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Establishment of Reference Intervals of Hematological Parameters and Evaluation of Age, Sex and Season Effect in the Preta de Montesinho Goat

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Área científica: Medicina e cirurgia de animais de produção e sanidade animal

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RESUMO

Um hemograma completo constitui uma série de testes que analisam as células sanguíneas, fornecendo informações sobre o número, tamanho, forma e amplitude de distribuição das mesmas. Trata-se de um teste básico, mas essencial, que os Médicos Veterinários podem realizar para obter rapidamente informações importantes sobre o perfil hematológico de um animal ou rebanho. A cabra Preta de Montesinho é uma raça autóctone portuguesa que atualmente está classificada como ameaçada. As raças autóctones devem ser preservadas porque, regra geral, são os animais que melhor estão adaptados às condições específicas da sua região, exibindo geralmente resistência a certas doenças, além de serem importantes reservatórios de diversidade genética única. Para uma raça em risco de extinção, como a Preta de Montesinho, manter a saúde dos animais para garantir a sua reprodução e aumentar o número de exemplares é essencial. Portanto, estabelecer os intervalos de referência hematológica é particularmente importante. O objetivo desta tese foi determinar os intervalos de referência hematológica para a raça Preta de Montesinho e avaliar a possível influência da idade, sexo e estações do ano nesses intervalos. Foram analisadas 136 amostras de sangue recolhidas de animais saudáveis e os intervalos de referência para 23 parâmetros sanguíneos foram estabelecidos. Em relação à idade, foram detetadas diferenças estatisticamente significativas ($p < 0,05$) em 15 parâmetros. O sexo influenciou significativamente 8 parâmetros, dos quais 6 eram índices de eritrócitos. A estação do ano afetou 7 parâmetros, dos quais 6 eram índices de eritrócitos. Os intervalos de referência descritos aqui podem servir como ferramenta para identificar animais doentes, avaliar a resposta a terapias e selecionar animais saudáveis para a reprodução, contribuindo assim para a preservação da raça.

PALAVRAS-CHAVE: Hematologia, caprinos, Preta de Montesinho; intervalos de referência.

ABSTRACT

The complete blood count (CBC) is a series of tests that analyze blood cells, providing information about their number, size, shape, and distribution width. It is an essential and basic test that veterinarians can perform to quickly gain valuable insights into the hematological profile of an animal or herd. The Preta de Montesinho goat is an autochthonous Portuguese breed that is currently classified as endangered. Autochthonous breeds should be preserved because they are usually best adapted to their specific regional conditions, exhibit resistance to certain diseases, and serve as important reservoir 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. The goal of this thesis was 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. Blood samples were collected from 136 healthy animals and the reference intervals (RI) for 23 blood parameters were established. Regarding age, significant statistical differences ($p < 0.05$) were detected in 15 parameters. Sex significantly influenced 8 parameters, 6 of which were erythrocytes indices. Season affected 7 parameters, 6 of which were erythrocytes indices. The RIs described here can serve as tools for identifying sick animals, evaluating their response to therapy, and selecting healthy animals for reproduction, thereby contributing to the preservation of the breed.

KEYWORDS: Hematology; goats; Preta de Montesinho; reference intervals.

CASE-LOG

The first internship lasted 13 weeks and took place in the laboratory of Microbiology and Infectious Diseases (ICBAS) under the supervision of Professor João Mesquita. During this time, I primarily worked with ticks from Brazil and Luxembourg and collected fecal samples from zoo animals. I learned several laboratory techniques, including DNA extraction, PCR, and sample purification. Throughout this period, we screened the ticks for various tick-borne diseases and tested the fecal samples for seven zoonotic protozoa. As a result of these studies, I had the chance to write an article and contribute to others.

The second internship lasted 3 weeks and took place in Barcelos with CapêloVets LDA. During this time, I mostly accompanied veterinarians on routine consultations and clinical and surgical emergencies of farm animals. The activities I performed are summarized in Table 1.

The third internship, with the team from the Organização de Produtores para a Sanidade Animal (OPSA) in Bragança and at Centro Pedagógico Veterinário in Instituto Politécnico de Bragança (IPB), had the duration of 7 weeks, 4 of which were part of the curricular internship. Unfortunately, my time here did not go as planned because the start of the infectious disease eradication and surveillance program for 2024 was delayed by DGAV. Due to this issue, I spent more time at IPB, where I had the chance to observe some of the institution's work on reproduction programs, carry out part of the practical work for this thesis, and conduct fecal testing for parasites in the laboratory. Table 2 summarizes the activities performed at OPSA and table 3 the activities done in IPB.

The fourth and final internship lasted 12 weeks and took place in Cahir, Ireland, at Cahir Veterinary Clinic. This practice had two branches and a total of six Veterinarians dedicated to medicine and surgery of livestock animals where I did reproductive medicine and clinical and surgery emergencies. I also gained experience in small animal's medicine and participated in a four-day DIY insemination course (two days theorical and two days practical). Additionally, I spent two weeks on a sheep farm during lambing season where I had a chance to see and assist in multiple lambing's but also help in feeding the lambs and sheep and cleaning of the facilities. Table 1 summarizes the work related to farm animals during those 12 weeks and table 4 describes the activities carried out in medicine and surgery of small animals.

Table 1. Activities related to medicine and surgery of large animals.

		A	B
Bovine			
Theriogenology	Dystocia	2	2
	Reproductive ultrasound	91	195
	Metritis	1	19
	Vaginal prolapse	1	1
	Uterine prolapse	–	2
	Pyometra	–	1
	PRIDS	–	194
Gastroenterology	Neonatal diarrhea	8	2
	Simple indigestion	6	1
	Suspected vagal indigestion	1	1
	Gastric impaction in calf	–	2
	Suspected intussusception/intestinal volvulus	1	–
	Suspected foreign body	1	–
	Abomasal ulcer	1	–
	Acute ruminal acidosis	2	–
Pneumology	Pneumonia in calves	18	3
	Pneumonia in adults	3	3
Metabolic Disorders	Hypocalcemia	9	1
	Ketosis	3	–
Udder	Mastitis	13	10
	Milk microbiological control	28	–
Surgery	Right displaced abomasum	2	–
	Left displaced abomasum	5	–
	Castration	4	2
	Dehorning (wire/saw)	4	23
	Disbudding	–	118
	Excision of supernumerary teat	–	4
	Digital amputation	–	1
Musculoskeletal	Limb fracture	2	2
	Musculoskeletal injury in limb	2	1
	Hygroma	1	–
	Traumatic tail injury	1	1
	Traumatic spine injury	1	1
Neurology	Suspected Cauda equina syndrome	1	–
	Suspected meningitis	1	–
	Obturator nerve injury	2	–
Prophylaxis	Vaccination	110	46
	Deworming	10	–
	Blood sampling for mineral analysis	–	43
	Tuberculosis testing	–	1036
Other	Abscess drainage	3	1
	Conjunctivitis in calf	–	1
	Herd health calls	–	3
	Blood sampling for disease screening	–	167
	Euthanasia	3	1
	Necropsy	3	–
	Blood transfusion	1	–
	Beet intoxication	–	1
Dogs			
	Vaccination	6	–
Sheep			
	Deworming	8	–
	Suspected meningitis	1	–
	Limb fracture	–	1
	Vaccination	–	99*
	Hoof trimming	–	15*
	Lambing	–	53*
	Vaginal prolapse	–	1*
Goats			
	Vaccination	25	–
	Mastitis	2	–
Horses			
	Vaccination	–	2
	Hoof trimming + hoof fitting	–	3
	Laminitis	–	1
	Heart murmur	–	1
Chicken			
	Enteritis	–	1

A: CapêloVets LDA, in Barcelos, B: Cahir Veterinary Clinic, Ireland. *Performed in Ireland during the stay at the sheep farm.

