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A Retrospective Study of the Erythrocytic Values of Semi-Intensively Raised Philippine Sheep (*Ovis aries*) at a Farm in Los Baños, Laguna

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Abstract | The study was conducted to evaluate the erythrocytic values of semi-intensively raised Philippine sheep. Health records of 23 male and 18 female sheep with an age distribution of ≤1 to > 1 years were used. Mean, standard deviation, and p-values were computed, and the results were compared with reference intervals. The t-test and point-biserial correlation were used to compare and determine the relationship of age and sex with the erythrocytic values. All mean values aside from the MCHC were within the normal reference intervals. The HCT and MCV were significantly higher in sheep of ≤1 year in age ($p<0.05$). Sheep that are >1 year in age had a significantly higher MCHC ($p<0.05$). There were no significant differences in the erythrocytic values between male and female sheep ($p<0.05$). The HCT and MCV were positively and moderately correlated with age and the MCHC was negatively and moderately correlated with age ($p<0.05$). The data serves as the first reported erythrocytic values of Philippine sheep of varied ages using an automated hematology analyzer. Most of the erythrocytic values were within the reported reference ranges therefore, it may be used for future research regarding the establishment of normal erythrocytic reference values for the Philippine sheep.

Keywords | Automated hematology analyzer, Erythrocytes, Philippine sheep, Reference intervals

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INTRODUCTION

Livestock raising is an integral part of the livelihood and food security of countries worldwide. It significantly contributes to the rural economy of many developing countries (Hegde, 2019; Saputro *et al.*, 2023). Similar to goats, sheep serve as an economically important livestock species in many countries because they are multipurpose animals valued for their capacity to produce meat, milk, wool, and hide (Stayt, 2022; Hamid *et al.*, 2023). In several developing countries, these animals serve as the principal source of animal protein (Hegde, 2019). Sheep population has steadily increased from 1.060 billion heads in 2010 to 1.266 billion in 2021 (Mazinani and Rude, 2020; IWTO, 2022). The Organization for Economic Cooperation and Development and Food and Agriculture Organization (OECD-FAO)(2021) of the United Nations, even stated that in comparison to the average data from 2018 to 2020, the global production of mutton is projected to grow by 15.7% and it will account for 6% of the additional meat consumed globally by 2030.

The sheep population in the Philippines has been stable at around 30,000 heads since 1973, with the majority of the local sheep population coming from small backyard farms (Ozaraga *et al.*, 2001; Statistica Research Department, 2023). Despite its smaller population compared to goats, sheep rearing provides employment and serves as an additional and alternative source of income to small-scale farmers (Ozaraga *et al.*, 2001).

Herd health programs are essential in optimizing the health and productivity of sheep and decreasing the incidence of diseases. They provide diagnostic testing for the surveillance, control, and elimination of diseases and inefficiencies in management (Reyes *et al.*, 1993; Whyte *et al.*, 2011). Diagnostic testing involves the examination of blood, which acts as a reflector of the physiological, pathological, and nutritional status of an animal (Etim *et al.*, 2014; Lazwari *et al.*, 2024). Hematologic analysis includes a complete blood count (CBC), one of the most common diagnostic tests that serve as an important indicator of an animal's overall health status (Tibbo *et al.*, 2005). The CBC, along with the medical history and physical examination, aid in the diagnosis of disease conditions. It helps detect any deviation from the normal (Etim *et al.*, 2014).

For the proper interpretation of results of the CBC, comparison with reference values is necessary. Hematological reference intervals have long been reported, but these values often exclude variations within subpopulations defined by factors such as breed, age, sex, and environment. Several studies have reported that factors such as these result in quantifiable variations in hematological values of sheep (Etim *et al.*, 2014; Njidda *et al.*, 2014; Oramari *et al.*, 2014; Bezerra *et al.*, 2017; Rahman *et al.*, 2018). Studies from foreign authors have also reported differences in normal blood values between breeds indigenous to their country thereby leading them to establish their own reference intervals (Egbe-Nwiyi *et al.*, 2000; Tibbo *et al.*, 2005; Njidda *et al.*, 2014; Ahmadi-Hamedani *et al.*, 2016). Therefore, the establishment of baseline indices of blood parameters of sheep indigenous to the country is necessary.

