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Assessment of Pork Meat Quality Following Electrical Stunning with Varying Rest Times: An Analysis of Lactic Acid Content and pH Value

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ABSTRACT

The quality of pork is closely linked to the stress animals experience before slaughter. Implementing practices that ensure adequate rest and minimize stress in animals before slaughter can play a vital role in enhancing meat quality. The present study aimed to evaluate pork quality following electrical stunning with differing rest periods. A total of 24 landrace pigs, aged 5 to 7 months, were randomly assigned to four treatment groups, including pigs with no stunning and a rest period of 24 hours or more (P1), pigs with no stunning and a rest period of less than 24 hours (P2), pigs subjected to electric stunning with a rest of 24 hours or more (P3), and pigs subjected to electric stunning with a rest of less than 24 hours (P4). Muscle glycogen levels were measured using a L-lactic acid colorimetric assay kit, and pH was assessed using established methods. The present results indicated that lactic acid concentrations in pigs that rested for more than 24 hours were higher than those in pigs that rested for less than 24 hours. Lactic acid levels in the control group (without stunning) for a rest period of more than 24 hours were 2,197 ± 0.388 mmol/g protein, higher than in pigs with a rest period of less than 24 hours. Similarly, in pigs subjected to electric stunning, lactic acid levels during a rest period of more than 24 hours were 2,057 ± 0.391 mmol/g protein, higher than those in pigs with a rest period of less than 24 hours (1.622 ± 0.543 mmol/g protein). Meanwhile, pH was higher in P1 (6.04 ± 0.16) than in P2 (5.99 ± 0.14). However, pH in P4 (6.01 ± 0.45) was slightly higher than that in P3 (5.84 ± 0.21). Electrical stunning reduced muscle lactic acid levels and pH. Conversely, providing pigs with a longer rest period (> 24 hours) before slaughter has been associated with higher muscle lactic acid levels. Therefore, it is recommended to combine electrical stunning with sufficient rest periods (over 24 hours) and to keep the pigs in a spacious area before slaughter.

Keywords: Lactic acid, Meat quality, pH, Resting time, Stunning

INTRODUCTION

Global demand for meat increases each year. In Bali, Indonesia, pork is a particularly important commodity, essential as a food ingredient and for use in traditional ceremonies. As a processed food, pork is expected to have high quality. Consequently, persistent efforts are made to optimize quality at every stage, from pig farming to slaughtering (Veerman et al., 2013). Favourable-quality pork generally has a bright colour, a distinctive aroma, a slightly sweet taste, and a chewy consistency (Lawrie, 2022).

Meat quality can generally be influenced by pre- and post-slaughter factors (Aberle et al., 2020; Soeparno, 2024). Pre-slaughter factors include transporting livestock from the fattening area to the slaughterhouse and handling during the slaughterhouse (Aberle et al., 2020). Transporting livestock is a potential stressor because livestock often experience fatigue, fear, and satiety during transport (EFSA AHAW et al., 2022). The level of stress experienced by pigs can be affected by factors such as travel distance and duration, livestock behavior, transportation type, livestock density, travel time, climate or weather conditions, handling methods during transit, the quality of rest provided, and the pig's individual vulnerability to stress (Terlouw et al., 2021; Lawrie, 2022). In addition, the choice of slaughter method can affect pork quality (Terlouw et al., 2021; Pandey et al., 2024).

Common pig slaughter methods mostly involve non-stunning techniques, while a small percentage use the stunning techniques. Non-stunning techniques involve killing an animal without prior unconsciousness, mainly used for religious slaughter (EFSA, 2020). Conversely, stunning animals refers to rendering animals unconscious before slaughter (Zivotofsky and Strous, 2012). Theoretically, stunning aims to minimize pain, fear, and stress in the animal during slaughter, which can affect meat quality (Hindle et al., 2010). Stunning can be carried out electrically, mechanically, or using gas (Dong et al., 2023). Most slaughterhouses in Bali use a pig-stunning device mainly to make animal handling

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easier before slaughter, especially for stunning pigs. Pig stunning is performed with a long, electrically charged rod (Suardana et al., 2024a).