Table 2. Activities performed at OPSA (Bragança).

Bovine	
Vaccination (Bluetongue)	90
TPM (Pre-Movement Tests)	13
Deworming and ectoparasite control (Pour-on)	15
Identification of calves (ear tag)	2
Abscess drainage	1
Sheep	
Vaccination (Bluetongue)	1370
Vaccination (Enterotoxemia)	558
Blood collection for brucellosis testing	1044
Deworming and ectoparasite control (PO)	512
Deworming and ectoparasite control (IV)	518
Identification of lambs (ear tag and ruminal bolus)	44
Replacement of lost/damaged tags	36
Goats	
Vaccination (Enterotoxemia)	47
Blood collection for brucellosis testing	99
Deworming and ectoparasite control (Pour-on)	105
Deworming and ectoparasite control (IV)	47
Identification of kids (ear tag and ruminal bolus)	5
Replacement of lost/damaged tags	5
Vaginal prolapse	1
Horses	
Microchipping	4
Marking	5
Dogs	
Vaccination (Rabies)	7
Microchipping	5

Table 3. Activities performed at IPB.

IPB	
Ovariectomy	1
Complete blood count	120
Fecal collection	30
Conical Baermann glass method	29
Willis's method	22
Mini-FLOTAC® technique	26
Fecal sedimentation technique	18

Table 4. Activities related to medicine and surgery of small animals.

Dogs and cats		
Surgery	Spay/neuter	4
	Dental	3
	Removal of testicular mass	1
	Removal of ear	1
	Removal of eyelid papilloma	1
Routine consultations	Vaccination	21
	Librela® appointment	10
	Cytopoint® appointment	3
	Anal gland expression	2
	Annual check-up	1
	Nail trimming	3
	Passport appointment	3
Internal medicine	Gastroenteritis	3
	Foreign body	2
	Kennel cough	1
	Bronchopneumonia	2
	Diabetes	3
	Acute renal failure	4
	Auricular polyps	1
	Otitis	7
	Dermatological consultation	7
	Oncological consultation	5
	Eye ulcer	1
	Perianal hernia	1
	Autoimmune hemolytic anemia	1
	Suspected poisoning	1
	Traumatic emergency (hit by car)	3
	Traumatic emergency (fall)	2
	Limb laceration	1
	Traumatic oral injury	1
	Seroma	2
	Follow-up consultation	10
Other	X-ray	7
	Ultrasound	4
	Blood sampling	15
	Insulin curve	5
	Blood transfusion	1
	Euthanasia	3
Hamster		
	Pneumonia	1

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BAS	Basophils
CAP	Confederação dos Agricultores de Portugal
CBC	Complete blood count
CI	Confidence interval
CO_2	Carbon dioxide
DGAV	Direção Geral de Alimentação e Veterinária
DIY	Do it yourself
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EOS	Eosinophils
FAO	Food and Agriculture Organization of the United Nations
fL	Femtoliter
HCT	Hematocrit
HGB	Hemoglobin
IPB	Instituto Politécnico de Bragança
LRL	Lower reference limit
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MON	Monocytes
MPV	Mean platelet volume
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate

NEU	Neutrophils
NK	Natural killer cell
LYM	Lymphocytes
OPSA	Organização de Produtores para a Sanidade Animal
PCR	Polymerase chain reaction
PCT	Plateletcrit
PCV	Packed cell volume
PDW	Platelet distribution width
pg	picograms
PRID	Progesterone releasing intravaginal device
RBC	Red blood cell count
RDW	Red cell distribution width
RDW-CV	Red cell distribution width – coefficient of variation
RDW-SD	Red cell distribution width – standard deviation
RI	Reference interval
SD	Standard deviation
SPREGA	Sociedade Portuguesa de Recursos Genéticos Animais
URL	Upper reference limit
WBC	White blood cells
µm	Micrometer

1. INTRODUCTION

1.1. Introduction and aim of the study

Hematology is the field of medical sciences that studies the blood and blood-forming tissues and is considered an integral part of clinical laboratory diagnostics in veterinary medicine. The complete blood count (CBC) is a collection of tests that analysis blood cells, such as red blood cells, platelets, and white blood cells (lymphocytes, neutrophiles, basophiles, monocytes and eosinophiles). It gives information about the number of each cell, size, shape, and distribution width. It also determines the hemoglobin content of erythrocytes and identifies immature and abnormal blood cells. In small ruminants, the CBC must be performed with unclotted whole blood (the blood must be added to EDTA because other anticoagulants can create dilution errors or interfere with morphologic analyses) and, if it is not possible to analyze the samples right away, they should be refrigerated for a maximum of 24 hours. However, blood smears must be prepared immediately (Johns & Heller, 2021). The venipuncture site should be easy and safe to access (in goats, the jugular vein is the most used) (Newcomer et al., 2021). A good technique of sampling and a calm animal is very important because, if these conditions are not ensured, the results may not correspond to the real blood cells values due to artifactual changes like iatrogenic hemolysis, tissue thromboplastin contamination and platelet activation (Polizopoulou, 2010; Sharkey et al., 2020).

The CBC is an important and basic test that every veterinarian can perform to quickly obtain valuable information about the hematological profile of an animal or herd. Combined with a physical exam and other diagnostic tests, it may be enough to obtain a diagnosis, resulting in a treatment plan and/or a prognosis (Polizopoulou, 2010).

Based on the knowledge of a CBC of a healthy animal, we can determine when an animal is sick. That is why it is important to characterize the blood parameters of animals and even breeds because it is known that the breed is a factor that may alter the values considered normal for a species (Elmaz et al., 2012; Nicoleti et al., 2023). When a breed is at severe risk of extinction, this knowledge becomes even more significant because if we do not know the blood parameters, it is hard to identify an anemia or other blood diseases or even some disease or disorder that affects, for example, white blood cells.

Although goat farming has declined over the last decades, the Iberian Peninsula still represents almost 25% of the European goat census. In a small country like Portugal, that has 6 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. Among them, goats stand out as 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. As 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.

The Preta de Montesinho goat is an autochthonous Portuguese breed that is currently classified by the Food and Agriculture Organization of the United Nations (FAO) as endangered-maintained breed, which means that this goat is in risk of extinction but is being maintained by an active public conservation program or within a commercial or research facility. 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 preservation of the breed (Silva et al., 2023).

With that in mind, the goal of this thesis was to enhance the understanding of the least represented Portuguese goat breed, the Preta de Montesinho, by determining the hematological reference intervals (RI) and assessing the potential influence of sex, age, and seasons on these intervals.

