



Measurement of Stress Levels in Pre- and Post-Slaughter Cattle at Tanah Merah Slaughterhouse Samarinda, East Kalimantan Province, Indonesia

(Pengukuran tingkat stres pada sapi pra dan pasca penyembelihan di rumah potong hewan Tanah Merah Samarinda, Provinsi Kalimantan Timur, Indonesia)

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ABSTRACT. The heightened demand for domestic beef has emerged in response to an expanding populace and heightened public interest in meat consumption. The principal objective of this investigation was to assess cardiac activity, as inferred from heart rate data, through the application of rigorous statistical methodologies and meticulous sampling techniques. The study comprised 70 Bali cattle sourced from the Samarinda Slaughterhouse (RPH), with statistical analysis facilitated by the utilization of the Z Test. Examination of the heart rate data indicated a notable degree of variability. Upon conducting the Z Test, a statistically significant finding was ascertained with $p < 0.05$, signifying the acceptance of H1. This, in turn, signified that the heart rate data exhibited an elevation in stress levels. Conversely, H0 was categorically refuted, implying an absence of heightened heart rate between the enclosure environment and the site of slaughter. Further observations centered on urination and defecation within the sample, yielding an average incidence of 11.425% amongst the 70 Bali cattle, serving as an indicator of stress or discomfort. The evaluation of stress levels in cattle within the Tanah Merah Samarinda Animal Slaughterhouse, situated in East Kalimantan, corroborated a significant surge in stress during the transition of cattle from the enclosure zone to the slaughter and dispersal area. This phenomenon is attributed to the deficiency in knowledge among stockpersons concerning optimal livestock handling and the principles of animal welfare.

Keywords: cattle, pre slaughter, post slaughter, slaughterhouse, stress levels

ABSTRAK. Permintaan yang meningkat untuk daging sapi dalam negeri muncul sebagai respons terhadap pertumbuhan penduduk yang terus meningkat dan minat publik yang tinggi dalam konsumsi daging. Tujuan utama dari penelitian ini adalah untuk menilai aktivitas jantung, seperti yang disimpulkan dari data denyut jantung, melalui penerapan metodologi statistik yang ketat dan teknik pengambilan sampel yang cermat. Penelitian ini melibatkan 70 ekor sapi Bali yang diperoleh dari Rumah Potong Hewan (RPH) Samarinda, dengan analisis statistik dilakukan dengan menggunakan Uji Z. Pemeriksaan data denyut jantung mengindikasikan tingkat variasi yang signifikan. Setelah melakukan Uji Z, temuan yang signifikan secara statistik ditemukan dengan $p < 0,05$, menunjukkan penerimaan H1. Ini, pada gilirannya, menunjukkan bahwa data denyut jantung menunjukkan peningkatan tingkat stres. Sebaliknya, H0 secara tegas ditolak, mengimplikasikan ketiadaan peningkatan denyut jantung antara lingkungan kandang dan lokasi pemotongan. Pengamatan lebih lanjut terkait dengan buang air kecil dan buang air besar dalam sampel menghasilkan insiden rata-rata sebesar 11,425% dari 70 ekor sapi Bali, yang berfungsi sebagai indikator stres atau ketidaknyamanan. Evaluasi tingkat stres pada sapi di Rumah Potong Hewan Tanah Merah Samarinda, yang terletak di Kalimantan Timur, mengkonfirmasi peningkatan signifikan dalam stres selama proses pemindahan sapi dari zona kandang ke area pemotongan dan penyebaran. Fenomena ini dikaitkan dengan kurangnya pengetahuan di antara peternak tentang penanganan ternak yang optimal dan prinsip kesejahteraan hewan.

Keywords: sapi, pra penyembelihan, setelah penyembelihan, rumah potong hewan, tingkat stres

INTRODUCTION

The national demand for beef has increased. The high consumer demand has caused an increase in slaughtering, due to the growing population and increased interest in meat consumption. The East Kalimantan Provincial Government, through the Livestock and Animal Health Agency, has stated that the number of animal slaughterhouses in East Kalimantan

reached 50,445 by December 2015, with the largest numbers in two cities, Samarinda (approximately 14,014) and Balikpapan (approximately 11,685). Beef cattle are the largest contributors to the national meat production, making livestock farming a potentially profitable enterprise. The quality of beef needs careful monitoring and improvement to meet the criteria of being safe, healthy, whole, and halal (ASUH).

