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Granulocyte Histomorphometry, Granulocyte Counts, and Platelet Profiles Associated with Age and Sex in Sumbawa Horses

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ABSTRACT

The Sumbawa horse (*Equus caballus*) is an indigenous Indonesian horse breed traditionally maintained under an extensive grazing system, characterized by unrestricted grazing and exposure to natural climatic conditions. This study aimed to evaluate granulocyte histomorphometry, granulocyte counts, and platelet profiles in clinically healthy indigenous Sumbawa horses in association with age and sex, providing baseline hematological data for this indigenous horse breed. A total of 80 clinically healthy indigenous Sumbawa horses meeting the predefined inclusion criteria were selected using purposive sampling and enrolled in this cross-sectional study. Peripheral blood smears were used for the histomorphometry evaluation of neutrophils, eosinophils, and basophils, whereas granulocyte counts, platelet counts, and platelet indices, including mean platelet volume (MPV), platelet distribution width (PDW-CV), and plateletcrit (PCT), were analyzed with an automated hematology analyzer. Sex had no significant effect on granulocyte histomorphometry or granulocyte counts. However, female horses exhibited significantly higher platelet counts and PCT values, whereas MPV was significantly higher in males. Adult horses exhibited significantly larger neutrophils and basophils, together with higher neutrophil and eosinophil counts. In contrast, platelet counts and platelet indices did not differ significantly between age groups. These findings indicated that granulocyte structural and quantitative characteristics are more responsive to age-related physiological adaptation, whereas platelet variation is predominantly associated with sex.

Keywords: Granulocyte count, Granulocyte histomorphometry, Platelet indices, Sumbawa horse, Sex

INTRODUCTION

The Sumbawa horse (*Equus caballus*) is one of Indonesia's indigenous equine genetic resources and plays an important socio-economic, cultural, and livestock role in West Nusa Tenggara Province (Sukri et al., 2022). In addition to its genetic importance, the breed is widely used as a working pony that supports transportation and rural livelihoods in the region (Drought et al., 2023) and has recently attracted scientific attention for its immunological and hematological characteristics (Rate et al., 2025). Unlike most horse populations for which hematological reference intervals have been established under intensive management systems in temperate regions, Sumbawa horses are predominantly maintained under the traditional *lar* grazing system, an extensive husbandry practice characterized by year-round communal grazing. They also serve as working ponies that are integral to local transportation and rural livelihoods in West Nusa Tenggara (Drought et al., 2023). The traditional *lar* grazing system provides a unique ecological context that distinguishes Sumbawa horses from horses managed under intensive production systems (Sukri et al., 2022; Rate et al., 2025). Under this extensive management system, horses graze freely throughout the year and are continuously exposed to seasonal fluctuations in forage availability, climatic variation, environmental microorganisms, and naturally occurring antigens (Martinson et al., 2017; Drought et al., 2023). The traditional *lar* grazing system and continuous exposure to natural climatic conditions may contribute to physiological adaptation and variation in hematological characteristics that should be considered when interpreting hematological findings in indigenous horses. Consequently, hematological reference intervals established for horses maintained under intensive management conditions may not accurately reflect the physiological status of indigenous horses adapted to tropical grazing environments (Pratama et al., 2025). Establishing population-specific hematological reference data is therefore essential for accurate clinical interpretation and for advancing the understanding of hematological variation under extensive tropical grazing systems.

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Routine hematological examination provides valuable quantitative information regarding circulating blood cells; however, numerical cell counts alone provide only limited insight into cellular functional status (Brooks et al., 2022). Peripheral blood smear evaluation and quantitative histomorphometry analysis allow assessment of cellular dimensions, nuclear architecture, and morphological characteristics that reflect granulocyte maturation, activation, and physiological adaptation (Brooks et al., 2022; Pratama et al., 2025). Morphological alterations may occur before detectable changes in leukocyte numbers, providing early evidence of cellular responses to environmental and physiological stimuli. Therefore, integrating quantitative hematology with cellular histomorphometry provides a more comprehensive characterization of hematological features associated with innate immune function than either approach alone (Walton and Lawson, 2021). Granulocytes, including neutrophils, eosinophils, and basophils, are the principal effector cells of innate immunity and undergo coordinated structural and functional changes in response to infection, inflammation, and tissue injury (Marshall et al., 2018; Arnold and Munitz, 2024).

Accumulating evidence demonstrates that granulocytes and platelets interact closely during inflammatory and innate immune responses. Beyond their classical role in hemostasis, platelets actively regulate leukocyte recruitment, inflammatory amplification, endothelial activation, and immunothrombosis through continuous communication with granulocytes and vascular cells (Scherlinger et al., 2023). In horses, platelet activation has likewise been associated with systemic inflammatory responses and innate immune regulation (Ehrmann et al., 2021; Walton and Lawson, 2021). Platelet profiles in horses can be more comprehensively characterized by combining platelet count (PLT) with platelet indices, including mean platelet volume (MPV), platelet distribution width (PDW-CV), and plateletcrit (PCT) (Gasparyan et al., 2011; Budak et al., 2016). Simultaneous evaluation of granulocyte histomorphometry, granulocyte counts, platelet count (PLT), and platelet indices provides a comprehensive characterization of hematological profiles in horses by integrating complementary cellular and platelet-related parameters (Satué et al., 2020; Brooks et al., 2022). Moreover, physiological variables such as age and sex influence hematopoiesis, immune maturation, hormonal regulation, and platelet production, making them essential considerations when establishing clinically meaningful hematological reference intervals (Koupenova et al., 2015; Klein and Flanagan, 2016; Miller et al., 2021).

Despite these recognized biological interactions, studies simultaneously evaluating both granulocyte morphology and platelet characteristics in indigenous horses remain unavailable. Previous investigations in Sumbawa horses have independently described hematological profiles, granulocyte counts, histological characteristics of granulocytes, and morphometric features of agranulocytic leukocytes and erythrocytes (Candrianisa et al., 2024; Pratama et al., 2025). However, these studies evaluated hematological profiles, granulocyte counts, granulocyte histological characteristics, and leukocyte morphometric features separately, leaving unresolved how granulocyte morphology relates to granulocyte abundance and platelet profiles in clinically healthy Sumbawa horses. Consequently, the relationships among granulocyte histomorphometry, granulocyte counts, and platelet profiles under extensive tropical grazing conditions remain poorly understood. Addressing this knowledge gap is important because coordinated evaluation of structural and quantitative hematological biomarkers may provide biological information that cannot be obtained from isolated measurements alone.