1.2. Preta de Montesinho goat

The Preta de Montesinho goat (Figure 1 and 2) is the most endangered autochthonous Portuguese breed. According to a census conducted in 2024 by the Sociedade Portuguesa de Recursos Genéticos Animais (SPREGA), there were 1878 females and 73 males within 45 farmers. For reference, the same institution said that, in the same year, there were 12846 females and 321 males of the Serrana breed, the most represented Portuguese goat.

The Preta de Montesinho is a medium-sized goat, height at the withers in females is 66 cm and in males 77 cm. The coat is short, dark (dark brown to black), smooth and shiny. The head is consistently long, with a straight profile and a narrow and slightly domed forehead and a wide chamfer. The ears are usually semi-pendent and the horns, when present, are small, with a triangular base, smooth, directed backwards with parallel or slightly divergent stems. The neck is long and lightly muscled, the backline is almost straight, drooping croup and short tail. The torso is slightly arched and the abdomen regularly developed. The udder is well developed with long teats hanging down or slightly directed forward (Associação Nacional de Caprinicultores da Raça Serrana, 2016).

Figure 1. Preta de Montesinho goat in IPB.

Figure 2. Preta de Montesinho goat in IPB.

The name has a connection with the color of the fur (black) and with the Montesinho Natural Park that is in the northeast region of Trás-os-Montes, but in the past was also known as Antiga, Galega, Bragançana or Preta. Although this goat is in extreme danger of extinction, in the last few years, we have seen a slow but steady increase in the numbers of representatives of this breed not only in their natural territory but also south of that region.

The Preta de Montesinho goat has only been recognized as a breed since 2009. In 1998, during the implementation of the herd book for the Bravia breed, another Portuguese goat, it was observed that some animals in remote areas did not exhibit the characteristics of the Bravia goat, nor of any other recognized or ancestral breed. Consequently, in 1999 and again in 2004, studies were conducted on these animals, characterizing them genetically, morphologically, and functionally. Between 1999 and 2004, there was a significant decline in the number of animals and farmers, accompanied by increasing mischaracterization, likely due to the introduction of males from different breeds. Over the years, the population continued to dwindle due to factors such as desertification, aging farmers, reduced milk collection in the area, and the closure of many cheese shops, leading to the

disappearance of most large farms (Confederação dos Agricultores de Portugal & Direcção Geral de Alimentação e Veterinária, 2021).

Fortunately, in 2009, when the Preta de Montesinho became a recognized breed, farmers began receiving government support, primarily in the form of financial aid, which helped slow the decline of this breed.

Previously, there were two production methods: one focused on meat and the other on milk. Animals selected for meat production were smaller and grazed in mountainous areas with scarce food resources. In contrast, more fertile land was reserved for animals kept for milk to boost production. However, the isolation of these geographical areas made it difficult to sell the products, eventually leading to the near disappearance of the breed. For a time, this goat was also kept near residential areas, earning the nickname “the poor man's milking cow”. Nowadays, some farmers keep this goat for dual purposes (meat and milk), while small groups are integrated into farms that also have sheep, primarily herds of Churra Galega Bragançana, an autochthone Portuguese sheep (CAP & DGAV, 2021).

1.3. Blood cells

1.3.1 Erythrocytes

Erythrocytes or red blood cells, are the most common cells in the blood and are produced in the bone marrow by a process called erythropoiesis that takes approximately five days and is influenced by the secretion of erythropoietin, activated in response to blood loss or hemolysis. Succinctly, cells in the kidney monitor the oxygen level in the blood and if there is a decrease, these cells release erythropoietin that increases the erythrocytes production in healthy bone marrow. If the demand for erythrocytes is high, they may be released in the blood stream in an immature phase, the reticulocytes. Ruminants, unlike dogs and cats, do not present reticulocytes in the peripheral blood of healthy animals. In goats, the average life span of erythrocytes is about 125 days (Smith & Sherman, 2023). Removal of old or damaged erythrocytes occurs mostly in the spleen but also in the liver and bone marrow. Some are also eliminated by random clearance independently of cell age, by a mechanism that is not clearly understood (Olver et al., 2022).

Red blood cells are round, without nucleus and other organelles and stain pink to red with most common laboratory stains. As they do not have organelles, they cannot synthesize proteins, nucleic acids or produce energy via Krebs cycle and oxidative phosphorylation but they still need energy in the form of ATP, NADH and NADPH to be functional cells (although the energy demand of erythrocytes is

lower when compared to the other blood cells). Thus, the energy needed is supplied by anaerobic glycolysis. Erythrocytes in ruminants are nearly flat, making the central pallor less evident, unlike the biconcave erythrocytes in other animals, which appear pale in the thinner central area on blood smears (Smith & Sherman, 2023). Their cell membrane is flexible to allow erythrocytes to pass through small capillaries. Among all domestic mammal species, goat erythrocytes are the smallest, typically less than 4 µm, and often exhibit unusual shapes (poikilocytosis) and varying sizes (anisocytosis) (Smith & Sherman, 2023). Due to their small size, it is crucial to use a machine validated for goats when performing a CBC to avoid erroneous interpretations, as small erythrocytes may be mistakenly counted as platelets (Polizopoulou, 2010).

The main function of erythrocytes is the transportation of oxygen and carbon dioxide between the lungs and all the other body tissues. Red blood cells are composed of 60 to 65% water, 30 to 35% hemoglobin, with the remaining portion consisting of metabolic enzymes and inorganic material (Voigt & Swist, 2011). Hemoglobin is an iron-porphyrin-protein complex that can bind to four molecules of oxygen. This is because it consists of four heme molecules, each containing an iron porphyrin pigment, attached to globin, a protein made up of two peptide chains of approximately 150 amino acids each. Hemoglobin is synthesized within developing erythrocytes, coordinated with the developmental stages of erythroid precursors. Hemoglobin binds or releases oxygen in the tissues depending on the oxygen concentration of their microenvironment. Hemoglobin has a somewhat lesser affinity for carbon dioxide but does transport the excess from the tissues to the lung by the same principle (concentration of CO_2) (Voigt & Swist, 2011).

Anemia is a decrease and polycythemia (less common) an increase of the number, volume and/or hemoglobin content of erythrocytes (Voigt & Swist, 2011). They are not disease entities by themselves but should be considered as features or clinical effects of an underlying disease process. There are multiple types of anemia because there are also many causes and mechanisms, so the CBC and blood smears are essential exams to guide a diagnosis.

In a standard CBC, there are 8 parameters that relate to erythrocytes: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW) and reticulocyte count. A blood film should always follow a CBC to morphologically evaluate erythrocytes, to detect the presence of blood parasites and to ensure that CBC counts are accurate (Polizopoulou, 2010).

