Veterinarski glasnik 2025, 79(1), 49-64
UDC: 636.39.064:612.11
https://doi.org/10.2298/VETGL241015002M

Full Research article

ESTABLISHMENT OF REFERENCE INTERVALS OF HEMATOLOGICAL PARAMETERS AND EVALUATION OF AGE, SEX, AND SEASON EFFECTS IN THE PRETA DE MONTESINHO GOAT

Rafaela S. S. MOREIRA[1], João JACOB-FERREIRA[2], Liliana SANTOS[3], Ramiro VALENTIM[3], Paulo P. CORTEZ[1], Hélder QUINTAS[2,4*]

1 – Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
2 – Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
3 – Escola Superior Agrária, Instituto Politécnico de Bragança, Alameda de Santa Apolónia, 5300-252 Bragança, Portugal
4 – Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (LA SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

Received 15 October 2024; Accepted 02 December 2024
Published online: 30 January 2025

How to cite: Rafaela S. S. Moreira, João Jacob-Ferreira, Liliana Santos, Ramiro Valentim, Paulo P. Cortez, Hélder Quintas. Establishment of reference intervals of hematological parameters and evaluation of age, sex, and season effects in the Preta de Montesinho goat. Veterinarski Glasnik, 2025. 79(1): 49-64. https://doi.org/10.2298/VETGL241015002M

Abstract

The Preta de Montesinho goat is an autochthonous Portuguese breed classified as endangered. Autochthonous breeds should be preserved because they are usually well adapted to their specific regional conditions, exhibit resistance to certain diseases, and serve as important reservoirs of unique genetic diversity. For a breed like the Preta de Montesinho, maintaining the health of the animals to ensure their reproduction and increase their numbers is essential. Therefore, understanding the hematological reference interval is particularly important. This study aimed to determine the hematological reference interval for the Preta de Montesinho breed and assess the potential influence of age, sex, and seasons on these intervals.

*Corresponding author: e-mail: helder5tas@ipb.pt

A total of 136 blood samples were collected from healthy animals. The samples were analyzed using the Mindray BC–5000 Vet® automatic hematology analyzer, and reference intervals for 23 blood parameters were established applying the Reference Value Advisor v2.1 software. JMP® was utilized to determine statistically significant differences between the established groups.

Regarding age, statistically significant differences ($p < 0.05$) between young and old goats were detected in 15 parameters. Sex significantly influenced eight parameters, six of which were erythrocyte indices. The annual season (summer or winter) affected seven parameters and six erythrocyte indices. The reference intervals described here can serve as a tool to identify sick animals, evaluate responses to therapies, and select healthy animals for reproduction, thus contributing to the breed's preservation.

Key Words: hematology, goats, Preta de Montesinho, reference intervals

INTRODUCTION

The Preta de Montesinho goat is the most endangered autochthonous Portuguese breed that is currently classified by the Food and Agriculture Organization of the United Nations (FAO) as an endangered-maintained breed. This means the goat is at risk of extinction, but is being maintained by an active public conservation program or within a commercial or research facility. According to a census conducted by the Sociedade Portuguesa de Recursos Genéticos Animais (SPREGA), in 2024 there were 1878 females and 73 males maintained by 45 farmers.

In a breed like the Preta de Montesinho, actively maintaining the animals' health to ensure their reproduction and increase their numbers is crucial. Thus, it is essential to learn everything possible about the breed, and knowing the hematological reference interval is particularly useful for assessing health. This knowledge serves as a tool for identifying sick animals, evaluating their response to therapy, and selecting healthy animals for reproduction, thereby contributing to the breed's preservation (Silva et al., 2023).

Although goat farming has declined over recent decades, the Iberian Peninsula still represents almost 25% of the European goat census. In a small country like Portugal, which has six native goat breeds, it is concerning that only 12.5% of the national stock is native and that most of these animals are crossed with foreign breeds (Ginja et al., 2018). The crossbreeding of local and exotic livestock breeds has led and continues to lead to the extinction or endangerment of several native breeds in Portugal, similar to trends in other countries (Bruno-de-Sousa et al., 2010). Autochthonous breeds should be preserved, as they usually are best adapted to the specific conditions of their region or country, exhibit resistance to certain diseases, and serve as significant reservoirs of unique genetic diversity. These animals are often farmed in extensive systems and thrive on poor pastures and marginal agroforestry land. Goats are nearly the only animal capable of optimizing these resources while contributing to ecosystem management (Carolino et al., 2016). Efforts to farm these animals can unlock potential for the socioeconomic development of rural areas. In Europe, about 7% of caprine breeds have disappeared, and many more are on the verge of extinction (Martínez et al., 2015); it is extremely important to raise awareness, increase knowledge, and make efforts to increase the populations of native breeds.

This study aimed to establish the hematological reference intervals (RI) and evaluate the potential impact of sex, age, and seasons on these intervals in the underrepresented Portuguese goat breed, the Preta de Montesinho.

MATERIALS AND METHODS

Study Population

The animals in this study belonged to the Agro-Livestock Unit of Instituto Politécnico de Bragança (IPB). The production system for these animals was semi-extensive, with grazing on permanent meadows during the day and stabling at night. The animals were frequently monitored by the veterinary doctors of the IPB and received regular medical and prophylactic care, including proper anthelmintic treatments. The samples (see below) were collected in three different months (in June and November of 2023 and January of 2024) within the scope of an animal health program that aimed to eradicate and monitor animal diseases.

To study the effect of age and sex on the hematological parameters, the population was divided into males (n=9) and females (n=127) and young (age <18 months old, n=33) and adults (≥ 18 months old, n=103). Regarding season, the samples were divided into two different groups: winter cold season (n=136) and summer warm season (n=30).

