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Complete Blood Profile and Neutrophil-Lymphocyte Ratio of Electrical Stunning and Non-Stunning Swine

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Abstract. Examination of the blood profile is crucial because blood functioned as an important role in the circulatory system and can be used to evaluate the emergence of stress in animals. The purposes of this research are to examine the complete blood profile and examine the possibility of stress symptoms by measuring the ratio of neutrophils-lymphocytes as the indicator of stress in electrical stunned and non-stunned swine. There were 40 six-month life swine with a weight of 80-100kg. The swine grouped into stunning and non-stunning group. A total of 3 ml of blood sample occupied and placed to a contained anticoagulant Ethylene Diamine Tetra acetic Acid (EDTA) vacutainer. The total of erythrocytes, hemoglobin, PCV, MCV, MCH, leukocytes and differential leukocytes were calculated using routine hematological examination methods with autoanalyzer machines. Based on the results of the research, the complete blood profile of electrical stunning slaughtered swine was healthier and more reliable than non-stunning. The ratio of neutrophils-lymphocytes in electrical stunning slaughtered swine was lower than non-stunning. This condition indicated a lesser amount of stress on the use of electric stunning.

Keywords: Complete blood profile, N/L ratio, Swine, Electrical stunning, Non-stunning.

I. INTRODUCTION

Swine is one of the livestock widely kept in Bali and cannot be separated from the lives of the society. The need for swine farming commodities is quite potential to be developed as one of the livestock commodities, considering the island of Bali is inhabited by a majority of Hindus. Pork will always be a leading commodity in society, whether it is used as an ingredient in processed foods or as a means of traditional ceremonies. Based on data from the Animal Husbandry Service of the Province of Bali in 2021, the population of swine in Bali reached 409.960 heads and there will be a potential to increase [1].

Swine is also a popular livestock nourished by most of the society of Bali [2].

Swine is prolific. Prolific means the swine has the ability to have many piglets at each birth, which ranges from 8-14 in a year and enable to give birth twice [3]. This fact becomes the reason of the society increasingly interested in keeping swine. Swine itself will be more efficient in changing their food ingredients if the quality of the ration consumed also have good quality. Besides, swine is also an efficient producer of meat sources for nutritional fulfillment which because it has a fairly high conversion to feed. All feed ingredients enabled to be converted into meat and fat [4]. The percentage of swine carcasses is reached 65-80% which categorized as quite high. The reason is swine converted the food waste efficiently [5].

The swine slaughter can be applied through two methods. Those are non-stunning and stunning. The stunning method is taken on to facilitate immobilization of slaughtered animals and to ensure the safety of slaughterhouse officers [6]. The stunning enabled to be taken out through electric method by using captive bolt stun gun and gas. Electrical stunning is applied using an energized clamp on the head or body of the animal [7]. Electrical stunning in more than a six-week life of swine had a standard, that is 220 volts and 1.3 amperes for 3 seconds [8]. The aimed of this stunning is to minimizes fear, stress and pain in animals during the slaughtering process which can affect the quality of meat [9][10].

While blood functioned as a medium of transporting food substances and nutrients throughout the body. On the other hand, blood is also vulnerable as a medium for spreading disease. Examination of the blood profile is vital because blood has a very important function for living things, besides the examination also helps to monitor the incidence of a disease [11]. Physiological indicators commonly used in evaluating the emergence of stress in livestock include blood chemistry and hematology [12]. Stress conditions affects the changes in the number of erythrocytes, hematocrit values, hemoglobin levels, and leukocyte count. Erythrocytes, hemoglobin, and hematocrit (PCV) will be increased if the animals are in distressing or sore situation. This happens because the circulated catecholamines (epinephrine/norepinephrine) [13]. Stress affected the total leukocyte profile and caused a decrease in eosinophils [14] also monocytes increased [15]. Stress condition also caused an increase in the number of neutrophils in the blood circulation and triggered a decrease in the number of lymphocytes [16]. The N/L ratio is the tranquil stress indicator to detect. The higher the N/L ratio, the higher the level of stress [17].

Heretofore, the research related to complete blood profile and any changes in the ratio of neutrophils-lymphocytes, especially in electrical stunned and non-stunned swine has not been widely disclosed. Based on these problems, further research on the complete blood profile and the ratio of neutrophils-lymphocytes in electrical stunned and non-stunned swine is essential to be conducted. The aims of conducting this research are to complete the data and as the basis for further research on the method of slaughtering swine.

