Comparison of Neutrophil to Lymphocytes (N/L) Ratio in Pregnancy and Lactation of Thin-Tail Sheep

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Abstract. Thin-tailed sheep are the most widely kept sheep species in Indonesia. The neutrophils and lymphocytes (N/L) ratio can be a stress indicator for sheep. Stress conditions can cause animals to experience impaired growth, productivity, and behavioral changes. However, the study of thr N/L ratio during pregnancy and lactation in thin tailed sheep is still lacking and variations in physiological status such as pregnancy and lactation condition can cause differences in the N/L ratio. This study was conducted to determine the stress level on the physiological status of pregnant and lactating thin tailed sheep by looking at the difference in the N/L ratio. The blood samples used were seven pregnant sheep and five lactating sheep rearing in El Farm Ternak Domba Kambing dan Pendopo Sawah Kunden, Berbah, Sleman, Yogyakarta. The data obtained were analyzed using the Independent T-test method with the help of SPSS software version 16. The result of statistical analysis showed that the ratio N/L of pregnancy and lactation in thin tailed sheep was no significant difference (P>0.05). However, the average N/L ratio in pregnant conditions was higher, it was 0.61 ± 0.95, while in lactation conditions, it was 0.23 ± 0.30. The conclusion obtained from this study showed that the difference in the physiological status of pregnancy and lactation had no significant effect on the N/L ratio in ewes and the two physiological conditions of the studied sheep were still within normal limits.

1 Introduction

Sheep is one of the food commodities which can mainly provide the availability of meat with high protein content. It is also an adaptable animal. Generally, sheep usually walk in groups, eat grass, don't choose food, and have a less sharp sense of ability so they are easy to maintain and feed [1]. According to the Badan Pusat Statistik [2], the average sheep population increases every year. In 2016 the sheep population was 15,716,667 heads, in 2018 there were 17,611,392 heads, until 2020 the population reached 17,769,084 heads.

In Indonesia, two types of sheep often used for their meat, one of which is thin-tailed sheep. Thin-tailed sheep are widely kept and have the widest population distribution in Indonesia. This sheep has the characteristics of a small and thin tail, white fur color and black stripes around the eyes. In general, the female has no horns and the ram has small horns. Male sheep weigh around 30-40 kg, while females weigh between 15-20 kg [3]. To produce good meat products, necessary to have good maintenance management as well.

The gestation period is the time between marriage and birth. During this period, with the development of the fetus, the mother's uterus will also experience significant anatomical and physiological changes [4]. The older the gestational age, the value of the N/L ratio increases. Meanwhile, the N/L ratio at the beginning of lactation (between 30-60 days postpartum) was higher than at the end of lactation (after 60 days postpartum) [5].

The lactation period is when the animal is producing milk, usually after giving birth. In the early lactation phase, the N/L ratio is higher than the late lactation phase. In the early lactation phase, the mother must undergo milk synthesis and undergo postpartum uterine involution simultaneously.

Stress is a condition that can threaten and disrupt homeostasis in animals, hurting the welfare of the animal itself. If there is stress, this zone of homeostasis will be disturbed, and the body will try to return it to a state before the stress occurs. As a result, animals can experience impaired growth, productivity, and behavioral changes [6]. Stress hormones are released when the body is under normal conditions in small amounts, but when stress occurs, the hormone levels will increase drastically [7]. The process starts with the secretion of corticotrophin-releasing factor (CRF) by the brain's hypothalamus into the bloodstream to the pituitary gland. Here, CRF will stimulate the release of adrenocorticotrophin hormone (ACTH) by the pituitary, which stimulates the adrenal glands to release several hormones and one of them is cortisol. If the stressor received by the hypothalamus is strong, then the secretion of CRF increases. As a result, the secretion of the hormone cortisol will also increase.

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Routine blood checks can be done to determine the health condition of the sheep. Blood tests can also be used to determine the level of stress experienced by sheep. Stress indicators usually involve the ratio of neutrophils and lymphocytes [8]. In animals that are lactating, parturition, postpartum and old animals usually have a fairly high ratio value compared to normal. This also affects the increase in the hormone cortisol in the body and changes in the leukogram. If stress occurs, the body will respond by increasing the number of neutrophils (neutrophilia) and decreasing lymphocytes (lymphopenia) in the blood, then the ratio of neutrophils and lymphocytes changes.

