Profile, correlation, and regression of erythrocyte index in Indonesian local fat-tailed sheep at pregnancy periods various until lactation

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Abstract. Indonesian Local Fat-Tailed sheep called Sapudi are very adaptive to rural farming conditions. This study aimed to analyze the erythrocyte index in Sapudi ewes at various periods of pregnancy until lactation as basic information for breeding ewes. This research method was a case study with sampling criteria of physically healthy ewes and three periods of pregnancy, including early pregnancy (EP), mid-pregnancy (MP), late pregnancy (LP), and lactating ewes (LT). The status of non-pregnant ewes (NP) was used as a control. Data analysis used statistics descriptive of the IBM SPSS 19 program. The variables observed were MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobine), and MCHC (Mean Corpuscular Hemoglobine). The results showed that the profile of the erythrocyte index tended to differ in each period from pregnancy, lactation, and control. Statistically, the erythrocyte index was significantly different ($p < 0.05$) only in the MCHC variable; the correlation value and regression significance of MCHC with MCH and MCV were 0.99 and 0.44, while the correlation of MCV and MCH was 0.58. This study concluded that the erythrocyte index in MCHC significantly increased by 6.46% in the EP phase and tended to decrease in the MP, LP, and LT phases by 8.02%, 5.41%, and 7.1% compared to the control (NP). A positive correlation of MCHC and MCH with the regression equation $y = 4.40 + 2.05X$. The study recommended that the breeding management of the sheep should be divided into three minimums: non-pregnant ewes, early pregnant ewes, and middle pregnant until lactation ewes.

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1 Introduction

Sheep in Indonesia was 1.18% of the world's total sheep population in 2022, while China has the largest sheep population in the world at 14.69 [1]. It showed that sheep farming is significantly developed in Indonesia, especially in rural areas, as one of the contributors to the people's economy. However, sheep farming in Indonesia could be more optimal, as evidenced by a 10.06% population decline from 2021 to 2022 [2]. Efforts to increase the sheep population continue to be encouraged by the government and livestock associations engaged in sheep farming, including improving livestock seeds through crossbreeding and regulations that better support the development of sheep farming [3-5]. One of the factors that determines the increase in the sheep population is its productivity. The success of increasing sheep productivity is primarily determined by the fertility of the ewes and ram and their offspring [6-10].

In contrast, the number of lambs per lambing and died after birth were primarily determined by the health of the ewes during pregnancy until lactation [11]. Pregnancy and lactation are essential factors that affect the health of ewes and lambs in a sustainable breeding program. Unhealthy ewes at various parities significantly affect the mortality and birth weight of lambs [12]. The health of ewes during pregnancy and lactation was also influenced by many factors, including the availability of oxygen regulated by erythrocytes in the body to transport energy throughout the body so that there is a balance because inadequate energy imbalance in pregnant sheep is not only dangerous for the ewes but also for the survival and performance of the sheep before and after giving birth accompanied by low birth weight and perinatal death. The importance of erythrocytes as a crucial component in the circulatory system that is responsible for ensuring that every cell, tissue, and organ of the body gets sufficient oxygen supply so that there is energy balance in the mother's body during pregnancy can be described by MCV, MCH, and MCHC. Therefore, it was necessary to conduct research related to erythrocytes of sheep with various phases of pregnancy until lactation, describing the profile, correlation, and regression of the erythrocyte index in Sapudi ewes at various periods of pregnancy until lactation as basic information for breeding ewes based on the condition of erythrocytes of ewes at each reproductive status.

2 Materials and methods

The research method was a case study with purposive sampling of ewes. The material used consisted of 20 Indonesian local fat-tail ewes aged 3-4 years. The quantitative characteristic of Indonesian Local Fat-tail sheep was body weight of around 20.25-40.96 kg, body height and body length of around 63.21 cm and 76.15 cm, 57.45-60.60 cm and 53.65-54.75 cm and originating from the UPT for livestock and forage breeding in Jember, Indonesia. The ewe sheep were divided into five reproductive group phases, namely, not pregnant, early pregnancy (30-60 days), mid-pregnancy (60-120 days), late pregnancy (>120 days), and lactation (Figure 1). Each treatment group consisted of five ewes. The variables observed were erythrocyte index, including MCV, MCH, and MCHC. This study used a quantitative descriptive method. The data obtained were analyzed using one-way Analysis of Variance (ANOVA). If a significant difference exists ($P < 0.05$), it was continued with the least significant difference test. Profile, correlation, and regression analysis used the IBM SPSS 19 program.

