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Association of Sex and Age with Histomorphometric Features of Agranulocytic Leukocytes and Erythrocyte Indices in Sumbawa Horses

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ABSTRACT

The Sumbawa horse, an indigenous Indonesian breed, plays important ecological and cultural roles in the region, such as participation in traditional ceremonies, local transportation, and horse-racing competitions; however, scientific data on its hematological and cellular characteristics remain limited, hindering accurate clinical evaluation and breed-specific health management. This study aimed to provide baseline histomorphometric and hematological data by examining 80 clinically healthy Sumbawa horses, grouped by sex (male and female) and age (young and adult). Peripheral blood smears were prepared immediately after venipuncture and stained using the modified Diff Quick method. Morphometric parameters of lymphocytes and monocytes, including cell diameter, perimeter, cell area, and nucleus to cytoplasm ratio, were measured using calibrated digital image analysis. Complete blood count parameters, including mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width coefficient of variation (RDW-CV), and red cell distribution width standard deviation (RDW-SD), were obtained using an automated hematology analyzer. Females exhibited larger lymphocyte dimensions (17.79 ± 5.89 µm² versus 16.57 ± 6.29 µm², whereas males showed larger monocyte size (18.44 ± 7.60 µm² versus 14.88 ± 5.46 µm²). Lymphocyte counts and percentages were higher in females, while monocyte values and RDW-CV were higher in males. Age significantly influenced lymphocyte morphometry and leukocyte profiles, whereas its effects on monocytes were limited, with only monocyte area showing a significant difference between age groups. These findings indicated significant age-related increases in lymphocyte perimeter, area, and diameter, whereas most monocyte and erythrocyte indices remained relatively stable between age groups. Sex related differences were also evident, with females exhibiting significantly higher lymphocyte counts and percentages, and males showing significantly higher monocyte counts and greater erythrocyte variability. These reference values provide clear morphometric distinctions between groups of Sumbawa horses, with adults exhibiting larger lymphocyte dimensions and males showing higher monocyte counts and greater erythrocyte variability, thus offering a biologically calibrated basis for further physiological interpretation.

Keywords: Age, Agranulocytic leukocyte, Erythrocyte indice, Histomorphometry, Horse, Sex

INTRODUCTION

The Sumbawa horse is an indigenous Indonesian breed that plays an important role in transportation, agriculture, and cultural activities on Sumbawa Island. Despite its socioeconomic significance, scientific information on physiological and hematological characteristics specific to this breed remains limited (Sukri et al., 2022). Hematological parameters such as leukocyte profiles and erythrocyte indices are essential indicators of immune competence, metabolic adaptation, and overall health (de Siqueira and Fernandes, 2024). Because these values vary across equine breeds, establishing breed-specific reference ranges is necessary to support accurate clinical diagnosis and herd health monitoring (de Vega et al., 2024).

Age and sex are known intrinsic factors that influence hematological characteristics in horses. Age-related changes in leukocyte morphology and immune cell dynamics have been reported and are associated with maturation and immunosenescence (DeNotta and McFarlane, 2023). Likewise, sex-related variations in immune responses occur due to hormonal differences, in which estrogen enhances lymphocyte-mediated immunity while androgens modulate monocyte activity and innate immune function (Sciarra et al., 2023). Previous studies have also shown that erythrocyte indices such as mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) differ according to age, metabolic demands, and sex-specific physiological influences in ponies (Gaina et al., 2020). These intrinsic variations demonstrate that age and sex must be considered when interpreting hematological data in equine clinical practice (Silva et al., 2023).

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Histomorphometric assessment provides additional insight into the structural characteristics of blood cells that may not be detected through routine hematology (Walton and Lawson, 2021). Measurements such as cell diameter, perimeter, area, and the nucleus-to-cytoplasm ratio provide valuable insights into subtle morphological adaptations. Although previous studies have compared hematological variables among various horse breeds (Schaefer et al., 2024). Research focusing on the histomorphometry of agranulocytic leukocytes, particularly lymphocytes and monocytes, remains scarce. Furthermore, no published reference values exist for Indonesian indigenous horses, including the Sumbawa breed.