Sample Collection and Hematological Analyses

For animals to be selected for sample collection, they had to meet specific criteria established before the days of sample collection: they had to be healthy, routinely dewormed, without any treatment in the last six months, and with a body condition score appropriate to their production phase (between 2.5 and 3.5 on a scale of 5). The animals were examined on the day of sampling, and a physical exam was performed. Any animal that had lesions, abscesses, was lethargic, or had a fever was excluded to ensure that only healthy animals were included in the study, preventing any disruption of results. Additionally, animals that were excited or agitated during sampling were also excluded.

Samples were collected as part of the disease testing integrated with the animal health program in force in Portugal. The animals were grabbed calmly, and the blood was collected via the jugular vein using an 18-gauge needle (BD PrecisionGlide™) into K3EDTA vacuum tubes (BD Vacutainer) with a 4 mL capacity. Immediately after the blood draw, the tubes were inverted gently several times to ensure adequate anticoagulant mixing. No later than 3 h after collection, the samples were processed with the Mindray BC-5000 Vet® that works with three technologies, tri-angle laser scatter, focused flow, and chemical dye.

The parameters obtained were red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV,) mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width – coefficient of variation (RDW-CV), red cell distribution width – standard deviation (RDW-SD), white blood cell count (WBC), differential total count and percentage of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophil (EOS), basophils (BAS), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). –

The animal study protocol was approved by the Ethics Committee by Polytechnic Institute of Bragança — P520717-R633322-D1864723.

Statistical Analyses

Statistical analysis was performed with Microsoft Office Excel® (version 2305 Build 16. 0. 16501. 20074), Reference Value Advisor v2.1 and JMP® Statistical Discovery version 7 software. Data collected was recorded in Excel®. Reference Value Advisor v2.1 was applied to determine RIs and confidence interval (CI) limits at 90%, analyze data normality with the Anderson-Darling test, and check for the presence of outliners with the Tukey and Dixon-Reed methods. After these tests and based on the distribution, either the nonparametric or robust method, with or without Box-Cox transformation, was utilized, and the RI and the 90% CI for the lower and upper limits were determined (Geffré et al., 2011; Silva et al., 2023).

The JMP® was used to verify if there were statistically significant differences between the different groups established (young-adults, female-male and warm-cold season; see above). The Shapiro-Wilk test was performed to test sample normality. Then, results were subjected to an analysis of variance (ANOVA) to investigate the influence of sex, age, and season (Silva et al., 2023). Differences were considered significant when $p < 0.05$.

RESULTS

Reference Intervals for Preta de Montesinho goat

A total of 136 animals were included in this study, and 23 hematological parameters were determined. In Table 1, the data is expressed as the mean, median, standard deviation (SD), the minimum and maximum value of each parameter, RI, the lower reference limit (LRL) at 90% CI, and the upper reference limit (URL) at 90% CI.

Table 1. Hematological reference intervals for the population of Preta de Montesinho goats included in the study (n=136).

Parameters/Units	N	Mean ± SD	Median	Min–Max	RI	LRL 90% CI	URL 90% CI
WBC (10^3/µL)	136	13.5±3.6	13.1	4.56–29.57	7.9–21.7	4.6–8.4	19.5–29.6
NEU (10^3/µL)	136	6.2±2.6	5.6	1.28–18.85	2.3–13.3	1.3–3.1	11.6–18.9
LYM (10^3/µL)	136	6.4±2.3	5.8	1.94–14	2.8–12.2	1.9–3.7	11.1–14.0
MON (10^3/µL)	136	0.3±0.1	0.2	0.05–0.8	0.1–0.6	0.1–0.1	0.5–0.8
EOS (10^3/µL)	136	0.5±0.6	0.3	0.01–5.92	0.1–1.5	0.0–0.1	1.2–5.9
BAS (10^3/µL)	136	0.1±0.1	0.1	0.03–0.3	0.0–0.3	0.0–0.1	0.3–0.3
NEU (%)	136	45.7±12.2	45.8	23–73.7	24.1–70.0	23.0–26.0	64.1–73.7
LYM (%)	136	47.8±12.3	46.9	13.5–72	23.5–69.3	13.5–28.3	67.9–72.0
MON (%)	136	1.9±1.1	1.6	0.5–5.8	0.6–4.9	0.5–0.7	4.0–5.8
EOS (%)	136	3.6±3.0	2.7	0.1–20.1	0.4–11.2	0.1–0.7	8.7–20.1
BAS (%)	136	1.1±0.4	1.1	0.2–2.5	0.5–2.0	0.2–0.5	1.8–2.5
RBC (10^6/µL)	136	17.6±3.0	18.1	7.9–25.92	10.5–23.4	7.9–12.4	21.8–25.9
HGB (g/L)	136	99.9±15.0	101.6	50.3–135.4	63.6–127.7	50.3–72.0	122.5–135.4
HCT (%)	136	29.6±4.3	29.7	14.9–40	19.7–38.1	14.9–21.8	36.8–40.0
MCV (fL)	136	17.1±2.7	16.9	11.5–27.8	12.2–22.7	11.5–13.1	21.8–27.8
MCH (pg)	136	5.7±0.7	5.6	4.4–7.7	4.5–7.1	4.4–4.8	6.9–7.7
MCHC (g/L)	136	338.4±19.6	337.0	278–387	301.0–381.7	278.0–308.0	368.0–387.0
RDW–CV (%)	136	24.0±2.4	23.6	20.6–41.8	21.1–30.0	20.6–21.5	27.3–41.8
RDW–SD (fL)	136	17.2±3.2	16.7	12.4–38.5	13.1–24.3	12.4–13.6	22.1–38.5
PLT (10^3/µL)	136	219.4±171.0	153.0	26–711	30.9–632.0	26.0–40.0	549.0–711.0
MPV (fL)	136	2.4±0.1	2.4	2.2–2.7	2.2–2.7	2.2–2.2	2.6–2.7
PDW (%)	136	12.9±0.5	13.0	11.7–13.6	11.7–13.5	11.7–11.8	13.3–13.6
PCT (%)	136	0.1±0.0	0.0	0.007–0.162	0.0–0.1	0.0–0.0	0.1–0.2

