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Comparative studies of meat quality traits and the proteome profile between low pH and high pH muscles in *longissimus dorsi* of *Berkshire*

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Running title: Proteomic changes between low and high pH muscle of *Berkshire*

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Abbreviations: **2DE**, two-dimensional gel electrophoresis; **MALDI-TOF/MS**, matrix-assisted laser desorption ionization time-of-flight mass spectrometric; **LD**, *longissimus dorsi*; **CRYAB**, alpha-crystallin B chain; **DUSP1**, dual specificity phosphatase 1; **VIM**, vimentin; **ATP5H**, ATP synthase subunit d, mitochondrial; **PRDX6**, peroxiredoxin6; **UCTH14**, ubiquitin carboxyl-terminal hydrolase14; **HSP-b1**, heat shock protein beta-1; **cyt c**, cytochrome c; **PHB**, prohibitin;

CA3, carbonic anhydrase 3; **SUCLA**, succinyl-CoA ligase [ADP-forming] subunit beta; **WHC**, water holding capacity; **WBSF**, Warner-Bratzler shear force; **Ct**, cycle threshold, **PSE**, pale, soft and exudative; **NPPC**, national pork producers council; **CIE**, commission international de l'Eclairage.

ABSTRACT

This study was conducted to assess the molecular mechanism of meat quality between low and high pH muscles using two-dimensional gel electrophoresis (2DE) with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) on *longissimus dorsi* (LD) muscles from *Berkshire* species, assigned to high (5.92 ± 0.02) and low pH groups (5.55 ± 0.03). The higher pH group had a lower lightness, yellowness, drip loss, shear force, and a higher NPPC (national pork producers council) color than the low pH group. The meat quality changes were related to the altered protein expression between the two groups. Fourteen protein spots were identified by MALDI-TOF/MS and among them, nine proteins involved in meat quality attributes significantly increased: the alpha-crystallin B chain (CRYAB), dual specificity phosphatase 1 (DUSP1), vimentin (VIM) X1 and X2, ATP synthase subunit d, mitochondrial (ATP5H), peroxiredoxin6 (PRDX6), ubiquitin carboxyl-terminal hydrolase14 (UCHL14), and cytochrome c (cyt c). Moreover, the proteins' translation efficiency was analyzed by their mRNA expression via qPCR. An increase in the mRNA levels of ATP5H, PRDX6, and UCHL14 is consistent with protein expressions. These results may provide valuable information to decipher the molecular mechanism behind meat quality of low and high pH muscles.

Keywords: meat quality, proteomics, *Berkshire*, muscle pH, drip loss.

INTRODUCTION

Consumption of meat has been globally increasing during the last fifty years due to the rise in developing countries' income levels. Specifically, pork is the most frequently eaten meat accounting for nearly more than half of the meat consumption in many countries. Most consumer demands for quality meat have been increasing, so high-quality meat production is important to satisfy the buying public and to render the pork industry competitive. In order to produce high quality meat, it is necessary to understand the meat quality characteristics and the factors to control them (Kim et al. 2016). These include pH, color, tenderness, water holding capacity (WHC), and chemical composition

The pH measurement is a one of the most direct ways to determine meat tenderness (Van Laack et al., 2001). A number of authors reported that a higher pH is associated with darker color, reduced drip loss, better WHC (higher water holding and water binding capacity as well as increased firmness and tenderness (Warner, 1994; Eikelenboom et al., 1996). The muscle in a live pig maintains a neutral 7.0 -7.2 pH; after slaughter it decreases because of incomplete oxidation, lactic acid accumulation, as well as acidification (Pearce et al., 2011), since muscles may convert glycogen into lactate as proposed by Przybylski et al. (2006). The preferred ranges for initial (pH_{45min}) and ultimate pH (pH_{24h}) were 6.3-6.7 and 5.7-6.1, respectively (PIC, 2003). A lower pH (5.4) effectively stimulated protein denaturation, protein solubility, protein oxidation, and decreased the WHC through breakdown of the structural and myofibrillar proteins in contrast to a higher pH (6.5) which caused loss of biological functions (Barbut et al., 2008). Additionally, normal early postmortem muscle pH decline could indicate good quality pork, as well as an ultimate meat with a higher pH of a darker color and less drip loss. Conversely, denatured protein could occur with a greater pH decline, responsible for WHC, meat texture, and

