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Haematological changes to assess stress arising from different methods of slaughtering in pigs

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ABSTRACT

This study aimed to investigate the blood profile and neutrophil and lymphocyte ratio (NLR) in pigs subjected to stunning and non-stunning. Forty pigs, aged between 6-8 months and weighing 80-100 kg, were used to assess haematological changes associated with different slaughter techniques and to evaluate stress levels. The samples were divided into two groups, the first group was subjected to stunning (with a captive bolt), and the other served as a non-stunning (control). Blood samples were collected from each pig using EDTA anticoagulant tubes, with 3 mL of blood collected per sample. These samples were then analyzed using an auto-haematology analyzer to measure various parameters including RBC, HB, PCV, MCV, MCH, WBC, neutrophils, lymphocytes, monocytes, and eosinophil, as well as the ratio of neutrophils and lymphocytes. The results showed that stunning pigs with a captive bolt exhibited significantly higher levels of RBC, PCV, and lymphocytes with a value of 8.144±1.66 ($\times 10^6/\mu L$); 41.5±13.62(%) and 41.805±7.82(%), compared to non-stunning pigs with a value of 7.209 ± 1.22 ($\times 10^6/\mu L$); 36.65±6.12(%); and 22.2±12.24 (%), respectively. However, stunning pigs exhibited lower WBC and neutrophils, measuring 13.825 ± 8.56 × $10^3/\mu L$ and 41.75±16.64(%) with captive bold, compared to non-stunning pigs with a value of 20.375±9.83 ($\times 10^3/\mu L$) and 63.30±19.04 (%) respectively. The NLR index was higher in non-stunning pigs, with a value of 2.851, compared to stunning pigs subjected to a captive bolt, with an index of 1.006. In conclusion, stunning with a captive bolt was found to reduce the stress level of pigs before slaughter, potentially leading to improvements in meat quality.

Keywords: Blood profile, Captive bolt stunning, NLR, Non-stunning, Pig

Pigs are highly valued as an important aspect of livestock farming, specifically in Bali, which ranks as the province with the 6th largest pig population in Indonesia, reaching a total of 449,859 in 2022 according to the data from the 2024/3/22. This prominence is underscored by the demographic makeup of Bali, where the majority adheres to non-Muslim beliefs (Kusnandar 2021). Consequently, this religious and cultural context is in line with the prevalent use of pork in various ceremonies and for consumption needs by the community.

Slaughtering pigs is an important step in ensuring the production of safe, healthy, and high-quality meat (Terlouw *et al.* 2008). However, stress experienced before slaughtering has significant implications on meat quality. This stress often leads to common issues such as carcass injuries, animal weight loss, and a decrease in meat quality due to an increase in *p*H level (>5.8), resulting in meat tenderness and color (dark meat) (Lomiwes *et al.* 2014). Stress, typically resulting from inadequate slaughtering practices, is a prevalent factor affecting meat quality. Specifically, pre-slaughtering stress is identified as a crucial factor that can affect the quality of meat, potentially resulting in characteristics such as Pale Soft Exudative (PSE) or Dark Firm Dry (DFD) (Sabow *et al.* 2015).

According to (OIE 2011) there are primarily two methods of slaughtering: stunning and non-stunning. Both methods have implications for the welfare of the pig that is going to be slaughtered. Subsequently, stunning involves rendering the animal unconscious before slaughtering, a process that ensures the safety of operators, simplifies the handle of animals to be slaughtered, and most importantly reduces stress levels (Becerril-Herrera *et al.* 2009). One common stunning method is the use of a captive bolt, which induces concussion and trauma to the brain (Finnie 1997, Hindle *et al.* 2010).

Analyzing an animal's blood profile is a valuable method to determine its health status. Stressful conditions can cause changes in the circulation or tissues, leading to changes in the red and white blood cell counts. Stress or discomfort causes an increase in erythrocytes, haemoglobin, and haematocrit of the animal as a result of the release of catecholamines (epinephrine/norepinephrine) (Rosita

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et al. 2015). Furthermore, it increases the total leukocyte profile specifically in the neutrophil and lymphocyte ratio (NLR) which are important indicators of stress level. The higher the NLR ratio, the higher the stress level of animals (Kusnadi 2009). Introducing the captive bolt method as a new mechanical stunning device for pigs in Bali was a collaborative effort between the Animals Australia Foundation, the Department of Agriculture at Badung Regency, and Veterinary Public Health laboratory of the Faculty of Veterinary Medicine, Udayana University Bali. Therefore, this study aimed to analyze the complete blood profile as well as NLR as indicators of stress levels in pigs subjected to both captive bolt stunning and non-stunning methods. The analysis serves as a basis for improving pork quality and promoting animal welfare.