The RBC is the number of erythrocytes in the blood. The HGB is the concentration of hemoglobin and is expressed in g/L. This erythrocyte index is very important as it is the most direct method of determining blood oxygen transport capacity (Brockus et al., 2011). Hematocrit is very

similar to packed cell volume (PCV) as both represent the percentage of the volume of whole blood occupied by erythrocytes. In other words, they indicate the percentage of erythrocytes circulating in peripheral blood. The PCV is determined by centrifugation of a blood sample in a microhematocrit tube and is a simple test with a small inherent error (±1%) (Brockus et al., 2011) while HCT is a calculated number by most automated cell counters after determining the red blood cell count and the mean corpuscular volume (HCT = RBC X MCV). The potential error for the HCT can be higher because the initial automated cell counters were designed for humans, and most mammals have erythrocytes smaller than humans. However, the error ends up being low, as it is in the PCV, as nowadays machines are adapted to most animals. Values below the standard value for these three indices usually means anemia while high values usually related to dehydration and splenic contraction (Brockus et al., 2011).

MCV expresses the average erythrocyte volume expressed in femtoliters (fL). The MCH is the amount of hemoglobin, by weight, per erythrocyte expressed in picograms (pg) and is calculated based on the HGB concentration and the RBC. The MCHC determines the concentration of HBG in the average erythrocyte calculated from HBG concentration and HCT and is considered the most accurate erythrocyte index.

The RDW is the coefficient of variation of the red cell volume distribution. In human medicine, there has been a growing interest in this parameter and several studies have made been to study the influence of age and sex and the prognostic value of the parameter in certain cardiac diseases (Hoffmann et al., 2015). Some automated cell counters divide the RDW in red cell distribution width - standard deviation (RDW-SD) and red cell distribution width - coefficient of variation (RDW-CV). The former is measured as the absolute deviation of the RBC volume distribution curve (in fL, the same unit as MCV), while the former is a relative variation base on the volume of the erythrocytes expressed in percentage (Hoffmann et al., 2015). Most of the time, when only one RDW is measured it is the RDW-CV.

The reticulocyte count is expressed as a percentage, or it can be multiplied by the total erythrocyte count and expressed as reticulocytes per microliter. In goats, as previously mentioned, healthy animals typically have zero reticulocytes in their blood. However, in the case of anemia, this parameter becomes important to assess. The presence of reticulocytes in circulating blood is usually considered a positive prognostic sign of red blood cell regeneration, indicating that the anemia is regenerative (Voigt & Swist, 2011).

1.3.2. White blood cells

The main cells acting in the inflammatory and immune response are the leukocytes or white blood cells. There are 5 types of white blood cells divided in two groups: neutrophils, basophils, and eosinophils, which are granulocytes, and lymphocytes and monocytes, which are mononuclear cells.

In the blood cell count, the leukogram is the part that refers to the leukocytes and gives the overall picture of the cells with the total white blood cell (WBC) count, differential cell counts including the absolute numbers of each leukocyte type. The leukogram is not complete without a microscopic evaluation of the blood film. This evaluation is useful for confirming the WBC count and detecting morphologic alterations, which can assist in guiding the diagnosis alongside the leukogram, other laboratory tests, and the physical exam (Sharkey et al., 2020).

Unlike erythrocytes, most leukocytes do not circulate freely in the blood. Instead, they are found in pools that shift back and forth under disease or hormonal influences, with the blood vessels serving as a means of transportation between them. There are two pools in the bone marrow consisting of white blood cells not yet released into the bloodstream: the proliferation pool, which comprises the leukocytes still in formation, and the storage pool, consisting of mature cells stored in the bone marrow, ready for when needed. Within the blood vessels, there are also two pools: the circulating pool with freely moving cells (these are the cells collected when blood is drawn) and the marginal pool, consisting of cells stuck to or rolling along the walls of small vessels and not freely circulating. In the tissues, there is one pool, the tissue pool, corresponding to the leukocytes that have left the blood and are now functioning in the tissues (Voigt & Swist, 2011).

1.3.2.1. Neutrophils

The neutrophils formation in the bone marrow takes between 3 and 10 days to be concluded. Then, these cells circulate in the blood vessels for 6/7 hours and later they migrate to body tissues where they survive 2/3 days in a healthy animal. However, if needed to be present in the inflammatory response, they only last a few hours (Olver et al., 2022). In most domestic animals, the neutrophils are the most prevalent white blood cell, but they are sometimes surpassed by lymphocytes in ruminants (Smith & Sherman, 2023).

The neutrophils are easily recognized in a blood smear because they have a segmented nucleus with 3 to 5 lobes. The nuclear chromatin is coarse, clumped, and dark stained. The nucleus of a newly released or juvenile neutrophil has a more ribbonlike or band appearance and it stains less intensely. The cytoplasm can be clear or a variation between light blue to pink. The cytoplasmatic granules are

fine and dust-like and stain light pink to gray but are usually too small to be visible on the light microscope (Voigt & Swist, 2011).

These cells are the first line of defense against microorganisms and tissue trauma. Toxins release by pathogenic microorganisms attract neutrophil to the damaged tissue. Next, neutrophils engage in phagocytosis of bacteria and other microorganisms, as well as particulate material, ultimately leading to their destruction by the proteolytic enzymes contained within their granules. The neutrophiles also secrete proinflammatory mediators that call to the site even more leukocytes to control the infection but also to enhance the inflammatory process (Brockus et al., 2011; Olver et al., 2022).

Neutrophilia refers to an abnormal elevation in the neutrophil blood count, often linked with infections, particularly those caused by pyogenic bacteria, as well as inflammation, neoplasia, intoxications, and the presence of corticosteroids and epinephrine, whether endogenous or exogenous. Neutropenia, the decrease in circulating neutrophiles, occurs with bone marrow suppression (some causes are virus, neoplasia, and chemicals), bacterial infections and sequestration of cells in capillary beds in endotoxic, septic, or anaphylactic shock. A temporary neutropenia may be observed in response to infection but the total circulating count usually rebounds rapidly (Brockus et al., 2011; Olver et al., 2022).

1.3.2.2. Eosinophils

The formation of eosinophils is similar to the formation of neutrophiles, although under the influence of distinctive cytokines, and takes between 2 and 6 days. After formation, the majority of the eosinophiles stay in the bone marrow, in the storage pool. When in the blood stream, they circulate between 6 and 10 hours but persist in the tissues for several days (between 2 days and 2 weeks, if anti-apoptotic factors are present) (Olver et al., 2022).

Eosinophils are also granulated cells, with different granules in terms of size, shape, number and staining in the different species. In ruminants, they are small, round, numerous and stain intense red to lilac. The nucleus varies from elongated (band) to bilobed or trilobed, and this appearance is consistent across domestic species. The cytoplasm typically stains light blue (Voigt & Swist, 2011).

Eosinophils were once thought to be significant only in allergic reactions and parasitic infections, but it is now known that they have many functions, some of which are still not fully understood. Eosinophils are attracted to histamine, antigen-antibody complexes, and foreign or degraded body proteins associated with inflammation and allergic conditions, suggesting they may play a role in controlling or regulating these responses. Most parasites also trigger an eosinophil response: these cells bind to the parasites and degranulate, as the parasites are too large to be engulfed.

Eosinophils can also phagocytize, detoxify tissues from chemicals, and contribute to homeostatic and immune functions. They participate in T lymphocyte-mediated immunity by recognizing and presenting microbial, viral, and parasitic antigens, as well as superantigens (Olver et al., 2022) and appear to play some role in coagulation and fibrinolysis by activating some steps in the clotting cascade. (Voigt & Swist, 2011).