color (Lonergan et al., 2008). The biophysical status of muscle protein is a direct reflection of meat quality, assessed by proteome analysis for understanding the meat's quality traits and their interactions' biological basis (Hwang et al., 2004). Proteomics refers to a powerful approach for meat quality prediction and precise protein expression monitoring. Elucidating the molecular link between muscle proteins and meat quality traits is obviously more accurate than looking at a single protein. The 2DE and MS analysis of porcine exudates have shown that potentially rich protein biomarker sources of the WHC include stress response factors, metabolic enzymes, and structural proteins, such as the myosin light chain-1, troponin T, actin, the myosin regulatory light chain, α - β -tropomyosin, phosphor triose isomerase, phosphocreatine kinase, desmin, and transcription factors (Bernevic et al., 2011; Hwang et al., 2005; Wang et al., 2016). However, given the thousands of proteins in muscles, these are too few to comprehend meat quality traits' mechanisms. Furthermore, to our best knowledge, there is no readily available information about the molecular mechanism and the effect of different pH levels on *Berkshire* meat quality. In the Korean market, *Berkshire* pork has been sold as premium meat and is in high demand, because it has more desirable characteristics such as tenderness, juiciness, redness, and brightness than other breeds (Do et al., 2012). Therefore, the objective of this study was to compare meat quality traits and to identify proteins differently expressed between low (5.61 ± 0.05) and high pH (5.96 ± 0.01) from *Berkshire* LD muscles using 2DE and MALDI-TOF/MS. Ultimately, we aimed to understand the molecular mechanisms of the relationship between proteins and quality traits of different pH meats better.

MATERIALS AND METHODS

Experimental animals

A total of 100 castrated *Berkshire* pigs were raised in fattening pens (20 pigs per pen) for 120 days under the same conditions (Da-San-Jong-Don Breeding Farm Co. Ltd., Namwon City, Cheonbuk Province, Korea). The pigs were fed with commercial feed according to the regimens of Purina Ltd and slaughtered at a similar live weight (115 ± 7 kg). According to Kim et al. (2011), meat samples from the *Berkshire* pigs were divided into low and high pH groups on the basis of post-mortem LD muscle pH_{24h} values: the lowest and highest 10% pH_{24h}, each $n = 10$, respectively. The carcass weight was significantly ($P < 0.05$) increased in high pH group (91.40 ± 1.40) whereas it was decreased in low pH group (84.70 ± 1.76). All animal care protocols were approved by the Chonbuk National University Institutional Animal Care and Use Committee.

Meat quality

The ultimate LD muscle pH (pH_{24h}) was measured on the 10-rib face using a pH star probe (Horiba 6252-10D, Irvine, CA, USA). NPPC color and marbling data were collected according to the National Pork Producers Council (1999).

At 24 h postmortem, the color of the freshly cut meat surface (30 min blooming time) was recorded using a Minolta chromameter (CR-300, Minolta Camera Co., Ltd., Osaka, Japan) (Martin and Marjeta, 2007). The color measurements were performed at room temperature and reported as CIE L^* , a^* , and b^* ((Lightness (L^*), Redness (a^*) and Yellowness (b^*)).

The meat's WHC measurement was carried out in three different ways: drip loss, filter-paper fluid uptake, and cooking loss. Drip loss was measured by the gravimetric method of Honikel (1998). The samples were cut and weighed immediately to obtain their initial drip loss weight, suspended in an inflated plastic bag, hung for 48 h at 4 °C, and then reweighed. The drip loss

amount was calculated as the difference between the weight at the onset and after hanging. Filter-paper fluid uptake was measured as described by Kauffman et al. (1986). First, meat samples were exposed to the air for 15 min, filter paper (55 mm in diameter) pre-weighed, placed on the meat's surface for 2 s to absorb its fluid, and then weighed again. Finally, cooking loss was measured by the method of Honikel (1998). Samples were weighed and packed in a plastic bag, which was placed in a water bath at 80 °C until a temperature of 75 °C was reached. The samples were then cooled (1-5 °C) under running water for 30 min, dried with a paper towel, and weighed. The percentage of cooking loss was calculated as the difference in weight before and after boiling.