In principle, the slaughtering method plays a crucial role in determining meat quality (Pandey et al., 2024). Poor slaughtering methods can lead to pre-slaughter stress (Suardana et al., 2024b). Pre-slaughter stress can affect the amount of glycogen in the muscles and liver, which is used as muscle energy reserves during rigor mortis (EFSA, 2020). This anaerobic glycolysis process converts glycogen into lactic acid, and glycogen deficiency in stressed livestock can limit post-mortem glycolysis (Aberle et al., 2020). This process persists until either muscle glycogen stores are depleted or the pH drops sufficiently to inhibit glycolytic enzyme activity (Aberle et al., 2020). When glycogen stores are abundant, anaerobic glycolysis generates sufficient lactic acid to reduce the meat's final pH to its isoelectric point (pH at 5.4-5.6). Low muscle glycogen caused by stress reduces lactic acid production during anaerobic glycolysis, resulting in a higher-than-normal final pH and the development of dark, firm, and dry meat. Conversely, prolonged stress can cause a pronounced drop in pH, resulting in an abnormally low final pH and pale, soft, exudative pork (Rosenvolt and Andersen, 2003). Field findings from several slaughterhouses in Bali, Indonesia, indicated that stunning was rarely used in pig slaughter, and pigs often did not obtain adequate rest before slaughter. Pigs are either directly moved from the holding pen to slaughter or allowed a full day of rest before slaughter the following day. Therefore, the present study aimed to evaluate the effects of electrical stunning and different pre-slaughter resting times on pork quality.

MATERIALS AND METHODS

Ethical approval

The present study protocol was approved by the Ethics Committees on Animal Experiments, Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, Indonesia, with certificate No. B/4/UN14.2.9/PT.01.04/2025.

Study design

A total of 24 landrace pigs, aged 5 to 7 months, were included in the current study. A veterinarian previously assessed the pigs' health. Pigs were housed in traditional baskets (bangsung) and provided water *ad libitum*. The pigs were categorized into four treatment groups, including pig with no stunning, resting for 24 hours or more (P1), pigs with no stunning before slaughter that resting for less than 24 hours (P2), pigs were subjected to electrical stunning (100 V, 1.5 A) for 6-8 seconds, followed by a rest more than 24 hours (P3), and pigs were subjected to the same electrical stunning protocol, followed by a rest less than 24 hours (P4). The samples were obtained from the pig slaughterhouse in Darmasaba, situated in the Abiansemal district of Badung regency, Denpasar, Bali, Indonesia.

Colorimetric lactic acid test

The samples for the colorimetric lactic acid and pH tests were collected from the biceps femoris muscles of pigs in two separate collections. The samples were placed in a cooler box and brought to the laboratory. The samples were stored in a freezer before the test. The colorimetric lactic acid test was performed according to the manufacturer's instructions (Elabscience, 2024). To prepare the chromogenic reagent working solution, reagents 1, 2, and 3 from the kit were mixed in a 100:1:20 ratio. The working solution was freshly prepared. For sample preparation, 100 mg of muscle tissue was weighed and placed into a microtube labeled with the respective sample code. Muscle tissue was minced, and 30 mg of the sample was suspended in 270 µL of phosphate-buffered saline (PBS). The microtube was centrifuged at 1000 g for 10 minutes at 4°C, and the supernatant was collected for further analysis. Lactic acid concentration was measured by diluting the sample 1:1. A standard curve was created by serially diluting 5 µL of a 10 mmol/L standard solution in PBS to produce concentrations from 0 to 7 mmol/L in designated wells of the microplate. The remaining wells of the microplate were filled with 5 µL of the diluted sample. Then, 120 µL of chromogenic reagent was added to each well, mixed, and incubated at 37°C for five minutes. Afterward, 180 µL of stop solution was added to each well and mixed for five seconds. Optical density was then measured at 539 nm. Lactic acid concentration (mmol/g protein) was calculated using the formula provided in the assay kit (E-BC-K044-M, USA; Elabscience, 2024).