The present study is among the first to simultaneously evaluate granulocyte histomorphometry, granulocyte counts, platelet count (PLT), and platelet indices in clinically healthy indigenous Sumbawa horses while considering physiological variation associated with age and sex. This integrated approach enables comprehensive characterization of structural and quantitative hematological features within a single analytical framework. Therefore, this study aimed to evaluate age- and sex-related variation in granulocyte histomorphometry, granulocyte counts, PLT, and platelet indices in clinically healthy Sumbawa horses maintained under the traditional *lar* grazing system.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Indonesia (Approval No. B/211/UN.14.2.9/PT.01.04/2024). Written informed consent was obtained from the owners of all privately owned Sumbawa horses before sample collection. Although the horses roamed freely on communal grazing land under the traditional *lar* grazing system, each horse had an identifiable owner. All procedures involving animals were conducted in accordance with institutional guidelines for animal welfare and research ethics.

Study design

A cross-sectional observational study was conducted between June and August 2025 to evaluate the associations of age and sex with granulocyte histomorphometry, granulocyte counts, and platelet profiles in clinically healthy Sumbawa horses.

Study area and animals

The study was conducted in Sumbawa regency, West Nusa Tenggara Province, Indonesia (Figure 1). Horses were sampled from Sumbawa Regency between June and August 2025 during the dry season, when ambient temperatures typically ranged from approximately 22°C at night to 31°C during the daytime. The horses were maintained under the traditional *lar* grazing system, in which they grazed freely on communal rangelands during the day and were confined to simple shelters at night. The grazing areas were dominated by Napier grass (*Pennisetum purpureum*), Setaria grass (*Setaria sphacelata*), signal grass (*Brachiaria decumbens*), and native savanna grasses, including *Digitaria* spp., *Chloris* spp., and *Panicum maximum*. The horses primarily consumed these natural forages and received cut forage and rice bran once daily as dietary supplements. All horses were managed under the same feeding regimen regardless of age or sex (Pratama et al., 2025). The study included 80 clinically healthy Sumbawa horses. Eligible animals were selected using purposive sampling and subsequently categorized by sex (male or female) and age (young or adult), generating four study groups of 20 horses each.

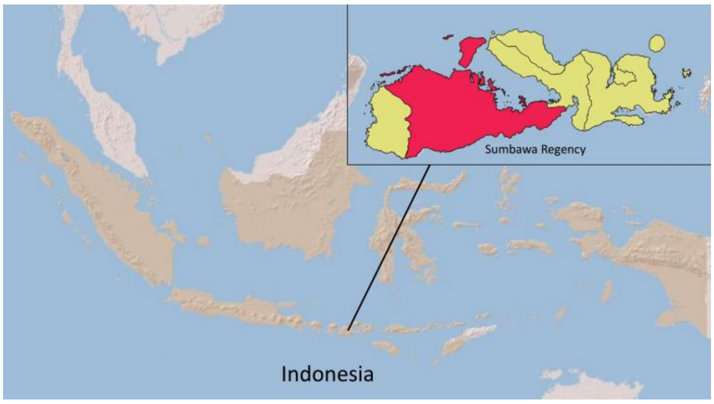

Figure 1. Geographic location of the study area in Sumbawa Regency, Indonesia. The map was prepared by the authors using GADM version 4.1 administrative boundaries (GADM, 2025) and the Esri Shaded Relief basemap.

Animal selection and veterinary clinical examination

Prior to enrolment, all horses underwent a comprehensive clinical examination performed by licensed veterinarians to confirm their health status. The clinical examination consisted of body condition scoring, measurement of rectal temperature, heart rate, respiratory rate, capillary refill time, mucous membrane color, and hydration status, together with evaluation of the respiratory, gastrointestinal, musculoskeletal, and other body systems. Body condition was assessed using the Henneke Body Condition Scoring system (Henneke et al., 1983), and only horses with normal body condition scores were eligible for inclusion.

All physiological variables measured during the clinical examination fell within the normal reference ranges for healthy horses (Hall et al., 2018; Walton and Lawson, 2021), with rectal temperatures ranging from 37.2 to 38.3°C, heart rates from 28 to 48 beats/min, respiratory rates from 8 to 14 breaths/min, and capillary refill times of less than two seconds. The inclusion criteria comprised clinically healthy Sumbawa horses maintained under the traditional *lar* grazing system that exhibited normal physical examination findings and physiological parameters. Horses presenting poor body condition, abnormal vital signs, evidence of systemic disease, gastrointestinal or respiratory disorders, or other clinically significant abnormalities were excluded from the study. All clinical examinations and blood sample collections were performed by licensed veterinarians following standardized procedures to ensure consistency in animal selection and sample collection. According to owner interviews and available health records, none of the horses had previously received any vaccination or anthelmintic treatment.

Faecal examination

To further verify the health status of the animals, gastrointestinal parasite burden was assessed by fecal egg count analysis using the McMaster technique (Nielsen, 2022; Lejeune et al., 2023). In accordance with current recommendations for defining clinically healthy equine populations in hematological investigations (Nielsen, 2021), only horses with negative fecal egg counts were considered eligible for inclusion. Horses with positive fecal egg count results indicative of gastrointestinal parasitism were excluded from further analysis.