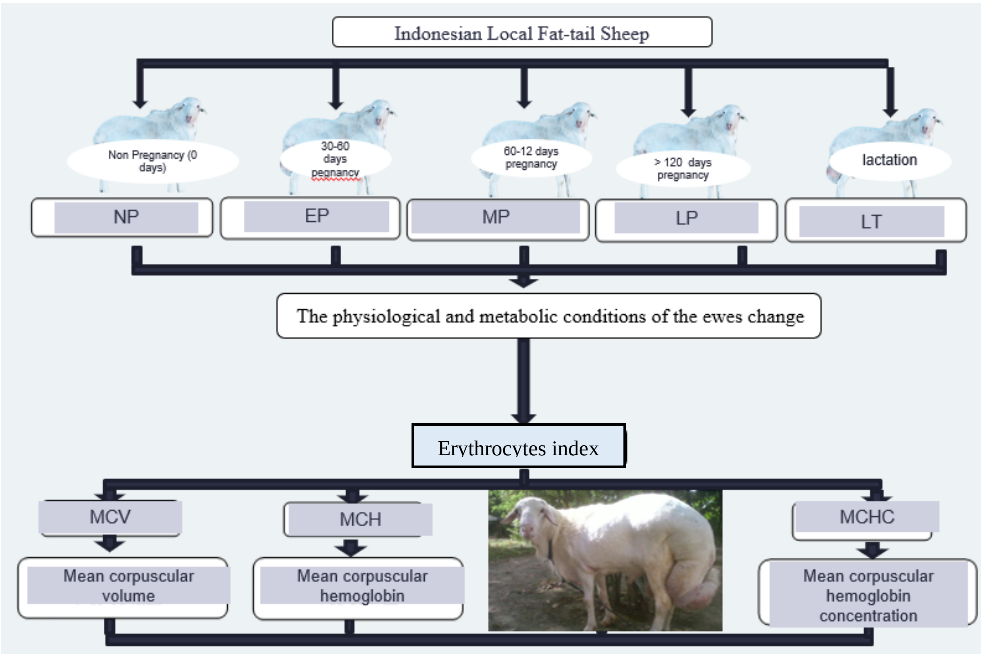


Fig 1. Reproductive group phases and variables observed in Indonesian Local Fat-tail sheep

Blood was taken from the jugular vein of the sheep. Previously, the jugular area, precisely 1/3 of the upper neck, was disinfected with alcohol (etical clearance number 002/EC-FKH/Int/2019). Furthermore, damming and blood collection were carried out. 2 ml of blood was taken with a 5 ml syringe and immediately inserted into a vacutainer containing EDTA anticoagulant. Then, the vacutainer was inserted into an ice box containing an ice pack. After sampling, the samples were taken as 20 sheep samples and then stored at a temperature of 8 °C. Samples could be analyzed for erythrocyte index using the Horiba ABX Micros ES60 haematology analyzer.

3 Results and discussions

3.1 MCV (Mean Corpuscular Volume)

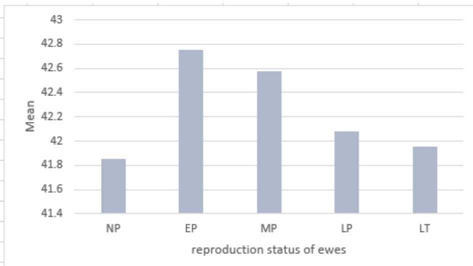
MCV describes the average size of red blood cells (erythrocytes), the results of which in Indonesian Local Fat-tail ewes can be seen in the following MCV profiles.

Table 1. MCV profile at various phases of pregnancy and lactating ewes.

Items	MCV (fL)				
	NP	EP	MP	LP	LT
Mean	41.85 ± 1.05	42.75 ± 0.29	42.58 ± 0.48	42.08 ± 0.79	41.95 ± 0.44
VP	1.10	0.08	0.23	0.63	0.19
Range	2.40	0.70	1.10	1.60	0.90

Notes: NP = non-pregnancy, EP =early pregnancy, MP = mid-pregnancy LP = late pregnancy, LT = lactation, VP = variation of phenotype

Table 1 shows the average MCV of ewe ewes from various phases of pregnancy and lactation ranged from 41.85-42.75 fL, a value that is close to the value of the results of research on ewes 40.38-42.17 fL [13]. Although the statistical test showed that the MCV value was not significantly different (*P>0.05*) seen from the mean, median, mode, maximum, and minimum values, the highest in the early pregnancy period (EP) showed the highest tendency, followed by the SD value and the lowest phenotype variation and range. The data meant that the MCV value in the EP phase tends to be the highest, followed by a relatively uniform value (Figure 2). Following the opinion that the lower the VP value for quantitative traits in sheep, the more uniform the quantitative traits; conversely, the higher the VP, the more diverse quantitative traits [14].



