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Correlation of Meat pH and Muscle Fiber Characteristics, Cortisol Level, and *Tenascin C* Gene Expression in Pigs

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ABSTRACT

The effects of meat pH on muscle fiber characteristics, cortisol level, and *Tenascin C* (*TNC*) gene expression were examined. The muscle samples (n=100) were randomly collected from the *Longissimus thoracis et lumborum* (LTL) to determine meat pH at 24 hours (meat pH24h) post-mortem. Muscle samples (five samples per group) with divergent meat pH levels (low versus high) were selected to study muscle fiber characteristics and mRNA expression based on quantitative real-time polymerase chain reaction (qRT-PCR). Blood samples (five samples per group) of the two meat pH levels were taken for serum cortisol analysis. The results showed that there was no significant differences between the groups for the muscle fiber characteristics of total number of fibers, muscle fiber diameter, cross-section area, perimysium thickness, and endomysium thickness. Different meat pH24h values did not affect the cortisol level. The mRNA expression of the *TNC* gene was significantly ($p<0.05$) downregulated in the low meat pH24h group compared to the high meat pH24h group. In conclusion, meat pH24h was unrelated to the cortisol level and muscle fiber characteristics. However, the *TNC* gene might play a role in meat pH24h in pigs.

Keywords: cortisol; meat pH; muscle fiber characteristics; TNC

INTRODUCTION

Meat quality may result in economic losses in the meat processing industry. One of the most important indicators of the effect of pre-mortem stress on meat quality is the pH value of the meat (initial and final pH levels, as well as the rate of pH decrease) (Lawrie & Ledward, 2014). Acute stress before slaughter can result in pale, soft, and exudative (PSE) meat with a low pH (pH < 5.3) at 24 hours post-mortem. Chronic stress can result in dark, firm, and dry meat with a high pH (pH > 6.0) at 24 hours post-mortem (Gonzalez-Rivas *et al.*, 2020). Animal stress was linked to high cortisol and creatine kinase levels that were associated with a high pH at 24 hours post-mortem (D'eath *et al.*, 2010). An increase in the measured meat pH at 45 minutes post-mortem was accompanied by the increased water-holding capacity (WHC) and plasticity, as well as a lower drip loss. A higher meat pH at 48 hours post-mortem had a greater impact on WHC, free drip loss, tenderness, and water content than a lower meat pH (Jankowiak *et al.*, 2021).

For several decades, genetic improvement of animal species has been carried out very successfully based on the selection of economically important quantitative traits such as yield and product quality (Meuwissen *et al.*, 2016). Molecular information removes some of the limitations of phenotypic selection by allowing selection at the genotype (DNA) level, resulting in rapid, more accurate, and cheaper selection (Samorè & Fontanesi, 2016). Integrating structural and functional genomics is the current challenge for genetic research on meat quality traits. Bioinformatics is becoming increasingly important for analyzing expression data, with the ultimate goal of extracting biologically meaningful information from a list of differentially expressed genes (Davoli & Braglia, 2007). The candidate genes are evaluated by considering the relationship between the traits of interest and known genes (Koopaei & Koshkoiyeh, 2011). A candidate gene approach seeks to identify genes based on their potential roles in physiology. Additional approaches, such as a gene expression analysis of the phenotypic divergence of interest, have been incorporated into the candidate gene analysis, whereby a putative candidate gene is one that can be statistically detected among the genes controlling large components of inheritable gene expression variation (Schellander, 2009).

The *Tenascin C* (*TNC*) gene has roles in extracellular matrix molecules (ECMs; Yilmaz *et al.*, 2022), with ECMs including collagens, proteoglycans, and glycoproteins (Nishimura, 2015). The expression of ECM molecules, such as heparan sulfate proteoglycans, affects muscle development and growth properties that, in turn, influence muscle structure and meat quality (Velleman, 2012; Wang *et al.*, 2016). The main composition of connective tissue, known as extracellular matrix protein, is also important for meat texture (Ahmad *et al.*, 2021; Zheng *et al.*, 2022) because proteoglycans are negatively charged and likely determine the water binding capacity of skeletal muscle. The extracellular matrix may be important for meat quality (Von Lengerken *et al.*, 2002). One study identified three SNPs in the *TNC* gene and found significant associations between identified polymorphisms and meat and carcass quality traits in Duroc × Pietrain and commercial Pietrain populations (Kayan *et al.*, 2011). Understanding the biological roles of genes regulating meat pH is critical to understanding the molecular genetic mechanism and can help the meat industry to achieve its goal of increasing high meat quality. Meat pH might be correlated to the muscle fiber characteristics, cortisol level, and *TNC* gene expression in pigs. Therefore, the present study aimed to evaluate the effects of meat pH24h on the muscle fiber characteristics, cortisol level, and *TNC* gene expression.