Blood collection and sample preparation

Whole blood samples were aseptically obtained from the jugular vein by licensed veterinarians using sterile Venoject® needles and collected directly into EDTA-coated vacuum tubes. Approximately 8 mL of whole blood was obtained from each horse between 08:00 and 11:00 h to minimize potential diurnal variation in hematological parameters. Immediately after collection, blood tubes were gently inverted 8-10 times to ensure complete mixing with the anticoagulant. Fresh blood samples were immediately used to prepare peripheral blood smears, whereas the remaining EDTA-anticoagulated blood was transported to the laboratory in a portable cooler maintained at 4 ± 1°C and processed within two hours after collection to minimize cellular deterioration and hemolysis (Arnold et al., 2019). The blood smears were air-dried, fixed in absolute methanol for three minutes, and subsequently stained using the Modified Diff-Quik staining kit (MDT IndoReagen®, Indonesia; Catalog No. 710623-MD) according to the manufacturer's instructions and standard veterinary hematology procedures (Brooks et al., 2022).

Histomorphometry analysis

Granulocyte histomorphometry was examined using an Olympus CX33 light microscope (Olympus Corporation, Tokyo, Japan) under 400× and 1000× magnification. Granulocytes were identified according to their characteristic nuclear morphology, cytoplasmic appearance, and specific cytoplasmic granules (Brooks et al., 2022). Morphometric analysis included neutrophils, eosinophils, and basophils. Only well-preserved cells exhibiting intact cellular morphology, distinct nuclear configuration, clearly defined cytoplasmic boundaries, and absence of staining artifacts were included in the analysis. Cells showing overlapping structures, indistinct cellular boundaries, poor staining quality, or smear preparation artifacts (e.g., cell distortion, folding, or mechanical damage) were excluded from the analysis in accordance with standard veterinary hematology procedures (Brooks et al., 2022).

Because eosinophils and basophils are naturally present at low frequencies in the peripheral blood of clinically healthy horses, these cell types were not consistently observed in every blood smear (Brooks et al., 2022; DeNotta and McFarlane, 2023). Therefore, morphometric analysis of eosinophils and basophils was performed only when well-preserved cells fulfilling the predefined morphological criteria were available. Digital morphometric measurements were obtained using EPview software (version 4.0; Olympus Corporation, Tokyo, Japan). The evaluated variables included cell area (μm²), cell perimeter (μm), and cell diameter (μm). All histomorphometric measurements were performed by a trained laboratory technologist following a standardized measurement protocol to ensure consistency throughout the study. Blood smear preparation, staining, and morphometric measurements were performed according to standardized laboratory protocols to minimize analytical variation (Stirn and Freeman, 2022).

Haematological analysis

Whole blood specimens were processed using an automated hematology analyzer (VetScan HM5; Abaxis, Union City, California, USA). The hematological variables evaluated included neutrophil, eosinophil, and basophil counts, as well as platelet count (PLT), mean platelet volume (MPV), platelet distribution width coefficient of variation (PDW-CV), and plateletcrit (PCT). The analyzer was calibrated and subjected to routine internal quality-control procedures, including background verification and analysis of commercial quality-control materials, according to the manufacturer's instructions before sample analysis to ensure analytical accuracy and measurement reliability. Routine internal quality-control procedures were maintained throughout the analytical process to ensure consistency and reliability of hematological measurements (Stirn and Freeman, 2022).

Statistical analysis

Descriptive statistics were calculated for all variables and are presented as mean ± standard deviation (SD). Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test prior to comparative analysis. Comparisons between age groups (young versus adult) and sex groups (male versus female) were performed using the independent-samples t-test. All histomorphometry and hematological variables were analyzed by the independent-samples t-test. All statistical analyses were performed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Differences in all histomorphometry and hematological variables between age and sex groups were considered statistically significant at $p < 0.05$.

RESULTS

Microscopic examination of granulocytes from Sumbawa horses reared under the traditional *lar* grazing system demonstrated characteristic morphological features for each granulocyte type across different age (young and adult) and sex (male and female) groups. Neutrophils (Figure 2) were round in shape and contained nuclei segmented into three to five lobes of varying sizes. Their cytoplasm appeared pale pink with fine granules. Eosinophils (Figure 3) were also round, with segmented nuclei and large pink-orange granules. The cytoplasm contained numerous large, densely packed eosinophilic (globular) granules. Basophils (Figure 4) exhibited a round cell shape with abundant dark blue to purple metachromatic cytoplasmic granules. Their nuclei were indistinct, poorly segmented, and often appeared irregular. Morphological observations indicated that these structural characteristics were consistently observed in both young and adult horses, regardless of sex.

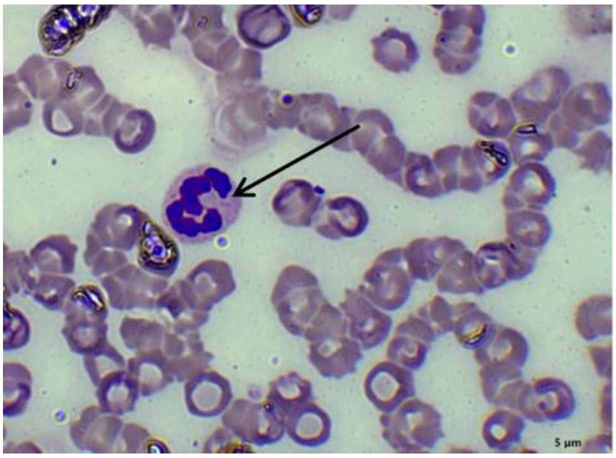

Figure 2. Representative neutrophil in a peripheral blood smear of a Sumbawa horse (arrow), Indonesia.

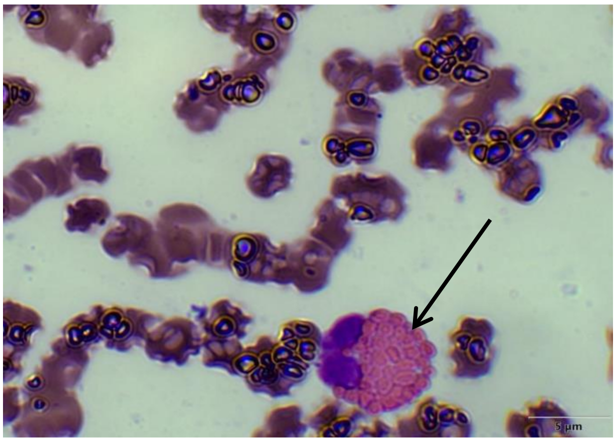

Figure 3. Representative eosinophil in a peripheral blood smear of a Sumbawa horse (arrow), Indonesia.

