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Investigative Baseline Reference on the Status of Pork pH, Shear Force, Colour, Drip and Cooking Loss in RYR1 Mutation Free, Commercial 3-way Crosses in Malaysia

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ABSTRACT

This paper attempts to provide findings of an investigative study on the baseline status of the pork quality in Malaysia. With consumer preferences changing towards the selection of good quality meat for consumption, there is a need to establish an investigative reference for the operators in the industry to gauge the performance of their animals and pork quality. This is also important to increase the competitiveness among producers to continuously improve the pork quality available to consumers. In this study, 30 commercial three-way crossed female pigs were randomly selected from government accredited abattoirs from east and west Malaysia and longisimus dorsi were collected for the determination of pH, drip loss, cooking loss, shear force and colour. All animals were screened for the RYR1 gene and the results were then compiled with statistical analysis to obtain an investigative baseline pork quality data in Malaysia. The average pork quality obtained from this study falls within the category of Red, Soft and Exudative (RSE), with an average ultimate pH of 5.83, drip loss more than 5% and L* values at 45.94. We have proposed an investigative baseline meat quality data for Malaysian pork from the average commercial pork quality data obtained. The proposed investigative pork quality baseline data in Malaysian is comparable in terms of studies done in other established countries and/or with international standards and falls within the RSE category of acceptable quality. It provides an investigative benchmark for researchers and end-producers to judge the

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quality of pork in an objective manner, both for consumption and for export purpose. Moreover, continuous selection against the RYR1 gene has successfully removed the gene from the sample size above, but constant random monitoring is still advisable if farms aim to ensure the elimination of this gene from their herd.

Keywords: Commercial 3-way cross pigs, drip loss, investigative pork quality baseline reference, pH; L* colour value, red soft exudative (RSE) meat, Ryanodine Receptor 1 (RYR1) mutation

INTRODUCTION

With consumer preferences changing towards the selection of good quality pork for consumption, there is a need to establish a reference for the operators in the industry to gauge the performance of their animals and the pork quality produced. This is also important to increase the competitiveness between producers to continuously improve the pork quality available to consumers.

Malaysia has approximately 772 farms and around 0.23 million sows in the production line, pushing the ex-farm value of the swine industry to an estimated RM2.5 billion in 2016 (Department of Veterinary Services, Malaysia, 2017). With a pork consuming population (PCP) of 30% out of 32 million, Malaysia remains self-sufficient in pork, where the local production supplies up to 95 % of the domestic consumption. In 1998, the industry culled off 1.1 million pigs during the Nipah virus outbreak. Designated breeder stock farms allocated by the Malaysian government, which supply cross-bred breeder animals to local farmers since 1926, were abolished (Singh & Fong, 2014). Subsequently, the genetic diversity of breeder flocks was maintained by importing breeder stock from the USA, Canada, Denmark and several other countries (Department of Veterinary Services, Malaysia, 2017). For nearly two decades since, the swine industry in Malaysia has been running on individual farms' self-developed swine herd breeding programmes, where the advantage lies with large private sectors with their own research and development facilities. Many resort to import breeder animals from well-known sources, for use in their own farms or for sale to other local commercial farmers (Singh & Fong, 2014). Other local farmers rely solely on local breeding companies for supply of purebred replacement animals to curb inbreeding problems. However, information on the current standard of commercial pork quality remains unknown to the general public.

In the 1980s and 90s, there was a major global emphasis placed on leaner pork, and intense genetic selection for fast growing, and lean animals was preferable. Due to consumer preferences, the pork industry has made significant progress in altering the composition of carcasses, to increase the lean-to-fat ratio of pork carcasses. Lean carcass, with high yielding cuts, attractive appearances and stability during cold storage are some of the characteristics considered by the industry as aspects of high-quality pork. Conversely, the selection for increased lean muscle mass led to the selection for animals with halothane positive genes (Ryanodine Receptor 1 mutation; halothane gene mutation; Porcine Stress Syndrome gene mutation), which are highly susceptible to stress, which often increase the incidence of PSE meat. Stress in halothane positive pigs (also known as pigs carrying the RYR1 gene), both homozygous and heterozygous for the gene, triggers a higher rate of post-mortem anaerobic glycolysis, leading to low pH early post-mortem (Rosenvold & Anderson, 2003). When in combination with high temperatures, the high protein denaturation rate which occurs induces the development of PSE meat, with dramatic effects on the water holding capacity (WHC) or the drip loss (DL).