MATERIALS AND METHODS

Meat Sample Preparation

The meat samples were obtained from a local meat supplier in Thailand, which involved no direct contact with live animals; therefore, ethical approval was not required. In total, 100 muscle samples were randomly collected from crossbred pigs [Duroc × (Large White × Landrace)]. The average slaughter weight was 112.13 ± 4.81 kg, measured at a commercial abattoir following the standard slaughtering procedures of the Department of Livestock Development, Thailand. The meat pH value was measured using a spear-type electrode (pH Spear, Eutech Instruments, Singapore) in the *Longissimus lumborum* between the 13th and 14th ribs at 24 h post-mortem (pH24h). The pH meter was calibrated using buffers of 4.20 and 7.10. Then, high and low meat pH24h groups were randomly selected from the 10% lower and 10% upper ranges of values of meat pH24h to provide 100 samples for mRNA expression and histological and cortisol analyses. The muscle samples were immediately collected from the *Longissimus thoracis et lumborum* (LTL), then kept at −80 ^{o}C for mRNA expression and fixed in 10% buffered neutral formalin for histological analysis. Blood samples were transferred in a tube without ethylenediamine tetra-acetic acid (EDTA) for cortisol analysis.

Histological Analysis

The samples were selected based on low and high meat pH24h groups (n= 5 per group). Each sample was cut into 0.5 × 0.5 × 1.0 cm pieces and immediately fixed in 10% buffered neutral formalin solution for 24 hours. Then, each sample was dehydrated in alcohol and cleared in xylene. Next, the samples were infiltrated and embedded in paraffin (Khoshoii *et al.*, 2013). Sections (3 μm thickness) were cut and prepared using hematoxylin and eosin (H&E) stain for general histological study. The stained cross-sections were viewed and photographed using a light microscope (Olympus FSX100, Tokyo, Japan) at 10× (objective lens) and 10× (eyepiece). Five photographs of different cross-sections from each muscle were taken. The samples were analyzed using the Image-J software (National Institute of Mental Health, Bethesda, MD, USA). The mean number of fibers per area was obtained by counting the total number of fibers (TNF) in five areas (each area being 582,007 μm^2) per sample. Approximately 300 fibers in five random microscope fields of view for each muscle were measured to estimate the muscle fiber diameter (measured in microns) and cross-section area (measured in square microns). The thickness of the endomysium and perimysium were determined on each sample. The structural elements were measured in an area of the fiber bundle. On each image, 40 measurements were made of the thickness of the endomysium, and 10 measurements were made of the thickness of the perimysium (both measured in microns). The mean thickness was estimated from the measured values (Koomkrong *et al.*, 2017).

Cortisol Analysis

Blood samples were collected from animals after the slaughtering process based on the low and high meat pH24h groups (n= 5 per group). Blood samples were transferred in a tube without EDTA. The non-anticoagulated blood was centrifuged at 3,000 rpm for 10 min to separate the serum. The serum samples were collected into microtubes and stored at -20 °C until the determination of cortisol concentration based on chemiluminescent microparticle immunoassay using an IMMULITE 1000 automated immunoassay system (Siemens Healthcare Diagnostics Inc., Flanders, NJ, USA).