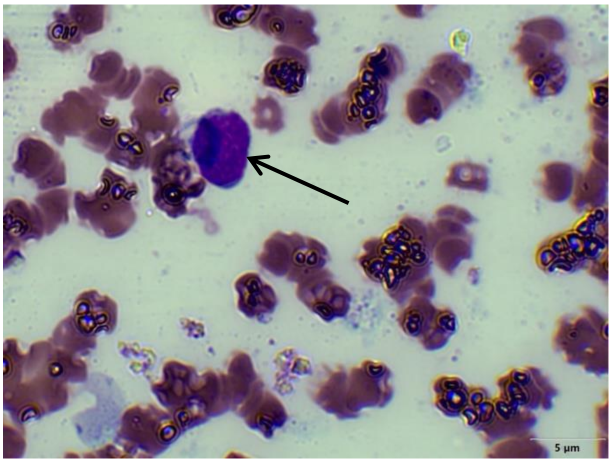

Figure 4. Representative basophil in a peripheral blood smear of a Sumbawa horse (arrow), Indonesia.

Histomorphometry of granulocytes based on sex

No significant sex-related differences were observed in the area, perimeter, or diameter of neutrophils, eosinophils, or basophils (Table 1; $p > 0.05$). Although male horses tended to exhibit slightly larger neutrophil dimensions than females, these differences were not statistically significant. Likewise, eosinophil and basophil morphometric parameters remained comparable between sexes.

Histomorphometry of granulocytes based on age

Age significantly influenced the histomorphometry characteristics of neutrophils and basophils (Table 2). Adult horses exhibited significantly greater neutrophil area, perimeter, and diameter than young horses ($p < 0.05$). Basophils demonstrated the same age-related trend, with adult horses exhibiting significantly larger area, perimeter, and diameter than young horses ($p < 0.05$). In contrast, eosinophil morphometric characteristics did not differ significantly between age groups ($p > 0.05$).

Granulocyte counts and platelet profiles based on sex

Neutrophil, eosinophil, and basophil counts did not differ significantly between male and female horses (Table 3; $p > 0.05$). In contrast, platelet-related variables showed significant sex-associated variation. Female horses exhibited significantly higher PLT and PCT, whereas male horses exhibited significantly higher MPV. PDW-CV did not differ significantly between sexes ($p > 0.05$).

Granulocyte counts and platelet profiles based on age

Adult horses exhibited significantly higher neutrophil and eosinophil counts than young horses (Table 4; both $p < 0.05$), whereas basophil counts did not differ significantly between age groups ($p > 0.05$). None of the platelet-related variables, including PLT, MPV, PDW-CV, and PCT, differed significantly between young and adult horses ($p > 0.05$).

Table 1. Histomorphometry characteristics of granulocytic leukocytes (neutrophils, eosinophils, and basophils) in clinically healthy Sumbawa horses according to sex

Indicator		**Male (Mean ± SD)**	**Female (Mean ± SD)**
Neutrophil	Area (μm^2)	23.02 ± 8.53	22.92 ± 9.65
	Perimeter (μm)	16.50 ± 3.06	16.34 ± 3.50
	Diameter (μm)	5.24 ± 0.97	5.20 ± 1.11
Eosinophil	Area (μm^2)	38.13 ± 15.64	39.27 ± 14.60
	Perimeter (μm)	21.43 ± 4.49	21.81 ± 4.25
	Diameter (μm)	6.82 ± 1.43	6.95 ± 1.34
Basophil	Area (μm^2)	21.61 ± 9.85	23.13 ± 9.95
	Perimeter (μm)	16.11 ± 3.44	16.66 ± 3.67
	Diameter (μm)	5.13 ± 1.09	5.30 ± 1.17

Table 2. Histomorphometry characteristics of granulocytic leukocytes (neutrophils, eosinophils, and basophils) in clinically healthy Sumbawa horses according to age

Indicator		**Young (Mean ± SD)**	**Adult (Mean ± SD)**
Neutrophil	Area (μm^2)	20.01 ± 7.53 ^{a}	25.93 ± 9.57 ^{b}
	Perimeter (μm)	15.60 ± 2.87 ^{a}	17.75 ± 3.33 ^{b}
	Diameter (μm)	4.97 ± 0.91 ^{a}	5.65 ± 1.06 ^{b}
Eosinophil	Area (μm^2)	37.54 ± 13.88 ^{a}	39.86 ± 16.23 ^{a}
	Perimeter (μm)	21.34 ± 4.10 ^{a}	21.90 ± 4.62 ^{a}
	Diameter (μm)	6.80 ± 1.29 ^{a}	6.97 ± 1.47 ^{a}
Basophil	Area (μm^2)	18.65 ± 7.12 ^{a}	26.08 ± 10.89 ^{b}
	Perimeter (μm)	15.01 ± 2.98 ^{a}	17.76 ± 3.57 ^{b}
	Diameter (μm)	4.78 ± 0.95 ^{a}	5.65 ± 1.14 ^{b}

^{a,b} Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Table 3. Granulocyte counts and platelet profiles in clinically healthy Sumbawa horses according to sex

Indicator	**Male (Mean ± SD)**	**Female (Mean ± SD)**
NEU (10^9/L)	4.38 ± 1.70 ^{a}	4.81 ± 1.84 ^{a}
EOS (10^9/L)	0.30 ± 0.17 ^{a}	0.26 ± 0.19 ^{a}
BAS (10^9/L)	0.03 ± 0.03 ^{a}	0.03 ± 0.03 ^{a}
PLT (10^9/L)	203.33 ± 71.88 ^{a}	301.45 ± 104.43 ^{b}
MPV (fL)	7.77 ± 0.61 ^{a}	7.50 ± 0.42 ^{b}
PDW-CV (%)	29.98 ± 2.27 ^{a}	29.55 ± 1.66 ^{a}
PCT (%)	0.15 ± 0.05 ^{a}	0.22 ± 0.08 ^{b}

^{a,b} Different superscript letters within the same row indicate significant differences ($p < 0.05$). NEU: Neutrophil count, EOS: Eosinophil count, BAS: Basophil count, PLT: Platelet count, MPV: Mean platelet volume, PDW-CV: Platelet distribution width, PCT: Plateletcrit.