In the 1990s, Denmark, The Netherlands, Sweden and Switzerland had eradicated the presence of the Halothane gene from their selection lines (Rosenvold & Anderson, 2003), while in the U.S., vertically integrated pork production companies began to reduce or eliminate pigs with the halothane gene in the late 1990s, which resulted in calmer pigs that are less likely to die during transport (Grandin, 1992). However, the use of the halothane stress gene still occurs in marketing systems, such as in Malaysia, where producers are paid on the basis of the largest loin eye and the thinnest backfat. Payment systems of this kind encourage the producers to select pigs for maximum quantity of lean meat, instead of good quality pork, which has low PSE occurrence (Grandin, 1992).

This study was designed to obtain the mean average value of the commercial pork meat quality characteristics across the local commercial three-way crosses, as an investigative baseline reference data for important pork quality parameters in Malaysia. Screening for the RYR1 mutation was also done to ensure that the quality of the pork attained is not influenced by the presence of this stress activated gene.

MATERIALS AND METHODS

Animals

Commercial crossbreds (Duroc X Landrace X Large White) (n=30) were randomly selected from several government model pig farms, certified under the Livestock Farm Accreditation Scheme “SALT”, practising proper Standard Operation Procedures (SOP). All animals underwent a 12-hour on-farm fasting time before transportation to one accredited slaughter house adhering to the Good Management Practices (GMP) references and have various HACCPs to monitor for food safety, meat quality and traceability in all products (Veterinary Health Mark, VHM Accreditation). The lorries used had natural ventilation and the animals all underwent transportation time of less than three hours. Stocking density of the lorries was approximately 0.425 m^2/100 kg pig and hydraulic lifts were used for loading and unloading. Transportation speed was at 60-70 km/h and it was done without stops, at the ambience temperature of approximately 29°C. All pigs were guided to the stunning area without the use of electric prods or whips and were handled as calmly as possible. Pigs were slaughtered, dressed and fabricated via automation processes and the samples were collected accordingly. The process of slaughtering, dressing and fabricating the pigs was carried out at accredited slaughter houses adhering to the Good Management Practices (GMP) references and have various HACCPs to monitor for food safety, meat quality and traceability in all products (Veterinary Health Mark, VHM Accreditation).

Sampling Procedures and Measurements

At 45 minutes post slaughter, pH and temperature were measured at 3 rib points (fourth, seventh, and tenth thoracic ribs), 7 cm away from the mid cutline, with a portable pH meter (Hanna Instruments, Woonsocket, RI, USA). At 24 hours post slaughter, ultimate pH and temperature were measured at the similar sites. Samples from the longissimus dorsi (LD) were collected for meat quality laboratory evaluations between the last thoracic to the fifth lumbar vertebrae, from the left-hand side of each carcass. LD samples for meat quality analysis were stored at -20°C until analysis.

Laboratory Evaluations

Drip loss of the LD was measured using the hanging bag method (Honikel, 1998) for 24 hours at 4°C. Cooking loss of individual standardised chops of 2.54 cm, cooked in water bath until the internal temperature reaches 75°C, were expressed as a percentage of the initial weight (Honikel, 1998). Warner Bratzler Shear Force (WBSF) of each sample was determined by running 10 cylinder cores of 1.27 cm in diameter and 2 cm in length through a HD plus a texture analyser (Stable Micro System, Surrey, UK.), using a V blade (pre-test speed: 3.0 mm s^{-1}; test speed: 1.0 mm s^{-1}; post-test speed: 3.0 mm s^{-1}) with a down stroke distance of 30.0 mm (Ruiz De Huidobro, Miguel, Blázquez, & Onega, 2005; Van Oeckel, Warnants, & Boucqué, 1996). The resistance of the cores to shearing was recorded every 0.01 s and plotted by a computer in a force-deformation plot. Objective colour (L*, a* and b*) was measured on the cut surface at the height of the last lumbar after 60 minutes blooming period with ColorFlex® colorimeter (Hunter Associates Laboratory, Reston, USA). The CIELAB L* was measured with a Hunter Labscan (HLS) equipped with D65 illuminant and 10° standard observer, with a measuring aperture of 30 mm (Kauffman et al, 1993).

All samples were assessed by applying Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) primer pairs, which was composed of detection of PSS mutations: PSS-F 5′- GAC ATC ATC CTT CTG GCT TCC -3′ and PSS-R: 5′- ATA GTT GAT GAG GTT TGT CTG C -3′ yield 221 bp normal products. PCR 221 bp sequence lying within an exon 17 (18,475 to 18,695) was amplified from the RYR1 gene (Brenig & Brem, 1992). After denaturation for 5 minutes at 94°C, amplications were carried out for 35 cycles at 94°C×30 s, 56°C×45 s and 72°C×30 s with a final extension step of 5 minutes at 72°C in a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA). The PSS genotype of PCR products was separately confirmed by PCR-RFLP. For RFLP analysis 10 µl of the 221 bp PSS fragment was digested with 5 units of HhaI at 37°C for 2 h. The digested DNA fragment was then separated by electrophoresis on 12% polyacrylamide gel in 1×TBE. The gel was pre-stained with redsafe (2 µg/ml) and visualized under UV light. All extraction of RYR1 PCR products were sent for sequencing to confirm the absence or presence of the mutation and its cutting sites.