mRNA Expression Study using qRT-PCR

The differential expression of the *TNC* gene was performed based on the low and high meat pH24h groups (n= 5 per group). Total RNA was isolated from 20 mg of muscle samples using a QIAamp RNA Mini Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations. The purity of the extracted RNA was measured using a spectrophotometer (LKB622, Biodrop, England). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed using a MyGo Pro® real-time PCR instrument (IT-IS Life Science Ltd; Middlesbrough, UK) with a reaction mixture using a QuantiNova SYBR Green RT-PCR Kit (Qiagen; Hilden, Germany), consisting of 10 μL of 2X QuantiNova SYBR Green RT-PCR Master Mix, 1 μL of each 10 μM (0.5 μM) forward and reverse primers, 0.2 μL of QN SYBR Green RT Mix, 5 μL of template and

2.8 µL of nuclease-free water to make a total reaction volume of 20 µL. The two-step amplification program involved pre-denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 sec and then annealing and extension at 60 °C for 10 sec. All samples were studied in duplicate as technical replications and the results were calculated as the mean of the two replications for further analysis. The final results were reported as the relative expression level after normalization of the transcript using *TBP* (*TATA sequence binding protein*) as the housekeeping gene. The PCR primers are shown in Table 1.

Statistical Analysis

Statistical analysis of the differences between the low and high meat pH24h groups for cortisol level, muscle fiber characteristics, and qRT-PCR results were analyzed based on t-tests (SAS Inst. Inc., Cary, NC, USA). Statistically significant differences were determined at $p<0.05$. The results were displayed as least squares means with the standard error of the mean. Pearson correlation coefficients between meat pH24h, muscle fiber characteristics, and cortisol level were calculated using the PROC CORR procedure in the SAS package.

RESULTS

Meat pH24h and Cortisol Level

The overall mean was 5.85 ± 0.03. The average meat pH24h was 5.48 ± 0.12 for the low meat pH24h group and 6.18 ± 0.08 for the high meat pH24h group, respectively. There was no significant difference in the cortisol levels between the low- and high-meat pH24h groups (Figure 1). There was a negative correlation between meat pH24h and cortisol (r^2= -0.066), as shown in Table 2.

Muscle Fiber Characteristics

The muscle fiber characteristics are summarized in Figure 2. There were no significant differences between groups for the muscle fiber characteristics of total number of fibers, muscle fiber diameter, cross-section area, perimysium thickness, and endomysium thickness (Table 3). However, the low meat pH24h group had a higher number of fiber and greater endomysium thickness than the high meat pH24h group. The low meat pH24h group had lower values for the muscle fiber diameter, cross-section area, and perimysium thickness than the high meat pH24h group. The correlation between meat pH24h and muscle fiber characteristics is presented in Table 2. Meat pH24h was significantly negatively correlated with endomysium thickness (r^2= -0.634).

mRNA Expression of *TNC* Gene

The meat samples with divergent meat pH24h phenotypic traits were selected for mRNA expression study. The relative expression levels of the *TNC* gene are represented in Figure 3. The expression level of the *TNC* gene in the high meat pH24h group was significantly higher than in the low meat pH24h group.

DISCUSSION

Cortisol or corticosterone levels are important indicators of stress (acute or chronic) in several animal species (Casal *et al.,* 2017; Creutzinger *et al.,* 2017; Gong *et al.,* 2015). In response to acute stress, an animal's body responds by releasing several stress hormones into the bloodstream, including cortisol, corticosterone, and adrenaline (Ghassemi Nejad *et al.,* 2022). Other studies have reported that animal stress was linked to high cortisol and creatine kinase levels that were associated with a high pH level at 24 hours post-mortem (D'eath *et al.,* 2010; García-Torres *et al.,* 2021; Lu *et al.,* 2018). However, the present study identified no significant difference in the cortisol levels between the low and high meat pH24h groups. Nonetheless, this could not be used to confirm that the animals had been stressed during processing; further study is required to investigate additional parameters, with other studies revealing that the stress indicators included lactate, glucose, cortisol, adrenocorticotropic hormone, creatine kinase, aspartate amino transferase, and alanine amino

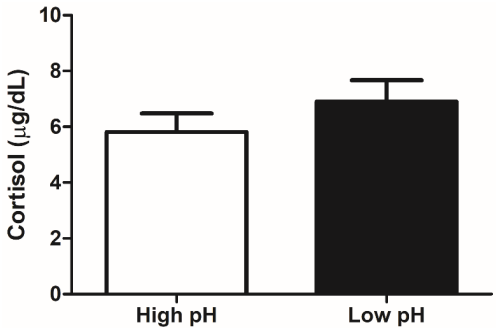

Figure 1. Cortisol levels in high and low meat pH24h groups in commercial pigs (n= 5 per group). Cortisol levels were not significantly different (p>0.05) between high and low meat pH24h groups. Error bars indicate ± standard error.