In the Philippines, studies on the evaluation of red blood cell values particularly the red blood cell (RBC) count, hemoglobin (Hgb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of indigenous sheep are few, limited to the research by Cajilog (1966), Ducusin *et al.* (1995), and Gaffud (2011). In terms of erythrocyte parameters, Ducusin *et al.* (1995) and Gaffud (2011) both reported on the RBC count and PCV using hemocytometer and microhematocrit methods, respectively. The study by Ducusin *et al.* (1995) had a sample size of 56 adult sheep and 40 lambs that were all apparently healthy and Gaffud (2011) included 36 adult sheep and two lambs, regardless of health status. The study by Cajilog (1966), which had a sample size of 37 sheep (31 adult sheep and six lambs), reported on MCV, MCH, and MCHC using Boddie's and Wintrobe's formulae.

Data on these parameters obtained using a hematology analyzer as well as values for the red blood cell distribution width (RDW) have not yet been reported in the country. Hence, there is a need to conduct further studies which focus on evaluating normal erythrocyte values of sheep under local conditions. This study will serve as baseline data for future research regarding the establishment of normal erythrocyte reference values of the Philippine sheep. This study will also provide updated information on the relationship of factors such as age and sex on erythrocytic values of Philippine sheep.

MATERIALS AND METHODS

Study population and area of study

Health records of 41 clinically healthy Philippine sheep semi-intensively raised at the University of the Philippines Veterinary Teaching Hospital Los Baños (UP-VTH LB), Laguna, Philippines in July 2021 were used in the study (Appendix A). According to the farm's management practices, the sheep were provided with Mulato grass (*Brachiaria ruziziensis*) at 30 g/kg body weight in the morning through cut-and-carry and allowed to graze in a fenced pasture composed of Para grass (*Brachiaria mutica*) and Mombasa grass (*Megathyrsus maximus*) in the afternoon. The stocking density was 8.25 livestock units per hectare. The herd was housed in a screened pen housing measuring 5x15 m^2 with galvanized iron roofing and cemented flooring. Clean drinking water was provided *ad libitum*. Vitamin and mineral supplementation were not given in the three months prior to sample collection. Deworming of the herd using 15 mg/kg albendazole was done once, two weeks before routine sample collection for health check. Out of a total population of 55 sheep in the farm, only records from 41 sheep determined to be healthy upon physical and clinical examination, and with negative results from both fecal examination (McMaster flotation technique) and blood parasite examination were included. The mean leukocytic and thrombocytic values of the herd were within the reference intervals. Based on the records, these included 23 males and 18 females, all intact, with the age distribution in years of ≤1 (n=16) and >1 (n=25), specifically 2 (n=15), 3 (n=7), and 4 (n=3). The youngest sheep included was 7 months old whereas the oldest sheep was 4 years old at the time of sample collection. Pregnancy diagnosis was made based on record keeping, physical examination, and ultrasonography. Ewes that had given birth from July 2021 to December 2021 were excluded from the study. The environmental temperature experienced by the herd throughout the three-day sample collection was from 25 °C to 27 °C with a daily rainfall of 13.7 mm to 19.5 mm, 87% to 93% relative humidity, and 13 km/h to 20 km/h wind speed.

The ear tag number, age, and sex of Philippine sheep were obtained from individual electronic health records. The erythrocytic values such as RBC count ($x10^6/\mu L$), HCT (%), Hgb (g/dL), MCV (fL), MCH (pg), MCHC (g/dL), and RDW (%) were also obtained from the records. These values were taken in July 2021 during an annual routine health check, using an 18-parameter hematology analyzer (Mythic 18 Vet, Orphee, Switzerland).

Data analysis

The mean and standard deviation (mean ± SD) for both the age (≤ 1 and > 1 year), and sex (male and female) groups were computed. At 5% level of significance, t-test was used to compare the mean ± SD among age and sex groups. Additionally, point-biserial correlation analysis was used to determine the relationship of age and sex with the erythrocytic values.

Ethical clearance

Ethical approval was not required for this study, as it involved only the retrospective analysis of existing health records and did not include any direct animal handling or experimentation.

RESULTS

Erythrocytic values of the Philippine sheep

The erythrocytic values (mean ± SD) of the Philippine sheep in comparison with the reference intervals published by Byers and Kramer (2010) and University of California Davis Veterinary Medicine (2001) are presented in Table 1. All the mean values obtained were within the reference intervals except for the MCHC (28.24 ± 0.383 g/dL) which was slightly below the reference range.