Given the unique genetic background, management systems, and environmental conditions in which Sumbawa horses are raised, generating specific physiological reference data is essential. The novelty of the present study lies in its combined analysis of leukocyte histomorphometry and erythrocyte indices in relation to age and sex, addressing the current lack of breed-specific data in Sumbawa horses and highlighting its importance for clinical diagnosis and health management, representing the first comprehensive baseline dataset for Sumbawa horses. Therefore, this study aimed to evaluate the histomorphometric features of agranulocytic leukocytes and erythrocyte indices in clinically healthy Sumbawa horses based on sex and age to enhance clinical interpretation and strengthen equine health management in this indigenous population.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Indonesia, under approval letter number B/211/UN.14.2.9/PT.01.04/2024. The authors considered the farmers' ethical concerns and obtained their consent before conducting the present study.

Study design

This observational cross-sectional study was conducted on Sumbawa Island, West Nusa Tenggara Province, Indonesia. A geographical map of the study area has been added as Figure 1. The sampled horses were managed under a semi-extensive system in which they grazed freely during the day and were confined to simple shelters or holding areas at night. The main plant species available in the grazing areas included Napier grass (*Pennisetum purpureum*), Setaria grass (*Setaria sphacelata*), signal grass (*Brachiaria decumbens*), and native savanna grasses such as *Digitaria* spp., *Chloris* spp., and *Panicum maximum*. Their diet consisted primarily of fresh rangeland grasses, supplemented with cut forage and rice bran provided once daily. All horses received the same feeding regimen regardless of age or sex, ensuring comparable nutritional conditions throughout the study.

Figure 1. The location of the study, Sumbawa Island, Indonesia

All horses included in this study were confirmed to be clinically healthy based on a veterinary physical examination conducted prior to blood collection. The assessment included evaluation of body condition, mucous membrane color, capillary refill time, rectal temperature, heart and respiratory rates, and the absence of gastrointestinal or respiratory abnormalities. A visual body condition assessment following the principles of the Henneke Body Condition Scoring

system (Henneke et al., 1983) was performed, and all horses showed normal body condition without signs of emaciation or obesity. In addition to the clinical evaluation, a fecal egg count was performed using the McMaster method to assess gastrointestinal parasite status (Lejeune et al., 2023; Mohammedsalih et al., 2025). Only horses with negative fecal egg counts were included in the study, ensuring that all sampled individuals met the criteria for being classified as healthy (Nielsen, 2021)**.** Physiological parameters recorded at examination were within normal ranges, including body temperature (37.2-38.3°C), heart rate (28-48 beats/min), respiratory rate (8-14 breaths/min), and capillary refill time (< 2 seconds). A total of 80 clinically healthy Sumbawa horses were selected using availability-based sampling consistent with previously published hematological studies. The animals were grouped by sex (male and female) and age (young and adult), forming four groups of 20 horses each, including young females, young males, adult females, and adult males.

Blood collection and sample preparation

Blood was collected from each horse (8 mL) through jugular venipuncture using sterile Venoject® needles and EDTA-coated vacuum tubes of the same volume. After collection, the tubes were labeled with the horse identification code and inverted 8–10 times to ensure proper anticoagulant mixing. Fresh blood was immediately used to prepare peripheral blood smears, while remaining samples were stored in a portable cooler at 4 ± 1°C and transported to the laboratory within two hours to prevent hemolysis (Rhodes et al., 2024). Peripheral blood smears were air-dried, fixed in absolute methanol for three minutes, and stained using the Modified Diff-Quik method (IndoReagen®, Indonesia; Catalog No. 710623-MD, manufacturer's protocol provided in the References). Microscopic examinations were performed using an OLYMPUS CX33 light microscope (Olympus Corporation, Tokyo, Japan) at 400× and 1000× magnifications.