pH test

Five grams of meat were mixed with 5 mL of distilled water and homogenized in a mortar. A calibrated pH meter (using pH 4.0 and 7.0 buffers) was inserted into the mixture to measure the pH. The measurement was performed three times, and the mean value was recorded (Dorleku et al., 2025).

Data analysis

The data on muscle lactic acid levels and pH were analyzed descriptively, with means and standard deviations reported. The means of the two treatments were compared using the General Linear Model Univariate (ANOVA) in

SPSS 25 for each dependent variable. Significant differences in p-value less than 5% ($p < 0.05$) were subjected to post hoc analysis using Duncan's multiple range test (Santoso, 2018).

RESULTS AND DISCUSSION

Muscle lactic acid levels

Table 1 presents the results of muscle lactic acid tests on landrace pork subjected to non-stunning and electrical stunning, with resting periods of either less than 24 hours or more than 24 hours. The present results indicated that lactic acid concentration was higher when the rest period exceeded 24 hours, with the control treatment (without stunning) measuring 2,197 ± 0.388 mmol/g protein, compared to P2, which was 2,069 ± 0.308 mmol/g protein. Likewise, pigs in P3 had higher lactic acid content at 2,057 ±0.391 mmol/g protein than pigs in P4 at 1,622 ± 0.543 mmol/g protein.

Table 1. Muscle lactic acid concentration on landrace pork subjected to non-stunning and electrical stunning with different resting periods

Treatment	Rest duration	Repetitions	Optical density value	Concentration (mmol/g protein)
Without stunning	**< 24 hours**	1	2.945	2.057
		2	2.867	2.007
		3	3.814	2.620
		4	2.670	1.880
		5	2.412	1.712
		6	3.070	2.139
		Average	**2.963 ± 0.476**	**2.069 ± 0.308**
	≥ 24 hours	1	3.682	2.534
		2	3.494	2.413
		3	2.938	2.053
		4	2.861	2.003
		5	2.214	1.585
		6	3.775	2.595
		Average	**3.161 ± 0.599**	**2.197 ± 0.388**
Electrical stunning	**< 24 hours**	1	2.828	1.982
		2	3.045	2.122
		3	3.043	2.121
		4	0.967	0.778
		5	1.839	1.342
		6	1.911	1.388
		Average	**2.272 ± 0.839**	**1.622 ± 0.543**
	≥ 24 hours	1	2.637	1.858
		2	2.148	1.542
		3	3.892	2.671
		4	2.652	1.868
		5	3.241	2.249
		6	3.089	2.151
		Average	**2.943 ± 0.604**	**2.057 ± 0.391**

The current results indicated that longer rest periods were associated with higher muscle lactic acid levels, although the difference was not statistically significant ($p > 0.05$). The present findings indicated that stunning and resting time had no significant effects on the reduction of muscle lactic acid levels ($p > 0.05$). Furthermore, the interaction between stunning treatment and resting time was not significant ($p > 0.05$).

It is well established that animals provided with adequate rest periods are afforded sufficient time to refill their glycogen reserves. Consequently, these animals have higher glycogen reserves than those with shorter rest intervals (Immonen and Puolanne, 2000). Elevated glycogen reserves in animals immediately after slaughter increase glycogen breakdown into pyruvate, ultimately leading to higher lactic acid accumulation (Aberle et al., 2020). The present results indicated that electrical stunning did not significantly increase muscle glycogen reserves in pigs compared to the control group ($p > 0.05$).