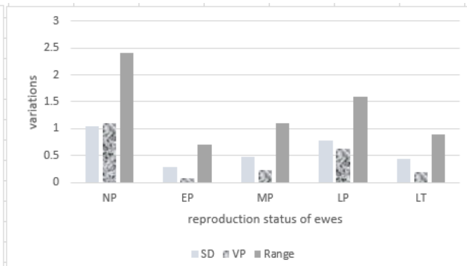


Fig 2. Mean and variation of MCV from various phases of pregnancy and lactation.

The MCV results of ewes tended to increase in pregnant and lactating ewes compared to non-pregnant ewes. However, the increase was not significant (P > 0.01), and the results were similar to research on Nguni, Boer, and Non-descript goats in South Africa [15], and in Balinese cattle[16]. Although insignificant, the mean MCV percentage tended to increase in pregnant and lactating ewes (EP, MP, LP, and LT) compared to non-pregnant ewes were 2.1%, 1.7%, 0.5%, and 0.2%, respectively. This is supported by the opinion that small ruminants had different hematologic included MCV in the lactation phase [17]. Strengthening this with another research [13], if the MCV increased, it would indicate a regenerative anaemia response caused by the erythrocyte hemolysis process. High MCV values may also be caused by the release of immature erythrocyte cells into the blood circulation system.

3.2 MCH (Mean Corpuscular Hemoglobin)

MCH describes the average haemoglobin in each erythrocyte; the profiles obtained from various pregnant and lactating ewes are shown in Table 2 below.

Table 2. MCH profile at various phases of pregnancy and lactating ewes.

Items	MCH (pg)				
	NP	EP	MP	LP	LT
Mean	13.95 ± 2.03	15.15 ± 0.73	12.95 ± 0.99	13.20 ± 0.63	12.98 ± 0.46
VP	4.11	0.54	0.98	0.39	0.22
Range	4.50	1.60	2.20	1.50	1.00

Notes: NP = non-pregnancy, EP =early pregnancy, MP = mid-pregnancy LP = late pregnancy, LT = lactation, VP = variation of phenotype

The results of statistical analysis showed that there was no significant difference (*P>0.05*) in various phases of pregnant and lactating ewes of Indonesian Local Fat-tail ewes, in accordance with research conducted on pregnant and lactating Baladi goats [18]. Table 2 shows that the highest average MCH was in EP, and the lowest in LT, with diversity values (SD, VP, range) both in EP, MP, LP, and LT far below NP. It indicated that the average MCH value in pregnant and lactating ewes (EP, MP, LP, and LT) tended to be more uniform compared to the control (NP). Deviation percentage in MCH values of the ewes reproduction status (EP, MP, LP, and ET) compared to non-pregnant status (NP), the results were EP was 8.6% higher compared to NP (control), whereas MP, LP, and LT were lower compared to NP with values of -7.1%, -5.3%, and -7.02%, respectively (Figure 3).