Erythrocytic values of the Philippine sheep based on sex

The comparison of erythrocytic values of the Philippine sheep between sex groups are presented in Table 2. The results revealed that there were no statistically significant differences in the values between male and female sheep (p<0.05).

Erythrocytic values of the Philippine sheep based on age

The erythrocytic values of the Philippine sheep based on age groups are presented in Table 3. Results revealed that the HCT, MCV, and MCHC were significantly different between the two age groups (p<0.05). At a 5% level of significance, sheep of at most one-year-old had a significantly higher HCT (39.76 ± 0.898 %) and

Table 1: Mean ± S.E. of erythrocytic values of Philippine sheep raised at a farm in Los Baños, Laguna and the reference values for sheep.

Variable	Mean	S.E.	Minimum	Maximum	Reference values
RBC count ($x10^6/\mu L$)	10.03	0.167	7.08	12.45	9.5-15.0
Hgb (g/dL)	10.35	0.165	7.1	12.2	9.0-15.0
HCT (%)	37.10	0.666	28	43.7	27.0-45.0
MCV (fL)	37.11	0.535	32	48.6	28.0-40.0
MCH (pg)	10.33	0.067	9.7	11.4	8.0-12.0
MCHC (g/dL)	28.24	0.383	21	37.3	31.0-34.0
RDW (%)	17.72	0.198	15.9	21	16.0-22.0

*Byers and Kramer (2010), and University of California, Davis Veterinary Medicine (2001). S.E., standard error; RBC count, red blood cell count; Hgb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width.

Table 2: Mean ± standard error of erythrocytic values by sex, and their comparisons using t-test.

Variable	Female	Male	Alternative hypothesis	p-value
	Mean± S.E.	Mean± S.E.		
RBC count ($x10^6/\mu L$)	10.22± 0.328	9.88± 0.150	F < M	0.8412
			F ≠ M	0.3177
			F > M	0.1588
Hgb (g/dL)	10.52± 0.319	10.21± 0.158	F < M	0.8161
			F ≠ M	0.3677
			F > M	0.1839
HCT (%)	37.87± 1.174	36.51± 0.752	F < M	0.8413
			F ≠ M	0.3175
			F > M	0.1587
MCV (fL)	37.20± 0.763	37.04± 0.758	F < M	0.5582
			F ≠ M	0.8835
			F > M	0.4418
MCH (pg)	10.32± 0.103	10.34± 0.090	F < M	0.4225
			F ≠ M	0.8449
			F > M	0.5775
MCHC (g/dL)	28.38± 0.653	28.12± 0.464	F < M	0.6305
			F ≠ M	0.6305
			F > M	0.7390
RDW (%)	17.51± 0.317	17.89± 0.252	F < M	0.1705
			F ≠ M	0.3409
			F > M	0.8295

* Significant at α = 0.05

Table 3: Mean ± standard error of erythrocytic values by age group, and their comparisons using t-test.

Variable	≤ 1 year old	> 1 year old	Alternative hypothesis	p value
	Mean± S.E.	Mean± S.E.		
RBC count (x10⁶/µL)	10.09± 0.325	9.98± 0.184	(≤1 y.o.) < (>1 y.o.)	0.6207
			(≤1 y.o.) ≠ (>1 y.o.)	0.7586
			(≤1 y.o.) > (>1 y.o.)	0.3793
Hgb (g/dL)	10.51± 0.319	10.24± 0.180	(≤1 y.o.) < (>1 y.o.)	0.7775
			(≤1 y.o.) ≠ (>1 y.o.)	0.4451
			(≤1 y.o.) > (>1 y.o.)	0.2225
HCT (%)	39.76± 0.898	35.41± 0.763	(≤1 y.o.) < (>1 y.o.)	0.9996
			(≤1 y.o.) ≠ (>1 y.o.)	0.0008 *
			(≤1 y.o.) > (>1 y.o.)	0.0004 *
MCV (fL)	39.68± 0.881	35.46± 0.427	(≤1 y.o.) < (>1 y.o.)	1.0000
			(≤1 y.o.) ≠ (>1 y.o.)	< 0.0001*
			(≤1 y.o.) > (>1 y.o.)	< 0.0001*
MCH (pg)	10.43± 0.111	10.27± 0.083	(≤1 y.o.) < (>1 y.o.)	0.8656
			(≤1 y.o.) ≠ (>1 y.o.)	0.2689
			(≤1 y.o.) > (>1 y.o.)	0.1344
MCHC (g/dL)	27.02± 0.809	29.02± 0.270	(≤1 y.o.) < (>1 y.o.)	0.0045 *
			(≤1 y.o.) ≠ (>1 y.o.)	0.0091 *
			(≤1 y.o.) > (>1 y.o.)	0.9955
RDW (%)	17.36± 0.317	17.95± 0.248	(≤1 y.o.) < (>1 y.o.)	0.0747
			(≤1 y.o.) ≠ (>1 y.o.)	0.1495
			(≤1 y.o.) > (>1 y.o.)	0.9253