Table 1. Primer sequences used for qRT-PCR analysis of *Tenascin C* gene

Gene	Primer sequence	Tm (°C)	Product	Reference
TNC	Fw: 5′-ACAATGAGATGCGGGTCACAG-3′	60	185 bp	(Kayan *et al.,* 2011)
	Rw: 5′-CGCTGACAGGAATGCTCTTCTT-3′			
TBP	Fw: 5′-GATGGACGTTCGGTTTAGG-3′	60	124 bp	(Kayan *et al.,* 2013)
	Fw: 5′-AGCAGCACAGTACGAGCAA-3′			

Note: *TNC= Tenascin C; TBP= TATA sequence binding protein.*

Table 2. Correlations between meat pH24h, muscle fiber characteristics, and cortisol level in commercial pigs

	pH24h	TNF	MFD	CSA	PT	ET	Cortisol
pH24h	-						
TNF	-0.126						
MFD	0.187	-0.834**					
CSA	0.330	-0.882***	0.928***				
PT	0.284	-0.434	0.195	0.223			
ET	-0.634*	-0.219	0.097	-0.088	-0.057		
Cortisol	-0.066	-0.394	0.533	0.597	-0.223	-0.402	-

Note: pH24h indicates the meat pH value in the *Longissimus thoracis et lumborum* (LTL) at 24 h post-mortem. TNF= the total number of fibers; MFD= muscle fiber diameter (μ); CSA= cross-section area (μm²); PT= perimysium thickness (μm); ET= endomysium thickness (μm). * p<0.05, ** p<0.01, *** p<0.001.

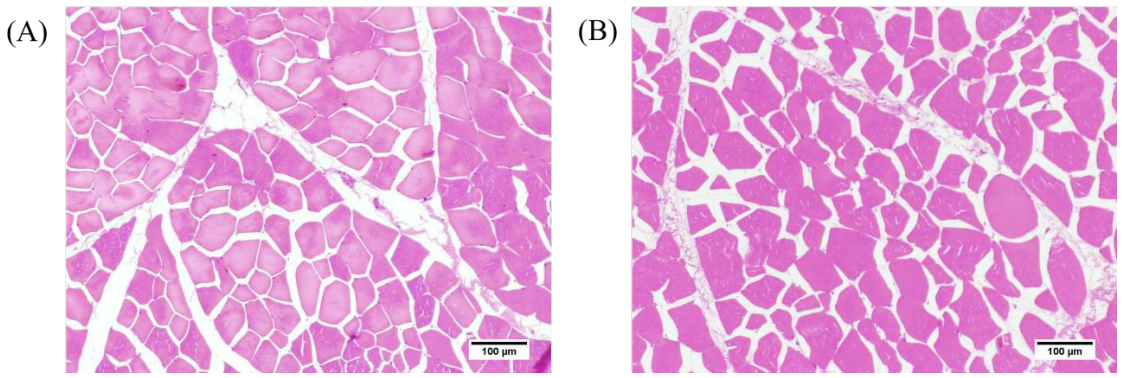

Figure 2. Histological cross-section of *Longissimus thoracis et lumborum* (LTL) muscle in commercial pigs stained with H&E and studied under a microscope (10X) (A) High meat pH (B) Low meat pH. Scale bar= 100 μm

Table 3. Muscle fiber characteristics of high and low meat pH groups in commercial pigs

Traits	High meat pH	Low meat pH	p-value
Total number of fibers	185.70±18.15	202.70±1.11	0.533
Muscle fiber diameter (μm)	48.19±3.31	47.11±2.34	0.795
Cross-section area (μm²)	2,931±332.70	2,487±275.90	0.334
Perimysium thickness (μm)	16.28±1.78	14.51±1.42	0.459
Endomysium thickness (μm)	2.37±0.29	3.50±0.77	0.205