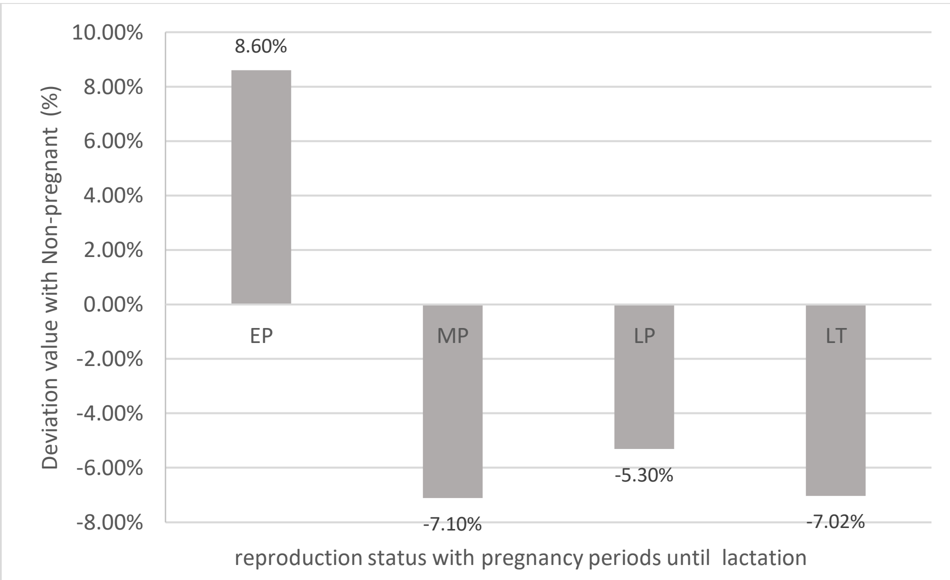


Fig 3. Percentage of MCH deviation values in pregnant and lactating ewes compared to control (non-pregnant ewes).

Figure 3 showed that EP had a higher positive MCH value than non-pregnant mothers (control). In contrast, MP, LP, and LT (medium gestation to lactation) have MCH values that decrease (negative) compared to non-pregnant mothers. It meant physiological differences in early pregnant mothers (EP) compared to the condition of mid—and late-pregnant ewes to lactation, including non-pregnant ewes. There was an indicated difference in oxygen-carrying capacity in the circulatory system between pregnant and lactating goats [19], and there was an indication of iron deficiency in MP and LP of pregnant phase and lactating goats [20]. The implication study is that the breeding management of Indonesian local fat-tailed sheep should be divided into three minimums: non-pregnant ewes, early pregnant ewes, and middle pregnant until lactation ewes.

3.3 MCHC (Mean corpuscular haemoglobin concentration)

MCHC describes the concentration or average level of haemoglobin in one erythrocyte. In this study, the following MCHC profile was produced.

Table 3. MCHC profile at various phases of pregnancy and lactating ewes.

Items	MCHC (g/dL)				
	NP	EP	MP	LP	LT
Mean	33.28a ± 4.04	35.43ab ± 1.81	30.60ac ± 1.99	31.48ac ± 1.03	30.90ac ± 0.78
VP	16.31	3.28	3.97	1.06	0.61
Range	9.10	4.00	4.30	2.10	1.80

Notes: NP = non-pregnancy, EP =early pregnancy, MP = mid-pregnancy LP = late pregnancy, LT = lactation, VP = variation of phenotype

The results of statistical analysis showed that the average MCHC was very significantly different (*P<0.01*) in pregnant and lactating ewes, where EP was significantly higher compared to MP, LP, and LT but a little bit different with NP (non-pregnant or control animals). The results of the study are in accordance with MCHC in pregnant and lactating donkeys [21], pregnant and lactating Dorper sheep [22], pregnant and lactating Nellore cows [23] and pregnant and lactating Santa Ines sheep [24]. These results indicate that there was an increase in the total oxygen capacity circulating between pregnant and lactating ewes [22].

Based on the mean values in Table 3, the MCHC value in the EP group was the highest, while the lowest value was in the MP group, followed by its diversity value in the pregnant and lactating ewes groups (EP, MP, LP, LT) which was lower compared to the non-pregnant or control mother group (NP). Deviation percentage in MCH values of the ewes reproduction status (EP, MP, LP, and ET) compared to non-pregnant status (NP), the results were EP was 6.46% higher compared to NP (control), while MP, LP, and LT were lower compared to NP with values of -8.02%, -5.4.1%, and -7.10%, respectively (Figure 4).