Agranulocyte histomorphometry

Morphological identification of lymphocytes and monocytes was based on nuclear shape, cytoplasmic features, and overall cell structure. Histomorphometric measurements, including cell diameter, cell perimeter, cell area, and nucleus-to-cytoplasm ratio, were performed using EP View software Version 4.0. For each horse, 50 lymphocytes and 30 monocytes were examined to obtain representative morphometric values. Microscopic examination was carried out using an OLYMPUS CX33 light microscope at 400× and 1000× magnifications. Morphological identification and histomorphometric measurements were performed on lymphocytes and monocytes, considering nuclear shape, cytoplasmic structure, and cell size. Digital morphometric evaluation was conducted using EP View software (version 1.4, Poland), which allowed accurate and standardized measurement of cellular dimensions (Maśko et al., 2021).

Automated hematological analysis

EDTA blood samples were analyzed using the VetScan HM5 automated hematology analyzer (Abaxis, Union City, California, USA). Leukocyte parameters measured included total white blood cell counts, along with lymphocyte and monocyte counts and their respective percentages. Erythrocyte indices obtained included mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width–coefficient of variation (RDW-CV), and red cell distribution width–standard deviation (RDW-SD). The analyzer was calibrated and quality-controlled before each analytical session according to manufacturer guidelines.

Statistical analysis

Descriptive statistics were used to summarize leukocyte differentials and histomorphometric measurements. Differences in hematological and morphometric parameters according to age and sex were analyzed using two-way analysis of variance (two-way ANOVA). Tukey's post-hoc test was applied when significant effects were detected. Statistical analyses were conducted using IBM SPSS Statistics Version 29.0 (IBM Corp., Armonk, NY, USA). A p-value less than 0.05 was considered statistically significant.

RESULTS

Histological observations of Sumbawa horses based on sex (male and female) and age groups (young and adult) revealed no distinct morphological differences in the characteristics of lymphocytes and monocytes ($p > 0.05$). In general, lymphocytes (Figure 2) exhibited large, darkly stained, round to oval nuclei with slight indentations. The chromatin appeared coarse and prominent, while the cytoplasm formed a thin, light-blue rim surrounding the nucleus. Some large lymphocytes contained azurophilic granules within the cytoplasm. Meanwhile, monocytes (Figure 3) showed nuclei that were round to kidney-shaped or ovoid with noticeable indentations. The chromatin appeared less condensed, finely

textured, and paler compared to lymphocytes. The cytoplasm was wide, light blue, and occasionally contained fine vacuoles or azurophilic granules. Morphometric measurements demonstrated variations between age and sex groups, both in terms of mean values and statistical significance across certain parameters.

The histomorphometric analysis of agranulocytic leukocytes in Sumbawa horses based on sex revealed significant differences between females and males ($p < 0.05$; Table 1). The mean lymphocyte perimeter, area, and diameter were significantly higher in females compared to males ($p < 0.05$). Conversely, monocyte morphometric parameters were greater in males than in females, with a perimeter of 14.98 ± 3.43 µm, an area of 18.44 ± 7.60 µm², and a diameter of 4.80 ± 1.18 µm, all showing significant differences ($p < 0.05$). These findings suggest a sex-related variation in agranulocyte cell size, where lymphocytes tend to be larger in females, whereas monocytes are larger in males.

