before slaughter, as the concentration is generally higher than that required to attain normal ultimate pH (5.5 to 5.6). Therefore, management strategies such as minimizing the stress during the preslaughter process, duration of transportation, and optimum conditions may offer additional opportunities for reducing the incidence of dark cutting.

**Figure 5.** Mean glucose concentrations of reactions buffer and cutaneous trunci (glycolytic muscle) or masseter (oxidative muscle; n = 6 per muscle) in lamb and beef or pectoralis major (glycolytic muscle) or sartorius (oxidative muscle; n = 6 per muscle) in chicken and turkey. Data are means ± SEM. *P ≤ 0.05 within time.
lairage time are further encouraged by these results to prevent muscle glycogen depletion and maintain sufficient muscle glycogen at slaughter (>critical threshold of 45 to 55 mmol/g at slaughter) required to achieve optimum ultimate pH (5.5 to 5.6; Monin 1981). Our data indicates that this phenomenon occurs because ruminant muscles with a higher proportion of glycolytic fibers with increased glycolytic capacity will utilize glycogen to acidify the muscle post-mortem until a separate factor (likely PFK-1 pH inactivation) terminates pH decline (England et al., 2014). Conversely, these results also indicated that increasing glycogen stores in more oxidative muscles with low glycolytic capacity is unlikely to further acidify the elevated ultimate pH of those muscles as the post-mortem glycojenolysis and glycolysis will terminate in the presence of excess glycogen. Ultimately, these data indicated that in postmortem skeletal muscle, understanding glycolytic capacity may be critical to understanding and predicting ultimate pH in meat.

As a final note, these results also open new areas of investigation using the in vitro glycolytic buffer system. Previously, the studies utilizing this buffer system were exclusively focused on pork quality problems (England et al., 2014, 2015, 2016; Matarneh et al., 2015, 2017; Scheffler et al., 2015). These new results indicate that beef, lamb, chicken, and turkey skeletal muscle all metabolize the glycolytic and glycogenolytic metabolites provided in the buffer system. Additionally, the pHu values reached by each species are consistent with in vivo values reported in the literature (Bendall 1973; de Fremery and Pool 1960; Tarrant and Sherington 1980). Therefore, further investigations into the underlying biochemical mechanisms controlling meat quality of beef, lamb, chicken,
and turkey are possible to solve species specific deviations in meat quality unique to each species.

Conclusions

The data presented here indicate that postmortem glycolysis and pH decline stop prematurely in the oxidative muscles resulting in increased residual glycogen, elevated pHu, and decreased lactate formation as compared to glycolytic muscles in beef, lamb, chicken, and turkey. In agreement with the previous findings in pigs, these data suggest that postmortem muscle glycolysis and pH decline terminate prematurely at higher pHu even in the presence of excess glycogen in oxidative muscles across livestock species. Therefore, future research should focus on inherent muscle factors (proportion of muscle fiber types, glycolytic capacity, and buffering capacity) altering postmortem muscle glycolysis and pHu, to reduce the incidence elevated pHu and associated economic losses to the meat industry.

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