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Research Article

Hematological Profile and Leukocyte Differentiation of Madura Cattle and their Crosses at Different Sexes and Ages

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Abstract | This study analyses the Haematological profile, and leukocyte differentiation of Madura cattle and its crossbreeds of different sex and age. The results are expected to provide a better understanding of the condition of livestock raised by the community. Blood samples were obtained from 60 heads of Madura and Limura (Limousine x Madura) cattle, which were differentiated by sex and age. Blood samples were collected into tubes and immediately stored in a cold box at approximately 4˚C. Data analysis was conducted at the Veterinary Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia. A statistical analysis was conducted using analysis of variance using Proc Mixed with general linear model (GLM) using SAS studio for academics Online Edition. This study showed the Haematological profile and leucocyte differentiation of male and female Madura cattle of different ages were generally similar to their crossbred Limura cattle and within the normal range. In conclusion, Madura cattle tend to be more adaptive or responsive to various exposure factors as indicated by higher erythrocyte and leucocyte counts than their crossbred counterparts Limura cattle.

Keywords | Differentiation, leukocyctes, hematological, Limura cattle, Madura cattle

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INTRODUCTION

Based on the Decree of the Minister of Agriculture No. 3735/Kpts/HK.040/11/2010, Madura cattle are designated as Indonesian local cattle breeds. Madura cattle is a small type of beef cattle is one of the indigenous beef cattle germplasms and susceptible to dry agroecosystem environment and well developed on the island of Madura. The productivity ability of Madura cattle that has grown and developed well in the dry land is resistant to disease and poor feed. The genotypic diversity of Madura cattle is quite diverse and based on the results of Sutarno and Setyawan's (2015) research, Madura cattle have a high carcass with good meat quality, with a carcass percentage of Madura cattle of 52.22% (Prihandini, Maharani, and Sumadi, 2020). Madura cattle have socio- cultural values used or competed as Kerapan cattle and Sonok cattle (display).

Madura cattle is a local beef cattle great potential to be developed as a cross between Madura cattle and Limousin cattle that called Limura. Limura cattle only entered Madura island in 2000 through artificial insemination. Limousin cattle introduction into Madura island through artificial insemination. The introduction of exotic cattle such as limousine cattle through crossbreeding with Madura cattle needs to be considered for production and repro-

duction performance, given that limousine cattle are less adapted to dry, hot areas, and lack of feed. Limura cattle have a better exterior body and body weight than Madura cattle, because Limura cattle are a combination of Madura and Limousin cattle (Volkandarie et al., 2013). However, the reproductive performance of Madura cattle is better than its crosses (Kutsiyah et al., 2003). Madura cattle have better reproductive efficiency and fertility index than Limura cattle (Omitasari, 2017).

Studies on the physiological basis conducted on Madurese cattle including their crosses are relatively fewer than local cattle breeds. Knowledge of physiological status is essential as a reference for implementing of various maintenance systems in livestock. The Haematological profile is one of the physiological variables that can be used to determine livestock's health status, production, and welfare (Bezerra et al., 2017; Brunel et al., 2018). The proper blood profile can support the physiological processes of livestock to provide optimal performance (Ali et al., 2013). In addition, an ideal physiological profile can be increasing livestock production, reproduction, and productivity (Bezerra et al., 2017; Ratnawati et al., 2023). The livestock's hematological profile parameters commonly examined are erythrocytes, leucocytes, hemoglobin, haematocrit, erythocyte index, and platelets. Previous studies have examined the Hematological profiles of several cattle breeds, including beef and dairy cattle, and their relationship with age, sex, breed, feed, infection, reproductive status, and environmental maintenance. (Suprayogi et al., 2017; Perumal et al., 2022; Yekti et al., 2023).

Currently, there is limited research on the Haematological profile of Madura cattle. Ramli Wahyudin. (2015) has reported haematocrit, leucocyte, erythrocyte, monocyte, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and lymphocyte cell count concentrations in Madura cattle fed onggok-based diets, however, Haematological profiles for various ages and sexes in Madura cattle and their crossbreds are not yet available. Therefore, this study aims to provide a complete Haematological profile of Madura cattle and their crossbreds, and the results are expected to provide a better understanding of the condition of livestock raised by the community.