The Warner-Bratzler shear force (WBSF) was determined by taking meat sample blocks of approximately 300 g. Three representative 1.27 cm diameter cores were removed from each steak parallel to the muscle fibers after cooling. Shear force values were determined with a Warner-Bratzler shear attachment using an Instron Universal Testing Machine (Model 3342; Instron Corporation, Norwood, MA, USA) with the following operating parameters: 50 kg load cell and a 200 mm/min cross-head speed. Each core sample was sheared once across the center of the core perpendicular to the muscle fibers. The shear force value was the mean of the maximum forces required to shear each set of core samples and expressed as kg of force (kgf).

Protein extraction

Samples were removed immediately after sacrifice and stored in liquid nitrogen. Hundred mg of samples were homogenized with a 1 ml sample preparation buffer (7M urea, 2M thiourea, 4% (w/v) CHAPS, 2% (v/v) IPG buffer pH 3-10, 40 mM DTT) in ice. After centrifugation (15,000 rpm for 20 min at 4 °C), the supernatant was collected. The 2D clean-up kit (GE Healthcare, NJ, USA) was employed for the sample clean up prior to the protein assay following the

manufacturer's instructions. The protein concentration was measured using the 2D Quant kit (GE Healthcare, NJ, USA).

Two-dimensional gel electrophoresis (2DE)

Isoelectric focusing was operated using an Immobiline Dry-strip pH 3-10 nonlinear, 24 cm strip (GE Healthcare, Uppsala, Sweden). The 800 µg of proteins were mixed in a rehydration buffer (7M urea, 2 M thiourea, 2% (w/v) CHAPS, .5% (v/v) IPG buffer pH 3-10, 0.002% bromophenol blue) up to 450 µl. Each 450 µl sample was added to a strip and covered with mineral oil. The strips were rehydrated (Protein IEF Cell, Biorad, USA) for 15 h at room temperature. Afterward, the proteins were separated by IEF at 100 V for 1 h (step), 200 V for 1 h (step), 200 V for 1 h (step), 500 V for 1 h (step), 1,000 V for 1 h (gradient), 10,000 V for 3 h 45 min (gradient), 30,000 Vh (EttanIPGphor 3, GE Healthcare) (50 µA per each gel strip). Prior to SDS-PAGE, the strips were in the equilibration buffer A (6 M urea, 75 mM Tris-HCl pH 8.8, 29.3% (v/v) glycerol, 2% (w/v) SDS, 0.002% (w/v) bromophenol blue, 1% (w/v) DTT) for 15 min and then in equilibration buffer B (same buffer replacing DTT with 2.5% (w/v) iodoacetamide) for 15 min. After equilibration, the strips were placed on vertical 12.5% polyacrylamide gels' tops and sealed with a 1% low-melting agarose solution. SDS-PAGE was run on an Ettan DALT system (Ettan DALT six electrophoresis unit, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 10 mA for the first 1 h and then at 40 mA until the blue line reached the gel's bottom in a refrigerator. After electrophoresis, the gels were subjected to silver staining for which a fixing solution was applied (45% methanol, 5% acetic acid) for 4 h sensitized with 0.2% sodium triosulfate for 1 min, stained with 0.5% silver nitrate solution for 30 min, developing (0.5% formaldehyde, 20% Na₂CO₃) until protein spots were visible and then stopped developing (20% acetic acid). After the 2DE stain, gels were scanned by a Bio-5000 plus (Microtec Co.) scanner

and analyzed using the Image Master 2D Platinum 7 software (GE Healthcare). All spots were automatically detected and manually edited. The selection of spots was chosen from more than twice up- or downregulated proteins as quantified by the Image Master 2D Platinum program.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS)