Eosinophilia is uncommon in large animals but can occur due to allergies, parasitism, chronic infections, recovery stages of acute infections, and may be associated with an increase in basophils and mast cells. Eosinopenia is rare to determine because normal counts in most animals can be zero. However, absolute eosinopenia has been associated with stress and corticosteroids (Brockus et al., 2011).

1.3.2.3. Basophils

Basophils are rare cells, making them the least understood type of leukocyte. The formation, in the bone marrow, is similar to the other leukocytes, taking about 2 days and a half. After, they circulate in the blood stream for 6 hours and the lifespan in the tissues is up to 2 weeks (Olver et al., 2022).

The basophil has an elongated, often coiled nucleus that may be partially segmented. Its cytoplasm stains gray to blue, and in ruminants, it is completely filled with dark purple to nearly black basophilic granules that obscure the nucleus. In terms of numbers, ruminants and horses tend to have more basophils compared to other animals (Voigt & Swist, 2011). Basophils and mast cells are effector cells of immediate hypersensitivity reactions and allergic diseases. They have a minimal phagocytic potential but still have a protective role against helminths, because they stimulate the T lymphocytes. Basophils also respond along with the eosinophils in multiple functions and provide protective functions with roles in innate immunity, host defense and inflammation (Brockus et al., 2011; Olver et al., 2022).

An increase in basophils may be associated with eosinophilia (common in allergic reactions and parasites) and corticosteroids while basopenia is physiological (Olver et al., 2022).

1.3.2.4. Lymphocytes

This leucocyte has a more complex formation than the others: there are various sites of production and maturation such as bone marrow, lymph nodes, spleen, gut-associated lymphoid tissue (Peyer's patches, tonsils, and appendix) and thymus in fetuses and neonates. There are short lived and

long lived or memory lymphocytes, with lifespan varying between days to up 20 years. Unlike other leukocytes, lymphocytes have significant potential for proliferation and recirculate among tissues in a process called “trafficking” (Sharkey et al., 2020). This process is important because, by moving through the body, lymphocytes have a higher chance of encountering a microorganism or an antigen that initiates an immunological response (Olver et al., 2022).

Lymphocyte morphology varies: the most frequently observed are mature lymphocytes, the smallest leukocytes, with a thin rim of blue-staining cytoplasm surrounding a round or slightly indented nucleus with aggregated chromatin. Medium and large lymphocytes also exist in all species but are more common in ruminants. These lymphocytes have more abundant cytoplasm, which may contain variously colored azurophilic granules. Their nucleus is indented, and the chromatin is lighter (Voigt & Swist, 2011).

Lymphocytes are the primary cells involved in the immune response. There are three types of lymphocytes: B cells, T cells, and natural killer (NK) cells. B lymphocytes are responsible for producing antibodies as part of the humoral immune system. T lymphocytes handle the cell-mediated portion of the immune response and have regulatory functions (signaling other leukocytes) or cytotoxic functions. NK cells, which have different surface markers than other lymphocytes, mediate cytotoxic interactions with target cells (Voigt & Swist, 2011; Olver et al., 2022).

Lymphocytosis, the increase in the number of lymphocytes, is mostly associated with chronic antigenic stimulation, especially by viruses, blood parasites, and tumors. Lymphopenia can be caused by chronic stress, endotoxemia and viruses that suppress the immune system (Brockus et al., 2011).

1.3.2.5. Monocytes

Monocyte production takes about 3 to 4 days. Once formed, they are released into the circulation, where they typically persist longer than other leukocytes, remaining in the bloodstream for up to 2 days. They also migrate into the tissues, where they differentiate into various subtypes, including fixed tissue macrophages and free and inflammatory macrophages, depending on the tissue type or the nature of the inflammatory process. The lifespan of macrophages varies from days to months (Olver et al., 2022).

They are usually the largest circulating leukocytes. The nucleus varies its form (can be round, oval, elongate, kidney bean or has multiple indentations), the chromatin is lacy or reticular and appears smoother, with less clumping than in other white blood cells. The cytoplasm is abundant, stains blue-grey, and has a foamy or ground glass appearance. Vacuoles are common (and may indicate activation

of the cell), and inclusions, consisting of phagocytosed particles or even whole cells, may be present (Voigt & Swist, 2011).

The main function of monocytes and macrophages is phagocytosis: they phagocytize and destroy organisms that cannot be controlled by neutrophils, especially fungi, protozoa, intracellular organisms, and some bacteria. They also inhibit the growth of intracellular pathogens, control iron metabolism, and, depending on the stimuli, macrophages can develop into proinflammatory M1 macrophages or anti-inflammatory M2 cells (that assist in the resolution of inflammation and tissue repair) (Sharkey et al., 2020).

Monocytosis is associated with corticosteroids or indicates chronic infection or inflammation (because they are not as rapidly mobilized as the other leukocytes), recovery from an acute process or diseases that stimulate a granulomatous type of inflammatory response, such as tuberculosis, brucellosis, and most internal fungal diseases. Monocytopenia does not have clinical significance (Olver et al., 2022; Voigt & Swist, 2011).

1.3.3. Platelets

Platelets are discoid, small, anuclear cells with reddish granules. They are produced in the bone marrow by an extension of megakaryocyte cytoplasm. This production takes between 4 and 5 days and, in most animals, they circulate in the blood for 5 to 9 days before being cleared by macrophages in the spleen and liver. Their main function is coagulation, maintenance of vascular integrity and control of hemostasis (Olver et al., 2022), but they also participate in the inflammatory response because they release vasoactive chemicals, produce cytokines, and interact with neutrophiles (Olver et al., 2022; Voigt & Swist, 2011).

On most automated blood cell counters, there are four parameters related to platelets: the platelet count, which indicates the number of circulating platelets in the blood; mean platelet volume (MPV), representing the average size of the circulating platelets and often inversely proportional to the platelet count; platelet distribution width (PDW), indicating the variation in platelet size expressed as a percentage (higher percentages denote more pronounced variation); and plateletcrit (PCT) or thrombocrit, which is the hematocrit of platelets, signifying the circulating platelet mass or the percentage of blood volume composed of platelets, calculated from the platelet count and the MPV (Brockus et al., 2011; Sharkey et al., 2020).

A number of platelets below the reference number is called thrombocytopenia and puts the animal at risk of bleeding because coagulation is impossible without enough platelets. The signs of

thrombocytopenia start with petechias and ecchymosis and can lead to abundant hemorrhage if the platelet count drops below 10 000 to 20 000/µL but the animal can also have nonspecific signs due to underlying disorders that causes the thrombocytopenia (Olver et al., 2022; Sharkey et al., 2020). It can be caused by decrease or ineffective production of platelets, increased platelet consumption or destruction or platelet sequestration which in turn can be caused by some medications, toxins, viruses, bacteria, blood parasites, and some coagulation disorders such as disseminated intravascular coagulation. Blood loss is more an outcome of thrombocytopenia than a cause (Sharkey et al., 2020). Thrombocytosis is an increase in the number of platelets and is associated with inflammatory disorders and neoplasia but usually is not related with increased risk of thrombosis (Brockus et al., 2011).