WBC: white blood cell count, **NEU:** neutrophils, **LYM:** lymphocytes, **MON:** monocytes, **EOS:** eosinophils, **BAS:** basophils, **RBC:** red blood cell count, **HCT:** hematocrit, **HGB:** hemoglobin, **MCV:** mean corpuscular volume, **MCH:** mean corpuscular hemoglobin, **MCHC:** mean corpuscular hemoglobin concentration, **RDW-CV:** red cell distribution width – coefficient of variation, **RDW-SD:** red cell distribution width – standard deviation, **PLT:** platelet count, **MPV:** mean platelet volume, **PDW:** platelet distribution width, **PCT:** plateletcrit, **SD:** standard deviation, **Min:** minimum, **Max:** maximum, **RI:** reference interval, **LRL:** lower reference limit, **URL:** upper reference limit, **CI:** confidence interval.

Influence of age on hematological parameters

Considering age, the initial population of 136 animals was divided into two groups: the young group, consisting of animals less than 18 months old (n=33), and the adult group, consisting of animals 18 months or older (n=103).

Table 2 summarizes the effect of age on the mean hematological parameters. Differences between groups were considered statistically different when $p < 0.05$. That said, mean WBC, LYM, BAS, LYM (%), BAS (%), RBC, MCHC, and RDW–CV were higher in younger animals, while, mean NEU, NEU (%), MON (%), HCT, MCV, MCH, and RDW–SD were higher in older animals.

Table 2. Effect of age on hematological parameters in the Preta de Montesinho goat.

Parameters/ Units	Young (n=33)		Adult (n=103)		p–Value
	Mean ± SD	RI	Mean ± SD	RI	
WBC ($10^3/\mu L$)	14.7±3.2	8.7–21.8	13.1±3.7	6.6–23.3	0.0304
NEU ($10^3/\mu L$)	5.0±1.5	2.6–8.9*	6.6±2.8	2.2–14.1	0.0016
LYM ($10^3/\mu L$)	8.8±2.5	3.9–14.1	5.6±1.7	2.6–10.4	<0.0001
BAS ($10^3/\mu L$)	0.2±0.1	0.1–0.3	0.1±0.1	0.0–0.3	0.0001
NEU (%)	34.3±9.2	21.6–57.2	49.3±10.7	29.1–71.6	<0.0001
LYM (%)	59.6±9.3	31.5–73.2	44.0±10.6	21.8–64.8	<0.0001
MON (%)	1.5±0.8	0.6–4.2	2.0±1.1	0.6–5.4	0.0225
BAS (%)	1.2±0.4	0.6–2.3	1.0±0.4	0.5–1.9	0.0175
RBC ($10^6/\mu L$)	19.3±2.6	13.4–24.1*	17.1±3.0	9.9–22.6	0.0002
HCT (%)	27.7±3.4	18.7–33.6	30.2±4.5	19.1–38.6	0.0058
MCV (fL)	14.5±1.7	11.4–18.5	17.9±2.4	13.7–23.0	<0.0001
MCH (pg)	5.1±0.4	4.2–6.0*	5.9±0.6	4.9–7.1	<0.0001
MCHC (g/L)	353.6±18.9	307.3–385.7*	333.5±17.3	299.0–370.0	<0.0001
RDW–CV (%)	24.9±2.0	21.7–30.2	23.7±2.5	21.1–29.2	0.0149
RDW–SD (fL)	15.4±1.9	12.2–20.4	17.8±3.3	13.6–25.3	0.0001

RBC: red blood cell count, **HCT:** hematocrit, **MCV:** mean corpuscular volume, **MCH:** mean corpuscular hemoglobin, **MCHC:** mean corpuscular hemoglobin concentration, **RDW-CV:** red cell distribution width – coefficient of variation, **RDW-SD:** red cell distribution width – standard deviation, **WBC:** white blood cell count, **NEU:** neutrophils, **LYM:** lymphocytes, **BAS:** basophils, **MON:** monocytes, **SD:** standard deviation, **RI:** reference interval.
***** Use with caution because possible outliners were detected (Geffré et al, 2011). ****** The sample size is too small ($n < 40$) to compute a nonparametric reference interval. The parametric analysis does not give enough confidence to formulate adequate RIs.

Influence of sex on hematological parameters

Considering sex, the initial population was divided into females (n=127) and males (n=9).

Table 3 summarizes the effect of sex on the mean hematological parameters. Differences between groups ($p < 0.05$) were found for mean EOS (%), BAS (%), and RBC, HGB, HCT, MCV, MCH, and MCHC. Mean RBC, HGB, HCT, and MCHC were higher in males, while mean EOS (%), BAS (%), MCV, and MCH were higher in females.

Table 3. Effect of sex on hematological parameters in the Preta de Montesinho goat.