MATERIALS AND METHODS

Ethical approval

The study procedures were done according to the operational standard (SNI ISO 17025: 2017) at Agricultural Quarantine Agency, Pamekasan, and controlled by the veterinarian.

Experimental design

A total of 60 heads Madura and Limura (Limousin X Madura) cattle were used in this research as well as the blood samples. This research was conducted in Pademawu Timur village, Pademawu sub-district, Pamekasan district. This research was conducted in February - March 2022.

Blood collection

Blood samples were collected using a 5 ml syringe and inserted into tubes containing ethylenediamine tetra-acetic acid (EDTA). Blood samples were immediately stored in a cold box at about 4°C and transferred to the laboratory for further examination. Hematological profile examination of Madura cattle and their crossbreds was conducted at the Veterinary Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia. The variables examined were erythrocyte, leucocyte, hemoglobin, haematocrit, erythrocyte and platelet counts. Leucocyte differential counts were performed by observing the morphology of leucocytes in blood smear preparations. The leukocyte differential counts observed included the total number of lymphocytes, monocytes and granulocytes in 100 leukocyte cells.

Statistical analysis

A statistical analysis was conducted using analysis of variance using Proc Mixed with general linear model (GLM) using SAS studio for academics Online Edition (https://odamid-apse1.oda.sas.com/SASStudio/). An error was expressed as standard error mean (SEM). At the end, probabilities values were subjected in Duncan Multiple Range Test. The following model was used (Adli et al., 2022; Sholikin et al., 2023).

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} was parameters observed, μ was the overall mean, T_i the number of blood taken, and eij the amount of error number.

RESULTS AND DISCUSSION

Leucocytes and their differentiation

The Haematological profiles of Madura cattle and their crosses are presented in Table 1. Leukocytes are active components of the body's defence system formed partly in the bone marrow and partly in lymphoid organs such as the thymus, bursa and lymph (Sugiharto, 2014). Leukocytes function as the body's defence, fighting infection directly and the toxins produced will be neutralized by antibodies in the blood plasma. The number of leucocytes in individual's body are different and changes according to body's condition. Changes in leucocyte composition can occur in stress, age, nutritional status, and physiological activity (Dellman and Brown, 1992).

Table 1: Hematology profile of Madura and Limura (Limousine x Madura) cattle

Parameters	Breed	Sex											
		Female						Male					
		Calf	SEM	Young	SEM	Adult	SEM	Calf	SEM	Young	SEM	Adult	SEM
Leukocyte (10^3 / mm^3)	Madura	9.28	0.84	14.74	6.14	8.6	1.06	11.38	1.18	10.6	2.83	9.3	0.83
	Limura	11.7	0.92	17.06	4.00	9.64	1.36	18.52	5.14	9.56	1.25	10.48	1.00
Erythrocyte (10^6 / mm^3)	Madura	8.8	0.77	9.04	0.76	5.66	5.66	8.56	0.70	8.46	0.57	8.72	0.72
	Limura	9.66	0.91	8.24	0.52	8.46	0.67	8.18	0.76	8.6	0.41	8.7	0.80
Haemoglobin (g/dL)	Madura	10.64	1.03	10.98	1.72	9.8	1.57	11.58	0.52	10.8	1.05	11.88	0.90
	Limura	16.06	2.83	10.64	0.87	11.28	0.45	9.45	1.04	11.84	1.05	10.38	0.87
Haematocrit (%)	Madura	30.50	1.97	30.52	3.02	30.6	1.93	33.44	1.64	31.232	1.19	31.84	1.03
	Limura	37.42	1.60	33.16	2.34	31.68	1.30	29.68	1.67	33.06	1.51	32.06	1.21
Thrombocytes (10^3/mm^3)	Madura	202.60	13.21	190.00	48.02	152.2	32.63	144.4	45.66	177.4	33.47	243.6	40.25
	Limura	348.80	56.23	205.4	58.65	208.2	9.52	177.5	28.39	201.6	74.91	173.4	30.24