MATERIALS AND METHODS

This study adheres to animal welfare guidelines and has received approval from the Institutional Animal Welfare Research Ethics Board at the Faculty of Veterinary Medicine.

Experimental site: The experiment was carried out in July-September 2022 at a pig slaughterhouse located in the Bali.

Sample collection: In this study, 40 heads of landrace pigs aged between 6-8 months and weighing between 80-100 kg were used. Additionally, 3 heads of other pigs were included in a pre-slaughter study to establish the baseline profile of pigs in the study location. The samples were grouped into two, i.e. stunning with the captive bolt, and non-stunning as a control with 20 samples per group. About 3 mL of blood samples were collected from each pig using a tube containing ethylene diamine Tetra-acetic Acid (EDTA) as an anticoagulant. These samples were then analyzed using an auto haematology analyzer at the Denpasar Veterinary Center (BBVet) Laboratory. The number of erythrocytes (RBC), haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH), Packed Cell Volume (PCV), White Blood Cell (WBC), neutrophils, lymphocytes, monocytes, eosinophil, and neutrophils lymphocytes ratio (NLR) were calculated.

Data analysis: The normality of the data was tested using the Shapiro-Wilk test, followed by the mean analyzed using the General Linear Model in SPSS-25 software, and presented in table or graphics (Santoso 2018).

RESULTS AND DISCUSSION

Haematological profiles have been used widely to identify nutritional status, health status, and also to evaluate animal stress (Brucka-Jastrzębska *et al.* 2020). Blood metabolic profile tests are commonly used for assessing the clinical health of animals due to their simplicity and cost-effectiveness (Madreseh-Ghahfarokhi *et al.* 2020). A previous study found that both captive bolts and non-stunning pigs exhibited increased blood levels prior to slaughtering, suggesting stress experienced by the pigs. The results of a preliminary study, acting as baseline data before the evaluation of the captive bolt-stunning effects on the blood profile of landrace pigs are presented in Table 1. Blood profile of landrace pigs that were subjected to stunning with captive bolts and non-stunning before slaughtering is depicted in Table 2.

Table 1. Blood profile of landrace pigs pre-slaughtered in the slaughterhouse at Taman sub-village, Darmasaba village, Abiansemal sub-district, Badung regency, Bali

Variable	Blood profile
	Mean±SD
RBC ($\times10^6$/µL)	5.98±0.39
HB (g/dL)	11.4±0.26
PCV (%)	30.57±1.80
MCV (fL)	51.17±1.05
MCH (Pg)	19.03±0.74
WBC ($\times10^3$/µL)	28.53±5.75
Neutrophils (%)	71.0±7.0
Lymphocytes (%)	20.33±5.69
Monocytes (%)	8.67±2.89
Eosinophil (%)	0
NLR	3.79±1.61

Note: Mean of each blood variable as a result of three-time measurements.

Based on the data presented in Table 2, it is shown that the RBC values, PCV values, and hemoglobin levels increased in both the group of pigs subjected to stunning with a captive bolt and the non-stunning group compared to their respective values before treatment (Table 1). The MCV value decreased in the non-stunning pigs group, while there was a slight increase in the stunning pigs increase group. Additionally, a slight decrease in MCH values was observed in both groups when compared with the MCH values of pigs before treatment. The pigs subjected to stunning had higher RBC and PCV values

Table 2. Blood profile of the landrace pig subjected to stunning with captive bolt and non-stunning at Taman sub-village, Darmasaba village, Abiansemal sub-district, Badung regency, Bali

Variable	Non-stunning	Captive bolt stunning	P-value
	Mean±SD	Mean ± SD	
RBC ($\times10^6$/µL)	7.209±1.22	8.144±1.66	0.050*
HB (g/dL)	13.505±2.05	14.795±2.68	0.096
PCV (%)	36.65±6.12	41.805±7.82	0.026*
MCV (fL)	50.97±3.35	51.59±3.05	0.544
MCH (Pg)	18.835±1.37	18.275±1.15	0.171
WBC ($\times10^3$/µL)	20.375±9.83	13.825±8.56	0.006*
Neutrophiles (%)	63.3±19.04	41.75±16.64	0.0005*
Lymphocytes (%)	22.2±12.24	41.5±13.62	0.000*
Monocytes (%)	11.9±6.24	13.6±7.53	0.442
Eosinophil (%)	2.60±5.40	3.15±2.74	0.074

Note: *, Significantly different (P<0.05). Mean of each blood variable as a result of three-time measurements.

than the non-stunning group, with P-values of 0.050 and 0.026, respectively. The results showed that the captive-bolts stunning method of slaughtering has a significant effect (P<0.05) on the value of RBC and PCV. WBC and neutrophil values dropped, but lymphocytes and monocytes increased in both stunning and non-stunning pigs. The total value of eosinophils in the group of non-stunning pigs was lower than in the group of stunning pigs.