Table 4. Granulocyte counts and platelet profiles in clinically healthy Sumbawa horses according to age

Indicator	**Young (Mean ± SD)**	**Adult (Mean ± SD)**
NEU (10^9/L)	3.97 ± 1.46 ^{a}	5.21 ± 1.85 ^{b}
EOS (10^9/L)	0.24 ± 0.18 ^{a}	0.33 ± 0.14 ^{b}
BAS (10^9/L)	0.03 ± 0.03 ^{a}	0.03 ± 0.04 ^{a}
PLT (10^9/L)	243.30 ± 97.15 ^{a}	261.48 ± 106.88 ^{a}
MPV (fL)	7.70 ± 0.48 ^{a}	7.57 ± 0.58 ^{a}
PDW-CV (%)	29.72 ± 1.95 ^{a}	29.81 ± 2.06 ^{a}
PCT (%)	0.18 ± 0.07 ^{a}	0.19 ± 0.08 ^{a}

^{a,b} Different superscript letters within the same row indicate significant differences ($p < 0.05$). NEU: Neutrophil count, EOS: Eosinophil count, BAS: Basophil count, PLT: Platelet count, MPV: Mean platelet volume; PDW-CV: Platelet distribution width, PCT: Plateletcrit.

DISCUSSION

This study simultaneously evaluated granulocyte histomorphometry, granulocyte counts, and platelet profiles in clinically healthy Sumbawa horses maintained under the traditional *lar* grazing system while assessing the influence of age and sex. Overall, age exerted a greater influence on granulocyte characteristics than sex, as reflected by the significantly larger neutrophil and basophil dimensions and higher neutrophil and eosinophil counts in adult horses. In contrast, granulocyte morphology and differential counts were largely comparable between males and females, whereas sex-related variation was mainly confined to platelet indices. These findings suggest that aging predominantly influences granulocyte structure and abundance, whereas platelet biology exhibits greater sexual dimorphism. By integrating granulocyte morphometry with granulocyte counts and platelet profiles, this study extends previous investigations in Sumbawa horses and complements existing literature on hematological variation among different horse breeds. Similar age- and sex-related variations in hematological characteristics have been reported in Spanish Purebred horses (Satué et al., 2020). Likewise, studies involving Arabian, Thoroughbred, and local horses demonstrated that hematological profiles are influenced by breed, age, and sex, although the affected parameters differ among breeds (Sawesi et al., 2023). Consistent with these findings, Yavuz et al. (2025) also reported significant age- and sex-related variation in hematological parameters in Arabian and Thoroughbred racehorses. In line with these observations, the present study demonstrated that age and sex also influence granulocyte morphometry, granulocyte counts, and platelet profiles in Sumbawa horses, further emphasizing the importance of establishing breed-specific reference values. These findings complement previous evidence of age- and sex-related variation in immunoglobulin M (IgM) concentrations in Sumbawa horses maintained under the traditional *lar* grazing system (Rate et al., 2025) and extend existing knowledge by providing baseline reference data on granulocyte morphometry, hematological, and platelet parameters for interpreting innate immune status and hematological adaptation in Sumbawa horses maintained under extensive tropical grazing conditions (Candrianisa et al., 2024; Pratama et al., 2025).

Effects of sex on granulocyte histomorphometry

Granulocyte histomorphometry characteristics were comparable between male and female Sumbawa horses, indicating that sex exerts minimal influence on granulocyte morphology under normal physiological conditions. Similar neutrophil, eosinophil, and basophil area, perimeter, and diameter suggest that granulocyte maturation appears to be largely independent of physiological differences in endocrine status. Comparable findings have been reported in healthy Spanish Purebred and Sumbawa horses, where sex has little influence on leukocyte-related hematological characteristics (Satué et al., 2020; Candrianisa et al., 2024; Pratama et al., 2025).

The absence of morphometric differences is biologically plausible because mature granulocytes complete their differentiation before entering the circulation, and their morphology generally remains stable unless altered by inflammation, toxic change, or accelerated granulopoiesis. Consequently, physiological differences between males and females are more likely to influence granulocyte function than cellular structure (Walton and Lawson, 2021; Brooks et al., 2022). Likewise, the similar eosinophil and basophil morphology observed in clinically healthy, parasite-free horses indicates minimal granulocyte activation under normal physiological conditions (Brosnahan, 2020).

These findings suggest that sex-specific reference intervals for granulocyte histomorphometry measurements are unlikely to be required in healthy Sumbawa horses. Despite significant sex-related differences in platelet parameters, granulocyte morphology remained stable, indicating that leukocyte structural characteristics are more tightly conserved than platelet-related variables. The preservation of granulocyte morphology despite sex-related differences in platelet parameters further supports the complementary role of granulocyte histomorphometry in hematological evaluation.

Effects of age on granulocyte histomorphometry

Unlike sex, age significantly influenced the morphology of selected granulocyte populations ($p < 0.05$). Adult Sumbawa horses exhibited significantly greater neutrophil and basophil area, perimeter, and diameter than young horses, whereas eosinophil morphometric characteristics remained unchanged. These findings indicate that age-related structural adaptation is cell-specific and reflects differential responses of granulocyte populations during immune maturation. Similar age-associated variation in equine hematological characteristics has been reported previously, emphasizing age as an important determinant of immune development (Satué et al., 2020; Candrianisa et al., 2024).

The larger neutrophil dimensions observed in adult horses are consistent with progressive maturation of innate immune function. As the predominant circulating phagocytes, neutrophils are continuously exposed to environmental microorganisms and naturally occurring antigens, particularly under extensive grazing conditions. Likewise, basophils exhibited comparable age-related enlargement despite representing only a minor leukocyte population, suggesting that structural adaptation during immune maturation extends beyond the predominant granulocyte population. Because these morphometric changes occurred without corresponding increases in basophil counts, cellular morphology may respond to age independently of circulating cell abundance (Walton and Lawson, 2021; DeNotta and McFarlane, 2023).