Parameters/ Units	Female (n=127)		Male (n=9)		p–Value
	Mean ± SD	RI	Mean ± SD	RI	
EOS (%)	3.7±3.1	0.4–11.3	1.7±1.0	**	0.0465
BAS (%)	1.1±0.4	0.5–2.0	0.8±0.3	0.3–1.6	0.0405
RBC (10^6/µL)	17.3±2.9	10.3–22.0	21.8±1.8	16.7–26.2	<0.0001
HGB (g/L)	98.9±14.9	63.3–128.5	114.5±8.2	94.3–134.9	0.0022
HCT (%)	29.3±4.4	19.6–37.5	32.6±2.8	25.2–38.7*	0.0284
MCV (fL)	17.2±2.7	12.3–22.8	15.1±2.0	11.6–21.5	0.0211
MCH (pg)	5.8±0.7	4.5–7.1	5.3±0.4	4.3–6.2	0.0329
MCHC (g/L)	337.4±19.4	301.0–379.0	351.8±19.6	304.9–401.6	0.0338

EOS: eosinophils, **BAS:** basophils, **RBC:** red blood cell count, **HGB:** hemoglobin, **HCT:** hematocrit, **MCV:** mean corpuscular volume, **MCH:** mean corpuscular hemoglobin, **MCHC:** mean corpuscular hemoglobin concentration, **SD:** standard deviation, **RI:** reference interval.
***** Use with caution because possible outliners were detected (Geffré et al, 2011). ****** The sample size is too small (n < 40) to compute a nonparametric reference interval. The parametric analysis does not give enough confidence to formulate adequate RIs.

Influence of season on hematological parameters

Regarding the influence of seasons, the samples were also divided into two groups, i.e., collected in the cold season (n=106) or warm season (n=30).

The effect of seasons is condensed in Table 4. Differences between the groups were considered significant when p < 0.05. Thus, the mean RBC, HGB, HCT, MCV, MCH, RDW-CV, and MPV were statistically different between groups. Mean RBC, HGB, HCT, and MCV were higher during the winter cold season than during the warm season.

Table 4. Effect of season on hematological parameters in the Preta de Montesinho goat.

Parameters/ Units	Cold season (n=106)		Warm season (n=30)		p–Value
	Mean ± SD	RI	Mean ± SD	RI	
RBC (10^6/µL)	17.9±2.6	11.8–22.6	16.5±4.0	8.2–24.9	0.0188
HGB (g/L)	103.2±13.3	66.4–130.5	88.4±15.2	57.8–121.0*	<0.0001
HCT (%)	30.6±3.9	19.8–38.6	25.8±3.8	16.9–33.2*	<0.0001
MCV (fL)	17.3±2.6	13.2–23.0	16.2±2.9	10.4–22.3	0.0422
MCH (pg)	5.8±0.6	4.8–7.0	5.5±0.7	4.2–7.2	0.0290
RDW–CV (%)	23.6±1.8	21.1–28.9	25.3±3.7	21.3–35.6	0.0006
MPV (fL)	2.37±0.1	2.2–2.7	2.43±0.1	**	0.0191

RBC: red blood cell count, **HGB:** hemoglobin, **HCT:** hematocrit, **MCV:** mean corpuscular volume, **MCH:** mean corpuscular hemoglobin, **RDW-CV:** red cell distribution width – coefficient of variation, **MPV:** mean platelet volume, **SD:** standard deviation, **RI:** reference interval.
***** Use with caution because possible outliners were detected (Geffré et al, 2011). ****** The sample size is too small (n < 40) to compute a nonparametric reference interval. The parametric analysis does not give enough confidence to formulate adequate RIs.

DISCUSSION

The evaluation of hematological parameters is commonly used to assess the health status of farm animals and to characterize different animal breeds, and goats are no exception. Studies like the one here conducted have been carried out since the last century. There are several articles dating back to the late 1970s that evaluate the influence of age on hematology parameters (Edjtehadi, 1978), sex (Somvanshi et al., 1987), season, gestation (Pospisil et al., 1987), and even compare different breeds of goats (Mbassa & Poulsen, 1992). Nowadays, with more sophisticated and better calibrated automated cell counters, these studies continue to be conducted and with more accurate results.

It is well documented that there are plenty of factors that can interfere with the erythrocyte, leukocyte, and platelet blood indices, for example, infectious diseases, parasites (Mpofu et al., 2020), body condition score (Shawaf et al., 2021), and many more. Hence, in this study, only dewormed animals were included that had adequate body condition scores, and the healthy animals were subjected to a physical exam prior to sampling.

We determined 23 blood parameters for the Preta de Montesinho goat. WBC and NEU counts were slightly above than those reported for other different goat breeds, while the other leukocyte indices were within the normal ranges established in other studies (Agradi et al., 2022; Mohammed et al., 2016). Research conducted on four Omani goat breeds showed that three of them had higher mean WBC values than the value we determined for the Preta de Montesinho goat. However, RIs were higher in one breed, similar in another, and lower in two. All four Omani breeds had higher mean and RI values for NEU (%) (Al-Bulushi et al., 2017) than we found in the current study. The mean WBC values established here could be physiologically high in Preta de Montesinho goats, as has been demonstrated for other breeds. Alternatively, despite only seemingly healthy animals being included in this study, the high WBC values could have been due to a subclinical disease, infection, or intoxication (Olver et al., 2022).

Regarding the parameters related to erythrocytes and hemoglobin, HGB, HCT, and MCHC were consistent with the RIs found in the literature (Newcomer et al., 2021; Olver et al., 2022). However, the mean RBC count in our study was slightly higher than the ones previously published (Arfuso et al., 2016; Mohammed et al., 2016). Depending on the source, the mean MCV and MCH values reported by us are either similar (Arfuso et al., 2016) or marginally lower (Mohammed et al., 2016; Olver et al., 2022) than previously published. Most of the studies did not mention the RDW or did not differentiate between RDW-SD and RDW-CV. When studies included RDW, they generally referred to the RDW-CV. That said, the RDW-CV reported in our study was the same as other studies (Karaşahin et al., 2022) or lower (Al-Bulushi et al., 2017).