The animal slaughterhouse in Tanah Merah, Samarinda, East Kalimantan serves as a distribution center and plays an important role as a link in obtaining good and consumable meat. The Tanah Merah Samarinda animal slaughterhouse still falls into category 1, meaning that the

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slaughtering is still done on the floor and the slaughtering business at the slaughterhouse have not been equipped carcass cooling facilities, resulting in warm carcasses. The government continues to encourage and create slaughterhouses that comply with the (safe, healthy, whole, halal) regulations. Thus, the slaughterhouse should have good slaughtering standards, similar to those of category 2 slaughterhouses, which have carcass cooling facilities and produce chilled and frozen carcasses (Campo *et al.*, 2014; Velarde *et al.*, 2014; Nakyinsige *et al.*, 2012). On average, 18 to 20 cattle are slaughtered at the Tanah Merah Samarinda East Kalimantan animal slaughterhouse nightly. Some slaughterhouses still use conventional methods in the slaughtering process, such as tying up the cattle and pulling them strongly so that they fall to the floor and are then slaughtered. Rough handling during slaughtering will cause stress to the animal and result in low-quality of meat. Animal handling during slaughtering must be well regulated to maintain standards, as animal welfare is part of the meat quality (Mpamhanga and Wotton, 2015). According to previous research (Mpamhanga and Wotton, 2015; Zimmerman *et al.*, 2013), the effects of stress and fatigue on animals before slaughter will have a negative impact on meat quality, referred to as Dark Firm Dry (DFD). DFD occurs due to stress, injury, disease, or fatigue in the animal before slaughter.

Animal welfare, also known as the care and treatment of animals, is a matter of conscious awareness with regards to the feelings of animals and how they should be treated without causing harm or suffering (Hultgren *et al.*, 2014). This concept is generally concerned with the treatment of animals in breeding, transportation, and slaughter. The "five freedoms" concept, which is referred to from regulations in Europe, outlines the rights of animals in relation to animal welfare (Losada-espinos *et al.*, 2018). These rights include freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury, or disease, freedom to express normal behavior, and freedom from fear and distress. Animal welfare is important because it affects the growth, reproduction, and lifespan of animals. Furthermore, it can also reduce the incidence of disease and increase the welfare of animals in studies (Støier *et al.*, 2016; Bourguet *et al.*, 2011). It has been found that improving animal welfare practices positively impacts animal pathology and disease resistance (Muchenje *et al.*, 2009). Situations where animal welfare is not met can

also have an impact on international relations, as seen in recent years with the temporary suspension of beef imports from Australia to several slaughterhouses in Indonesia due to animal abuse concerns (Farouk *et al.*, 2016).

Given the background outlined, the purpose of this research is to measure the level of stress in pre-slaughter or post-slaughter cattle, beginning with the handling process of livestock from the holding area to the post-slaughter stage, based on the measurement of heart rate, urination, and defecation in the Tanah Merah slaughterhouse in Samarinda, East Kalimantan.

MATERIALS AND METHODS

Data Sources and Samples

The materials were used in this research consist of 70 males Bali cattle with an age of 4 to 5 years that were to be slaughtered and an inspection form. The Bali cattle located in the Tanah Merah Samarinda slaughterhouse in East Kalimantan originate from Nusa Tenggara Timur and Sulawesi. The tools employed in this research include a stethoscope, a stopwatch, writing instruments, and a camera.

Handling Procedures

The research procedure was initiated with the observation of 70 Bali cattle, starting from the holding pen area, the slaughtering area, the changing and slaughtering area, until the death of the livestock at the Tanah Merah Slaughterhouse in Samarinda, East Kalimantan. The observation was conducted in the night time. Each night, 7 to 10 Bali cattle were observed during the 11 days of slaughter. The results of the observation were recorded in the pre-prepared examination form sheet. The assessment of stress levels in cattle in this study was conducted using a form that included the measurement of heart rate before and after handling, urination and defecation. The observations were divided into two, namely antemortem and postmortem. Antemortem or pre-slaughter observations included the measurement of heart rate before and after handling. Meanwhile, postmortem or post-slaughter observations included urination and defecation.