In contrast, eosinophil morphology remained stable despite the slightly higher eosinophil counts observed in adult horses. Because eosinophil differentiation is primarily regulated by antigenic stimulation associated with parasitic infection and allergic responses rather than chronological age, similar cellular dimensions were expected in the absence of major stimulatory factors. The exclusion of horses with detectable gastrointestinal parasitism further supports the interpretation that eosinophil morphology remained under normal physiological regulation rather than environmental or pathological influence (Brosnahan, 2020).

Overall, these findings demonstrated that age influences granulocyte morphology more profoundly than sex and that individual granulocyte populations respond differently to immune maturation. The selective enlargement of neutrophils and basophils, together with the unchanged eosinophil morphology, extends previous descriptions of leukocyte morphology in Sumbawa horses and provides baseline reference information for interpreting age-related granulocyte development in indigenous horses maintained under tropical grazing conditions.

Effects of sex on granulocyte counts and platelet profiles

Unlike platelet-related variables, circulating granulocyte counts were comparable between male and female Sumbawa horses, indicating that sex has little influence on granulopoiesis or peripheral granulocyte homeostasis under normal physiological conditions. Similar neutrophil, eosinophil, and basophil counts have been reported in healthy horses, suggesting that granulocyte populations are maintained through tightly regulated hematopoietic mechanisms rather than sex alone (Satué et al., 2020; Candrianisa et al., 2024). Because all horses were clinically healthy, maintained under identical grazing conditions, and free of detectable gastrointestinal parasitism, environmental factors capable of altering granulocyte populations were minimized, supporting the interpretation that the comparable granulocyte counts reflected normal physiological regulation rather than differences in innate immune competence (Walton and Lawson, 2021; Brooks et al., 2022).

In contrast, platelet indices exhibited clear sexual dimorphism. Female horses had significantly higher PLT and PCT values, whereas males exhibited significantly greater MPV, consistent with physiological variation in thrombopoiesis and platelet turnover rather than pathological alteration (Koupenova et al., 2015; Gaina et al., 2020; Satué et al., 2020). PDW-CV remained comparable between sexes, and all platelet-related variables were within physiological reference ranges, indicating normal biological variation. Overall, these findings show that sex influences platelet profiles more readily than granulocyte populations, with granulocyte morphology and abundance remaining stable despite measurable differences in platelet production and circulating platelet mass.

Effects of age on granulocyte counts and platelet profiles

Age exerted a greater influence on circulating granulocyte populations than on platelet-related variables. Adult Sumbawa horses exhibited significantly higher neutrophil and eosinophil counts than young horses, whereas basophil counts and all platelet indices remained comparable between age groups. Combined with the enlarged neutrophil and basophil dimensions observed in adult horses, these findings support the interpretation that aging is associated with both structural and quantitative granulocyte characteristics, whereas platelet-related variables appear to remain relatively stable. Together, these structural and quantitative changes are consistent with progressive maturation of innate immune function in adult horses. As the principal phagocytic cells of innate immunity, neutrophils continuously respond to environmental stimuli throughout life, whereas eosinophils are influenced by long-term exposure to environmental antigens despite the absence of detectable gastrointestinal parasitism in the present study. These findings support the concept that immune maturation continues beyond early growth and is accompanied by selective changes in circulating granulocyte populations (Gaina et al., 2020; Miller et al., 2021; Satué et al., 2020; DeNotta and McFarlane, 2023). Consistent with this interpretation, Rate et al. (2025) reported age-related variation in immunoglobulin M (IgM) concentrations in Sumbawa horses maintained under the traditional *lar* grazing system, suggesting that both cellular and humoral immune components undergo age-related adaptation in this indigenous horse breed.

In contrast, basophil counts remained unchanged despite the significant enlargement of basophil dimensions observed in adult horses, suggesting that structural adaptation may occur independently of changes in circulating cell numbers. This discrepancy highlights the complementary value of combining histomorphometry and hematological analyses, as subtle cellular adaptations may not be detected through differential leukocyte counts alone (Walton and Lawson, 2021).

Unlike granulocyte populations, PLT, MPV, PDW-CV, and PCT remained stable across age groups, indicating that thrombopoiesis and platelet turnover are maintained within relatively narrow physiological limits throughout adulthood (Satué et al., 2017; Gaina et al., 2020). Overall, these findings demonstrated that age-related hematological adaptation in

Sumbawa horses is characterized primarily by coordinated changes in granulocyte morphology and abundance, whereas platelet physiology remains comparatively stable. These observations establish baseline reference data for interpreting age-related hematological variation in indigenous horses maintained under tropical grazing conditions.

Influences of the traditional lar grazing system

The traditional *lar* grazing system provides an important ecological context for interpreting the hematological characteristics of Sumbawa horses. Unlike intensively managed horses, Sumbawa horses graze freely on communal rangelands throughout the year and are continuously exposed to seasonal climatic variation, diverse forage resources, environmental microorganisms, and naturally occurring environmental antigens associated with pasture-based management (Martinson et al., 2017; Drought et al., 2023). Similar effects of environmental and management conditions have been reported in other horse populations. Francesca Aragona et al. (2023) demonstrated that housing systems and seasonal variation significantly influenced platelet counts and most leukocyte populations, whereas Deniz et al. (2024) reported significant effects of climatic variation on leukocyte populations, including neutrophils, lymphocytes, and monocytes, as well as platelet counts. Likewise, Sawesi et al. (2023) showed that management practices, including exercise and dietary supplementation, affected several hematological indices, while Ono et al. (2021) reported seasonal variations in hematological profiles in indigenous Noma horses. Collectively, these findings support the interpretation that continuous exposure to the traditional *lar* grazing system may contribute to physiological adaptation and variation in the hematological characteristics of Sumbawa horses and should be considered when establishing reference intervals for clinically healthy indigenous populations.