Platelet indices are also commonly neglected, but comparing with the few articles that included them, the parameters (MPV, PCT and PDW) were below those previously reported (Mohammed et al., 2016; Singh Nathawat et al., 2023). Regarding the PLT, although the upper limit of the RI was within the values presented in other studies, the lower limit was below (Johns & Heller, 2021; Smith & Sherman, 2023). There are several reasons that can explain these platelet indices. Firstly, the lower RIs we observed might be physiological for Preta de Montesinho goat, as breed is a well-documented factor influencing hematological parameters (Arfuso et al., 2016). Additionally, platelets often appear in the peripheral blood in clusters of various sizes (Smith & Sherman, 2023), which could cause automated cell counters to count several platelets as one or misidentify them as other types of blood cells. Furthermore, sampling in some animals might have been slow or traumatic, or the blood inadequately mixed with the anticoagulant, which could have resulted in platelet activation and/or clumping. This clumping can decrease the measured platelet count generated by hematology analyzers (Sharkey et al., 2020). Although this study only included apparently healthy animals that were regularly checked by veterinarians, infectious diseases are a well-known cause of low platelet indices (Johns & Heller, 2021). A limitation of this study was the absence of blood smears and manual blood cell counts, which would have either confirmed the determined values or indicated that the low indices were due to platelet activation or clumping.

To evaluate the influence of age on hematological parameters, the initial population was divided into two different groups (younger or older than 18 months). This age threshold was chosen because 18 months is the average age at which goats begin their productive life, that is, the age at first parturition. The literature indicates that erythrocyte parameters in goats are labile, changing markedly over the goat's lifetime (Smith & Sherman, 2023). We proved this and more, as we found statistically significant differences in 15 of the 23 parameters assessed. The mean WBC, LYM, and BAS (count and percentage), and RBC, MCHC, and RDW-CV were higher in young than in old goats, while the NEU (count and percentage), MON (percentage), and HCT, MCV, MCH, and RDW-SD were lower in the young goats.

In relation to the leukocyte parameters, some studies did not find age influenced the WBC (Yaqub et al., 2013). However, most studies found that the WBC is higher in younger than in older animals (Agradi et al., 2022; Arfuso et al., 2016; Yaqub et al., 2013). Studies cite the fact that there are age-dependent variations in the immune systems of animals, with younger goats appearing to have a robust protective system that offers rapid and potent defense against infectious agents (Piccione et al., 2014), which seems to corroborate the current findings. The higher LYM fraction and percentage in young animals and the elevated NEU parameters in older animals (our study), align with literature stating that lymphocytes are the predominant leukocyte in younger goats, while in older goats, the percentage of neutrophiles is equal to or slightly higher than the percentage of lymphocytes (Antunović et al., 2020).

The findings for the erythrocyte indices were also validated by other studies: mean RBC decreased with age (Agradi et al., 2022; Antunović et al., 2020; Arfuso et al., 2016; Karaşahin et al., 2022) and so did the MCHC (Agradi et al., 2022) while the mean HCT, the MCH and MPV increased with age (Agradi et al., 2022; Karaşahin et al., 2022; Yaqub et al., 2013). This physiological variation could be attributed to the increased oxygen-carrying capacity of younger goats compared to older ones, due to higher metabolic activity of the young animals. The RDW-CV (RDW-CV = [RDW-SD/MCV] × 100) declining with age can be explained by poikilocytosis, which seems to be more present in young animals (Agradi et al., 2022). Although RDW-SD did not show the same trend, this difference lies in the calculation methods for both parameters.

Most studies mentioned generally aligned with our findings, but a few reported different results. For instance, some studies found that age significantly influences HGB values (Agradi et al., 2022) and platelet indices (Arfuso et al., 2016), which were not observed in this study. Additionally, Arfuso et al. (2016) reported that MCV decreases with age, while MCHC and HCT increase, which was the opposite of our findings.

To assess the influence of sex, the goats were divided into males and females. The mean EOS (%), BAS (%), MCV, and MCH were higher in females, while mean RBC, HCT, HGB, and MCHC were superior in males. In the other parameters, statistically significant differences were not determined.

A study performed on Hair goats in 2022 concluded that sex had an influence on MCV and MCH (higher in females) and on MHCH and RDW (elevated in males) (Karaşahin et al., 2023). In Angora goats, the RBC was higher in males than in females, and sex did not influence the platelet indices (Polat et al., 2018). We found the same differences in RBC, MCV, MCH, and MHCH in our study, and we also concluded that sex does not have an influence in platelet parameters. However, we did not find statistically significant variations between male and female regarding the RDW. A clear explanation for the higher values in males, particularly in RBC, HCT, and HGB, is the stimulating effect of androgens on erythropoiesis.

An interesting study carried out in 2023 investigated not only the effect of sex on the hematological values but also the effect of breeding season (Karaşahin et al., 2023). That study found that RBC, HGB, HCT, MCH, and MCHC were higher in males than in females, but also that some of these were only statistically different in the breeding season. The results suggested that males mate more frequently and require more oxygen due to higher liveliness. Greater physical activity occurs during mating, and the generated heat raises the metabolic rate and triggers bodily changes, such as thermoregulation and increased erythrocyte production from the spleen to meet the oxygen deficit. Additionally, respiratory capacity, cardiac output, and heart rate must rise to supply more blood to muscles. These factors lead to a general increase in the erythrocyte indices during the breeding season (Karaşahin et al., 2023).

Although we found statistically significant differences between the two sexes, the RIs established for males should be considered with caution because, as the number of studied individuals was low (n=9), we cannot state that our sample was representative of the Preta de Montesinho male population. To improve the accuracy of these results, similar studies with a higher number of individuals are needed. Nevertheless, in a breed that only had 73 males registered in 2024, increasing the number of these animals while maintaining the criteria of selection (healthy, dewormed, animals with known medical history) is very difficult to achieve.