Proteins were subjected to in-gel trypsin digestion (Shevchenko et al., 1996). Excised gel spots were de-stained with a 100 μ l solution (50% MEOH/DW) followed by ammonium bicarbonate (200 mM) for 20 min. The gel pieces were dehydrated with 100 μ l of acetonitrile, dried in a vacuum centrifuge, 50 μ l 10 mM DTT in 0.1 M ammonium bicarbonates at 56 °C added, and then shaken for 30 min. Afterward, 50 μ l 55 mM iodoacetamide in .1 M ammonium bicarbonate was added, incubated for 20 min in the dark, the supernatant removed, and then 200 μ l acetonitrile and 200 μ l 0.1 M ABC solution added, which was repeated three times (ABC and acetonitrile process). The dried gel pieces were rehydrated with 20 μ l of 50 mM ammonium bicarbonate containing 0.2 g modified trypsin (Promega) for 45 min on ice. After removal of the solution, 30 μ l of 50 mM ammonium bicarbonate was added to perform the digestion process overnight at 37 °C. The peptide solution was desalted using the ZipTip C18 column (PIERCE Co.). Mass spectrometric measurements were analyzed with the Bruker Ultraflex III MALDI-TOF machine (Burker Daltonics, Germany) to detect protein sequences. A description of the instruction's conditions is shown in Table 1.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total cellular RNA was extracted from muscles using a TriZol reagent and the prepared RNA's purity was checked with the μ Drop plate (Thermo Fisher scientific, USA). The cDNA was synthesized with the iScriptTM cDNA Synthesis Kit from BIO-RAD, using 2 μ g of the total RNA.

The reverse transcription polymerase chain reaction assays were performed with the CFX96™ Real-Time PCR detection system (BIO-RAD). The cDNA was amplified with each gene and the reaction carried out according to the manufacturer's instructions (BIO-RAD). Real-time PCR was performed using a cDNA equivalent from each sample's total RNA in an amount of 10 ng with primers specific for CRYAB, DUSP1, ATP5H, PRDX6, UCTH14, heat shock protein beta-1 (HSP-b1), cyt c, beta-enolase X1, prohibitin (PHB), 60S ribosomal protein L29, carbonic anhydrase 3 (CA3), succinyl-CoA ligase [ADP-forming] subunit beta (SUCLA), DUSP1, and a housekeeping gene, β -actin (Table 2). The statistical analysis of the real-time PCR results was calculated by using the Δ Ct (cycle threshold) value ($Ct_{\text{gene of interest}} - Ct_{\text{reporter gene}}$). Relative gene expressions were obtained by $\Delta\Delta$ Ct methods ($\Delta Ct_{\text{sample}} - \Delta Ct_{\text{calibrator}}$). The conversion between $\Delta\Delta$ Ct and relative gene expression levels is as follows: Fold induction = $2^{-\Delta\Delta Ct}$, where $2^{-\Delta\Delta Ct}$ is the relative gene expression (Livak and Schmittgen, 2001).

Statistical analysis

All values were expressed as means $\pm$ SE. The data were statistically evaluated by Students' test for unpaired comparisons. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Meat quality, identification, and comparison of differentially expressed proteins

The present study evaluated the influence of two different pH levels on the proteomic profile and pork meat quality traits. Meat quality, including its color (CIE L*, a* and b*), drip loss, shearing force, and cooking loss are the primary consumer acceptance determinants during purchase. We first examined the meat quality traits according to the pH. Compared to a low pH muscle, the high pH muscle had a significantly ($P < 0.05$) improved meat color (CIE L* and b*), a lower drip loss, a lower shear force, and a higher NPPC color which can be used as meat quality indicators or markers (Table 3). These results are accordance with Nam et al. (2012) who observed that the decreased pH rate reflects a better meat color (CIE L* and b*), lower drip and cooking loss in porcine LD muscles. The findings demonstrate clearly that the high pH muscles contain superior meat quality traits when compared to low pH muscles.

To compare the proteomic profile possibly related to functional pH differences of muscles the high-resolution MALDI-TOF/MS was used. Based on our proteomic results, the expression of fourteen proteins was identified as potentially important meat quality regulators: CRYAB, DUSP1, VIMX1 and X2, ATP5H, PRDX6, UCTH14, HSP-b1, cyt c, β -enolase X1, PHB, 60S ribosomal protein L29, CA3, and SUCLA (Fig. 1 and Table 4). Among them, nine proteins spots were overabundant in high pH groups: CRYAB, DUSP1, VIMX1, VIMX2, ATP5H, PRDX6, UCTH14, HSP-b1, and cyt c. In the low pH group muscle the five protein spots β -enolase X1, PHB, CA3, 60S ribosomal protein L29, and SUCLA significantly increased when compared to high pH muscles. The proteins PRDX6 and HSP-27 positively correlate with color stability and also function as tenderness biomarkers (Joseph et al., 2012; Cassar-Malek and Picard, 2016). Moreover, PRDX6 is a powerful antioxidant that regulates and restores a normal redox state to