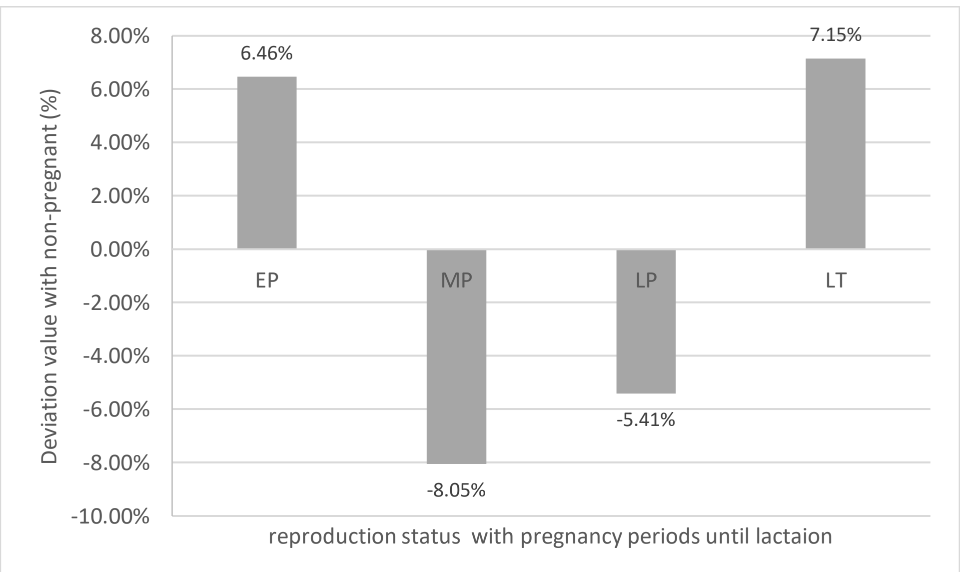


Fig 4. Percentage of MCHC deviation values in pregnant and lactating ewes compared to control (non-pregnant ewes).

Figure 4 shows that EP has a higher positive MCHC value than non-pregnant mothers (control). At the same time, MP, LP, and LT (medium pregnancy to lactation) have decreased MCHC values (negative) compared to non-pregnant mothers. There were physiological differences in early pregnant mothers (EP) compared to mid- and late-pregnant ewes to lactation, including non-pregnant ewes. The physiological difference implied that the breeding management of Indonesian local fat-tailed sheep should be divided into three minimums: non-pregnant ewes, early pregnant ewes, and middle pregnant until lactation ewes.

3.4 Correlation and regression of erythrocyte index

The regression analysis showed that there is a sufficient correlation (r = 0.44 and 0.58) between MCV and MCH and MCV and MCH. At the same time, the relationship between MCHC and MCH is very close (0.99). Table 4 provides more details**.**

Table 4. Correlation, determination coefficient and regression of erythrocyte index.

Items	Relation		
	MCHC with MCH	MCHC with MCV	MCH with MCV
r	0.99	0.44	0.58
R^2	0.97	0.18	0.33
Regression equation	Y = 4.40+2.05 X	Y = - 41.0+1.74 X	Y= -32.10+1.08 X

Table 4 shows that the coefficient of determination (R^2) value is very high (0.97) in the relationship between MCHC and MCH, meaning that the MCHC value is influenced by 97% of MCH. In contrast, the relationship between MCHC and MCV and MCH and MCV has a moderate coefficient of determination (0.18 and 0.33), meaning that the MCHC value is influenced by MCV by 18%, and the relationship between MCH and MCV by 33%. The regression equation that could be implemented in the field of MCHC with MCH was Y= 4.40 +2.05 X, which meant that an increase of 1 unit in MCH would be followed by increasing MCHC by 2.05. The high value of the coefficient of determination and significance of the regression equation was also reinforced by the data distribution pattern in the Q-Q plot analysis between MCH and MCHC, which showed relative similarity. In contrast, MCV has a slightly different pattern from MCH and MCV (Figure 5).

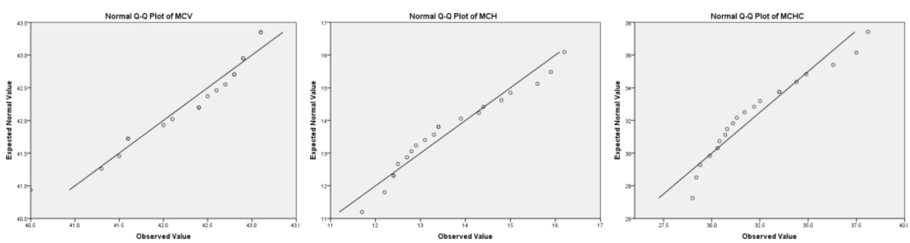


Fig 5. Q-Q plot of MCV, MCH, and MCHC in non-pregnant, pregnant, and lactating dams.

Figure 5 shows that the MCV, MCH, and MCHC values in non-pregnant, pregnant, and lactating dams are normally distributed. MCV, MCH, and MCHC values can be used to diagnose anaemia in livestock [25-27]

4 Conclusions

This study concludes that the erythrocyte index in MCHC significantly increased by 6.46% in the EP phase and tended to decrease in the MP, LP, and LT phases by 8.02%, 5.41%, and 7.1% compared to the control (NP). A positive and very high correlation in MCHC and MCH with the regression equation Y = 4.40 + 2.05X. The correlation between MCHC and MCV, MCV and MCH was moderate. The study recommended that the breeding management of Indonesian local fat-tailed sheep should be divided into three minimums: non-pregnant ewes, early pregnant ewes, and middle pregnant until lactation ewes.