This study aims to determine the difference in stress levels in pregnant and lactating female thin-tailed sheep through analysis of the ratio N/L and expected to provide information in determining stress levels in sheep based on the analysis of the ratio N/L in the blood.

2 Material and Method

2.1 Location and Research Time

The study began on August 8, 2021, and ended on November 26, 2021. Sampling of sheep blood was carried out on August 9, 2021, at 07.00-12.00 WIB. The temperature at the location is around 32◦C with humidity of 56%, wind speed of 11 km/h, and rainfall of 10%, with location coordinates at -7,8067559,110,4644625. The neutrophil/lymphocyte data analysis was carried out from October 10 to November 26, 2021.

2.2 Animal Test

The sheep selected as samples were adapted to the desired physiological conditions. This study used twelve female thin-tailed sheep samples that randomly picked. Then the samples were divided into two groups, seven pregnant females and five lactating females, which were selected at random. The age range of pregnant ewes is between 10 months to 3 years, with an average body weight of 35 kg to 50 kg. The age range of lactating ewes is between 18 months to 3 years, with an average body weight of 35 kg to 50 kg. This research has obtained a Certificate of Ethical Eligibility from the EC Commission of the Faculty of Veterinary Medicine UGM No. Certificate: 00017/04/LPPT/VII/2021.

2.3 Blood Sampling

Blood samples were taken through the jugular vein using a 10 ml syringe with a needle size of 21 G 1". The collected blood was put into an EDTA tube and then placed in a cooler box to prevent damage to the sheep blood sample.

2.4 Leukocyte Count

Blood hematology calculations were used Automatic Hematology Analyzer mindray BC-2800 Vet with Impedance 3 diff working principle. The total leukocyte count was done manually using a Hemocytometer Count with Turk's reagent. After that, the differential leukocyte count was performed manually by making blood smears fixed with methanol and stained with Giemsa absolute for 3 minutes, then rinsed. The results of the staining of the blood smear were calculated using a Differential Cell Counter under a microscope with a magnification of 1000x.

2.5 Statistical Analysis

The research data wew collected in Microsoft Office Excel. Then, statistical analysis was carried out using the SPSS 16. The Normlaity Test waWS used to determine whether the data were normally distributed. Independent T-Test analysis was performed with two differen variables. Significance can be seen through the magnitude of the value of Sig. (2-tailed).

3 Result and Discussion

Examination of the N/L ratio was obtained from the comparison between the absolute neutrophil and absolute lymphocyte values. The results of the descriptive analysis of the average N/L ratio of pregnant and lactating ewe thin-tailed sheep are presented in **Table 1**.

Table 1. N/L ratio of pregnant and lactating in ewe thin-tailed sheep.

Category	N	N/L Ratio	Reference	Std Error
Pregnancy	7	0.61 ± 0.95^{a}	0,30-0,50*	0.28
Lactation	5	0.23 ± 0.30^{a}	0,30-0,50*	0.03

The same superscript (a,a) in the same column showed no significant difference in the two physiological conditions of thin-tailed sheep ($P > 0.05$)
*[14]

The results, show that the N/L ratio in pregnant ewes was 0.61 ± 0.95 and in lactating ewes 0.23 ± 0.30. This shows that the N/L ratio in pregnant and lactating thin-tailed sheep did not increase significantly, even in lactating it was lower than normal. The normal N/L ratio in sheep ranges from 0.30 – 0.50 [9]. The N/L ratio can indicate stress when it exceeds 1.5 [10]. If stress symptoms occur in one or both groups, this will trigger an increase in cortisol and cause neutrophilia and lymphopenia.When the neutrophil count increases and the lymphocyte count decreases, the N/L ratio increases. An increase in the N/L ratio can also be caused by the health and nutritional conditions of the animal itself [5, 11].