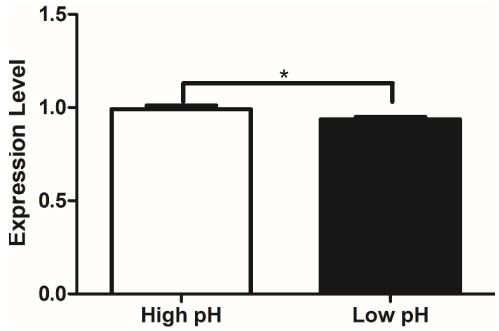

Figure 3. Normalized expression levels of *Tenascin C* (*TNC*) gene transcript in *Longissimus thoracis et lumborum* (LTL) muscle between high and low meat pH24h groups in commercial pigs (n= 5 per group). The expression level was expressed as the ratio of Cq values of *TNC* gene with the housekeeping gene *TATA sequence binding protein* (*TBP*). The mRNA expression levels were significantly (* = p<0.05) different between high and low meat pH24h groups.

transferase levels. For example, lactate dehydrogenase can be used to predict meat quality, while cortisol, alanine amino transferase, and albumin can be used for the prediction of carcass quality (Čobanović *et al.*, 2020; Dokmanovic *et al.*, 2015). In addition, the serum creatine kinase activity and cortisol level were higher in blood samples collected immediately before transportation, suggesting a strong response to stress by the animal in the pre-slaughter treatment (Śmiecińska *et al.*, 2011). To improve meat quality, it is necessary to adjust the handling practices to reduce stress during processing (Carrasco-García *et al.*, 2020). Other factors that could greatly impact meat pH24h are the slaughtering method and cooling conditions (Park *et al.*, 2002). Fiber type characteristics were reported to be mainly associated with the post-mortem metabolic rate and meat quality traits (Ryu & Kim, 2005). The cross-sectional area, muscle fiber diameter, and muscle fiber number between the biopsied and post-mortem samples were highly represented by genetic correlation (Kim *et al.*, 2018). The genetic correlation was reportedly moderate-to-high between the biopsied samples and

drip loss, lightness, and meat color (Lee *et al.,* 2022). Higher pH24h values had a highly associated impact on water holding capacity, free drip loss, tenderness, water content, and color (Jankowiak *et al.,* 2021). Muscle fiber cell degradation could be assessed based on the endomysium thickness (Tippala *et al.,* 2021), with this finding perhaps being an indicator that a lower meat pH involved water loss, leading to an increase in the endomysium thickness.

Meat pH at 24 hour post-mortem is used as an indicator for measuring meat quality, with meat pH being related to water-holding capacity, tenderness, and meat color (Hamoen *et al.,* 2013; Hopkins *et al.,* 2014). Meat pH and drip loss were reported to have a highly significant negative correlation (Jankowiak *et al.,* 2021). Another study revealed that meat pH24h could be classified into 3 groups of acid meat, normal meat, and DFD meat (Dark Firm Dry) for pH ranges of <5.6, 5.6–5.9, and >6.0, respectively (Jankowiak *et al.,* 2021). The present classified the meat pH24h into 2 groups, with the average for the low meat pH24h group being 5.48 ± 0.12, while for the high meat pH24h group, it was 6.18 ± 0.08. *The Tenascin C* (*TNC*) gene has roles in ECMs (Yilmaz *et al.,* 2022), where the ECM molecules include collagens, proteoglycans, and glycoproteins (Nishimura, 2015). Proteoglycans have a negative charge density (high pH) that can hold water in muscle tissue cells (Velleman, 2000). Accordingly, a low meat pH will increase protein denaturation of muscle fiber (Scheffler & Gerrard, 2007). The present study found that the expression level of the *TNC* gene in the high meat pH24h group was significantly higher than in the low meat pH24h group, which be evidence to support the role of the *TNC* gene, where its high expression influences the level of proteoglycan that, in turn, increases the meat pH and holds water in muscle cells. Additionally, the *TNC* gene is located in the *Sus scofa* chromosome (SSC)1, with other studies reporting that the quantitative trait loci affecting the meat pH was mapped onto SSC1 (Gao *et al.,* 2021; Jennen *et al.,* 2007). From these results, it could be postulated that cortisol and muscle fiber characteristics are not involved in meat pH24h, while the *TNC* gene might be a potential positional and functional candidate for meat pH24h.