Statistical Analysis

The data obtained from 70 Bali cattle were analyzed using Quantitative Descriptive Analysis in accordance with the observation of Stress Level Measurement on Bali cattle before and after Slaughter in the Tanah Merah Slaughterhouse

(RPH) in Samarinda, East Kalimantan. The obtained data was calculated in statistical form. The method adopted from Selevan (Hemsworth *et al.*, 2011), which stipulated the use of a stethoscope to detect heart rate, urination, and defecation. The statistical analysis technique used in this research was the Z-Test. According to (Lepschy *et al.*, 2008), the Z-Test is used to compare the means of two variables in one group. The Z-Test is used as a comparative test or difference when the scale of three variables is Quantitative. Before conducting the hypothesis test, a Descriptive Test, Normality Test, and Homogeneity Test were first carried out using the SPSS (Statistical Packages for Social Science) computer program version 20.0 for Windows.

RESULTS AND DISCUSSION

Measurement of Pre-Slaughter Stage Homogeneity Test

This study was conducted at the Tanah Merah Samarinda Animal Slaughterhouse in Kalimantan Timur located on Jl. Poros Samarinda-Bontang, KM 21. The cattle Slaughterhouse has five pens which are used for rest and holding areas prior to slaughter. Animal Slaughterhouses are important in the meat industry as the animals must be fasted before being slaughtered.

The stress level of the animals greatly affects animal handling, which in turn affects the final meat quality, leading to a decrease in quality and dark cutting beef due to high pH levels. To measure the stress level of the animals before and after handling during slaughter, parameters such as heart rate, urination and defecation behavior can be observed. Stressors can be physical, such as heat, noise, transportation and lack of or restricted food, or psychological, such as castration, isolation or social mixing, restraint and handling (Wibowo *et al.*, 2022; Lambooi *et al.*, 2012).

According to previous study (Bozzo *et al.*, 2018; Kovács *et al.*, 2015), heart rate has been proven to be an indicator of physical and emotional stress. Many studies have concluded that major events, such as an increased in numbers of animals on farms, rough and winding road transportation, changes in vehicle speed, and unfamiliar slaughterhouses, have resulted in significant stress levels. In acute stress situations, heart rate is often used as an indicator of stress and the autonomic response to the animal's well-being (Prunier *et al.*, 2013; Chambers and Granin, 2001; Ramli, 2014).

Animal welfare is typically defined as a reference to an animal's ability to adapt to changes in environmental conditions. When the environment exerts pressure on an animal to a certain extent, new defense mechanisms will be activated to face the new conditions (Njisane and Muchenje, 2017; Ceballos *et al.*, 2018). This response mechanism is referred to as stress response. Recent studies (Mach *et al.*, 2008), have highlighted that when a cow experiences stress or fatigue before being slaughtered, the glycogen content in the muscle will diminish, causing the concentration of lactic acid formed to not reach the pH level of 5.6, resulting in meat that appears dark, firm, and dry, known as dark firm dry (DFD). According to (Kovács *et al.*, 2015) where the frequency of heartbeats is affected by age, gender, and weight in cattle, and the frequency of heartbeats from psychophysiological status and physical activity. Physical restraint can cause stress in animals which is a hypothesis for why the heart rate increases (Kovács *et al.*, 2015; Ceballos *et al.*, 2018). Some authors reported (Kovács *et al.*, 2014), where the heart rate frequency is also affected by physical body activity, exercise, and environmental conditions such as ambient temperature and air humidity.

The rough treatment carried out at the Tanah Merah Samarinda animal slaughterhouse in East Kalimantan 100% does not meet the standards of animal welfare performed by pen officers during the stage of guiding from the pen area to the slaughtering place and the change is treated roughly and hastily, with forced pulling, kicking, hitting, twisting of tails, causing pain and suffering to the animal. The term stress refers to the behavior, physiological, and emotional status of animals faced with situations that pose a threat to their bodily function or mental condition (Wibowo *et al.*, 2022; Sant and Paranhos, 2013).