protect the cell or organism against oxidative stress. It is overexpressed in the female pigs' *longissimus lumborum* which can directly reduce phospholipid hydroperoxide and increase formaldehyde detoxification as compared to castrated male pigs (Manevich and Fisher, 2005). Furthermore, higher PRDX6 levels were obtained in indoor pigs which are associated with lower myofibrillar protein amounts related to less oxidative and more glycolytic pathways compared to outdoor pigs (Kwasiborski et al., 2009). The high PRDX6 level present in tender meat might have been exposed to less oxidative stress than tough meat (Jia et al., 2009). Hence we presumed that the high pH group could possess better antioxidant properties, an increased glycolytic activity, and might be more tender, because the PRDX6 expression level was higher than in the lower pH group (Fig. 1 and Table 4). These protein expression results were consistent with our obtained mRNA expression levels.

The PHB (spot 204) expressions displayed different patterns in both pH groups: It increased in low pH muscles and downregulated in the high pH ones (Fig. 1 and Table 4), inconsistent with the mRNA expression. The mRNA expression of PHB significantly increased in high pH muscles compared to the low pH muscles. A previous study, consistent with mRNA expression, found that, PHB was high in *Lantang* pigs but decreased in *Landrace* pigs. Gondret et al. (2014) reported that the PHB expression was high for up to 18 days in adipocyte than older pigs. However, the precise functions of PHB in skeletal muscles remain uncertain and are worthy of further research (Li et al., 2013). Moreover, PHB is associated with ROS production; there were interlinks between PHB and cyt c; the PHB drop increased the expression of both HSP-b1 and cyt c (Mao et al., 2012; Chen et al., 2005). This is accordance with our current study, i.e. the protein expression of PHB decreased whereas the cyt c expression was significantly elevated in the high pH as compared to the low pH group (Fig. 1 and Table 4). However, the exact functions

of PHB in meat traits are still unclear and require further investigations to understand them with regard to meat quality.

Spots 234 and 478 were identified as HSP-b1 and cyt c, and there were differently expressed in the low and high pH groups (Fig. 1 and Table 4): A higher protein expression of HSP-b1 and cyt c was found in the latter category. The HSP family is known for its role to protect cells from stress and for its physiological importance to maintain cellular homeostasis. But HSP-b1 has different functions in muscles: It is a candidate related to meat quality and the increased expression of this protein could provide better resistance against acute heat stress and protein degradation in cattle (Liu and Steinacker, 2001). Kim et al. (2008) reported that HSP-b1 downregulation, which is positively correlated with shear force, might lead to a rapid degradation of muscle proteins. Additionally, it decreases when the muscle pH declines, which can damage myofibril structure and lead to increased drip loss. The HSP-b1 also prevents apoptosis and could be signaling molecules for the immune system (Li et al., 2013). All these findings concur with our experiment: The HSP-b1 was significantly increased in high pH muscles, which indicates that they had a lower drip loss and a greater disease resistance than low pH muscles. This study examined if its protein expression is associated with gene expression, but there were inconsistencies between the proteins and the mRNA expressions.

The release of cyt c from mitochondria into the cytoplasm is a key marker of the apoptosis process initiation. The cyt c content in the mitochondria fraction rapidly decreased, but activated caspase-3 and caspase-9 leading to the initial apoptosis phase which may greatly influence meat color during the early *post mortem* period (Huang et al., 2016). The high calcium level in pork meat results from the mitochondria integrity releasing a cyt c, then interacting with caspase-9, and finally triggering apoptosis. Moreover, the cyt c expression increased in PSE (pale, soft and

exudative) meat. This suggests that PSE meat has a higher potential to undergo apoptosis more intensely than RFN (reddish, firm and non-exudative) meat (Guo et al., 2016). In the present study, Fig. 1 and Table 4 show that the cyt c protein expression was elevated in high pH in contrast to the low pH *Berkshire* LD muscles. The expressions of the proteins and the mRNA were inconsistent, because the cyt c markedly increased in the low pH group, but decreased in the high pH group.