2. MATERIALS AND METHODOS

2.1. Study Population

The animals in this study belonged to the Veterinary Pedagogical Center of the Polytechnic Institute of Bragança (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 and other workers of the IPB and received regular medical and prophylactic care, including proper anthelmintic treatments. The samples were collected in three different moments (in June and November of 2023 and January of 2024) within the scope of the animal health program that aimed to eradicate and monitor animal diseases. The study was approved by the ethics committee of IPB.

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 in two different groups, cold season (n=136) and warm season (n=30).

2.2. Sample Collection and Hematological Analyses

For animals to be selected for sample collection, they had to meet specific criteria established prior to the days of 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). On the day of sampling, the animals were examined, and a physical exam was performed and any animal that had lesions, abscesses, was lethargic or had fever was excluded to ensure that only healthy animals were include 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 in the animal health program in force in Portugal. The animals were grabbed calmly, and the blood collection was obtained via jugular vein using an 18-gauge needle (BD PrecisionGlide™) into K3EDTA vacuum tubes (BD Vacutainer) with a capacity of 4 mL of blood. Immediately after the blood draw, the tubes were inverted gently several times to ensure adequate anticoagulant mixing. The samples were processed until 3 h after collection 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.

2.3. Statistical Analyses

In this study, the 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%, to analyze data normality with the Anderson–Darling test and to check the presence of outliners with Tukey and Dixon–Reed method. 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 before (young-adults, female-male and warm-cold season). The Shapiro–Wilk test was performed to test sample normality, and then samples 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 $p < 0.05$.

3. RESULTS

3.1. Reference Interval for Preta de Montesinho goat

A total of 136 animals were included in this study and 23 hematological parameters were determined. In table 5, 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 5. Hematological reference intervals for the population of Preta de Montesinho 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

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, WBC: white blood cell count, NEU: neutrophils, LYM: lymphocytes, MON: monocytes, EOS: eosinophils, BAS: basophils, 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.

3.2 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 6 summarizes the effect of age on the hematological parameters. Differences between groups were considered statistically different when $p < 0.05$. That said, WBC, LYM, BAS, LYM (%), BAS (%), RBC, MCHC, RDW–CV were higher in younger animals, while, NEU, NEU (%), MON (%), HCT, MCV, MCH, RDW–SD were higher in older animals.

Table 6. Effect of age on hematological parameters in Preta de Montesinho goat.

	Young (n=33)		Adult (n=103)		
Parameters/Units	**Mean ± SD**	**RI**	**Mean ± SD**	**RI**	***p*–Value**
WBC (10^3/µL)	14,7±3,2	8,7–21,8	13,1±3,7	6,6–23,3	**0,0304**
NEU (10^3/µL)	5,0±1,5	2,6–8,9*	6,6±2,8	2,2–14,1	**0,0016**
LYM (10^3/µL)	8,8±2,5	3,9–14,1	5,6±1,7	2,6–10,4	**<0,0001**
MON (10^3/µL)	0,2±0,1	**	0,3±0,1	0,1–0,6	0,1662
EOS (10^3/µL)	0,5±0,4	0,1–2,0	0,5±0,5	0,1–1,5	0,7443
BAS (10^3/µL)	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**
EOS (%)	3,4±2,4	0,6–12,3	3,7±3,2	0,4–11,6	0,6276
BAS (%)	1,2±0,4	0,6–2,3	1,0±0,4	0,5–1,9	**0,0175**
RBC (10^6/µL)	19,3±2,6	13,4–24,1*	17,1±3,0	9,9–22,6	**0,0002**
HGB (g/L)	98,1±12,7	66,2–119,6	100,5±15,7	62,2–130,8	0,4288
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**
PLT (10^3/µL)	222,4±139,8	34,0–592,2	218,5±180,5	28,4–663,6	0,9102
MPV (fL)	2,4±0,1	**	2,4±0,1	2,2–2,7	0,4685
PDW (%)	12,9±0,5	**	12,9±0,5	11,7–13,5	0,9116
PCT (%)	0,1±0,0	**	0,1±0,0	0,0–0,2	0,8423

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, WBC: white blood cell count, NEU: neutrophils, LYM: lymphocytes, MON: monocytes, EOS: eosinophils, BAS: basophils, PLT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit, 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. Bold values indicate *p*-values < 0.05, representing parameters with statistically significant differences.

3.3. Influence of sex on hematological parameters

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

Table 7 summarizes the effect of sex on the hematological parameters. Differences between groups ($p < 0,05$) were found for EOS (%), BAS (%), RBC, HGB, HCT, MCV, MCH and MCHC. The mean for RBC, HGB, HCT, MCHC was higher in males, while EOS (%), BAS (%), MCV and MCH was higher in females.

Table 7. Effect of sex on hematological parameters in Preta de Montesinho goat.

	Female (n=127)		Male (n=9)		
Parameters/Units	**Mean ± SD**	**RI**	**Mean ± SD**	**RI**	***p*–Value**
WBC (10^3/µL)	13,3±3,6	7,8–21,6	15,6±2,4	7,3–23,9	0,0671
NEU (10^3/µL)	6,1±2,6	2.2–13,6	7,8±2,2	1,8–13,1	0,0670
LYM (10^3/µL)	6,3±2,4	2,7–12,3	7,1±2,0	4,0–14,1	0,3333
MON (10^3/µL)	0,2±0,1	0,1–0,6	0,3±0,2	0,0–1,3	0,0805
EOS (10^3/µL)	0,5±0,5	0,1–1,6	0,3±0,2	**	0,2505
BAS (10^3/µL)	0,1±0,1	0,0–0,3	0,1±0,0	0,0–0,2	0,2636
NEU (%)	45,4±12,4	24,0–70,3	49,5±8,5	27,2–70,3	0,3296
LYM (%)	47,9±12,5	23,0–69,3	45,9±9,0	27,3–71,5	0,6431
MON (%)	1,9±1,1	0,6–5,0	2,1±0,9	0,3–5,1	0,6065
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**
RDW–CV (%)	24,1±2,5	21,1–30,1	24,1±1,6	19,8–27,8	0,8835
RDW–SD (fL)	17,3±3,3	13,0–24,3	15,9±1,8	13,1–30,0	0,2208
PLT (10^3/µL)	217,1±169,9	30,4–632,0	251,8±193,7	**	0,5589
MPV (fL)	2,4±0,1	2,2–2,7	2,4±0,1	**	0,7959
PDW (%)	12,9±0,5	11,7–13,5	13,0±0,2	**	0,4407
PCT (%)	0,1±0,0	0,0–0,1	0,1±0,0	0,0–0,3	0,5168

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, WBC: white blood cell count, NEU: neutrophils, LYM: lymphocytes, MON: monocytes, EOS: eosinophils, BAS: basophils, PLT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit, 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. Bold values indicate *p*-values < 0.05, representing parameters with statistically significant differences.

3.3. Influence of season on hematological parameters

Regarding the influence of seasons, the animals were also divided in two groups, cold season (n=106), and warm season (n=30).

The effect of seasons is condensed in table 8. Differences between the groups were considered significant when $p < 0,05$, thus, the RBC, HGB, HCT, MCV, MCH, RDW-CV, and MPV are statistically different. RBC, HGB, HCT and MCV were found to be higher during the winter.