The results of studies are in line with several previous findings which showed a decrease in the number of eosinophils. This decrease is believed to be mediated through the effect of anterior pituitary and adrenocortical hormones, which increase during stressful situations (Karpoor *et al.* 2011). According to the findings by Titiasari *et al*. 2019 it was reported that an increase in leukocytes correlates with infection, age, health, environment, and stress. Another study also stated that bone marrow diseases or damage, viral infections, serious systemic diseases, chemotherapy, and radiation decreased leukocyte counts as well as resulted from prolonged stress (Aliviameita and Puspitasari 2019). An increase or decrease in one or more types of white blood cells can affect an increase or decrease in the total white blood cells (Maheswari *et al.* 2013). The measurement of stress levels in pigs was also analyzed by examining the neutrophils and lymphocytes ratio in both pigs slaughtered using stunning and non-stunning pigs (Table 3).

Table 3. Neutrophils-lymphocytes ratio (NLR) of the landrace pig subjected to stunning with captive bolt and non-stunning at Taman sub-village, Darmasaba village, Abiansemal sub-district, Badung regency, Bali.

Treatment	Mean±SD	Min	Max	P-value
Non-stunning	4.838±4.71	0.272	17.40	0.0001*
Captive bolt stunning	1.272±0.97	0.149	3.889	

Note: *, Significantly different (P<0.05). Mean of each blood variable as a result of three-time measurements.

Based on the data presented in Table 3, it shows that the NLR value in pigs slaughtered using captive bolt is lower than the NLR value of non-stunning. The data are descriptively presented in Fig. 1.

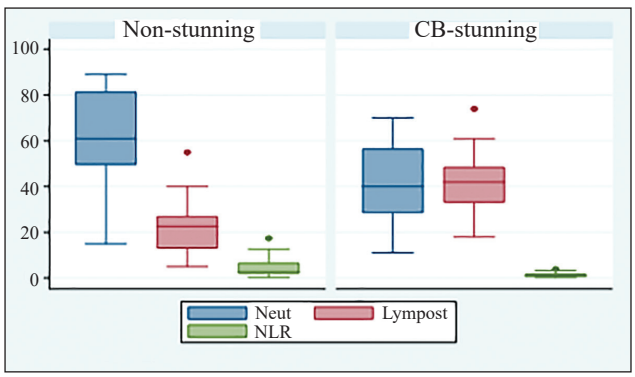

Fig. 1. Neutrophil, lymphocyte, and neutrophil-lymphocyte ratio (NLR) graph of the landrace pig subjected to stunning with captive bolt and non-stunning at Taman sub-village, Darmasaba village, Abiansemal sub-district, Badung regency, Bali.

Fig. 1 shows NLR in the non-stunning group was higher than in the captive bolt stunning group. The non-stunning group had a minimum number of neutrophils of 15% and a maximum of 89%, while lymphocytes had a minimum number of 5% and a maximum of 55%. The captive bolt stunning group had a minimum neutrophil number of 11% and a maximum of 70%, while lymphocytes had a minimum number of 18% and a maximum of 74%. Table 3 shows that the NLR value is significantly different (P<0.05) between stunning and non-stunning pig groups. This showed that the difference in treatment between the two groups influenced the stress level in animals, as observed in the NLR value. Furthermore, the blood profile and NLR value were also confirmed by the results of the Giemsa stain between neutrophil and lymphocyte cells of non-stunning and stunning with a captive bolt which is shown in Fig. 2.

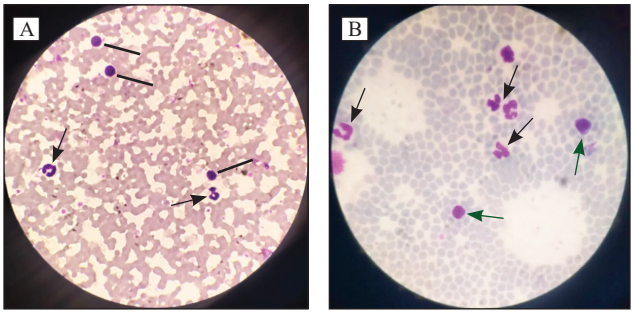

Fig. 2. The Giemsa-blood stain both; A. Blood with captive bolt stunning; B. Non-stunning. (Black arrows show neutrophil cells and blue arrows show lymphocyte cells) (Giemsa stain, 100× magnification).