II. MATERIALS AND METHODS

The object of this research was swine. The technique used was electrical stunning and non-stunning slaughtered swine. There were several criteria in selecting the swine. Those are 40 six-month life swine with a weight of 80-100kg. A total of 40 swine were divided into two groups consisting 20 swine with electric stunning and 20 swine without stunning.

Research samples were taken at two abattoirs in Darmasaba. Sampling was carried out from 01.00 to 02.00 WITA. 3 mL of whole blood was taken using a disposable syringe. Blood was placed into an EDTA tube, then homogenized and labeled slowly. Furthermore, the tube is stored in a cooling box filled with an ice pad. The sample storage was transferred to a 5°C tempered refrigerator. The tube was brought to the Denpasar Veterinary Center Laboratory at 08.00 WITA using a cooling box to test the complete blood profile and the ratio of neutrophils-lymphocytes.

The blood was examined at the Denpasar Veterinary Center Laboratory. Complete blood counted using hematology examination with a hematology analyzer machine (Rayto RT-7600 Auto Hematology Analyzer®). This tool calculated the value of erythrocytes, hemoglobin (Hb), average cell volume (MCV), MCH, PCV, white blood cell count, and the percentage of neutrophils-lymphocytes.

The data from this research analyzed using the Saphiro Wilk test, then further tested with parametric and non-parametric tests according to the normal distribution. The results of the analysis are presented in the form of tables/figures.

III. RESULTS AND DISCUSSION

Complete Blood Profile of Electrical Stunning and Non-Stunning Swine

Observations of 20 samples, each of which were treated by electric stunning or non-stunning can be seen in Table 1.

Table 1. Swine blood of electrical stunning and non-stunning slaughtered swine.

Variable	Non-Stunning	Electrical Stunning	p-Value
	Mean ± SD	Mean ± SD	
RBC ($\times 10^6$/µL)	7.209 ± 1.22	9.085 ± 1.99	0.001*
HB (g/dL)	13.505 ± 2.05	16.075 ± 3.39	0.006*
PCV (%)	36.65 ± 6.12	46.05 ± 9.87	0.0006*
MCV (fL)	50.97 ± 3.35	50.895 ± 2.48	0.936
MCH (Pg)	18.835 ± 1.37	17.695 ± 1.03	0.007*
WBC ($\times 10^3$/µL)	20.375 ± 9.83	17.755 ± 8.24	0.213
Neutrophiles (%)	63.3 ± 19.04	41.65 ± 18.61	0.0008*
Lymphocytes (%)	22.2 ± 12.24	40.95 ± 16.39	0.0001*
Monocytes (%)	11.9 ± 6.24	11.95 ± 4.00	0.976
Eosinophil (%)	2.60 ± 5.40	5.45 ± 4.79	0.003*

Note: *significantly different ($P < 0.05$)

The calculation results in Table 1. showed that the mean total of erythrocytes, hemoglobin levels and PCV values in the group of non-stunning swine are respectively $7.209 \pm 1.22 \times 10^6$/µL; 13.505±2.05 g/dL; 36.65±6.12%, the electrical stunning swine mean are $9.085 \pm 1.99 \times 10^6$/µL; 16.075 ± 3.39 g/dL; 46.05 ± 9.87%. Analysis of the significance of total erythrocytes, hemoglobin levels and hematocrit values with the Wilcoxon test showed that the group of non-stunning swine and the group of electrical stunning swine, P values were 0.001 respectively; 0.006 and 0.0006. These results indicated that the mean total of erythrocytes, hemoglobin levels and hematocrit values of the two groups were significantly different ($P<0.05$). This means, the method of slaughter has a significant effect on the value of erythrocytes, hemoglobin, and PCV.

Based on Table 1., the group sample of non-stunning had average 18.835 ± 1.37 Pg of MCH, while the group sample of electrical stunning had average 17.695 ± 1.03 Pg of MCH. Statistical test results showed that the treatment of non-stunning and electric stunning was significantly different ($P<0.05$). It can be concluded that the method of slaughter has a significant effect on MCH.