Statistical Analysis

The average meat quality for all the samples was tabulated to obtain the average meat quality parameters reported as the investigative baseline data of pork quality in Malaysia, reported with its standard error of means.

RESULTS

The average live weight of all pigs weighed on farm was 105.5 ± 6.88 kg. Upon arriving at the abattoirs, the average live weight measured at 104.43 ± 5.91 kg, giving an average travelling loss of 0.72%. After slaughtering, the average hot carcass weight was found to be at 89.59 ± 5.43 kg across all samples, giving an average dressing percentage of 85.79% and a 4% average chill loss when the carcasses were stored in chillers prior to distribution. Table 1 shows the average pork quality parameters of commercial interest obtained for Malaysian commercial three-way cross. This is proposed to be the investigative baseline data for pork quality in Malaysia. Table 2 shows the meat quality categories and criteria used in this study, according to Kauffman et al (1992), whereby, it is concluded that Malaysian Pork Quality standards are consistent with RSE (Reddish-pink, Soft and Exudative) category.

Results from PCR-RFLP showed that all samples were negative for the RYR1 mutation, where the site of cleavage for the HhaI was not mutated within the PCR product. Therefore, all RFLP PCR products exhibit two fragments of 145 bp and 76 bp (normal pig, C/C allele) on the gel, whereas mutant PCR-RFLP products exhibit one fragment of 221 bp (mutant pig, T/T allele) or exhibit three fragments of 221 bp, 145 bp and 76 bp (carrier pig, C/T allele). This is further confirmed with the virtual restriction of the sequencing results by using an online Restriction Mapper Version 3, available at http://www.restrictionmapper.org/.

Table 1
Investigative baseline data for the pork quality parameters of commercial three-way cross in malaysia (n=30)

Trait	Average ± SEM
Meat Quality (Loin)	
Loin pH $_{45\text{-min}}$	6.55 ± 0.06
Loin pH $_{ultimate}$	5.83 ± 0.03
Drip loss (loin), %	10.19 ± 0.65
Cooking loss (loin), %	27.43 ± 0.73
Warner Bratzler Shear Force, kg	3.41 ± 0.37
Colour Hunter L*	45.94 ± 0.47
Colour Hunter a*	6.39 ± 0.25
Colour Hunter b*	15.73 ± 0.26

Table 2
Assessment of meat quality classes (kauffman et al, 1992)

Meat quality	$pH_{ultimate}$	Drip loss (%)	L* value
PSE	<6.0	≥5	≥50
RSE	<6.0	≥5	42-50
RFN	<6.0	<5	42-50
PFN	<6.0	<5	≥50
DFD	≥6.0	<5	<42

$pH_{ultimate}$ – pH value measured 24 hours post-mortem; L* – lightness; PSE – pale, soft and exudative; RSE – reddish-pink, soft and exudative; RFN – red, firm and non-exudative; PFN – pale, firm and non-exudative; and DFD – dark, firm and dry meat

DISCUSSION

In recent years, the meat market has undergone changes with the growing awareness amongst consumers linking between diet and health, creating a demand on healthy meat products (brand recognition) which are of consistently high quality and have the ability to trace back to its origins.

With the increase in demand for pork due to population growth, import of frozen pork will be expected as the swine production in Malaysia remains stagnant. Following the Nipah disease outbreak, for about 20 years, there have been no official standards or baseline status on Malaysian pork quality. Hence, this data (Table 1) is recommended as a key investigative baseline reference for the status of pork quality in Malaysia, which is highly useful as initial baseline benchmark for the industry. Currently, accredited slaughter houses adhere to the Good Management Practices (GMP) references and have various HACCPs to monitor for food safety, meat quality and traceability in all products (Veterinary Health Mark, VHM Accreditation). Thus, the establishment of investigative baseline status of the pork quality in Malaysia is essential for future improvement.