The average N/L ratio in the two groups of sheep is not significantly different, as evidenced by a P-value > 0.05, with pregnant conditions having a higher N/L ratio than lactation condition. This value is contrary to the research that has been done by Sarmin *et al*., [12] in fat-tailed sheep, where the N/L ratio of pregnant ewes was 0.07 ± 0.00 and lactation was 0.31 ± 0.10. His research also found that the ratio between N/L was not significantly different ($P > 0.05$). Research by Nareswari *et al*., [13] in Sapera goats, found that the average N/L ratio in pregnant females was 0.19 ± 0.12 and lactation 0.47 ± 0.23. This

could be because the sampling in both studies was carried out randomly without considering the age of need and the duration of lactation experienced by the ewes. However, in a study conducted by Sarmin *et al*., [9] on PE goats, found that the fourth-month pregnant female had an N/L ratio of 2.89 ± 2.89 and a fourth-month lactation of 0.74 ± 0.55, in line with the research that has been done. According to Kanna *et al*., [10], the N/L ratio can indicate stress when it exceeds 1.5. So it can be said that the two groups of pregnant and lactating ewes studied did not show any indication of stress.

According to Bazzera *et al*., [5], who have researched Morada Nova and Santa Ines sheep, the type (breed) and reproductive stage affect the hemoglobin content in the blood, and gestational age lactation duration influence the rise and fall of the N/L ration. The older the gestational age, the value of the N/L ratio increases. Meanwhile, the N/L ratio at the beginning of lactation (between 30-60 days postpartum) was higher than at the end of lactation (after 60 days postpartum). This is one of the factors that cause the higher value of the N/L ratio of thin-tailed sheep in the pregnant phase compared to the lactation phase.

According to Pitri *et al*., [14], cortisol is released in very small amaounts thourghout the day under normal circumstances. However, cortisol levels can increase drastically under stress and can even increase 20 times [14]. The increase in the N/L ratio can occur due to the release of the hormone cortisol, which appears when the animal is under stress [6]. This will cause the bone marrow to release neutrophils so that in the blood circulation the number will increase. Neutrophils will increase when the body experiences trauma and inflammation (especially when suppuration) [15, 16]. In this study, pregnant conditions can trigger an increase in neutrophils caused by a decrease in immunity, so that the mother is more susceptible to infection. Research conducted by Sarmin *et al*., [9] found that the value of the N/L ratio in pregnant women at the final gestational age increased from the previous month. This can be caused by the rapid development of the fetus, the growing size of the fetus and the rapid growth of the mammary glands [17]. According to Duncanson [18], the increasing size of the fetus will cause pressure on the abdominal cavity, allowing the mother to experience trauma inside the abdomen. As we reach the end of gestation, cortisol in the fetus (fetal cortisol) will play many roles in the maturation of many organs and systems, including respiratory, renal, and cardiovascular. This causes the mother to need a lot of energy to maintain pregnancy and normal physiological conditions.

According to Bazzera *et al*., [5], in the early lactation phase, the N/L ratio is higher than the late lactation phase. This is because in the early lactation phase, the mother must undergo milk synthesis and postpartum uterine involution simultaneously [5]. In general, sheep uterine involution is complete on the 27th of the postpartum day and is preceded by the emergence of postpartum estrus [19]. Then, increase the value of neutrophils, causing an increase in the level of the N/L ratio in the sheep that are in the early phase of lactation. Meanwhile, in the final lactation phase, the N/L ratio tends to be low. In this phase, the body produces cortisol in small quantities to maintain prolactin levels so that milk synthesis continues normally.

The normality test result using Shapiro-Wilk are $P > 0.05$, indicating that the data is normally distributed. Analysis result using the Independent T-test method to the physiological condiiton of pregnant and lactating thin-tailed sheep on the N/L ratio are $P > 0.05$, which shows taht there was no significant difference in the N/L ratio in the two groups of sheep that were tested.

4 Conclusion

The difference in physiological status pregnancy and lactation had no significant effect on the N/L ratio in ewes. The two physiological conditions of the studied sheep were still within normal limits.

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