The profiles of the number of neutrophil and lymphocyte cells are presented in Table 1. The electrical stunning swine showed the number of neutrophil cells and lymphocytes was almost constant, 41.65 ± 18.61% for neutrophils and 40.95 ± 16.39% for lymphocytes. The number of neutrophils was seen to increase sharply in non-stunning swine, followed by a decrease in the number of lymphocytes. The number of neutrophils increased to 63.3 ± 19.04% and

the number of lymphocytes was found to decrease by about 22.2 ± 12.24%. Analysis of the significance of total neutrophils, and lymphocyte values by Wilcoxon test showed that the group of non-stunning swine and the group of electrical stunning swine, P values were 0.0008, respectively; and 0.0001. The results of statistical tests showed that the percentage of neutrophils and lymphocytes against the treatment of non-stunning and electrical stunning was significantly different (P<0.05). This means that the method of slaughter has a significant effect on the value of neutrophils and lymphocytes. Stressful conditions affected an increase in the number of neutrophils and a decrease of lymphocytes in the blood circulation [16]. Therefore, the high value of neutrophils indicated distressing condition in the group non-stunning swine.

The profile of the number of MCV, WBC, and Monocytes based on the results of statistical tests (Table 1.) the two treatments did not differ (P>0.05) so that the type of both treatment, non-stunning and electrical stunning swine, did not affect the values of MCV, WBC, and monocytes.

Neutrophil and Lymphocyte Ratio

A test on the ratio of neutrophils and lymphocytes were carried out to test stress levels. The test results can be seen in table 2.

Table 2. Neutrophil-Lymphocyte Ratio

Treatment	Mean ± SD	Min	Max	p-Value
Non-Stunning	4.838 ± 4.71	0.272	17.40	0.0003*
Electrical Stunning	1.353 ± 0.98	0.109	3.428	

Note: * significantly different (P < 0.05)

In line with Table 2., it can be stated that the ratio of neutrophils and lymphocytes in the treatment with electric stunning is lower than non-stunning. Descriptively as in the following Figure 1:

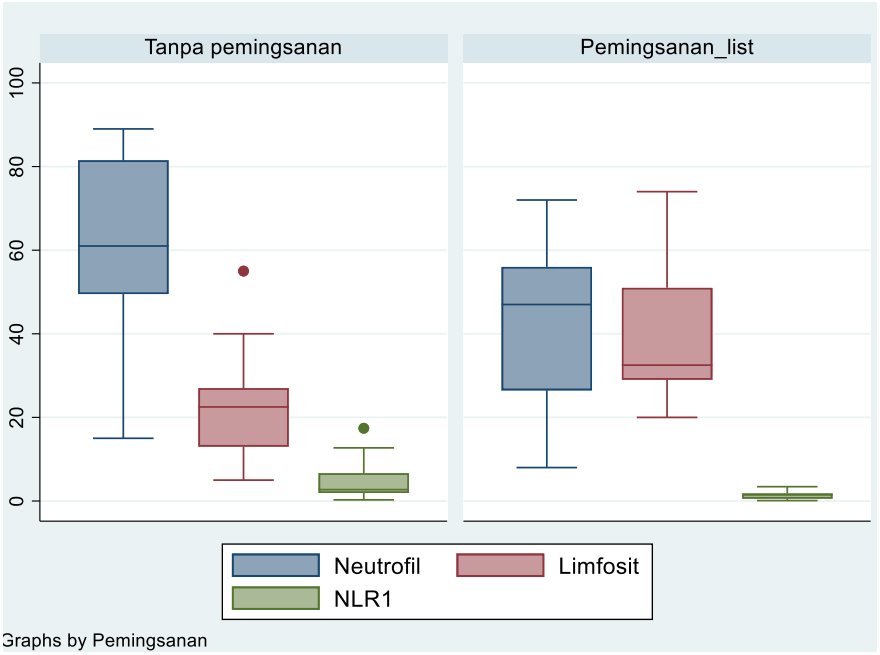

Figure 1. Neutrophil and Lymphocyte Ratio Chart

Based on Table 2., the lowest N/L ratio value was found in swine that were electrically stunned, which was 1.535±0.98, while the highest value was found in swine with non-stunning with a value of 4.838±4.71. The N/L ratio has not been found in swine. The higher the ratio, the higher the stress level as a form of adaptation to the environment [17].