Fig. 2 shows the amount of neutrophils, specifically band neutrophils, in the pigs blood subjected to stunning with a captive bolt and non-stunning. The results showed that the neutrophil for pigs subjected to stunning with captive bolt was lower than non-stunning.

Besides neutrophils and lymphocytes, eosinophils also play a crucial role as a measure of stress. The number of eosinophils can help distinguish between the leukocyte response caused by stress and a response caused by the presence of infection. The studies conducted in mammals have shown that stress can induce glucocorticoids to decrease the number of eosinophils (Zheng *et al.* 2009, Son *et al.* 2020). A similar phenomenon can also be observed in healthy humans who experience stress during examinations, as studies have shown a decrease in their eosinophil levels (Karpoor *et al*. 2011). The number of eosinophils decreases in response to acute stress because of several factors, such as 1. Inhibition of the release of mature eosinophils from the bone marrow; 2. Eosinophils can migrate to other body tissues, leading to their decrease or disappearance in the blood. Localization of the eosinophil generally at the lymph nodes, the site of inflammation, and spleen through diffuse intravascular margination or occurs due to damaged eosinophils; and 3. there is inhibition of eosinophil output

(Bass *et al.*1980, Hori *et al.* 2016). The higher stress levels observed in the group of non-stunning slaughtered pigs could be correlated with certain procedures performed before slaughtering. Specifically, before slaughter, the pigs snout, front, and hind legs were tied together, this process caused fear or stress in the pigs. The loud noise produced by pigs during the slaughtering process can cause stress to others before they are slaughtered. The differences in stress levels between the non-stunning pigs and those subjected to stunning with captive bolts are supported by the NLR results. Subsequently, the NLR value of the non-stunning pigs was higher compared to the group of stunning pigs, and these results indicated a higher stress level in the non-stunning group. For comparison, a previous study (Carrasco-Garcia *et al.* 2020) found that in cattle, the normal level of body resistance can be determined by an NLR value of around 1.5, and values higher than this threshold indicate that cattle are experiencing stress. The higher the ratio number, the higher the stress level as a result of adaptation to the environment (Kusnadi 2009).

Loud noises in abattoirs promote stress during the slaughtering process (Iulietto *et al.* 2018). Pigs slaughtered without stunning may experience pain throughout the process until they lose consciousness completely (Prayoga *et al.* 2020). Pain is the main stimulus that can cause animals to experience stress (Ulrich-Lai *et al.* 2006). Subsequently, stress in animals causes a response from the HPA axis that will stimulate the secretion of the hormone cortisol (glucocorticoids) then directly triggers the occurrence of neutrophilia (increased number of neutrophils) and lymphopenia (decreased number of lymphocytes) causing an increase in the NLR value (Abdelilah *et al.* 2019).

The study showed that a group of non-stunning pigs had a greater number of neutrophil cells than the group of captive bolt-stunning pigs. Meanwhile, the number of lymphocyte cells in the non-stunning group of pigs was lower compared to the stunning group. The results directly led to an increase in the NLR value and showed a higher stress level in the non-stunning group, or slaughtering with captive bolt stunning resulted in lower stress levels in the group. The study results are in line with the theoretical framework, which suggests that stunning is a method of slaughtering that helps minimize pain, fear, suffering, and stress in animals (Hindle *et al*. 2010).

Stunning before slaughtering improves meat quality compared to non-stunning. Recent studies support this finding, showing that meat from non-stunned animals exhibits higher cooking loss compared to meat from stunned pigs (Rita Adnyani *et al.* 2022). Following animal death by exsanguination, muscles become deprived of oxygen, initiating anaerobic glycolysis. Elevated levels of stress hormones before or during slaughter can deplete muscular glycogen reserves, as glycogen is hydrolyzed to lactic acid (Fenandez and Tornberg 2007, Carrasco-Garcia *et al*. 2020). Moreover, several problems caused by stress are carcass injuries, weight loss, and a decrease in meat quality as a result of an increase in *p*H (>5.8). Another effect of the high ultimate *p*H is meat tenderness and color (dark meat) (Lomiwes *et al*. 2014). Stress can produce meat with characteristic Pale Soft Exudative (PSE) or Dark Firm Dry (DFD) (Sabow *et al.* 2015). In conclusion, using captive bolts as a stunning tool could reduce stress levels in pigs, characterized by an increase in complete blood profile parameters such as RBC, PCV, and lymphocytes, along with a decrease in WBC and neutrophils compared to non-stunning, as well as their NLR value. This study predicted that the use of captive bolt-stunning would improve meat quality through a reduction of the pigs stress level.

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