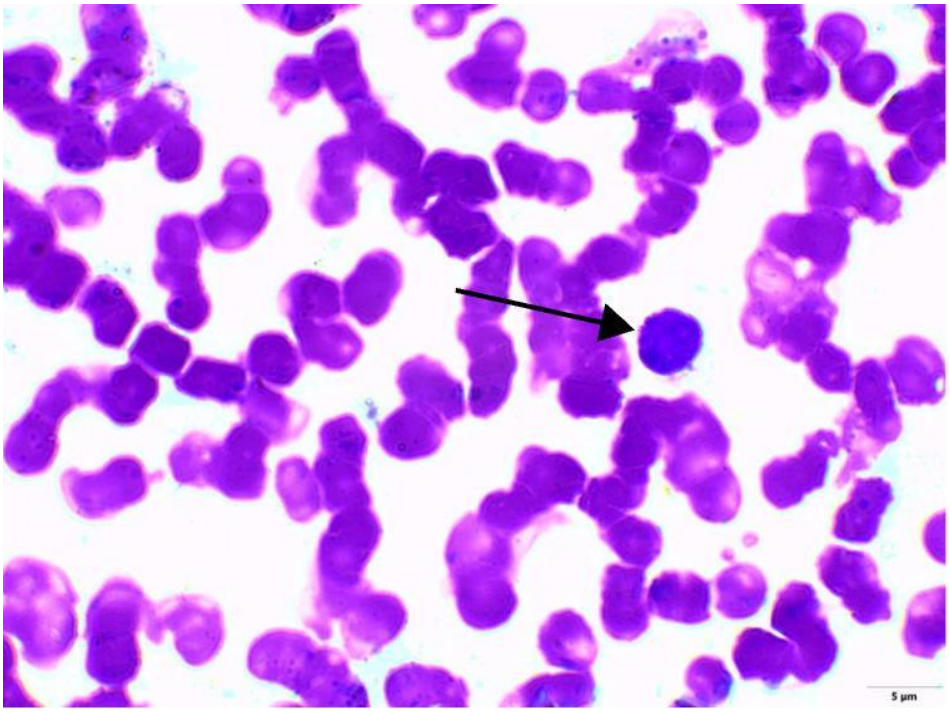

Figure 2. Representative lymphocyte in a peripheral blood smear of a Sumbawa horse (arrow). The lymphocyte shows a round, densely stained nucleus with a thin rim of cytoplasm.

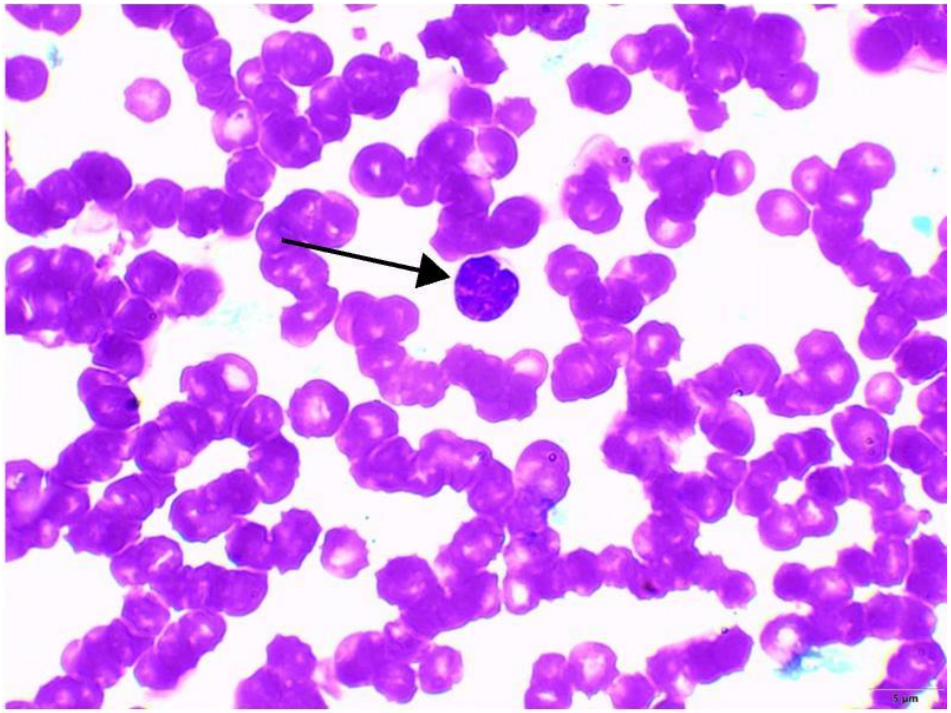

Figure 3. Representative monocyte in a peripheral blood smear of a Sumbawa horse (arrow). The monocyte shows a large kidney-shaped or horseshoe-shaped nucleus with lacy and fine chromatin.