In this research, higher N/L ratio values in the group of non-stunning swine indicated a high level of stress events. This statement is suspected to be occurred by the procedure performed prior to slaughter. The slaughter procedure with non-stunning began with tying the snout, elevating the forelegs and hind legs in swine. This supposition is desired to be a source of the swine's stress condition.

Pain is not sensed in slaughter with electric stunning because electric currents pass through the brain and cause disturbances in brain activity. So that, animals lose consciousness and are insensitive to pain. Pain is one type of stimulus affects animals to experience stress. Stress experienced by animals due to pain results in the emergence of the HPA Axis response which will stimulate the secretion of the hormone cortisol.

The increase in the N/L ratio is caused by the release of cortisol that occurs when the animal is in stress condition. High cortisol levels will cause the bone marrow to release neutrophils so that the number of neutrophils in the blood will increase. Increased levels of glucocorticoids can also reduce the number of lymphocytes, this causes circulating lymphocytes to stick to

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the endothelium of blood vessels, and then migrate from the blood circulation to other tissues, the lymphocytes are stored and not released again.

The results of the blood smear test, the comparison of neutrophils and lymphocytes between electrical stunning and non-stunning swine samples can be seen in Figure 2.

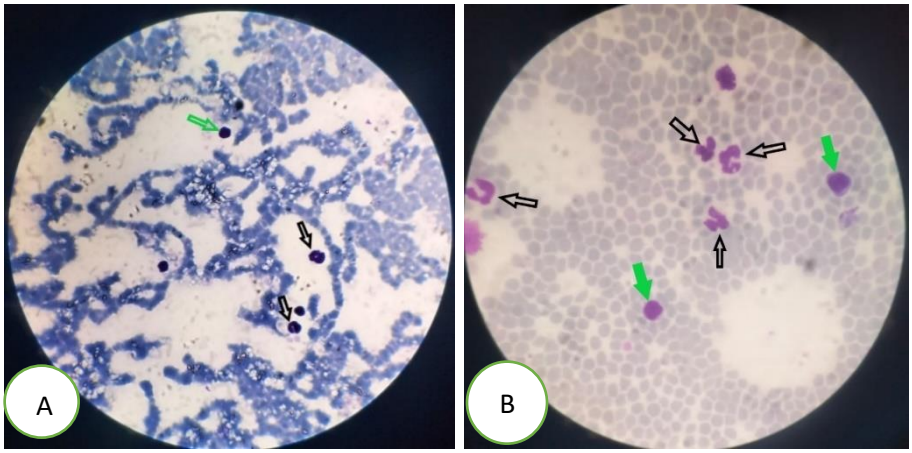

Figure 2. Blood test results. A: Electric Stunning, B: Non-Stunning. Black arrows: Neutrophils, Green arrows: Lymphocytes. (Giemsa stain, 100× magnification).

The results of blood smear observations were carried out on blood samples of swine slaughtered with electric stunning and non-stunning showed that neutrophils and lymphocytes blood cells were found. All these cells were found to have morphological features in accordance with existing theoretical references. Neutrophils have fine granules in the cytoplasm and the nucleus is droopy. The chromatic nucleus looks dense and clumped. Lymphocytes have a cell nucleus almost covering the cytoplasm. The results of the blood study in Figure 2. showed that the number of neutrophil cells in the group of non-stunning swine was higher than in the group of electric stunning swine. Meanwhile, the number of lymphocytes in the group of non-stunning swine was less than in the group of electric stunning swine. It indicated that the group of non-stunning swine had a higher stress level than the group of electric stunning swine.

IV. CONCLUSION

1. Electrical stunning swine showed some reliable blood parameters, especially in terms of total neutrophils (41.65±18.61%) and lymphocytes (40.95±16.39%) with the analysis results for both are significantly different ($P<0.05$).
2. The ratio value of N/L was lower in electrical stunning slaughtered swine, 1.353±0.98. Based on statistical test results, it was significantly different ($P<0.05$). This results also strengthened

by the results of preparate blood tests which showed escalation neutrophils in non-stunning treatment and had medium negative correlation ($P<0.05$) with stunning significantly.

V. SUGGESTION

The further research is necessary to be conducted. It is aimed to discover complete blood profile and the ratio of neutrophils-lymphocytes in electrical stunned and non-stunned swine. It can be taken out by extended research durations and increased frequency of blood tests to obtain a more complete value-series of hematological.

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