Assumption Testing Homogeneity Test

The purpose of the Homogeneity Test is to show that two or more groups of sample data come from populations with the same variation. In this study, the homogeneity among the three groups, namely the heartbeat of cattle in the pen, the slaughterhouse and the change, varies similarly.

Based on Table 1 above, the calculation results show the value of P for heart rate data is 0.622 ($p > 0.05$). Thus, the heart rate data variable is homogenous.

Table 1. Homogeneity test

Levene Statistic	Df1	Df2	Sig.
.475	2	207	.622

The rule of the homogeneity test is, the variable data is considered homogeneous if the p-value is greater than 0.05.

Normality Test

The test of normality is conducted to examine the distribution of the observed frequency of the studied variables compared to the theoretical frequency. The normality assumption test uses the Kolmogorov-Smirnov analytic statistical test because the sample size is larger than 50.

Table 2. The results of the normality test in the barn area

	Kolmogorov-Smirnov		
	Statistic	Df	Sig.
Barn Area	.249	70	.000

The rule used is that if $p > 0.05$, then the distribution is normal and if $p < 0.05$, then the distribution is not normal (Levesque and Corp, 2008). The results of the normality assumption test on the heart rate data of cattle in the barn area showed a p value of 0.00 with a statistical value of 0.249. The test results based on the rules indicated that the distribution of cattle heart rate values is normal because the rule used is if $p > 0.05$, the distribution is normal and if $p < 0.05$, the distribution is not normal (Levesque and Corp, 2008). The results of the normality assumption test on the heart rate data of cows at the slaughtering area showed a p value of 0.001 with a statistic value of 0.149.

Table 3. The results of the normality test in the slaughtering area

	Kolmogorov-Smirnova		
	Statistic	Df	Sig.
Slaughtering Area	.149	70	.001

The results of the normality assumption test on the changes in cow heart rate data showed a p value of 0.005 with a statistic value of 0.130.

Table 4. The results of the normality test for changes

	Kolmogorov-Smirnova		
	Statistic	Df	Sig.
Overthrow of animal	.130	70	.005

Based on the Table, it can be concluded that the distribution of cow heart rate data in the lairage area, slaughtering area, and changes has a normal distribution. Thus, data analysis can be

carried out parametrically because the conditions for parametric analysis are met.

Hypothesis Test

The hypothesis in this study is to determine the difference in cattle heart rate as an indication of stress at the Tanah Merah Samarinda slaughterhouse in East Kalimantan Province before and after changes. This study uses the Z test analysis technique with the rule that if $p < 0.05$, then H_1 is accepted and H_0 is rejected. Conversely, if $p > 0.05$, then H_1 is rejected and H_0 is accepted. Based on the Table presented above, the result of the statistical test reveals that the P value for the heart rate of cattle in the enclosure and at the slaughterhouse is $p = 0.00$, which is less than the commonly used significance level of 0.05.

This implies that a significant difference or increase in the heart rate of cattle was observed between the enclosure and the slaughterhouse. Previous research reported (Borell *et al.*, 2007), where the normal range of heart rate for cattle in the enclosure is between 60 to 80 beats per minute. However, the findings of the current study suggest that the heart rate of cattle in the slaughterhouse is not within this normal range. This indicates that cattle in the slaughterhouse may experience a greater level of stress, which can have implications for the well-being and quality of the meat produced (Losada-espinos *et al.*, 2018; Farouk *et al.*, 2016; Borell *et al.*, 2007; Hemsworth *et al.*, 2011).

The figure 1 shows that there is a significant difference on level of stress between cattle in the enclosure and at the slaughterhouse. The difference is evidenced by the heart rate data, which shows a p-value of 0.00 or less than 0.05. In the holding pen, the cattle are given adequate rest and handled well. However, at the slaughterhouse, The escalation in stress levels could be attributed to the cattle enduring vigorous and forceful handling, including being pulled, kicked, beaten, and whipped during transportation from the holding pen to the slaughterhouse. This is because the structures of the holding pen and slaughterhouse are separate, and the cattle are not transported through a designated pathway.