Table 1. Histomorphometric values of agranulocytic leukocytes (lymphocytes and monocytes) in Sumbawa horses based on sex in West Nusa Tenggara, Indonesia

Indicator	Female (Mean ± SD)	Male (Mean ± SD)
Lymphocyte perimeter (µm)	14.74 ± 2.48 [a]	14.19 ± 2.65[b]
Lymphocyte area (µm²)	17.79 ± 5.89[a]	16.57 ± 6.29[b]
Lymphocyte diameter (µm)	4.69 ± 0.79[a]	4.50 ± 0.84[b]
Monocyte perimeter (µm)	13.44 ± 2.43[a]	14.98 ± 3.43[b]
Monocyte area (µm²)	14.88 ± 5.46[a]	18.44 ± 7.60[b]
Monocyte diameter (µm)	4.28 ± 0.77[a]	4.80 ±1.18[b]

[a,b] Different superscript letters in the same row indicate significant differences ($p < 0.05$)

The histomorphometric analysis of agranulocytic leukocytes in Sumbawa horses based on age variation showed significant differences in most lymphocyte morphometric parameters but not in monocytes ($p < 0.05$; Table 2). The perimeter, area, and diameter of lymphocytes in adult horses (15.37 ± 2.20 µm; 19.20 ± 5.49 µm²; and 4.89 ± 0.69 µm) were significantly higher than those in young horses (13.56 ± 2.62 µm; 15.16 ± 6.06 µm²; and 4.31 ± 0.84 µm, $p < 0.05$). In contrast, monocytes in adult horses showed slightly higher mean values (14.41 ± 3.10 µm; 17.33 ± 7.71 µm²; and 4.59 ± 0.98 µm) than in young horses (14.01 ± 3.02 µm; 15.99 ± 5.79 µm²; and 4.50 ± 1.08 µm), though the differences were not statistically significant ($p > 0.05$). These results indicated that aging influences an increase in lymphocyte morphometric size but did not significantly affect monocyte dimensions in Sumbawa horses.

The leukocyte parameters and erythrocyte indices of Sumbawa horses according to sex are presented in Table 3. Female horses exhibited higher mean values of lymphocyte count (5.32 ± 2.08 ×10⁹/L), lymphocyte percentage (51.12 ± 16.04%), and mean corpuscular hemoglobin (MCH; 14.85 ± 3.15 pg) compared to males, with statistically significant differences ($p < 0.05$). Conversely, male horses showed higher monocyte count (0.33 ± 0.24 ×10⁹/L), monocyte percentage (3.87), and red cell distribution width (RDW-CV; 27.96) than females, also with significant differences ($p < 0.05$). No significant differences were observed in total white blood cell count (WBC#), mean corpuscular hemoglobin concentration (MCHC), and RDW-SD between sexes ($p > 0.05$). These findings indicated that sex variation influenced several leukocyte and erythrocyte parameters in Sumbawa horses.

Table 2. Histomorphometric values of agranulocytic leukocytes (lymphocytes and monocytes) in Sumbawa horses based on age in West Nusa Tenggara, Indonesia

Indicator	Young (Mean ± SD)	Adult (Mean ± SD)
Lymphocyte perimeter (µm)	13.56 ±2.62[a]	15.37 ± 2.20[b]
Lymphocyte area (µm²)	15.16 ± 6.06 [a]	19.20 ± 5.49[b]
Lymphocyte diameter (µm)	4.31 ± 0.84[a]	4.89 ± 0.69[b]
Monocyte perimeter (µm)	14.01 ± 3.02[a]	14.41 ± 3.10[a]
Monocyte area (µm²)	15.99 ± 5.79[a]	17.33 ± 7.71[b]
Monocyte diameter (µm)	4.50 ± 1.08[a]	4.59 ± 0.98[a]

[a,b] Different superscript letters in the same row indicate significant differences ($p < 0.05$)

Table 3. Leukocyte parameters and erythrocyte indices of Sumbawa horses according to sex in West Nusa Tenggara, Indonesia