Spot 473-1 is identified as CA3 and showed a significantly increased expression in low pH groups, but similarly decreased in the high pH groups (Fig. 1 and Table 4). It is well known that CA3 plays a significant role in skeletal muscles' cytoplasmic pH regulation and that it stimulates the rate of ATP synthesis (Kim et al., 2006). A previous study demonstrated that the mRNA expression of CA3 was negatively correlated with ultimate pH while being positively correlated with drip loss and the L^* value (Desai et al., 2015). Moreover, CA3 has been associated with meat color, pH, conductivity, and it is also close to WHC in different porcine muscles (Su et al., 2004). Similarly, the protein spot and mRNA expression of CA3 was high in the low pH group which implicates that an increased CA3 level could be related to its elevations of drip and cooking loss. The β -enolase X1 of the LD muscle was overexpressed in the low pH group compared to the high pH group (Fig. 1 and Table 3). The β -enolase downregulation would increase the pH, the water holding capacity, and decrease the drip loss in pectoral muscles. It has been reported that meat with a higher pH has a higher water holding capacity and a lowered drip loss because of β -enolase decreasing (Zheng et al., 2014). Significant increases in low pH muscles' expression of β -enolase X1 being related to elevated water holding capacity and drip loss decrease are consistent with our findings. Thus, the downregulation of β -enolase X1 in high

pH muscles enhances the filter paper fluid uptake, NPPC color, and decreases the drip loss in LD muscles of *Berkshire*.

VIM is an intermediate filament protein and acts as an antioxidant in most tissues that are involved in the development of pigs embryos' muscles (Otero 1997). Dunner et al. (2013) reported that VIM has been associated with meat traits including color, pH, drip loss, and tenderness. In contrast, the 2DE VIM X1 and X2 protein expressions were significantly increased in the high pH group when compared to the low pH group (Fig. 1 and Table 4). Our data confirm that high pH group's muscles that are positively related to meat quality traits may possess antioxidant properties.

CRYAB, as a main substrate of *post-mortem* proteolysis, is also a potentially good marker of tenderness and meat color (Paredi et al., 2012). The CRYAB protein is involved in the color formation of meat as illustrated by Sayd et al. (2006) who reported that darker meat from *Large White* × *Duroc* × *Hampshire* possessed an oxidative metabolism, indicated by an abundance of CRYAB mitochondrial enzymes. On the other hand, the downregulation of CRYAB expression could be related to progressive muscle atrophy and, thus, CRYAB is also a weight loss marker (Sayd et al. 2006). The CRYAB protein was expressed at spot 64, upregulated in the high pH group, and downregulated in the low pH group (Fig. 1 and Table 3). Our results indicate that CRYAB might play an important function in maintaining the dark red color (CIE a*) and tenderness of the high-pH group. Similarly, ATP5H expression was significantly decreased in the low pH, but increased in high pH group. The same results were observed for the mRNA expression. Zhang et al. (2010) reported that the higher ATP5H protein expression in bovine skeletal muscles leads to increased intramuscular fat, which is an important issue in the animal industry, because such meat is a preferred taste of people.

The expression levels of DUSP1 and the 60S ribosomal protein L29 protein spot from *Berkshire* are illustrated in Fig. 1 and Table 4. The expression level of 60S ribosomal protein L29 significantly increased whereas DUSP1 decreased in the low pH group. At a genetic level, the 60S ribosomal protein L29 was associated with meat marbling (accumulation of intramuscular fat). However, the precise functions of both 60S ribosomal protein L29 and DUSP1 in muscles remain uncertain and require further research.

The correlation between protein abundances and mRNA expression levels depend on several biological factors, such as the translation efficiency and a protein's half-life. Therefore, to manipulate meat quality genetically, it is important to determine the mRNA expression that regulates meat quality at a protein level. In order to determine the mRNA expression of proteins differentially expressed according by the pH, we performed an RNA qRT-PCR assay. Further, the qPCR analysis of these proteins at the mRNA levels demonstrated their differing role according to the pH; the expression levels of all target genes are shown in Fig. 2. The selected seven genes (ATP5H, 60S ribosomal protein L29, PRDX6, PHB, SUCLA, β -enolase X1, and UCTH14) were significantly upregulated in high pH LD muscles of *Berkshire*. Moreover, the mRNA expressions of CRYAB and CA3 were significantly downregulated in *Berkshire* high pH LD muscles. There were no significant differences in gene expressions of DUSP1 and HSP-b1 between low and high pH groups. Protein abundances of PRDX6, CA3, ATP5H, and UCTH14 were consistent with mRNA levels, while PHB, CRYAB, 60S ribosomal protein L29, β -enolase X1, and SUCLA were inconsistent, thus functioning as potential targets for the genetic manipulation of meat quality. This inconsistency may reflect unsynchronized abundances of mRNA and proteins (Zheng et al., 2014). The consistent between mRNA and protein level dependence on many biological factors, mainly transcription/translation efficiency and proteins