Furthermore, when the p value of the heart rate of cattle at the slaughtering area and deformation of cattle shows a value of $p = 0.00$ or $p < 0.05$, the hypothesis in this study, H_1 , is accepted and H_0 is rejected. This means that a difference or increase in the heart rate of cattle between the slaughtering area and deformation of

cattle was found. This study found that the cattle's heart rate under severe stress reached 100 to 120 beats per minute (BPM).

Table 5. Z test on cattle movement from lairage to slaughtering area

Cattle heart rate	Groups	Df	Sig. (2-tailed)	Mean Difference	Std Error Difference
	Lairage Area	138	.000	-25.429	1.125
	Slaughtering Area	134.441	.000	-25.429	1.125

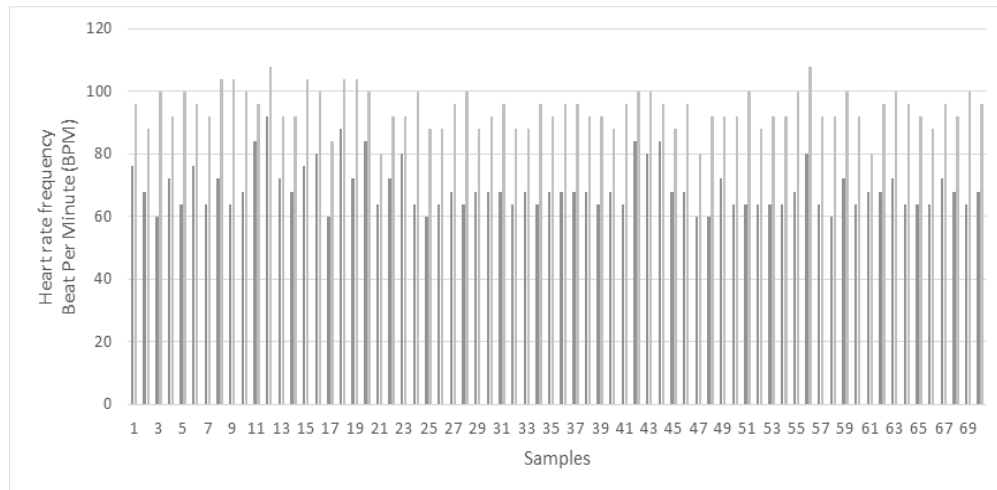


Figure 1. Cattle Heart Rate (■: lairage), (□: Slaughtering area)

The bar Graph 2 has depicted that the results of this study indicate a significant difference ($p < 0.05$) in stress levels between cattle at the cutting point and deformation, as shown by the increase in heart rate data with a value of $p = 0.00$ or $p < 0.05$. This means that a difference or increase in the heart rate of cattle was found between the slaughtering area and deformation of cattle (thrown down). This is due to the fact that at

the slaughtering area, cattle are subjected to extreme handling, witnessing other cattle being slaughtered, and receiving rough treatment, causing stress. In the process of deformation, cattle are tethered with ropes fastened to poles, subsequently subjected to forceful pulling and abrupt immobilization, resulting in a notable elevation in their heart rate and a pronounced state of heightened stress.

Table 6. Z test transposition of cattle from slaughtering area to deformation of animals

Cattle heart rate	Groups	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
	Slaughtering area	138	.000	-22.475	1.103
	Deformation of cattle	135.751	.000	-22.475	1.103