Limura (Limousine x Madura); SEM – standard error mean

Based on the study's results, it is known that the average number of total leucocytes of Madura cattle (Figure 1) are lower than that of Limura cattle (Figure 2) were presented insignificant difference ($p > 0.05$). This study is not much different from the number of leucocytes of Balinese cattle studied by Tirta Aliana et al. (2018) and Aceh cattle studied by Sofyan Hamny et al. (2020). However, the leucocyte count of Limura cattle is slightly higher than the normal leucocyte value of 4 - 12 (10^3 / mm^3) (Kessell, 2015). The high leucocyte value in Limura cattle is due to their low adaptability to the environment. The high temperature of the environment in the Madura region makes Limura cattle, which are the result of crossing with exotic cattle, still unable to adapt to the local environment. The average leucocyte counts at different sex and age levels for Madura and Limura cattle fluctuates. The number of leucocytes in the blood fluctuates greatly (Roland et al., 2014). The number and proportion of leucocytes in the blood represent the condition of leucocyte distribution. It also describes the immune system in the body (Dhabhar et al., 2012). An increase in the mean total leucocyte count can be caused by the physiological response of cattle to the environment, climate and feed. Stress is an adaptive mechanism of living things, including livestock. Stress levels vary widely between individuals and animal breeds. Roland et al. (2014) stated that stress is one of the causes of leucocytosis (high levels of leucocytes). Dhabhar et al. (2012) state that stress can cause mobilization of immune cells, especially leucocytes. Leukocyte mobilization causes changes in the dynamics of leukocyte counts. These changes affect the function of the immune system.

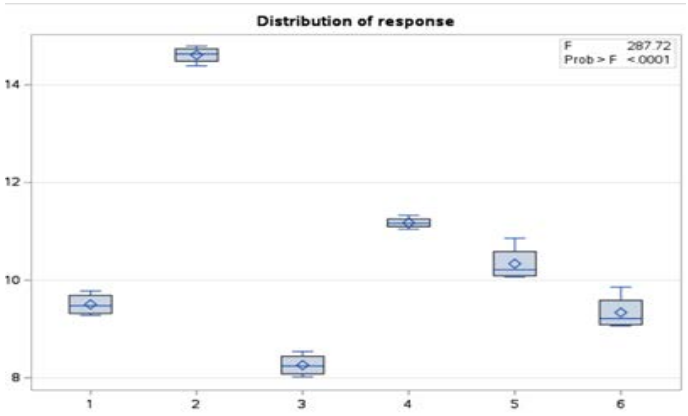

Figure 1: Leukocyte profile of Madura cattle (1) calf; (2) young; (3) adult (female); (4) calf, (5) young, (6) adult (male)

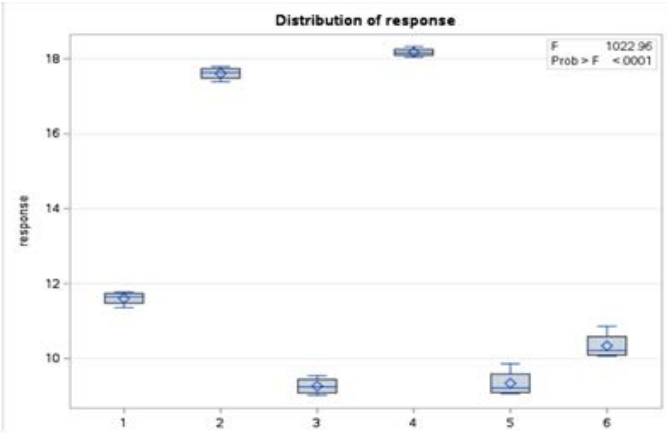

Figure 2: Leukocyte profile of Limura cattle (1) calf; (2) young; (3) adult (female); (4) calf, (5) young, (6) adult (male)