* Significant at α = 0.05

MCV (39.68 ± 0.881 fL) than sheep of two years and older, 35.41 ± 0.763 % and 35.46 ± 0.427 fL, respectively. In contrast, the MCHC was significantly higher in sheep of two years and older (29.02 ± 0.270 g/dL) than younger sheep (27.02 ± 0.809 g/dL) (p<0.05). There were no significant differences in the mean values of the remaining parameters, specifically the RBC count, Hgb, MCH, and RDW, between the two age groups (p < 0.05).

Correlation analysis between erythrocytic values and signalment (age and sex)

The correlation between erythrocytic values and signalment (sex and age) is presented in Table 4. At a 5% level of significance, the HCT, MCV, and MCHC were significantly correlated with age. The HCT and MCV were negatively and moderately associated with age, with p-values of 0.0008 and <0.0001, respectively (p<0.05). Sheep of at most one year of age were most likely to have a higher HCT and MCV than sheep that are two years and older. In contrast, the MCHC with a p-value of 0.0091 was positively and moderately correlated with age where sheep of two years and above were most likely to have a higher MCHC than those one year and younger (p<0.05). However, no observed correlation between sex and all erythrocytic parameter values obtained from the Philippine sheep (p<0.05).

Table 4: Point biserial correlation between sex and erythrocytic values, and age group (≤ 1 and >1) and erythrocytic values.

Variable		
Age vs.	**Coefficient**	**p-value**
RBC count (x10⁶/µL)	-0.0495	0.7586
Hgb (g/dL)	-0.1226	0.4451
HCT (%)	-0.5038	0.0008*
MCV (fL)	-0.6082	< 0.0001*
MCH (pg)	-0.1768	0.2689
MCHC (g/dL)	0.4027	0.0091*
RDW (%)	0.2292	0.1495
Sex vs.		
RBC count (x10⁶/µL)	-0.1600	0.3177
Hgb (g/dL)	-0.1444	0.3677
HCT (%)	-0.1601	0.3175
MCV (fL)	-0.0236	0.8835
MCH (pg)	0.0315	0.8449
MCHC (g/dL)	-0.0537	0.7390
RDW (%)	0.1526	0.3409

* Significant at α = 0.05

DISCUSSION

Most of the erythrocytic values in Philippine sheep were within the reference intervals established by Byers and Kramer (2010) and University of California Davis Veterinary Medicine (2001). The only parameter slightly below the reference range was the MCHC (28.24 ± 0.383 g/dL). A decreased MCHC reflects a reduction in hemoglobin concentration and may be associated with regenerative or non-regenerative anemia (Jones and Allison, 2007). Regenerative anemia in sheep is commonly due to parasitism and is characterized by immature erythrocytes with reduced hemoglobin content (Stockham and Scott, 2008; Johns and Heller, 2021). However, MCHC alone is insufficient to diagnose anemia, and supporting findings such as reticulocytosis, increased MCV, and basophilic stippling are required (Tvedten, 2022; Helmyati *et al*., 2023). Since the mean Hgb was within normal limits and no accompanying hematologic abnormalities typical of iron or copper deficiency (Jones and Allison, 2007; Barger, 2010) were observed, the slightly low MCHC may reflect minor physiological variation or previous subclinical parasitism. Blood smear evaluation remains important for confirming anemia and assessing regenerative responses.

Differences from earlier local studies by Cajilog (1966), Ducusin *et al*. (1995), and Gaffud (2011) may be attributed to differences in animal health status, environment, and laboratory methods. Earlier reports showed lower RBC counts, which were often attributed to parasitism (Cajilog, 1966; Gaffud, 2011) or seasonal/environmental influences (Ducusin *et al*., 1995; Mohammed *et al*., 2014). Other studies observed higher RBC or Hgb values during the dry season due to heat stress and possible dehydration (Nafisat *et al*., 2021), although Tadesse *et al*. (2022) suggested that sheep can adapt well to varying climatic conditions. Temperature and seasonal fluctuations significantly influence hematologic values (Khalil *et al*., 2022), and reference intervals ideally reflect animals from similar environmental conditions (Wood and Quiroz-Rocha, 2010). The present values likely reflect animals adapted to the environmental conditions in Laguna during sampling.