The impact of seasons was also analyzed. The sampling was performed in three different months, i.e., June and November of 2023 and January of 2024. Initially, a statistical analysis was conducted on samples collected in November and January, as weather conditions did not vary significantly during this period (late fall to early winter). The analysis confirmed that there were no statistically significant differences. Therefore, the 136 animals in this study were divided into two groups: cold season, which included samples collected in November and January and warm season, which corresponded to samples from June.

Statistically significant differences were found in seven parameters: mean RBC, HGB, HCT, MCV and MCH, that were higher in the cold season, and mean RDW-CV and MPV, which were lower in the cold season. Our findings were validated by other studies (Polat et al., 2018; Singh Nathawat et al., 2023).

Most erythrocyte indices were lower during the warm season than in the cold season. This phenomenon can be attributed to hemodilution, as it is documented that goats exposed to high temperatures increase their water consumption, leading to expansion of blood plasma volume (Polat et al., 2018). Additionally, high temperatures are known to cause peripheral vasodilation and redistribution of cardiac output, further contributing to hemodilution (Al-Haidary, 2004). Finally, another study (Habibu at al., 2017) indicated that oxidative stress caused by high ambient temperatures could denature and precipitate hemoglobin molecules in erythrocytes, leading to their degradation. This could also explain the lower values observed (Habibu at al., 2017).

Another explanation for the erythrocyte indices differing in warm and cold seasons could be the indirect influence of heat stress on the satiety center: thermal stress activates the rostral cooling center of the hypothalamus, which subsequently stimulates the medial satiety center (Yaqub et al., 2013). This stimulation inhibits the appetite center, resulting in decreased feed intake, which can cause nutritional deficits that could influence on the blood parameters (Yaqub et al., 2013). Another study indicated that erythrocyte values are generally elevated during colder seasons compared to warmer ones. This increase is attributed to the heightened use of concentrate feeding, which typically occurs in winter months when animals are often kept indoors or require supplemental feed due to inadequate pasture availability (Singh Nathawat et al., 2023). High erythrocyte levels may also be due to high metabolic rates in winter that stimulate erythropoiesis (Singh Nathawat et al., 2023).

Most researchers also found seasonal differences in WBC and some leukocyte parameters (Agradi et al., 2022; Ghosh et al., 2013; Polat et al., 2018), but in our study, we concluded that the season did not have a statistically significant impact on leukocyte indices. This could be explained by the smaller number of samples from the warm season (n=30), which could be insufficient to observe significant differences. Moreover, it would be interesting to repeat this study with samples collected in each season while also recording some relevant parameters related to the season, such as temperature, relative humidity, and temperature humidity index, to determine if more differences would be found.

Our study population consisted of 136 animals, which is an acceptable sample size considering that there are fewer than two thousand individuals of the breed. Nevertheless, it is important to consider the potential limitations of this research, especially when studying the impacts of age, sex, and season. The statistical analysis is less robust in groups with $n < 40$ (Geffré et al., 2011). In our study, a few RIs could not be computed due to the small sample size. While other RIs are provided, they should be interpreted with caution, as 1 to 3 potential outliers were identified, significantly affecting the RIs because of the limited sample size (Geffré et al., 2011).

Using a greater number of samples and involving other Preta de Montesinho goat farmers in the study could have enhanced its external validity. However, it is crucial to address biases, such as farm management systems and health conditions, as these could greatly affect hematological parameters in the Preta de Montesinho goat. Therefore, all animals included in future studies to establish hematological norms should be fed similarly, exposed to the same environmental and agricultural practices, and undergo regular deworming and veterinary evaluations.

CONCLUSION

Hematological reference intervals are a tool for preserving endangered animal breeds, and these results demonstrate that the RIs available in the literature are somewhat inadequate for the Preta de Montesinho goat. We found that younger Preta de Montesinho animals have higher WBC count, and RBC and MCHC than older goats, and males have higher erythrocyte indices than females. Seasons impact the animals' erythrocyte indices, these being generally lower in summer than in winter.

This study established reference values that could assist veterinarians in more accurately interpreting laboratory data and monitoring the health status of Preta de Montesinho goats, which could be a useful tool to help promote this breed's management and conservation efforts.

Acknowledgements

This work was funded by 0687_OVISPID_2_E Interreg España-Portugal (EU) Poctep

Authors' contributions

Conceptualization: HQ; methodology: RV and HQ; validation: JJF, RV, PPC and HQ; formal analysis: RSM and HQ; investigation: RSM, JJF, LS, RV, PPC and HQ; resources: PA, HQ, LC and ACC; data curation: RSM and HQ; writing – original draft preparation: RSM ; writing – review and editing: RSM, PPC and HQ; visualization – RSM, JJF, LS, RV, PPC and HQ; supervision: RV, PPC and HQ; project administration: HQ; funding acquisition: HQ. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

ORCID iDs

Rafaela S. S. Moreira https://orcid.org/0009-0003-8852-0608
João Jacob-Ferreira https://orcid.org/0000-0002-6683-4820
Liliana Santos https://orcid.org/0009-0009-6312-6552
Ramiro Valentim https://orcid.org/0000-0002-3514-988X
Paulo P. Cortez https://orcid.org/0000-0001-7132-6786
Hélder Quintas https://orcid.org/0000-0002-6934-1669

REFERENCES

Agradi, S., Menchetti, L., Curone, G., Faustini, M., Vigo, D., Villa, L., Zanzani, S.A., Postoli, R., Kika, T.S., Riva, F., Draghi, S., Luridiana, S., Archetti, I., Brecchia, G., Manfredi, M.T., Gazzonis, A.L. 2022. Comparison of Female Verzaschese and Camosciata delle Alpi Goats' Hematological Parameters in The Context of Adaptation to Local Environmental Conditions in Semi–Extensive Systems in Italy. Animals. 12(13), 173. https://doi.org/10.3390/ani12131703.