Firstly, pH_{45min} post slaughter is often used as an indicator of the early glycolytic rate in pig carcasses. Acute stress just prior to slaughter can lead to rapid rate of glycolysis and therefore rapid accumulation of lactate and a low muscle pH, resulting in pale, soft, exudative (PSE) pork. On the other hand, low muscle glycogen at slaughter can lead to insufficient lactic acid formation, high muscle pH and dark, firm dry (DFD) pork. The study found the classification done by Kauffman et al. (1992) to be the most suitable to explain the results obtained (Table 2). With the average $pH_{ultimate}$ at 5.83, drip loss more than 5% and L* values at 45.94, samples obtained from Malaysia averagely falls into the category of the RSE (Red, Soft, Exudative) category, which is considered as one of the good quality meat. This is also evident when the results are compared to the ideal $pH_{ultimate}$ suggested by NPPC Pork Quality Solutions Team, which is between 5.6-5.9. This is also consistent with other studies, where the $pH_{ultimate}$ 5.63 predicts good meat quality in pork (Kušec & Kralik, 2003). This may indicate the effectiveness of Malaysia's pre-slaughtering handling, the slaughtering process systems, as well as the post slaughter management in minimising the development of PSE (Pale, Soft and Exudative) and DFD (Dark, Firm and Dry) meat down the production line.

Many studies centred on the measurement of $pH_{ultimate}$ as an indicator of meat quality (Fernandez & Tornberg 1991). $pH_{ultimate}$ will only start to increase as a result of extreme depletion of glycogen (Pethick, Rowe & Tudor, 1995). The $pH_{ultimate}$ presented in the study was below the standard acceptable limit of 6.0. As presented, the ultimate pH falls within the normal range of 5.6-5.9, as suggested by NPPC Pork Quality Solutions Team, consistent with other studies, where the ultimate pH mean of 5.63 predicts good meat quality in pork (Kauffman et al., 1993; Kušec & Kralik, 2003).

Secondly, colour is one of the strongest attributes influencing consumer's visual judgement of meat quality. The three dimensions scale, which determines meat colour are lightness (L*), redness (a*) and yellowness (b*) and these are highly correlated with the visual perception of pink intensity (Lindahl, 2005; Ramos, Maloso, Delgado, & Francisquine, 2014). From this study, the colour of the meat in Malaysia tends to edge towards the darker hue, but it lies within the normal range of colour as of its surrounding countries, such as Thailand, with a mean L* value of 49 (Satsadeedech, Jiropas, Wattanachant, & Angkuraseranee, 2000).

Knowledge on the molecular mutation in the RYR1 and its association with MH (malignant hyperthermia) and PSS (Porcine Stress Syndrome) led to a development of simple, accurate and non-invasive test, which made it simpler to eliminate the mutated RYR1 gene from the population (Fujii et al., 1991). Generally, crossbreds with homozygotes for the normal NN allele of the Halothane gene showed darker muscle colour in the LD, when compared with the heterozygotes (Nn) or homozygotes (nn) (Apple, Stivarius, Reiman, Rakes, & Maxwell, 2002; Channon, Payne, & Warner, 2000; Hamilton, Payne, & Warner, 2000; Lindahl, 2005; Ohene-Adjei, Ellis, McKeith, & Brewer, 2003). Since the Halothane gene is known for its association with pale, soft and exudative (PSE) meat, the gene was tested for in the samples and was found to be negative. Therefore, in terms of consumer preference, the slight trade off in colour is negligible, especially when the overall meat quality is not compromised by the effects of the Halothane gene.

Furthermore, pork producers have made various attempts in order to accurately identify pig carcasses quality using rapidly known parameters available immediately during post-mortem for various down-line processing categorisation and price management. PH_{45min} can be used as a predictor to classify meat for further down-processing at the slaughter line (Kušec & Kralik, 2003). A correlation table was obtained from this experiment which shows significant correlation between pH_{45min} and colour Hunter a* values (r = -0.592; $p<0.01$). This suggests that the pH decline early post mortem can only be used to predict colour stability in Halothane gene free population.

Lastly, due to some undesirable relationships between meat quantity and quality, the balance on the emphasis on meat quality traits in the breeding programme is important to maximise sustainable genetic improvement of the swine carcass and also increase production efficiency in Malaysia. Moreover, with the export of swine meat from Malaysia dropping from RM75.88 million in 2013 to RM45.54 million in 2015, there is a need to increase the quality of the pork produced, as one of the measures to increase revenue obtained from the export of swine produces (Department of Veterinary Services, Malaysia, 2017). Hence, the initiation of a pork quality benchmark can relevantly serve as a guideline for farmers, regulatory personnel and pork retailers, aiming to improve the quality of pork harvested at the end of the processing line, be it for consumption or for future export of pork from Malaysia.

CONCLUSION

The proposed investigative pork quality reference falls within range to most standards found in various studies done internationally and falls in the RSE category, using the Kaufmann's scale (Kauffman et al., 1993), which is satisfactory in terms of consumer perception. Continuous selection against the RYR1 gene has successfully removed the gene from the sample size above, but constant random monitoring is still advisable if farms aim to ensure the elimination of this gene from their herd.

STATEMENT OF ANIMAL RIGHTS

Samples were taken in a humane manner, with minimum handling of live animals. Meat and liver samples and data were collected post-mortem.

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