half-life. Moreover, the genes could be present and be matured but they are not necessarily transcribed as well protein degradation (Celis et al., 2000). Some mRNA are transcribed but were not translated, and the number of mRNA copies does not necessarily reflect the number of functional protein molecules (Anderson and Seilhamer, 1997). A further verification of those proteomic results should be performed with western blot analysis.

this study we investigated meat quality traits between low pH and high pH groups of LD muscle from *Berkshire*.

CONCLUSION

In conclusion, meat quality traits such as (CIE L* (Lightness), CIE b* (Yellowness), Drip loss, shearing force and NPPC color) have differed between low pH and high pH groups of LD muscle from *Berkshire*. The results showed that high pH group had good meat quality than low pH group. Moreover, we have compared the meat quality trait's related proteomes of low pH and high pH muscles from *Berkshire* using 2DE and MALDI-TOF/MS. By proteomic analysis, we observed 14 candidate proteins that differed in spot density between low and high pH muscles. Nine protein spots (CRYAB, DUSP1, VIM X1 and X2, ATP5H, PRDX6, UCTH14, HSP-b1, and cyt c) which are major regulators of meat quality traits, metabolic enzymes, and the stress response factor significantly increased in high pH muscles. Additionally, β -enolase X1, PHB, 60S ribosomal protein L29, CA3, and SUCLA significantly decreased in the high pH group. Moreover, the mRNA expressions of ATP5H, PRDX6, and UCTH14 were consistent with the protein level, whereas this was not the case for β -enolase X1, PHB, 60S ribosomal protein L29, cyt c, CRYAB, and SUCLA. Such results indicate that the metabolic energy and stress-related proteins are important indicators for meat quality in high pH muscles. Our study provides

valuable information to understand the molecular mechanisms responsible for differences in meat quality between low and high pH pig muscles.

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Legends

Figure 1. Representation of two-dimensional electrophoresis (2-DE gel) profiles from LD muscles of *Berkshire* with a low pH (A) and a high pH (B) (arrows indicate the target proteins).

Figure 2. Transcriptional levels of differentially expressed proteomic profiles from LD muscles of *Berkshire* with a low pH and a high pH. *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$.

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367x233mm (96 x 96 DPI)

Table 1. Conditions of MALDI-TOF/MS

Item	Condition
Mode of operation	Reflector
Acquisition mass range	0-7000 Da
Extraction mode	Delayed
Number of lase shots	800
Polarity	Positive
Laser intensity	40%
Acquisition control	Manual
Laser rap rate	25.0 Hz
Accelerating voltage	20,000 V
Calibration type	Peptide STD II
Grid voltage	60.5%
Calibration matrix	CHCA
Guide wire voltage	1.0%
Low mass gate	400 m/z
Extraction delay time	400 nsec