Indicator	Female	Male
WBC# (×10⁹/L)	10.26 ± 2.40[a]	9.40 ± 2.35[a]
Lymphocyte# (×10⁹/L)	5.32 ± 2.08 [a]	3.96 ± 1.78[b]
Monocyte# (×10⁹/L)	0.23 ± 0.15[a]	0.33 ± 0.24[b]
Lymphocyte (%)	51.12[a]	41.55[b]
Monocyte (%)	2.38[a]	3.87[b]
MCH (pg)	14.85 ± 3.15[a]	12.89 ± 2.76[b]
MCHC (g/dL)	33.40 ± 5.15[a]	33.16 ± 4.58[a]
MCV (fL)	44.62 ± 7.11[a]	39.10 ± 7.27[b]
RDW-CV (%)	25.78[a]	27.96[b]
RDW-SD (fL)	39.94 ± 2.54[a]	40.64 ± 2.51[a]

WBC: White blood cell count, Lym: Lymphocyte count, Mon: Monocyte count, Lym: Lymphocyte percentage, Mon: Monocyte percentage, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, RDW-CV: Red cell distribution width–coefficient of variation, RDW-SD: Red cell distribution width–standard deviation; [a,b] Different superscript letters in the same row indicate significant differences ($p < 0.05$); Data are expressed as Mean ± SD

The leukocyte parameters and erythrocyte indices of Sumbawa horses according to age are presented in Table 4. Young horses exhibited significantly higher total white blood cell count (WBC; 10.59 ± 2.79 ×10⁹/L), lymphocyte count (6.11 ± 1.20 ×10⁹/L), and lymphocyte percentage (58.34 ± 10.79%) compared to adults ($p < 0.05$). In contrast, adult horses showed significantly higher monocyte count (0.36 ± 0.23 ×10⁹/L) and monocyte percentage (4.16%) than younger horses ($p < 0.05$). The red cell distribution width (RDW-CV) was also significantly higher in young horses (27.76%) than in adults (25.98%, $p < 0.05$). Meanwhile, there were no significant differences in MCH, MCHC, MCV, and RDW-SD between the two age groups ($p > 0.05$). These results indicated that leukocyte profiles were more affected by age variation than erythrocyte indices in Sumbawa horses.

Table 4. Leukocyte parameters and erythrocyte indices of Sumbawa horses according to age

Indicator	Young (Mean ± SD)	Adult (Mean ± SD)
WBC (×10⁹/L)	10.59 ± 2.79[a]	9.08 ±2.40[b]
Lymphocyte (×10⁹/L)	6.11 ± 1.20[a]	3.16 ± 1.59[b]
Monocyte (×10⁹/L)	0.22 ± 0.16[a]	0.36 ± 0.23[b]
Lymphocyte (%)	58.34[a]	34.33[b]
Monocyte (%)	2.08[a]	4.16[b]
MCH (pg)	13.66 ± 2.46[a]	14.08 ± 3.66[a]
MCHC (g/dL)	32.99 ± 4.07[a]	33.57 ± 5.54[a]
MCV (fL)	41.90 ± 8.58[a]	41.82 ± 6.74[a]
RDW-CV (%)	27.76[a]	25.98[b]
RDW-SD (fL)	40.69 ± 2.59[a]	39.89 ± 2.44[a]

WBC: White blood cell count, Lym: Lymphocyte count, Mon: Monocyte count, Lym: Lymphocyte percentage, Mon: Monocyte percentage, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, RDW-CV: Red cell distribution width–coefficient of variation, RDW-SD: Red cell distribution width–standard deviation; [a,b] Different superscript letters in the same column indicate significant differences ($p < 0.05$); Data are expressed as Mean ± SD

DISCUSSION

Histological observations of lymphocytes and monocytes in Sumbawa horses showed that sex and age did not produce major morphological differences, although minor variations in morphometric measurements were apparent, such as slightly larger lymphocyte area and diameter in females and greater monocyte size in males. Lymphocytes in horses typically had large, dark-staining nuclei that were round to oval, surrounded by a thin cytoplasmic rim, while monocytes exhibited larger, kidney-shaped or ovoid nuclei with paler cytoplasm (Brooks et al., 2022). Similar leukocyte morphology and minor breed-related variations have also been reported in other indigenous horse populations (Lester et al., 2015). Occasional azurophilic granules within the cytoplasm denote the presence of primary lysosomes containing hydrolytic enzymes such as myeloperoxidase, which participate in normal phagocytic function. In horses, the appearance of these granules at low frequency is considered a normal component of monocyte maturation and immune surveillance. Thus, their presence reflects physiological cellular activation rather than an indication of inflammation, infection, or pathological hematological alterations (Maśko et al., 2021; Walton and Lawson, 2021).