The predominance of age-related over sex-related variation supports the view that long-term environmental adaptation contributes more substantially to granulocyte adaptation than normal physiological differences between males and females. Adult horses exhibited enlarged neutrophils and basophils together with higher neutrophil and eosinophil counts, whereas granulocyte morphology and abundance remained largely comparable between sexes, with sex-related differences primarily confined to platelet parameters. Moreover, structural enlargement of granulocytes was not consistently accompanied by corresponding changes in circulating cell numbers, indicating that cellular morphology and routine hematological variables provide complementary information for identifying subtle physiological adaptations (Stokol, 2020).

CONCLUSION

This study simultaneously evaluated granulocyte histomorphometry, granulocyte counts, and platelet profiles in clinically healthy Sumbawa horses maintained under the traditional *lar* grazing system. Sex had no significant effect on granulocyte histomorphometry or granulocyte counts, whereas age significantly influenced neutrophil and basophil histomorphometry as well as neutrophil and eosinophil counts. In contrast, platelet profiles were primarily influenced by sex rather than age. These findings establish baseline hematological reference data for clinically healthy Sumbawa horses and highlight the importance of considering age and the traditional *lar* grazing system when interpreting hematological characteristics in indigenous horses. They also provide a valuable reference for clinical pathology and future comparative studies of hematological adaptation under tropical grazing conditions. Future studies should include multiple indigenous horse populations from different geographical regions and integrate functional immunological and molecular biomarkers, such as cytokines and oxidative stress indicators, to further elucidate hematological adaptation under diverse environmental and management conditions.

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Authors' contributions

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

Availability of data and materials

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

Competing interests

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

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. No AI tools were used to conduct this study and prepare the manuscript. The authors accept the full responsibilities of originality of the study before submission and throughout the entire peer-review process of publication.

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REFERENCES

Arnold IC and Munitz A (2024). Spatial adaptation of eosinophils and their emerging roles in homeostasis, infection and disease. Nature Reviews Immunology, 24(12): 858-877. DOI: https://www.doi.org/10.1038/s41577-024-01048-y

Arnold JE, Camus MS, Freeman KP, Giori L, Hooijberg EH, Jeffery U, Korchia J, Meindel MJ, Moore AR, and Sisson SC (2019). ASVCP guidelines: Principles of quality assurance and standards for veterinary clinical pathology (version 3.0): Developed by the American Society for Veterinary Clinical Pathology's (ASVCP) quality assurance and laboratory standards (QALS) committee. Veterinary Clinical Pathology, 48(4): 542-618. DOI: https://www.doi.org/10.1111/vcp.12810

Brooks MB, Harr KE, Seelig DM, Wardrop KJ, and Weiss DJ (2022). Schalm's veterinary hematology, 7th Edition. John Wiley & Sons., Hoboken, New Jersey, USA, pp. 993-1003. DOI: https://www.doi.org/10.1002/9781119500537

Brosnahan M (2020). Eosinophils of the horse: Part II: Eosinophils in clinical diseases. Equine Veterinary Education, 32(11): 590-602. DOI: https://www.doi.org/10.1111/eve.13262

Budak YU, Polat M, and Huysal K (2016). The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: A systematic review. Biochemia Medica, 26(2): 178-193. DOI: https://www.doi.org/10.11613/BM.2016.020

Candrianisa ARP, Suwiti NK, Nindhia TS, Setiasih NLE, Kendran AAS, and Suatha IK (2024). Histologic features and granulocyte white blood cell counts of young and adult Sumbawa horses reared under the Lar system. Buletin Veteriner Udayana, 16(4): 1148-1158. DOI: https://www.doi.org/10.24843/bulvet.2024.v16.i04.p21

Deniz Ö, Aragona F, Murphy BA, Tümer KÇ, Bozacı S, and Fazio F (2024). Climate change impact on blood haemogram in the horse: A three-year preliminary study. Frontiers in Veterinary Science, 11: 1482268. DOI: https://www.doi.org/10.3389/fvets.2024.1482268

DeNotta S and McFarlane D (2023). Immunosenescence and inflammaging in the aged horse. Immunity & Ageing, 20(2): 2-10. DOI: https://www.doi.org/10.1186/s12979-022-00325-5

Drought A, Arifianto D, Voss JT, McLay SJ, Jabbar A, Hitchens PL, and El-Hage CM (2023). Veterinary aid clinic assessments of working ponies in West Nusa Tenggara province, Indonesia: A retrospective study. Australian Journal of Veterinary Sciences, 55(1): 61-67. DOI: https://www.doi.org/10.4067/S0719-81322023000100061

Ehrmann C, Engel J, Moritz A, and Roscher K (2021). Assessment of platelet biology in equine patients with systemic inflammatory response syndrome. Journal of Veterinary Diagnostic Investigation, 33(2): 300-307. DOI: https://www.doi.org/10.1177/1040638720983791

Francesca Aragona F, Arfuso F, Fazio F, De Caro S, Giudice E, Monteverde V, Piccione G, and Giannetto C (2023). Circadian variation of peripheral blood cells in horses maintained in different environmental and management conditions. Animals, 13(11): 1865. DOI: https://www.doi.org/10.3390/ani13111865

Global Administrative Areas (GADM) (2025). GADM data, version 4.1. Database of global administrative areas (GADM). Available at: https://gadm.org/data.html

Gaina CD, Widi AY, and Saputra A (2020). Age-sex related in hematological values of sandalwood pony horses (*Equus caballus*) in East Sumba, NTT. Journal of Veterinary and Animal Sciences, 3(2): 103-109. DOI: https://www.doi.org/10.24843/JVAS.2020.v03.i02.p06

Gasparyan AY, Ayvazyan LP, Mikhailidis D, and Kitas GD (2011). Mean platelet volume: A link between thrombosis and inflammation?. Current Pharmaceutical Design, 17(1): 47-58. DOI: https://www.doi.org/10.2174/138161211795049804

Hall EJ, Carter AJ, Stevenson AG, and Hall C (2018). Establishing a yard specific normal rectal temperature reference range for horses. Journal of Equine Veterinary Science, 74: 51-55. DOI: https://www.doi.org/10.1016/j.jevs.2018.12.023