The present results contradicted the fact that stunning can reduce stress, leading to higher lactic acid levels due to anaerobic glycolysis. Stunning animals before slaughter minimizes pain, stress, and fear (Hindle et al., 2010). The lower lactic acid levels in the muscles of electrically stunned pigs might be linked to the extra stress the pigs endure during electrical stunning. This is because the electrical equipment used by the slaughterers has not been standardized in terms of shape and current, and the operators performing the stunning lack training. The electrical stunning device used during the present study consisted of a metal tip or clip that channels an electric current to the target. The electrical current was supplied via forceps attached to the back of the animal's ear at 100 V and 1.5 A. The exposure duration was 6-8 seconds. The standard electrical stunning parameters for pigs older than six weeks are 220 V and 1.3 A (EFSA, 2004). The electrical current frequency used for stunning livestock is 1,000-10,000 Hz (Simmons et al., 2001).

During the current experiment, the operator performed more than one stunning operation. This situation could have increased the stress in pigs already experiencing chronic stress from being placed in a blangsung. Overcrowding was an additional source of stress for the pigs. These conditions caused the pigs to release large amounts of glycogen while simultaneously reducing their glycogen stores, thereby decreasing lactic acid production via glycolysis (Aberle et al., 2020). The low lactic acid levels in electrically stunned pigs were associated with lower glycogen levels, aligning with the findings of Septianingsih (2023), who found higher glycogen concentrations (1.2 mg/g) in non-stunned pigs rested for more than 24 hours compared to stunned pigs (1.0 mg/g) under the same rest conditions.

After slaughter, muscle tissue begins anaerobic glycolysis as it turns into meat. During this process, glycogen is converted into lactic acid until glycogen is depleted or the pH drops enough to inhibit glycolytic enzyme activity (Aberle et al., 2020). When glycogen reserves are abundant, the subsequent accumulation of lactic acid is enough to lower the pH to the muscle’s isoelectric point, which typically ranges between 5.4 and 5.6 (Soeparno, 2024). In relation to meat quality, muscles with high glycogen and lactic acid levels tend to undergo more rapid postmortem glycolysis, resulting in a paler surface color, increased exudate loss, and greater protein denaturation than muscles with similar glycogen content but lower lactic acid concentrations (Terlouw et al., 2021).

pH value

The pH values of landrace pork meat, which were subjected to non-stunning and electrically stunning treatments, as well as resting periods of either more than 24 hours or less than 24 hours, are presented in Table 2. Based on the current findings, pigs that rested for less than 24 hours had a slightly lower pH (5.99 ± 0.14) than pigs that rested for more than 24 hours (6.04 ± 0.16). However, the difference was statistically insignificant ($p > 0.05$). The present results indicated that stunning and resting time had no significant impacts on pH ($p > 0.05$). In addition, the interaction between stunning and resting was not significantly different ($p > 0.05$). Conversely, for pigs subjected to electrical stunning, different results were noted. Pigs in P4 demonstrated a pH of 6.01 ± 0.45, whereas those in P3 had a pH of 5.84 ± 0.21.

Table 2. Post-mortem pH values of landrace pork meat between non-stunning and electrically stunning treatments with resting periods of < 24 hours and > 24 hours

Treatmant	Rest duration	Repetitions	pH value
Without stunning	**< 24 hours**	1	5.90
		2	5.98
		3	6.19
		4	5.81
		5	6.13
		6	5.95
		Average	**5.99 ± 0.14**
	≥ 24 hours	1	6.17
		2	5.93
		3	5.79
		4	6.19
		5	6.16
		6	5.98
		Average	**6.04 ± 0.16**
Electrical stunning	**< 24 hours**	1	6.17
		2	5.70
		3	5.97
		4	5.31
		5	6.47
		6	6.46
		Average	**6.01 ± 0.45**
	≥ 24 hours	1	5.62
		2	5.69
		3	6.01
		4	5.80
		5	6.18
		6	5.73
		Average	**5.84 ± 0.21**