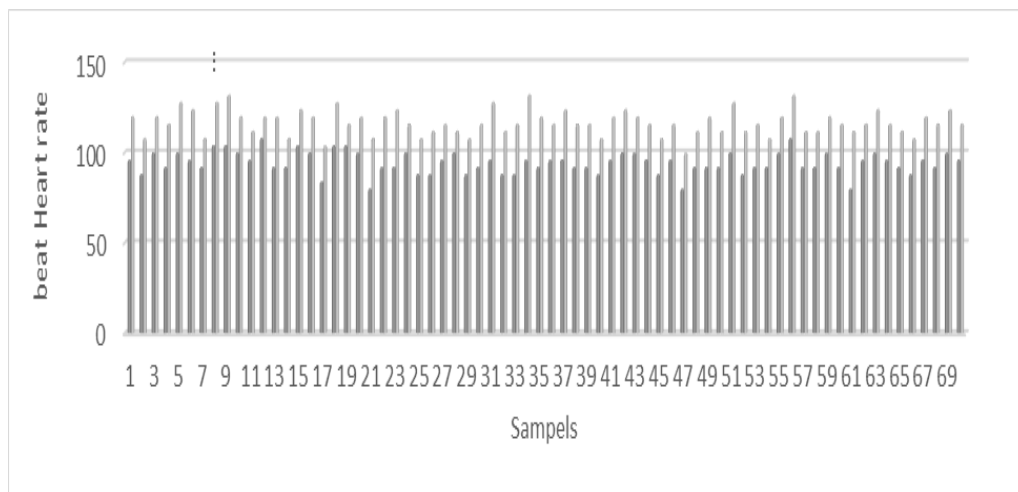


Figure 2. Cattle Heart Rate. (■: Slaughtering area), (□: Deformation of cattle)

Post Slaughter Measurement

Urination and Defecation Post Slaughtering

Stress experienced by cattle has given a negative effect on meat quality. Rough handling before slaughter is highly ineffective and the cattle will lose energy. Consequently, upon the cattle's slaughter, stress-induced behaviors manifest, such as the act of urination and defecation. The release of catecholamines and glucocorticoids (Produced by the medulla and the adrenal cortex, respectively). occurs within seconds to minutes after the stressor. They are the front-line hormones

in the fight to overcome the stressful situation. Both hormones are rapidly metabolized and excreted through urine and feces (Lepschy *et al.*, 2008). Urinary catecholamine metabolites were measured in livestock (Hay & Mormède, 2008). However, activity of the Autonomic nervous system (ANS) can be indirectly evaluated by heart rate variability (Kovács *et al.*, 2015). The findings presented in Table 7 suggest that among the cattle, 12.85% of the animals urinated and 10% defecated.

Table 7. Percentage of results of the physiological response indicator in the form of urination and defecation of faeces after slaughtering process

Physiological Response Indicator	Total head of cattle	Cattle Physiology response	Percentage
Urination	70	9	12.85%
Defecation of Faeces	70	7	10%
Percentage			11.425%

Nine cattle were observed to urinate, while seven cattle were observed to defecate. The results of the 11-day physiological response monitoring of 70 Bali cattle throughout the slaughter procedure at RPH Tanah Merah Samarinda revealed an average percentage of 11.425%. This observation suggests that urination and defecation can be considered indicative of the animals' stress or discomfort levels. These physiological responses are acknowledged as indicators of the animal's distress, both during and post the slaughter procedure. (Lepschy *et al.*, 2008).

In terms of animal welfare, it is crucial to familiarize the animal with its surroundings, the road surface, and the resting area to minimize the risk of injury. The physical environment should also provide a safe and comfortable place for rest and movement, which including the ability to change body posture and exhibit natural behavior. Cattle typically require a rest period of 12-24 hours in the holding pens (Diaz *et al.*, 2014). Furthermore, the resting livestock reduces stress, allowing more blood to drain and providing enough energy for the rigor mortis process to occur effectively (Sonoda *et al.*, 2017; Coombes *et al.*, 2014; Zybert *et al.*, 2014; La and Bovina, 2013).

Resting livestock is crucial, as exhausted animals that are immediately slaughtered without rest may result in dark meat, commonly referred to as "dark cutting meat," due to the animal's stress (Beef Stress Syndrome). The stress increases secretion of the adrenalin hormone, which can negatively impact glycogen metabolism in the muscles (Wibowo *et al.*, 2022).

CONCLUSIONS

This study assessed stress levels in cattle at the Tanah Merah Samarinda Abattoir in East Kalimantan by monitoring heart rate and analyzing post-slaughter excretions. The findings demonstrated that the cattle under study experienced stress, as evidenced by significant increases in stress levels and the excretion of urine and faeces. The assessment of stress levels revealed that the stockperson employed harsh and improper handling techniques, leading to animal discomfort and stress. Thus, the study recommends the implementation of proper handling techniques to minimize animal stress and improve animal welfare.

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