CONCLUSION

The present study found that the meat pH24h was not correlated to the cortisol level or muscle fiber characteristics. However, the meat pH24h was associated with the expression of the *TNC* gene, with this perhaps playing a role in the meat pH24h and being highly expressed in a high meat pH. Further study of the *TNC* gene should be undertaken to understand the mechanisms related to meat pH.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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REFERENCES

Ahmad, K., J.-H. Lim, E.-J. Lee, H.-J. Chun, S. Ali, S.S. Ahmad, S. Shaikh, & I. Choi. 2021. Extracellular matrix and the production of cultured meat. Foods 10:3116. https://doi.org/10.3390/foods10123116

Carrasco-García, A. A., V. T. Pardío-Sedas, G. G. León-Banda, C. Ahuja-Aguirre, P. Paredes-Ramos, B. C. Hernández-Cruz, & V. V. Murillo. 2020. Effect of stress during slaughter on carcass characteristics and meat quality in tropical beef cattle. Asian-Australas. J. Anim. Sci. 33:1656. https://doi.org/10.5713/ajas.19.0804

Casal, N., X. Manteca, R. Peña, A. Bassols, & E. Fàbrega. 2017. Analysis of cortisol in hair samples as an indicator of stress in pigs. J. Vet. Behav. 19:1-6. https://doi.org/10.1016/j.jveb.2017.01.002

Čobanović, N., S. D. Stanković, M. Dimitrijević, B. Suvajdžić, N. Grković, D. Vasilev, & N. Karabasil. 2020. Identifying physiological stress biomarkers for prediction of pork quality variation. Animals 10:614. https://doi.org/10.3390/ani10040614

Creutzinger, K. C., J. M. Stookey, T. W. Marfleet, J. R. Campbell, D. M. Janz, F. J. Marqués, & Y. M. Seddon. 2017. An investigation of hair cortisol as a measure of long-term stress in beef cattle: Results from a castration study. Can. J. Anim. Sci. 97:499-509. https://doi.org/10.1139/CJAS-2016-0206

D'Eath, R. B., S. Turner, E. Kurt, G. Evans, L. Thölking, H. Looft, K. Wimmers, E. Murani, R. Klont, & A. Foury. 2010. Pigs' aggressive temperament affects pre- slaughter mixing aggression, stress and meat quality. Animal 4:604-616. https://doi.org/10.1017/S1751731109991406

Davoli, R. & S. Braglia. 2007. Molecular approaches in pig breeding to improve meat quality. Brief. Funct. Genomic Proteomic 6:313-321. https://doi.org/10.1093/bfgp/elm036

Dokmanovic, M., M. Z. Baltic, J. Duric, J. Ivanovic, L. Popovic, M. Todorovic, R. Markovic, & S. Pantic. 2015. Correlations among stress parameters, meat and carcass quality parameters in pigs. Asian-Australas. J. Anim. Sci. 28:435. https://doi.org/10.5713/ajas.14.0322

Gao, G., N. Gao, S. Li, W. Kuang, L. Zhu, W. Jiang, W. Yu, J. Guo, Z. Li, & C. Yang. 2021. Genome-wide association study of meat quality traits in a three-way crossbred commercial pig population. Front. Genet. 12:614087. https://doi.org/10.3389/fgene.2021.614087

García-Torres, S., M. Cabeza de Vaca, D. Tejerina, M. P. Romero-Fernández, A. Ortiz, D. Franco, M. A. Sentandreu, & Oliván, M. 2021. Assessment of stressby serum biomarkers in calves and their relationship to ultimate pH as an indicator of meat quality. Animals 11:2291. https://doi.org/10.3390/ani11082291