Henneke D, Potter G, Kreider J, and Yeates B (1983). Relationship between condition score, physical measurements and body fat percentage in mares. Equine Veterinary Journal, 15(4): 371-372. DOI: https://www.doi.org/10.1111/j.2042-3306.1983.tb01826.x

Klein SL and Flanagan KL (2016). Sex differences in immune responses. Nature Reviews Immunology, 16(10): 626-638. DOI: https://www.doi.org/10.1038/nri.2016.90

Koupenova M, Mick E, Mikhalev E, Benjamin EJ, Tanriverdi K, and Freedman JE (2015). Sex differences in platelet toll-like receptors and their association with cardiovascular risk factors. Arteriosclerosis, Thrombosis, Vascular Biology, 35(4): 1030-1037. DOI: https://www.doi.org/10.1161/ATVBAHA.114.304954

Lejeune M, Mann S, White H, Maguire D, Hazard J, Young R, Stone C, Antczak D, and Bowman D (2023). Evaluation of fecal egg count tests for effective control of equine intestinal strongyles. Pathogens, 12(11): 1283. DOI: https://www.doi.org/10.3390/pathogens12111283

Marshall JS, Warrington R, Watson W, and Kim HL (2018). An introduction to immunology and immunopathology. Allergy, Asthma Clinical Immunology, 14(Suppl 2): 49. DOI: https://www.doi.org/10.1186/s13223-018-0278-1

Martinson KL, Siciliano PD, Sheaffer CC, McIntosh BJ, Swinker AM, and Williams CA (2017). A review of equine grazing research methodologies. Journal of Equine Veterinary Science, 51: 92-104. DOI: https://www.doi.org/10.1016/j.jevs.2017.01.002

Miller AB, Loynachan AT, Barker VD, and Adams AA (2021). Investigation of innate immune function in adult and geriatric horses. Veterinary Immunology and Immunopathology, 235: 110207. DOI: https://www.doi.org/10.1016/j.vetimm.2021.110207

Nielsen M (2022). Parasite faecal egg counts in equine veterinary practice. Equine Veterinary Education, 34(11): 584-591. DOI: https://www.doi.org/10.1111/eve.13548

Nielsen MK (2021). What makes a good fecal egg count technique?. Veterinary Parasitology, 296: 109509. DOI: https://www.doi.org/10.1016/j.vetpar.2021.109509

Ono T, Inoue Y, Hisaeda K, Yamada Y, Hata A, Miyama TS, Shibano K, Kitagawa H, Ohzawa E, and Iwata E (2021). Effect of seasons and sex on the physical, hematological, and blood biochemical parameters of Noma horses. Journal of Equine Science, 32(1): 21-25. DOI: https://www.doi.org/10.1294/jes.32.21

Pratama KFAD, Suwiti NK, Ardana IBK, Setiasih NLE, Dharmawan NS, Sulabda IN, and Besung INK (2025). Association of sex and age with histomorphometric features of agranulocytic leukocytes and erythrocyte indices in Sumbawa horses. World's Veterinary Journal, 15(4): 1007-1014. DOI: https://www.doi.org/10.54203/scil.2025.wvj102

Rate IMB, Suwiti NK, Suardana IBK, and Besung INK (2025). Effects of gender and age on immunoglobulin m profile of sumbawa horses raised in the traditional grazing system. Farm Animal Health and Nutrition, 4(1): 1-5. DOI: https://www.doi.org/10.58803/fahn.v4i1.65

Satué K, Hernández Á, Lorente C, Fazio E, and Medica P (2020). Age-and sex-related modifications of hematology in Spanish purebred horse. Journal of Equine Veterinary Science, 93: 103219. DOI: https://www.doi.org/10.1016/j.jevs.2020.103219

Sawesi OK, Elbaz AK, Mahmoud AS, Duro EM, Alteab AA, Milad KK, and Bennuor EM (2023). Hematological reference values of horses in Western Libya and their relationship to breed, age, and management. Open Veterinary Journal, 13(12): 1696-1707. DOI: https://www.doi.org/10.5455/OVJ.2023.v13.i12.18

Scherlinger M, Richez C, Tsokos GC, Boilard E, and Blanco P (2023). The role of platelets in immune-mediated inflammatory diseases. Nature Reviews Immunology, 23(8): 495-510. DOI: https://www.doi.org/10.1038/s41577-023-00834-4

Stirn M, and Freeman KP (2022). Quality management of hematology techniques. In: M. B. Brooks, K. E. Harr, D. M. Seelig, K. J. Wardrop, and D. J. Weiss (Editors). Schalm's veterinary hematology, 7th Edition. John Wiley & Sons., Hoboken, New Jersey, USA, pp. 1243-1254. DOI: https://doi.org/10.1002/9781119500537.ch137

Stokol T (2020). Hematology red flags: The value of blood smear examination in horses. Veterinary Clinics: Equine Practice, 36(1): 15-33. DOI: https://www.doi.org/10.1016/j.cveq.2019.11.001

Sukri A, Dewi IN, Primawati SN, Wangiyana I, Muttaqin Z, and Winaya A (2022). Revealing the genetic diversity of Sumbawa endemic horse using microsatellite-based DNA fingerprint. Biodiversitas, 23(8): 4152-4158. DOI: https://www.doi.org/10.13057/biodiv/d230837

Walton RM and Lawson CA (2021). Equine hematology. In: R. M. Walton and A. C. Valenciano (Editors), Equine hematology, cytology, and clinical chemistry, 2nd Edition. John Wiley & Sons., Hoboken, New Jersey, USA, pp. 9-26. DOI: https://www.doi.org/10.1002/9781119500186.ch2

Yavuz H, Paksoy Y, Selvi MH, and Altuğ N (2025). Determination of hematological and biochemical parameters in healthy Arabian and thoroughbred racehorses. Journal of Advances in VetBio Science Techniques, 10(1): 26-34. DOI: https://www.doi.org/10.31797/vetbio.1565704

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