Erythrocyte

The erythrocyte values of male and female Madura cattle at different ages were 8.56 ± 0.72; 8.46 ± 0.59; 8.72 ± 0.73; 8.8 ± 0.78; 9.04 ± 0.77; 5.66 ± 5.66 (10^6 /mm^3), respectively. While for Limura cattle (Madura x limousine) males and females at different ages were respectively 8.18 ± 0.77; 8.6 ± 0.42; 8.7 ± 0.81; 9.66 ± 0.91; 8.24 ± 0.51; 8.46 ± 0.68 (10^6 /mm^3) (Table 1). Erythrocytes (red blood cells) are one of the cellular components of blood that binds to haemoglobin and functions as an oxygen carrier (Aspinall and Cappello, 2015). Based on the study's results, it is known that the number of erythrocytes of male Madurese cattle is higher than female Madurese cattle, as well as the number of erythrocytes of male Madurese cattle is higher than the number of erythrocytes with female Madurese cattle. This indicates that gender affects the erythrocyte value in Madura cattle and Limura cattle in this study. The difference in erythrocyte count is thought to be due to gender and cattle breed. Research conducted by Roland et al. (2014) and Adam et al. (2015) showed that the sex of cattle affects the number of erythrocytes. With increasing age, the erythrocyte value is higher in male madura cattle, and Limura cattle. This is in accordance with the opinion of Widhyari et al. (2014) that Erythrocytes are produced in the spinal cord after birth and the number of erythrocytes tends to increase with the age of the cow until it reaches a stable value). However, this is not the case with female madura cows and female Limura cows, the increasing age of the erythrocyte value has decreased, this is likely due to nutritional factors. Nutritional factors also affect the number of erythrocytes in cattle. The more adequate nutrients in the feed will show a normal erythrocyte count and lies in the average high range of cattle blood. Frandson, (1992) stated that nutrients in feed such as iron, Cu, vitamins, and amino acids, affect erythrocyte count. The number of erythrocytes obtained in Aceh and Bali cattle was within the normal range. This indicates that the nutrients in the feed are adequate. According to Arut (2010), some minerals and vitamins play an important role erythropoiesis. Iron is required for hemi synthesis. Copper in the form of ceruloplasmin, is essential in the release of iron from tissues to plasma. Vitamin B6 (pyridoxine) is required as a cofactor in the first stage of enzymatic heme synthesis. Cobalt is essential in the synthesis of vitamin B_{12} by ruminants. Guyton and Hall (1997) added that vitamin B_{12} and folic acid deficiency could cause maturation failure in erythropoiesis, resulting in low blood erythrocyte count.

Haemoglobin

Haemoglobin values of male and female Madura cattle at different ages were 11.58 ± 0.52; 10.8 ± 1.07; 11.88 ± 0.91; 10.64 ± 1.04; 10.98 ± 1.73 respectively (Figure 3); 9.8 ± 1.57 (g/dL), while for Limura cattle (Madura x limousine) males and females at different ages were 9.45 ± 1.09; 11.84 ± 1.05; 10.38 ± 0.88; 16.06 ± 2.86; 10.64 ± 0.97; 11.28 ± 0.46 (g/dL), respectively (Table 1) (Figure 4). Haemoglobin has the same function as erythrocytes partially. The presence of haemoglobin is related to the normality of the metabolism of food substances because haemoglobin functions as a binder of O_2 throughout the body, CO_2 from the whole body to the outside of the body, and as a medium for iron metabolism. Haemoglobin is a red pigment, which is a complex protein containing iron that functions as a carrier of oxygen molecules (Aspinall and Cappello, 2015). The high haemoglobin value is thought to be related to the high number of erythrocytes in Madura and Limura cattle. Lawrence et al. (2017) and Sonjaya (2013) stated that an increase in haemoglobin correlates with an increase in erythrocytes. An increase in hemoglobin concentration occurs in response to the body's adaptation to high oxygen demand (Gravena et al., 2010). Haemoglobin value of Ma-

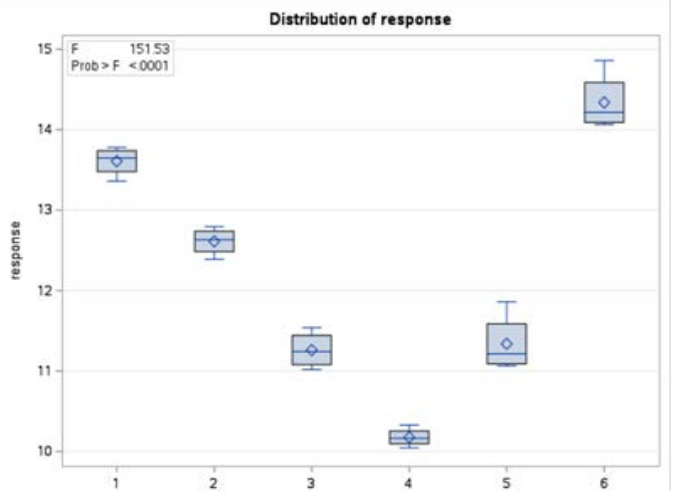

Figure 3: Haemoglobin profile of Madura cattle (1) calf; (2) young; (3) adult (female); (4) calf, (5) young, (6) adult (male)