Ghassemi Nejad, J., M. H. Ghaffari, M. Ataallahi, J.-H. Jo, & H.-G. Lee. 2022. Stress concepts and applications in various matrices with a focus on hair cortisol and analytical methods. Animals 12:3096. https://doi.org/10.3390/ani12223096

Gong, S., Y. L. Miao, G. Z. Jiao, M. J. Sun, H. Li, J. Lin, M. J. Luo, & J. H. Tan. 2015. Dynamics and correlation of serum cortisol and corticosterone under different physiological

or stressful conditions in mice. PLoS ONE 10:e0117503. https://doi.org/10.1371/journal.pone.0117503

Gonzalez-Rivas, P. A. S. S. Chauhan, M. Ha, N. Fegan, F. R. Dunshea, & R. D. Warner. 2020. Effect of heat stress on animal physiology metabolism, and meat quality: A review. Meat Sci. 162:108025. https://doi.org/10.1016/j.meatsci.2019.108025

Hamoen, J. R., H. M. Vollebregt, & R. G. M. Van Der Sman. 2013. Prediction of the time evolution of pH in meat. Food Chem. 141:2363-2372. https://doi.org/10.1016/j.foodchem.2013.04.127

Hopkins, D. L., E. N. Ponnampalam, R. J. Van De Ven, & R. D. Warner. 2014. The effect of pH decline rate on the meat and eating quality of beef carcasses. Anim. Prod. Sci. 54:407-413. https://doi.org/10.1071/AN12314

Jankowiak, H., A. Cebulska, & M. Bocian. 2021. The relationship between acidification (pH) and meat quality traits of polish white breed pigs. Eur. Food Res. Technol. 247:2813-2820. https://doi.org/10.1007/s00217-021-03837-4

Jennen, D., A. Brings, G. Liu, H. Jüngst, E. Tholen, E. Jonas, D. Tesfaye, K. Schellander, & C. Phatsara. 2007. Genetic aspects concerning drip loss and water-holding capacity of porcine meat. J. Anim. Breed. Genet. 124:2-11. https://doi.org/10.1111/j.1439-0388.2007.00681.x

Kayan, A., M. J. Uddin, H. Kocamis, D. Tesfaye, C. Looft, E. Tholen, K. Schellander, & M. U. Cinar. 2013. Association and expression analysis of porcine HNF1A gene related to meat and carcass quality traits. Meat Sci. 94:474-479. https://doi.org/10.1016/j.meatsci.2013.04.015

Kayan, A., M. Cinar, M. Uddin, C. Phatsara, K. Wimmers, S. Ponsuksili, D. Tesfaye, C. Looft, H. Juengst, & E. Tholen. 2011. Polymorphism andexpression of the porcine *Tenascin C* gene associated with meat and carcass quality. Meat Sci. 89:76-83. https://doi.org/10.1016/j.meatsci.2011.04.001

Khoshoii, A. A., B. Mobini, & E. Rahimi. 2013. Comparison of chicken strains: Muscle fibre diameter and numbers in pectoralis superficialis muscle. Glob. Vet. 11:55-58.

Kim, J. M., K. S. Lim, K. B. Ko, & Y. C. Ryu. 2018. Estimation of pork quality in live pigs using biopsied muscle fibre number composition. Meat Sci. 137:130-133. https://doi.org/10.1016/j.meatsci.2017.11.020

Koomkrong, N., N. Gongruttananun, C. Boonkaewwan, J. Noosud, S. Theerawatanasirikul, & A. Kayan. 2017. Fiber characteristics of pork muscle exhibiting different levels of drip loss. Anim. Sci. J. 88:2044-2049. https://doi.org/10.1111/asj.12859

Koopaei, H. K. & A. E. Koshkoiyeh. 2011. Application of genomic technologies to the improvement of meat quality in farm animals. Biotechnology Molecular Biology Review 6:126-132.

Lawrie, R. A. & D. Ledward. 2014. Lawrie's Meat Science. Woodhead Publishing Limited, Cambridge, England. p. 1020.