Table 8. Effect of season on hematological parameters in Preta de Montesinho goat.

	Cold season (n=106)		Warm season (n=30)		
Parameters/Units	**Mean ± SD**	**RI**	**Mean ± SD**	**RI**	***p*–Value**
WBC (10^3/µL)	13,8±3,6	8,2–23,1	12,5±3,4	5,9–19,9	0,0763
NEU (10^3/µL)	6,4±2,7	2,9–14,1	5,6±2,2	1,0–10,2	0,1566
LYM (10^3/µL)	6,5±2,4	2,8–12,6	6,1±2,2	2,7–11,8*	0,4904
MON (10^3/µL)	0,2±0,1	0,1–0,6	0,3±0,1	0,1–0,7*	0,5688
EOS (10^3/µL)	0,5±0,5	0,1–1,6	0,3±0,2	0,1–0,9	0,0745
BAS (10^3/µL)	0,1±0,1	0,0–0,3	0,1±0,1	0,0–0,3	0,2576
NEU (%)	46,0±12,3	24,2–71,4	44,5±11,8	18,9–68,9	0,5498
LYM (%)	47,3±12,4	22,0–69,3	49,5±12,0	27,1–77,9	0,3695
MON (%)	1,8±1,1	0,6–4,8	2,2±1,2	0,6–5,3	0,0886
EOS (%)	3,8±3,3	0,4–11.6	2,7±1,6	0,0–6,7	0,0551
BAS (%)	1,1±0,4	0,5–1,9	1,1±0,4	0,5–2,3	0,6747
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**
MCHC (g/L)	337,2±19,2	301,0–377,6	342,4±21,0	297,0–386,1	0,2027
RDW–CV (%)	23,6±1,8	21.1–28,9	25,3±3,7	21,3–35,6	**0,0006**
RDW–SD (fL)	17,1±2,6	13,5–24,2	17,6±4,8	11,8–26,8*	0,4383
PLT (10^3/µL)	225,5±178,3	28,7–657,7	198,0±142,8	31,7–590,0	0,4389
MPV (fL)	2,37±0,1	2.2–2,7	2,43±0,1	**	**0,0191**
PDW (%)	12,9±0,5	11,7–13,5	12,8±0,4	**	0,4621
PCT (%)	0,1±0,0	0,0–0,2	0,0±0,0	0,0–0,2	0,5383

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, WBC: white blood cell count, NEU: neutrophils, LYM: lymphocytes, MON: monocytes, EOS: eosinophils, BAS: basophils, PLT: platelet count, MPV: mean platelet volume, PDW: platelet distribution width, PCT: plateletcrit, 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. Bold values indicate *p*-values < 0.05, representing parameters with statistically significant differences.

4. 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 seventies that evaluate the influence of age on hematology parameters (Edjtehadi, 1978), sex (Somvanshi et al., 1987), season and gestation (Pospisil et al., 1987) and even compare different breeds of goats (Mbassa & Poulsen, 1992). In the present days, 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 erythrocytes, leukocytes and platelets 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, with adequate body condition score and healthy animals subjected to a physical exam prior to sampling.

We determined 23 blood parameters for Preta de Montesinho goat. WBC and NEU counts were slightly above than what is described for other different goat breeds, while the other leukocytes 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 one we determined for 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). The values established here may be physiologically higher in this breed of goats, like what has been demonstrated for other breeds. Alternatively, despite only seemingly healthy animals were included in this study, these values may be 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 RBC count in our study was slightly higher than the ones that are published (Arfuso et al., 2016; Mohammed et al., 2016). Depending on the source, the MCV and MCH values reported here could be either similar (Arfuso et al., 2016) or marginally lower (Mohammed et al., 2016; Olver et al., 2022). Most of the studies do not mention the RDW or do not differentiate between RDW-SD and RDW-CV. When studies include RDW, they generally refer to the RDW-CV. That said, the RDW-CV reported was the same as other studies (Karaşahin et al., 2022) or lower (Al-Bulushi et al., 2017).

Platelets indices are also commonly neglected, but comparing with the few articles that included them, the parameters (MPV, PCT and PDW) were below the 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 platelets indices. Firstly, the lower RIs 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 varying size (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, traumatic or inadequately mixed with the anticoagulant which may 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 platelets 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 WBC, LYM and BAS (count and percentage), RBC, MCHC, RDW-CV were higher in young goats while the NEU (count and percentage), MON (percentage), HCT, MCV, MCH and RDW-SD were lower.

In relation to the leukocytes' parameters, there are some studies that did not find age influence on the WBC (Yaqub et al., 2013). However, most of them found that the WBC is higher in younger animals (Agradi et al., 2022; Arfuso et al., 2016; Yaqub et al., 2013). Studies refer 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 findings here. The higher LYM fraction and percentage in young animals and the elevated NEU parameters in older animals, align with literature stating that lymphocytes are the predominant leukocyte in younger goats, while in older, neutrophiles have an equal or slightly increased percentage when compared to lymphocytes (Antunović et al., 2020).

The findings related to the erythrocytes' indices were also validated by other studies: 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 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. 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 does not show the same trend, this difference lies in the calculation methods for both parameters.

Although the studies mentioned generally align with our findings, there are a few that 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 was 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 EOS (%), BAS (%), MCV and MCH were higher in females, while RBC, HCT, HGB and MCHC were superior in males. In the other parameters, statistically significant differences were not determined.

A study performed in Hair goats in 2022 concluded that sex had an influence in MCV and MCH (higher in females) and in MHCH and RDW (elevated in males) (Karaşahin et al., 2023) and another carried out in Angora goats referred that the RBC was higher in males and that sex did not seem to have an influence in platelets indices (Polat et al., 2018). We found the same differences in RBC, MCV, MCH and MHCH in our study, we also concluded that sex does not seem to have an influence in the platelets' 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 was carried out in 2023 that investigated not only the effect of sex in the hematological values but also the effect of breeding season. That study found that the RBC, HGB, HCT, MCH and MCHC were higher in males but also that some of these were only statistically different in the breeding season. The results suggest that males mate more frequently and require more oxygen due to higher liveliness. Increased physical activity during mating and 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 erythrocytes' indices (Karaşahin et al., 2023).

Although we found statistically significant differences between the two groups, 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 registered 73 males in 2024, increasing the number of samples while maintaining the criteria of selection (healthy, dewormed, animals that the medical history is known) is very difficult to achieve.

The impact of seasons was also analyzed. The sampling was performed in three different moments, 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: RBC, HGB, HCT, MCV and MCH, that were higher in the cold season, and RDW-CV and MPV, which were lower in the same season. Our findings were validated by similar articles (Polat et al., 2018; Singh Nathawat et al., 2023).

Most erythrocyte indices were lower during the warm season when compared to 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. Additionally, other studies have indicated that oxidative stress caused by high ambient temperatures may 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 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. This stimulation inhibits the appetite center, resulting in decreased feed intake which can cause nutritional deficits that could have an influence in the blood parameters (Yaqub et al., 2013). Another study attributed the higher values in the cold season to increased concentrate feeding, which typically occurs during winter, when animals are often stabled or require additional

feeding due to insufficient pasture availability. It may also be due to higher metabolic rates that stimulate erythropoiesis (Singh Nathawat et al., 2023).