Al-Bulushi, S., Shawaf, T., Al-Hasani, A. 2017. Some hematological and biochemical parameters of different goat breeds in Sultanate of Oman "A preliminary study." Veterinary World. 10(4), 461–466. https://doi.org/10.14202/vetworld.2017.461-466.

Al-Haidary, AA. 2004. Physiological responses of Naimey sheep to heat stress challenge under semi-arid environments. Int J Agri Biol. 6(2):307–309.

Antunović, Z., Novaković, K., Klir, Ž., Šerić, V., Mioč, B., Šperanda, M., Ronta, M., Novoselec, J. 2020. Blood metabolic profile and acid-base status of Istrian goats – A critically endangered Croatian goat – in relation to age. Veterinarski Arhiv. 90(1), 27–38. https://doi.org/10.24099/vet.arhiv.0780.

Arfuso, F., Fazio, F., Rizzo, M., Marafioti, S., Zanghì, E., Piccione, G. 2016. Factors affecting the hematological parameters in different goat breeds from Italy. Annals of Animal Science. 16(3), 743–757. https://doi.org/10.1515/aoas-2015-0094.

Bruno-de-Sousa, C., Martinez, A.M., Ginja, C., Santos-Silva, F., Carolino, M.I., Delgado, J.V., Gama, L.T. 2011. Genetic diversity and population structure in Portuguese goat breeds. Livestock Science. 135(2–3), 131–139. https://doi.org/10.1016/j.livsci.2010.06.159.

Carolino, N., De Sousa, C.B., Carolino, I., Santos-Silva, F., Sousa, C.O., Vicente, A., Ginja, C., Gama, L.T. 2016. Biodiversidade caprina em Portugal. In: Bayona, J.E.V., Martínez, L.Z., Bermejo, J.V.D., Galván, G.R. (Eds.), Biodiversidad caprina iberoamericana. Ediciones Universidad Cooperativa de Colombia, pp. 57-74.

Edjtehadi, M. 1978. Age-associated changes in the blood picture of the goat. Zentralblatt Für Veterinärmedizin Reihe A. 25(3), 198–206. https://doi.org/10.1111/j.1439-0442.1978.tb00920.x.

FAO—Food and Agriculture Organization of the United Nations. World Livestock: Domestic Animal Diversity Information System (DAD-IS). 2023. https://www.fao.org/dad-is/browse-by-country-and-species/en/ (accessed on 24 May 2024).

Geffré, A., Concordet, D., Braun, J.P., Trumel, C. 2011. Reference Value Advisor: A new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Veterinary Clinical Pathology. 40(1), 107–112. https://doi.org/10.1111/j.1939-165X.2011.00287.x.

Ghosh, S., Singh, A.K., Haldar, C. 2013. Adaptive and ecological significance of the seasonal changes in hematological, biochemical and hormonal parameters in the tropical goat Capra hircus. Journal of Endocrinology and Reproduction. 113–122. https://www.researchgate.net/publication/264342301.

Ginja, C., Cortés, O., Gama, L.T., Delgado, J.V., Amills, M., de Sousa, C.B., Cañón, J., Capote, J., Dunner, S., Ferrando, A., Carpio, M.G., Gómez, M., Jordana, J., Landi, V., Manunza, A., Martin-Burriel, I., Barro, P.A., Rodellar, C., Santos-Silva, F., Martínez, A.M. 2018. Conservation of goat populations from southwestern Europe based on molecular diversity criteria. In: Sustainable Goat Production in Adverse Environments. Vol. 1, Springer International Publishing, pp. 509–533. https://doi.org/10.1007/978-3-319-71855-2_29.

Habibu, B., Kawu, M., Makun, H., Aluwong, T., Yaqub, L., Dzenda, T., Buhari, H. 2017. Influences of breed, sex and age on seasonal changes in haematological variables of tropical goat kids. Archives Animal Breeding. 60(1), 33–42. https://doi.org/10.5194/aab-60-33-2017.

Johns, J., Heller, M. 2021. Hematologic Conditions of Small Ruminants. Veterinary Clinics of North America – Food Animal Practice. 37(1), 183–197. https://doi.org/10.1016/j.cvfa.2020.10.004.

Karaşahin, T., Aksoy, N.H., Dursun, Ş., Bulut, G., Haydardedeoğlu, A.E., Çamkerten, İ., İlgün, R. 2022. Effects of age and sex on some hematological and biochemical parameters in Hair goats. Veterinary Research Forum. 13(1), 15–19. https://doi.org/10.30466/vrf.2020.120090.2841.

Martínez, A.M., Gama, L.T., Delgado, J.V., Canõn, J., Amills, M., de Sousa, C.B., Ginja, C., Zaragoza, P., Manunza, A., Landi, V., Sevane, N. 2015. The Southwestern fringe of Europe as an important reservoir of caprine biodiversity. Genetics Selection Evolution. 47(1). https://doi.org/10.1186/s12711-015-0167-8.

Mbassa, G.K., Poulsen, J.S.D. 1992. The comparative haematology of cross-bred and indigenous East African goats of Tanzania and breeds reared in Denmark. Veterinary Research Communications. 16.

Mohammed, S.A., Razzaque, M.A., Omar, A.E., Albert, S., Al-Gallaf, W.M. 2016. Biochemical and hematological profile of different breeds of goat maintained under intensive production system. African Journal of Biotechnology. 15(24), 1253–1257. https://doi.org/10.5897/ajb2016.15362.