Lee, S.-H., S. Kim, & J.-M. Kim. 2022. Genetic correlation between biopsied and post-mortem muscle fibre characteristics and meat quality traits in swine. Meat Sci. 186: 108735. https://doi.org/10.1016/j.meatsci.2022.108735

Lu, X., Y. Zhang, L. Qin, W. Ma, L. Zhu, & X. Luo. 2018. Association of ultimate pH and stress-related blood variables in cattle. Meat Sci. 139:228-230. https://doi.org/10.1016/j.meatsci.2018.02.004

Meuwissen, T., B. Hayes, & M. Goddard. 2016. Genomic selection: A paradigm shift in animal breeding. Anim. Front. 6:6-14. https://doi.org/10.2527/af.2016-0002

Nishimura, T. 2015. Role of extracellular matrix in development of skeletal muscle and postmortem aging of meat. Meat Sci. 109:48-55. https://doi.org/10.1016/j.meatsci.2015.05.015

Park, B. Y., S. H. Cha, Y. M. Yoo, J. H. Kim, H. S. Chae, I. N. Ahn, Y. K. Kim, J. M. Lee, & S. G. Yun. 2002. Comparison of pork quality by different postmortem pH24 values. J. Anim. Sci. Technol. 44:233-238. https://doi.org/10.5187/JAST.2002.44.2.233

Ryu, Y. & B. Kim. 2005. The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. Meat Sci. 71:351-357. https://doi.org/10.1016/j.meatsci.2005.04.015

Samorè, A. B. & L. Fontanesi. 2016. Genomic selection in pigs: state of the art and perspectives. Ital. J. Anim. Sci. 15:211-232. https://doi.org/10.1080/1828051X.2016.1172034

Scheffler, T. & D. Gerrard. 2007. Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. Meat Sci. 77:7-16. https://doi.org/10.1016/j.meatsci.2007.04.024

Schellander, K. 2009. Identifying genes associated with quantitative traits in pigs: integrating quantitative and molecular approaches for meat quality. Ital. J. Anim. Sci. 8:19-25. https://doi.org/10.4081/ijas.2009.s2.19

Śmiecińska, K., J. Denaburski, & W. Sobotka. 2011. Slaughter value, meat quality, creatine kinase activity and cortisol levels in the blood serum of growingfinishing pigs slaughtered immediately after transport and after a rest period. Pol. J. Vet. Sci. 14:47-54. https://doi.org/10.2478/v10181-011-0007-x

Tippala, T., N. Koomkrong, & A. Kayan. 2021. Influence of freeze-thawed cycles on pork quality. Anim. Biosci. 34:1375. https://doi.org/10.5713/ajas.20.0416

Velleman, S. 2000. The role of the extracellular matrix in skeletal development. Poult. Sci. 79:985-989. https://doi.org/10.1093/ps/79.7.985

Velleman, S. 2012. Meat science and muscle biology symposium: Extracellular matrix regulation of skeletal muscle formation. J. Anim. Sci. 90:936-941. https://doi.org/10.2527/jas.2011-4497

von Lengerken, G., S. Maak, & M. Wicke. 2002. Muscle metabolism and meat quality of pigs and poultry. Veterinarija Zootechnika 20:82-86.

Wang, F., Y. Zhang, J. Li, X. Guo, B. Cui, & Z. Peng. 2016. Contribution of cross- links and proteoglycans in intramuscular connective tissue to shear force in bovine muscle with different marbling levels and maturities. LWT - Food Science Technology 66:413-419. https://doi.org/10.1016/j.lwt.2015.10.059

Yilmaz, A., T. Loustau, N. Salomé, S. Poilil Surendran, C. Li, R. P. Tucker, V. Izzi, R. Lamba, M. Koch, & G. Orend. 2022. Advances on the roles of tenascin-C in cancer. J. Cell Sci. 135:jcs260244. https://doi.org/10.1242/jcs.260244

Zheng, Y. Y., Y. Chen, H. Z. Zhu, C. B. Li, W. J. Song, S. J. Ding, & G. H. Zhou. 2022. Production of cultured meat by culturing porcine smooth muscle cells *in vitro* with food grade peanut wire-drawing protein scaffold. Food Res. Int. 159:111561. https://doi.org/10.1016/j.foodres.2022.111561