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References

1. The World Population Review, Sheep population by country 2024, (2024) https://worldpopulation review.com /country-rankings /sheep-population-by-country.
2. Central Statistics Agency. Sheep population by Province in Indonesia (head), 2021-2023, (2024). https://www.bps.go.id/id/statistics-table/2/NDczIzI=/populasi-domba-menurut-provinsi.html.
3. F.A. Barakeh, A.O. Kashroum, R.A. Tarawneh, F.A. Al-Lataifeh, A.N. Al-Yacoub, M. Dayoub, K. Al-Najjar, Sustainable sheep and goat farming in arid regions of Jordan. Ruminants. **4**, 241-255 (2014). https://doi.orf/10/3390/ruminants4020017.
4. R. Ardiansyah, E. Miwandhono, Hasnudi, Analysis of development potential of sheep in Deli Serdang Regency, North Sumatra Province. Jurnal Peternakan Integratif. **9**, 71-78 (2021).
5. V.D. Nguyen, C.O. Nguyen, T.M.L. Chau, D.Q.D. Nguyen, A.T. Han, T.T.H. Le. Goat production, supply chains, challenges, and opportunities for development in Vietnam: a review. Animals. **13**, 2546 (2023). https://doi.org/10.3390/ani13152546.
6. H. Tufekci, V. Sejian, Stress factors and their effects on productivity in sheep. Animals. **13**, 2769 (2023). https://doi/org/10.3390/ani13172769.
7. N. Lecic, V.C. Petrovic, D.R. Muslic, N. Maksimovic, B. Cekic, I. Cosic, Influence of some factors on fertility and weight if seep and body weight development of lambs. Biotechnology in Animal Husbandry. **38**, 93-100 (2022). https://doi.org/10.2298/BAH2202093L.
8. N. Assan, Effect of genetic and non-genetic factors on growth traits in goats and sheep production. Scientific Journal of Zoology. **9**, 106-122 (2019). Doi:10.14196/siz.v9i1.539.
9. J.L.P. Pacheco, A.H. Molina, L.T. Rovira, M.V.S. Fernandez, C.G. Contreras, M.V. Gomez, P. Feyjoo, E. Caceres, M.F. Mateo, F. Hernandez, P.M. Ros, J.V.G. Martin, A.G. Bulnes, S. Astiz, Maternal metabolic demands caused by pregnancy and lactation: association with productivity and offspring phenotype in high-yielding dairy ewes. Animals. **9**, 295 (2019). Doi:10.3390/ani9060295.
10. K.H.A. Gubory, Effects of the presence of rams during pregnancy on lambing performance in ewes. Anim. Rerprod. Sci. **52**, 205-211 (1998). Doi:10.1016/s0378-4320(98)00095-5.
11. T. Clune, A. Lockwood, S. Hancock, A.N. Thompson, S. Beetson, A.J.D. Campbell, E. Glanville, D. Brookes, C. Trengove, R. O'Handley, K. Gavin, I.J. Caroline. Abortion and lamb mortality between pregnancy scanning and lamb marking for Maiden ewes in Southern Australia. Animals. **12**, 10 (2022). https: //doi.org/10.3390/ani12010010.
12. M.A.P.D. Sousa, S.N. Esteves, L.C.C. Daher, S.B. Gallo, V.S.d.A. Pareira, J.F.d. Fonseca, A.G.M.e. Silva, F.Z. Brandao, M.E.F. Oliveira, A.d.N. Barreto, G.B. Vergani, A.R. Garcia, Overnutrition of ewe in late gestation and the impact on placental efficiency and lamb's performance. Animals. **13**, 103 (2023). https://doi.org/10.3390/ani13010103.