Mpofu, T.J., Nephawe, K.A., Mtileni, B. 2020. Gastrointestinal parasite infection intensity and hematological parameters in South African communal indigenous goats in relation to anemia. Veterinary World. 13(10), 2226–2233. https://doi.org/10.14202/vetworld.2020.2226-2233.

Nathawat, S.M., Gupta, B., Gottam, G., Singh, M. 2023. Profiling of hematological parameters due to seasonal variation in Sirohi goat. The Pharma Innovation Journal. 7, 1890–1895. www.thepharmajournal.com.

Newcomer, B.W., Cebra, C., Chamorro, M.F., Reppert, E., Cebra, M., Edmondson, M.A. 2021. Sheep, Goat, and Cervid Medicine. 3rd ed., Elsevier.

Stayt, J, 2022. Hematology of Sheep and Goats, in: Brooks, M.B., Harr, K.E., Seelig, D.M., Wardrop, K.J., Weiss, D.J. (Eds.), Schalm's Veterinary Hematology. 7th ed., Wiley Blackwell. https://doi.org/10.1002/9781119500537.fmatter.

Piccione, G., Monteverde, V., Rizzo, M., Vazzana, I., Assenza, A., Zumbo, A., Niutta, P.P. 2014. Reference intervals of some electrophoretic and haematological parameters in Italian goats: Comparison between Girgentana and Aspromontana breeds. Journal of Applied Animal Research. 42(4), 434–439. https://doi.org/10.1080/09712119.2013.875914.

Polat, H., Pehlivan, E., Dellal, G. 2018. Annual change of haematological parameters in Angora goats. Biological Rhythm Research. https://doi.org/10.1080/09291016.2018.1557850

Pospisil, J., Kaset, F., Vahala, J. 1987. Basic haematological values in the Cameroon goat (Capra hircus). Biochem. Physiol. 88(3).

Sharkey, L.C., Burton, E.N., Heinrich, D.A., Johns, J.L., Heller, M. 2020. Large Animal Internal Medicine. 6th ed., Elsevier.

Shawaf, T., Al Bulushi, S., Al-Ali, M.A., Meligy, A.M.A., Salouci, M., Hussen, J. 2021. Investigation of some trace elements and hematological and biochemical parameters in the blood of emaciated Omani goats. Veterinary World. 14(7), 1960–1965. https://doi.org/10.14202/vetworld.2021.1960-1965.

Silva, G., Queiroga, F., Ferreira, M., Andrade, D., Silvestre-Ferreira, A.C. 2023. Establishment of reference intervals of hematological parameters and evaluation of sex and age effect in the Miranda Donkey. Animals. 13(14). https://doi.org/10.3390/ani13142331.

Smith, M.C., Sherman, D.M. 2023. Goat Medicine. 3rd ed., John Wiley & Sons.

Sociedade Portuguesa de Recursos Genéticos Animais [SPREGA]. 2024. https://www.fao.org/dad-is/browse-by-country-and-species/en/ (accessed on 18 April 2024).

Somvanshi, R., Biswas, J.C., Sharma, B., Koul, G.L. 1987. Haematological studies on Indian pashmina goats. Research in Veterinary Science. 42(1), 124–126. https://doi.org/10.1016/S0034-5288(18)30667-2.

Yaqub, L.S., Kawu, M.U., Ayo, J.O. 2013. Influence of reproductive cycle, sex, age and season on haematologic parameters in domestic animals: A review. Journal of Cell and Animal Biology. 7(4), 37–43. https://doi.org/10.5897/jcab12.063.

USPOSTAVLJANJE REFERENTNIH INTERVALA HEMATOLOŠKIH PARAMETARA I PROCENA UTICAJA STAROSTI, POLA I GODIŠNJEG DOBA KOD KOZA RASE PRETA DE MONTESINHO

Rafaela S. S. MOREIRA, João JACOB-FERREIRA, Liliana SANTOS, Ramiro VALENTIM, Paulo P. CORTEZ, Hélder QUINTAS

Kratak sadržaj

Koza rase Preta de Montesinho je autohtona portugalska rasa koja se klasifikuje kao ugrožena. Autohtone rase treba očuvati jer su obično dobro prilagođene specifičnim regionalnim uslovima, pokazuju otpornost na određene bolesti i predstavljaju važne rezervoare jedinstvene genetske raznovrsnosti. Kod rase poput Preta de Montesinho, očuvanje zdravlja životinja kako bi se obezbedila njihova reprodukcija i povećao broj jedinki od suštinskog je značaja. Stoga je razumevanje referentnih intervala hematoloških parametara posebno važno. Cilj ove studije bio je da se odrede referentni intervali hematoloških parametara za rasu Preta de Montesinho i proceni potencijalni uticaj starosti, pola i godišnjih doba na ove intervale.

Ukupno 136 uzoraka krvi prikupljeno je od zdravih jedinki. Uzorci su analizirani pomoću automatskog hematološkog analizatora Mindray BC–5000 Vet®, a referentni intervali za 23 parametra krvi utvrđeni su korišćenjem softvera Reference Value Advisor v2.1. Za statističku analizu razlika između definisanih grupa korišćen je softver JMP®.

U pogledu starosti, statistički značajne razlike ($p < 0,05$) između mladih i starih koza zabeležene su kod 15 parametara. Pol je značajno uticao na osam parametara, od kojih je šest bilo indeksa eritrocita. Godišnje doba (leto ili zima) uticalo je na sedam parametara, uključujući šest indeksa eritrocita. Ovde opisani referentni intervali mogu služiti kao alat za identifikaciju bolesnih životinja, procenu odgovora na terapije i odabir zdravih životinja za reprodukciju, čime se doprinosi očuvanju ove rase.

Ključne reči: hematologija; koze; Preta de Montesinho; refererntni intervali