Most of the researchers also found differences in WBC and some leukocytes parameters (Agradi et al., 2022; Ghosh et al., 2013; Polat et al., 2018) but, in this study, we concluded that the season did not have a statistically significant impact on the leukocytes' 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 could be that the timing of collection of samples is different. That said, it would be interesting to repeat this study with samples collected in each season while also recording some parameters related to the season, such as temperature, relative humidity and temperature humidity index, in order 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. In groups with $n < 40$, the statistical analysis is less robust, and a few RIs were not computable due to the small sample size. Other RIs are described but should be consulted with caution, as 1 to 3 possible outliers were detected, which significantly influence the RIs due to the sample size (Geffré et al., 2011).

Increasing the number of samples and involving other Preta de Montesinho goat farmers in the study could enhance its external validity. Additionally, addressing biases such as farm management systems and health conditions is crucial, as all animals included in this study were fed similarly, exposed to the same environmental and agricultural practices, and underwent regular deworming and veterinary evaluations.

5. CONCLUSION

The Preta de Montesinho goat is a unique breed found exclusively in Portugal, where caprine farming is associated with economically disadvantaged regions and agroforestry resources that are challenging for other species to use and, although conservation and improvement programs have been developed for this breed, there is still much to be done to prevent the extinction of these animals.

The establishment of reference values of hematological parameters is essential for evaluating the health and physiological status of farm animals and the findings of this study highlight the need for

a breed characterization, as it was confirmed that there were differences in the hematological RIs when compared to other breeds. Furthermore, we also concluded that age, sex and seasons had an impact in the blood parameters, proving that these factors should be considered when interpreting the parameters in order to ensure accuracy.

These results demonstrate that the RIs available in the literature were somewhat inadequate for this breed, emphasizing the need for studies with a greater number of sampled individuals, while simultaneously evaluating and monitoring the health of animals and herds. Consequently, this study established reference values that may assist veterinarians more accurately interpreting laboratory data and monitoring the health status of animals, which could be a useful tool to help promote the management and conservation efforts for this breed. Moreover, it could serve as a foundation for future research into this breed or other Portuguese breeds.

REFERENCES

Agradi S, Menchetti L, Curone G, Faustini M, Vigo D, Villa L, Zanzani SA, Postoli R, Kika TS, Riva F, Draghi S, Luridiana S, Archetti I, Brecchia G, Manfredi MT, Gazzonis AL (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). 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

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

Associação Nacional de Caprinicultores da Raça Serrana [ANCRAS] (2016). Raça Preta de Montesinho. Available online: http://www.ancras.pt/ (accessed on 18 April 2024)

Brockus CW, Latimer KS, Webb JL, Boudreaux MK, Spangler EA, Welles EG (2011). Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology (5th ed.). John Wiley & Sons, Inc.

Bruno-de-Sousa C, Martinez AM, Ginja C, Santos-Silva F, Carolino MI, Delgado JV, Gama LT (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 CB, Carolino I, Santos-Silva F, Sousa CO, Vicente A, Ginja C, Gama LT (2016). Biodiversidade caprina em Portugal. In Bayona JEV, Martínez LZ, Bermej, JVD, Galván GR (Eds.).

Biodiversidad caprina iberoamericana (1.ª ed., pp 57-74). Ediciones Universidad Cooperativa de Colombia.

Confederação dos Agricultores de Portugal, & Direcção Geral de Alimentação e Veterinária. (2021). Catálogo Oficial de Raças Autóctones Portuguesas.

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

Elmaz Ö, Saatcı M, Dağ B, Aktaş AH, Ata A, Gülay MŞ, Mamak N, Gök B (2012). Some descriptive characteristics of a new goat breed called Honamli in Turkey. Tropical Animal Health and Production, 44(8), 1913–1920. https://doi.org/10.1007/s11250-012-0156-7

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

Geffré A, Concordet D, Braun JP, 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 AK, 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 LT, Delgado JV, Amills M, de Sousa CB, Cañón J, Capote J, Dunner S, Ferrando A, Carpio MG, Gómez M, Jordana J, Landi V, Manunza A, Martin-Burriel I, Barro AP, Rodellar C, Santos-Silva F, Martínez AM (2018). Conservation of goat populations from southwestern Europe based on molecular diversity criteria. In Sustainable Goat Production in Adverse Environments (Vol. 1, pp. 509–533). Springer International Publishing. 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

Hoffmann JJML, Nabbe KCAM, van den Broek NMA (2015). Effect of age and gender on reference intervals of red blood cell distribution width (RDW) and mean red cell volume (MCV). Clinical Chemistry and Laboratory Medicine, 53(12), 2015–2019. https://doi.org/10.1515/cclm-2015-0155

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

Karaşahin T, Aksoy NH, Dursun Ş, Bulut G, Haydardedeoğlu AE, 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 AM, Gama LT, Delgado JV, Canõn J, Amills M, de Sousa CB, 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 GK, Poulsen JSD (1992). The comparative haematology of cross-bred and indigenous East African goats of Tanzania and breeds reared in Denmark. Veterinary Research Communications, 16.

Mohammed SA, Razzaque MA, Omar AE, Albert S, Al-Gallaf WM (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 TJ, Nephawe KA, 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

Newcomer BW, Cebra C, Chamorro MF, Reppert E, Cebra M, Edmondson MA (2021). Sheep, Goat, and Cervid Medicine (3rd ed.). Elsevier.

Nicoleti JL, Façanha DA, Kalil MA, Fonseca EP, Barral TD, Sampaio JR, Meyer R, Portela RW (2023). Hematological and clinical biochemistry profiles in Canindé goats infected by Corynebacterium pseudotuberculosis and bred in a tropical semi-arid region. Tropical Animal Health and Production, 55(1). https://doi.org/10.1007/s11250-022-03431-0

Olver CS, Harvey JW, dos Santos AP, Christian JA, Andreasen CB, Levine DN, Young KM, Layne EA, Kastil BC, Pohlman LM, Souza CD, Weiss DJ, Eren Mv, Warren AL, Yates RM, Boudreaux MK, Christopherson PW (2022). 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 PP (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

Polizopoulou ZS (2010). Haematological tests in sheep health management. Small Ruminant Research, 92(1–3), 88–91. https://doi.org/10.1016/j.smallrumres.2010.04.015

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

Sharkey LC, Burton EN, Heinrich DA, Johns JL, Heller M (2020). Large Animal Internal Medicine (6th ed.). Elsevier.

Shawaf T, Al Bulushi S, Al-Ali MA, Meligy AMA, 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 AC (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

Nathawat SM, 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

Smith MC, Sherman DM (2023). Goat Medicine (3rd ed.). John Wiley & Sons.

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

Somvanshi R, Biswas JC, Sharma B, Koul GL (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

Voigt GL, Swist SL (2011). Hematology Techniques and Concepts for Veterinary Technicians (2nd ed.). John Wiley & Sons Inc.

Yaqub LS, Kawu MU, Ayo JO (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

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