13. Y. Fanta, Y. Kechero, N. Yemane, Hematological parameters of sheep and goats fed diets containing various amounts of water hyacinth (*Eichhornia crassipes*). Front. Vet. Sci. **1**, 1286563 (2024). doi:10.3389/fvets.2024.1286563.
14. M. Mudawamah, N. Hairoh, M.Z. Fadli, M.W. Lestari, M.Y. Afroni, Y. Oktanella, G. Ciptadi, Phenotype variation, correlation, and regression of morphology characteristics in rams and ewes Garut Indonesian local sheep. International Journal of Agriculture, Environment and Bioresearch. **09**, 82-88 (2024). https://ijaeb.org/link9.php?id=917.
15. E.M. Idamokoro, V. Muchenje, A.J. Afolayan, P.J. Masika, Variations in some haemato-biochemical markers from a small flock of free ranging Nguni, Boer, and Nondescript goats during late gestation and early lactation periods. Indian Journal of Animal Research. **53**, 476-481 (2018). https://doi.org/10.18805/ijar.B-865.
16. A.A.S. Kendran, T.G.O. Pemayun, Hematology profile of Bali cattle during gestation period at Subang Breeding Center, Bandung, Bali. Buletin Veteriner Udayana. **12**, 161-166 (2020).
17. N.Y. Salem, Effect of lactation on hemato-biochemical and minerals constituents in small ruminant. Inter J Vet Sci. **6**, 53-56 (2017).
18. M.E. Azab, H.A.A. Maksoud, Changes in some hematological and biochemical parameters during prepartum and postpartum periods in female Baladi goats. Small Ruminant Research. **34**, 77-85 (1999). http://doi.org/10.1016/S0921-4488(99)00049-8.
19. B. Habibu, M.U. Kawul, H.J. Makun, T. Aluwong, L.S. Yaqub, M.S. Ahmad, M. Tauheed, H.U. Buhari, Influence of sex, reproductive status and foetal number on erythrocyte osmotic fragility, haematological and physiologic parameters in goats during hot dry season. Veterinarni Medicina. **59**, 479–490 (2014).
20. J.P. Crilly, P. Plate, Anaemia in lambs and kids reared indoors on maternal milk and the impact of iron supplementation on haemoglobin levels and growth rates. Animals. **12**, 1863 (2022). https://doi.org/10.3390/ani12141863.
21. F. Bonelli, A. Rota, M. Corazza, D. Serio, M. Sgorbini, Hematological and biochemical findings in pregnant, postfoaling, and lactating Jennies. Theriogenology. **85**, 1233-1238 (2016). https://doi.org/10.1016/j.theriogenology.2015.12.004.
22. B.P. Santarosa, G.N. Dantas, D.O.L. Ferreira, H.B. Hooper, A.C.R.C. Porto, S.M.F.C. Garcia, S.R.S. Surian, P.A.P. Pieruzzi, A.A.D. Silva, R.C. Goncalves, Comparison of hematological parameters between single and twin pregnancies in Dorper ewes during gestation, lambing, and postpartum. Animal Reproduction, Ciencia Rural, Santa Maria. **52**, e20201065 (2022). http://doi.org/10/590/0103-8478cr20201065.
23. W.D.C.D. Oliveira, T.P.D.E. Silva, M.J.D. Araujo, R.L. Edvan, R.L. Oliveira, L.R. Bezerra, Changes in hematological biomarkers of Nellore cows at different reproductive stages. Acta Scientiarum. **41**, 1-7 (2019). https://doi.org/10.4025/actascianimsci.v41i1.45725.
24. L.R. Bezerra, W.D.C. Oliviera, T.P.D. Silva, J.N.C. Torreao, C.A.T. Marques, M.J. Araujo, R.L. Oliviera, Comparative hematological analysis of morada nova and santa ines ewes in all reproductive stages. Pesquisa Veterinária Brasileira. **37**, 408-414 (2017). https://doi.org/10.1590/S0100-736X2017000400017.
25. T. Tathong, S. Khamhan, S. Soisungwan, C. Phoemchalard, Effects of hemp-derived Cannabidiol on blood variables, carcass characteristics, and meat quality of goats. Animals. **14**, 1718 (2024). https://doi.org/10.3390ani14121718.
26. S. Khamhan, T. Tathong, C. Phoemchalard, The effects of fresh hemp leaf suppkementation (*Cannabis sativa*) on the physiological and carcass characteristics and

meat quality in transported goats. Animals. **13**, 3881 (2023). https://doi.org/10.3390/ani13243881.

27. M. Zarkawi, A. Soukouti, Reference values of essential haematological parameters in Damascus does and bucks throughout the year. Archiva Zootechnica. **27**, 5-22 (2024). Doi:10.2478/azibna-2024-0001.