The lower pH in P3 indicated that more glycogen was converted into lactic acid during glycolysis. This could be caused by stress in pigs resulting from their housing conditions, such as being kept close to the slaughter area, as well as stress from electrical stunning performed without standardized procedures and by inadequately trained personnel. These conditions can increase stress in pigs immediately before slaughter. Theoretically, stress in animals should promote muscle metabolism, but glycogen remains abundant. Therefore, when the animal dies, the abundant glycogen is converted to lactic acid through anaerobic glycolysis, resulting in a low pH in the muscle (Rosenvolt and Andersen, 2003; Lawrie, 2022). In practice, optimizing this process for meat quality requires specific training; however, a previous study in a Bali pig abattoir in Indonesia found that technical understanding may be limited, as 47.06% of operators had only a junior high school education. This was followed by operators with senior high school or elementary school education (41.18%) and a small proportion with a college education (5.88%). Moreover, most operators (69.23%) believed that stunning was not associated with reducing stress in pigs (Suardana et al., 2024a). In contrast, calmer pigs are believed to have larger glycogen stores and to convert more glycogen into lactic acid, resulting in a greater drop in pH (Lawrie, 2022). Regarding meat quality, insufficient muscle glycogen from prolonged stress leads to a lower final pH, resulting in pork with undesirable qualities. Meat with a final pH of 5.5-5.7 is considered normal and typically appears bright red. However, a rapid decline in pH to approximately 5.3-5.6 can result in pale, soft, and exudative meat, characterized by pale coloration, a soft texture, and excessive surface exudate. On the other hand, a slow or incomplete decline in postmortem pH leads to an abnormally high ultimate pH, usually 6.5-6.8 or higher than 6.2, resulting in dark, firm, and dry meat. This condition is identified by its darker color, firmer texture, and less surface moisture (Aberle et al., 2020).

Correlation between muscle lactic acid levels and pH

The present results indicated no significant correlation across the meat quality parameters ($p > 0.05$). The current findings demonstrated that muscle lactic acid levels had a very weak positive relationship with pH ($r = 0.181$), which was not statistically significant ($p > 0.05$). The relationship between lactic acid levels in muscles and pH, as well as meat quality, was closely correlated. In unstressed animals, glycogen reserves remained high, allowing normal lactic acid formation and a reduction in pH. A strong correlation was observed between lactic acid levels, a pH of 5.5-5.7, and meat quality. This relationship led to bright color, acceptable texture, optimal water retention, and stable meat quality. A rapid decrease in pH leads to protein denaturation, reducing water-holding capacity and producing pale, soft, and exudative meat. In contrast, minimal lactate buildup maintains a high pH, resulting in dark, firm, and dry meat (Lawrie, 2022). The current results indicated that subjecting pigs to electric stunning before slaughter in the study area did not increase glycogen recovery. This was likely because the stunning was not performed correctly or effectively, considering that the equipment and procedure are crucial for truly improving meat quality.

CONCLUSION

Electrical stunning reduced muscle lactic acid levels and pH. Conversely, providing pigs with a longer rest period (>24 hours) before slaughter has increased muscle lactic acid levels. It is advisable to pair electrical stunning with adequate rest periods (more than 24 hours) and to house the pigs in a spacious area before slaughter. Moreover, the operator should receive proper training before slaughtering to achieve optimum, favourable pork quality. Further investigations are required to assess the impact of standardized electrical stunning parameters and operator training on pork quality by measuring additional stress indicators, such as cortisol levels.

DECLARATIONS

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Authors' contributions

I Wayan Suardana conceived and designed the study. Hamong Suharsono and I Nyoman Suyasa conducted the trial and collected the samples. Komang Januartha Putra Pinatih analyzed the data. All authors have read and approved the final edition of the manuscript.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors explicitly and transparently disclose that no AI tool was used throughout the article.

Availability of data and materials

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Competing interests

The authors have not declared any conflict of interest.

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