Table 2. Primer sequences for qRT-PCR

Gene name	Product size (bp)	strand	Sequence (5'-3')
crystallin alpha B (CRYAB)	171bp	Sense antisense	CGAGGAACTCAAGGTCAAGG AGGACCCCATCAGATGACAG
dual specificity phosphatase1 (DUSP1)	203bp	Sense antisense	ATCTCCCCCAACTTCAGCTT GTGATGGGGCTCTGAAGGTA
beta-enolase X1	319 bp	Sense antisense	CCAGGCAGTTGAGAAGAAGG TCACTTGGCCTTTGGGTAC
ATP synthase subunit d, mitochondrial (ATP5H)	220 bp	Sense antisense	TCATACCCCGAAACCAGAAG GCATCCACCAGAGCAGTGTA
peroxiredoxin6 (PRDX6)	336 bp	Sense antisense	TCATGGGGCATTCTTTTCTC CAGCTTTTATCGGGACCAA
ubiquitin carboxyl-terminal hydrolase 14 (UCHL1)	451 bp	Sense antisense	TGTTTTAAAGCTGGGGGTTG ATGACTGACCTCAGGGTTGG
Prohibitin (PHB)	257 bp	Sense antisense	ACATCACCTGCGTATCCTC GGTCAGATGCGTCAGAGACA
heat shock protein beta-1 (HSP-b1)	289 bp	Sense antisense	CCGGTGTTTCACTCGAAAAT AGCACAGAGAGTTGGCAGGT
60S ribosomal protein L29	401 bp	Sense antisense	CTTGCCAAGAAGCACAACA CCAGGAATCACACCAGTCCT
carbonic anhydrase3 (CA3)	202 bp	Sense antisense	GCTTCGCCAGTTTCATCTTC GAACTCGCCTTTCTCACGTC
succinate CoA ligase [ADP-forming] subunit beta, (SUCLA)	482 bp	Sense antisense	GACTGGACCCAGGAAGATGA GCTTGCTTGGCTAAGGTCAC
cytochrome c (cyt c)	203 bp	Sense antisense	AATGGGCAATGTTGAGAAGG ACGCCATCAGTGTCTCCTCT
β -actin	114 bp	Sense antisense	TCTGGCACCACACCTTCT TGATCTGGGTCATCTTCTCAC

Table 3.Effect of pH on meat quality in porcine *longissimus dorsi* muscles

	pH Low (n = 10)	pH High (n = 10)
pH (24-hour)	5.61 ± 0.05	5.96 ± 0.01*
Color		
CIE L* (Lightness)	51.27 ± 0.58	48.18 ± 0.59*
CIE a* (Redness)	15.66 ± 0.34	16.17 ± 0.15
CIE b* (Yellowness)	4.60 ± 0.21	3.91 ± 0.24*
Filter-paper fluid uptake (mg)	71 ± 6.40	74 ± 4.98
Drip loss (%)	3.14 ± 0.42	1.29 ± 0.16*
Cooking loss (%)	13.55 ± 0.87	12.66 ± 0.72
Shearing force (N)	2.75 ± 0.20	2.08 ± 0.11*
NPPC color	3.19 ± 0.16	3.95 ± 0.15*
NPPC marbling	2.2 6± 0.25	1.6 ± 0.14

Note: Mean values are presented as mean ± SE. *significant as compared with a low pH at $P < 0.05$.

Table 4. Identification of proteome changes between low pH and high pH in porcine *longissimus dorsi* muscles

Spot no.	Description of protein	Expression pattern	Fold change	score	Coverage (%)	Mass	pI	Species	NCBI accession no.
64	Alpha-crystallin B chain	↑	2.47	60	42	20,116	6.76	Susscrofa	gi 545848505
87	Dual specificity phosphatase 1	↑	3.57	99	37	25,287	6.9	Susscrofa	gi 927206798
109	Beta enolase isoform X1	↓	3.00	83	47	48,155	8.52	Susscrofa	gi 545859898
154	vimentin isoform X1	↑	2.41	81	48	53,692	5.06	Susscrofa	gi 545853401
154	Vimentin isoform x2	↑	2.41	82	52	49,248	5.09	Susscrofa	gi 545853404
155	ATP synthase subunit d, mitochondrial	↑	2.45	121	56	18,561	5.99	Susscrofa	gi 347658971
182	Peroxiredoxin6	↑	2.31	76	48	25,078	5.73	Susscrofa	gi 47523870
203	Ubiquitin carboxyl-terminal hydrolase 14, partial	↑	2.81	126	30	56,893	5.39	Susscrofa	gi 927226365
204	Prohibitin isoform X1	↓	2.72	113	42	29,923	5.40	Susscrofa	gi 350590415
234	Heat shock protein beta-1	↑	2.48	71	41	22,985	6.23	Susscrofa	gi 55926209
326	60S ribosomal protein L29	↓	3.23	152	81	17,544	11.92	Susscrofa	gi 47522730
473-1	Carbonic anhydrase 3	↓	3.39	60	33	29,678	7.72	Susscrofa	gi 56711366
473-2	Succinyl-CoA ligase [ADP-forming] subunit beta	↓	2.38	52	24	46,571	6.08	Susscrofa	gi 311266259
478	Cytochrome c	↑	2.34	87	79	11,810	9.52	Susscrofa	gi 194018698

Note: The expression patterns are as follows: (↑) high expressed protein in high pH group; (↓) low expressed protein in high pH group