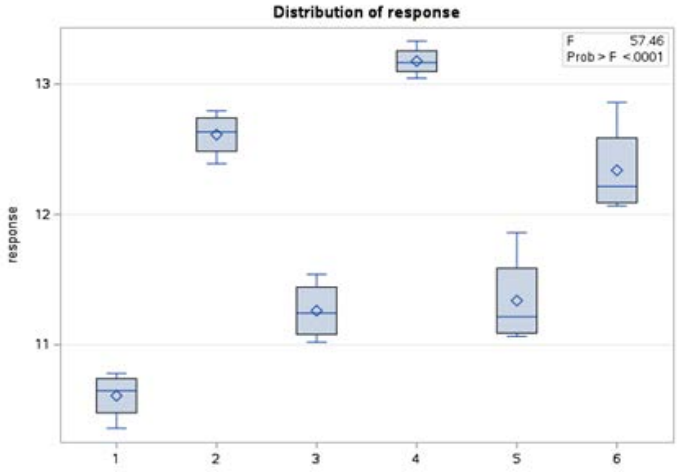

Figure 4: Haenoglobin profile of Limura cattle (1) calf; (2) young; (3) adult (female); (4) calf, (5) young, (6) adult (male)

dura cattle is lower than that of Limura cattle, but the value is still within the normal range of 24 - 46% Kessell (2015). Optimal metabolic conditions are not always followed by high production performance, differences influence this in the level of nutritional adequacy of feed and individual livestock factors in converting protein into meat. Klinkon et al. (2015) reported that the hemoglobin level of male Simmental cattle fed a mixture of field grass silage, corn stover silage, and sunflower seed meal was 13.47±0.47 g/dL. Sarker et al. (2010) reported that Hanwoo bulls (endemic to Korea) had a blood haemoglobin content of 13.25±0.17 g/dL. Weiss and Wardrop (2010) added that the normal value of haemoglobin in cattle has an interval between 8.4-12 g/dL.

Haematocrit

The mean haematocrit values of male and female Madura cattle at different ages were 33.44 ± 1.64; 31.232 ± 1.20; 31.84 ± 1.04; 30.5 ± 1.98; 30.52 ± 3.07 respectively; 30.6 ± 1.94 (%), while for Limura cattle (Madura x limousine) males and females at different ages (calf, young, and adult) were respectively 29.68 ± 1.67; 33.06 ± 1.50; 32.06 ± 1.25; 37.42 ± 1.61; 33.16 ± 2.35; 31.68 ± 1.30 (%) (Table 1). Hematocrit (in percentage) is the ratio of the number of erythrocytes compared to the total blood volume (Cunningham, 2002). Based on the study's results, hematocrit in male and female Madura cattle with different ages was relatively stable in the range of 30.5 ± 1.98 - 33.44 ± 1.64. In contrast, the haematocrit value in male and female Limura cattle was 9.68 ± 1.67 - 37.42 ± 1.61. There is a difference in hematocrit value between Madura cattle and Limura cattle. This difference is thought to be influenced by race and gender factors. The high number of erythrocytes is also the cause of the high haematocrit value in Limura cattle compared to Madura cattle. Sonjaya (2013) stated that the number of erythrocytes affects the haematocrit value. A study by Syam et al. (2016) on beef cattle showed that feed type could affect hematocrit value. Cattle fed by forage and urea molasses block led to lower hematocrit values than those given additional concentrate. Based on interviews with farmers, we revealed that the Madura and Limura cattle used in this study were only fed on grass without additional concentrate, still the quantity of feed given to Limura cattle was greater than that of Madura cattle. Ariana et al. (2018) also stated that nutritional factors can cause changes in blood profile. An increase in haematocrit value has limited benefits because it can increase blood viscosity, slowing blood flow in capillaries and increasing heart work (Cunningham, 2002). Normal haematocrit values in cattle are 21 - 30% (Weiss and Wardrop, 2010). Calves aged 2 weeks to 6 months have a haematocrit value of 23 - 42% (Lumsden et al., 1980). Calves aged 0 - 8 weeks have a haematocrit value of 26.79 - 27.30%.