Sex influenced leukocyte morphometry in this study, with females exhibiting larger lymphocyte perimeter, area, and diameter, whereas males had larger monocytes. This pattern in horses may be associated with hormonally driven immune modulation, where estrogens have been reported to support lymphocyte enlargement, and androgens may contribute to monocyte maturation in equine species (Benashour et al., 2024). Age also affected leukocyte dimensions in horses, as adult horses showed larger lymphocytes than juveniles, while monocyte size remained relatively stable. This suggests that lymphocyte enlargement is associated with age-related immune maturation and increased antigenic exposure, whereas monocytes appear to maintain more consistent structural characteristics across age groups (Miller et al., 2021).

Hematological findings paralleled the morphometric observations. Females had higher lymphocyte counts, lymphocyte percentages, and MCH in this study, a pattern consistent with reports in mares, where estrogen enhances adaptive lymphocyte activity (Jaworska et al., 2022). Conversely, males exhibited higher monocyte values and RDW-CV, which aligns with earlier studies reporting greater innate immune activation and erythrocyte size variability in stallions (Benashour et al., 2024). No significant sex differences were observed in total WBC, MCHC, or RDW-SD in horses, suggesting that these core hematological parameters are largely regulated by physiological mechanisms that are not strongly influenced by sex-related hormonal differences. This stability indicates that baseline leukocyte and

erythrocyte characteristics remain consistent between males and females, even though other immune traits may vary (Candrianisa et al., 2024; Rate et al., 2025).

These results highlight that sex and age selectively influence leukocyte and erythrocyte characteristics in Sumbawa horses. The minor variations observed in lymphocyte and monocyte morphology, as well as in erythrocyte indices, likely represent normal adaptive physiological mechanisms rather than pathological changes. This stability is clinically important because it helps establish reliable reference expectations for Sumbawa horses, allowing veterinarians to distinguish normal biological variation from early indicators of disease during routine hematological evaluation. Considering sex and age is crucial for interpreting hematological and immunological profiles, as these intrinsic factors influence baseline values that support clinical assessment, disease surveillance, and herd management in horses (Gaina et al., 2020; Rate et al., 2025). These findings also provide useful reference information for evaluating the health status of Sumbawa horses under field conditions.

CONCLUSION

Sex and age influence both the morphometry of agranulocytic leukocytes and hematological profiles in Sumbawa horses. Female horses exhibited larger lymphocyte dimensions and higher lymphocyte counts, percentages, and mean corpuscular hemoglobin, whereas males showed larger monocyte dimensions, higher monocyte counts, percentages, and RDW-CV. Age primarily affected lymphocyte size, with adults showed increased morphometric values compared to juveniles, while monocyte morphology remains relatively stable. These findings highlight the importance of considering sex and age when interpreting hematological and immunological parameters in Sumbawa horses. Future research may incorporate additional physiological, nutritional, and environmental variables to further refine the understanding of sex- and age-related hematological and morphometric patterns in Sumbawa horses.

DECLARATIONS

Authors' contributions

Kadek Ferdy Agastia Dwi Pratama conceptualized the study, designed the experiments, and conducted data collection and analysis. Ni Ketut Suwiti, Ida Bagus Komang Ardana, Nengah Kerta Besung, Ni Luh Eka Setiasih, Nyoman Sadra Dharmawan, and I Nyoman Sulabda contributed to blood sample preparation, laboratory management, statistical analyses, and drafting of the manuscript. All authors have read and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Availability of data and materials

All data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Ethical considerations

This study was conducted using original data and did not involve any duplication, copy or plagiarism of previously published studies. The authors did not use AI to write the text of this article.

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