Leukocyte

Based on the result of the leukocyte differentiation of Madura and Limura (Limousine x Madura) cattle were presented whole insignificant differences ($p > 0.05$) (Table 2). Either Madura and Limura cattle were presented insignificant differences across lymphocyte (%), monocyte (%), and granulocyte (%) (Table 2). Leukocyte are an envelope of phospholipids, proteins, and glycoproteins that repair mechanical tissue damage such as bleeding. However, the Madura cattle at the young stage presented better compared with Limura cattle both male and female (Table 2). Weiss and Wardrop (2010) stated that several factors, including bleeding, iron deficiency, and inflammation influence the increase in platelet concentration. The same finding was reported by Sarker et al (2010) that the leukocyte concentration of Hanwoo bulls in the same treatment showed a significant difference in concentration. The results of leucocyte differentiation of Madura cattle showed that the variable number of lymphocytes and monocytes had the same range of values as Limura cattle but showed fluctuations in values at different sex and age levels. Although fluctuations occur, they are still within the normal range. In contrasts the percentage of granulocytes in Limura cattle shows a higher value than in Limura cattle. According to Smith and Mankoewidjojo (1988), the normal values of lymphocytes; monocytes; and granulocytes of cattle are 42-61%; 2.5-13.5%; and 5-26%, respectively. Dharmawan (2002) reported the normal range of lymphocytes is 45- 75%; and monocytes is 2-7%. It was further reported that the normal values of bovine lymphocytes and monocytes are (1.6-8.1) x10^3µl; and (0-0.7) x10^3µl (Roland et al., 2014). The number of lymphocytes in the blood circulation can be affected by the level of production, recirculation, and use or destruction of lymphocytes. A decrease in the number of lymphocytes can be due to stress and stress due to environmental factors, hot cage temperature, and narrow cage size, Stress in cattle will stimulate an increase in the secretion of glucocorticoid hormones which results in a decrease in the number of lymphocytes in the blood). The fluctuation of monocyte percentage in Madura and Limura cattle at different sex and age levels indicates physiological disturbances in the cattle body. Monocytes are the forerunner of macrophages that are still in the blood circulation. Suppose there is an infectious agent in a tissue. In that case, monocytes with amoeboid movement come out of the blood vessels to become macrophages that are ready to phagocyte the microb or foreign agent (Berata, 2010). Macrophages are defence cells that also play a role in helping to activate other leukocytes such as lymphocytes and basophils. Therefore, the increase in monocytes in this study is thought to be due to synergism with an increase in the percentage of lymphocytes (Peinado et al., 1999).

Table 2: Leukocyte differentiation of Madura and Limura (Limousine x Madura) cattle

Parameter	Breed	Sex											
		female						Male					
		Calf	SEM	Young	SEM	Adult	SEM	Calf	SEM	Young	SEM	Adult	SEM
Lymphocyte (%)	Madura	58.80	1.47	61.00	2.89	60.00	2.21	59.20	1.27	58.40	2.00	60.60	3.90
	Limura	62.00	4.40	63.20	5.87	62.60	3.62	58.60	2.71	60.00	5.41	65.00	5.25
Monocyte (%)	Madura	13.60	3.50	12.40	3.50	11.00	1.56	10.80	1.30	11.40	2.76	14.00	1.85
	Limura	10.60	1.80	12.20	2.47	11.40	2.31	13.50	2.87	11.60	2.71	12.60	3.35
Granulocyte (%)	Madura	27.60	3.03	28.20	1.91	29.00	3.75	30.00	0.87	30.20	3.43	25.00	3.65
	Limura	27.40	6.07	26.60	6.51	26.00	4.51	37.50	2.35	28.40	3.81	22.40	5.44

Limura (Limousine x Madura); SEM – standard error mean

CONCLUSION

The Haematological profile and leucocyte differentiation of male and female Madura cattle of different ages are generally similar to their crossbred counterparts and within the normal range. Madura cattle tend to be more adaptive or responsive to various exposure factors as indicated by higher erythrocyte and leucocyte counts compared to their crossbred counterparts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

NOVELTY STATEMENT

There has been no research published about Hematological Profile and leukocyte differentiation of Madura cattle and their crosses at different sexes and ages.

AUTHOR'S CONTRIBUTION

Desi Kurniati collecting data, doing the research, preparing manuscript; Suyadi Suyadi conceptualization, supervision, review the manuscript; Veronica Margaretha Ani Nugiartiningsih supervision; Kuswati Kuswati conceptualization, review the manuscript, conceptualization. All authors read and approved the final version of the manuscript in the present journal

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