Compared with previous local reports, differences in methodology may also explain variation. Earlier studies used hemocytometer and microhematocrit techniques (Ducusin *et al*., 1995; Gaffud, 2011) or manual calculations (Cajilog, 1966), while this study used an automated analyzer, which provides more precise measurements and minimizes artifacts (Fathima *et al*., 2019; McCartney, 2022). The RDW values recorded here, which fell within reference ranges (University of California Davis Veterinary Medicine, 2001), are the first locally reported values. Although RDW is not widely used clinically in veterinary medicine, it is useful for anemia classification and for detecting early changes in erythrocyte size (Weiss and Tvedten, 2012; Salvagno *et al*., 2014; Martinez *et al*., 2019).

No significant differences in erythrocytic values were found between male and female sheep. This aligns with findings by Ducusin *et al*. (1995), Tibbo *et al*. (2005), Mostaghini *et al*. (2005), Oramari *et al*. (2014), and Carlos *et al*. (2015). Other authors reported higher erythrocyte indices in males due to androgen effects on erythropoiesis (Egbe-Nwiyi *et al*., 2000; Yaqub et al., 2013; Badawi and Al-Hadithy, 2014; Al-Samarai and Al-Jbory, 2017;), while some reported higher values in females (Tibbo *et al*., 2005; Ahmadi-Hamedani *et al*., 2016). Reproductive status also influences hematologic results, especially in estrus, pregnancy, and lactation (Soliman and Selim, 1966; Adili *et al*., 2013; Caesar *et al*., 2020; Brown *et al*., 2020). Because none of the ewes in this study were pregnant and because the number of females was small, sex-related differences may not have been detectable. Differences may also relate to variations in body size and metabolic rate (Kedang *et al*., 2022).

Age-related patterns observed in this study such as higher HCT and MCV in sheep ≤1 year old and higher MCHC in older sheep are consistent with previous reports. Young animals often exhibit higher metabolic rates and larger erythrocytes due to the presence of fetal RBCs (Ullrey *et al*., 1965; Egbe-Nwiyi *et al*., 2000; Gaffud, 2011; Meneghini *et al*., 2016; Panousis *et al*., 2017; Rahman *et al*., 2018). Higher HCT in younger sheep may also reflect increased excitability and stress responses associated with restraint (Panousis *et al*., 2017; Brown *et al*., 2020). The decline in HCT with age is consistent with physiological senescence and reduced capacity to maintain erythrocyte mass (Ullrey *et al*., 1965; Brown *et al*., 2020). The higher MCHC in older sheep corresponds to maturation-related changes in hemoglobin composition, including the transition from fetal to adult hemoglobin (Brun-Hansen *et al*., 2006). Some studies, however, have reported no significant age-related differences (Carlos *et al*., 2015; Al-Samarai and Al-Jbory, 2017; Drebee and Abdul-Razak, 2020).

Correlation analysis also supported these age-related trends. The HCT and MCV were negatively correlated with age, consistent with previously described developmental changes in erythropoiesis and metabolic rate (Jabbar et al., 2012; Yaqub *et al*., 2013; De Souza *et al*., 2018). In contrast, the positive correlation between MCHC and age is consistent with the disappearance of fetal hemoglobin and gradual increase in adult hemoglobin (Brun-Hansen *et al*., 2006; Ullrey *et al*., 1965).

CONCLUSION

Most of the hematological parameters had values within the reference interval aside from a slightly decreased MCHC. The observation of higher HCT and MCV values in sheep of at most one year of age, and an MCHC value greater in sheep of two years and above, demonstrate a significant impact of age on some erythrocytic parameters of sheep. However, the present study did not reveal significant differences in erythrocytic values between sexes.

To the best of the authors' knowledge, this is the first reported RDW value of the Philippine sheep and first report on the erythrocytic values of clinically healthy Philippine sheep of varied ages using an automated hematology analyzer. The results may serve as a preliminary data for future research regarding the establishment of normal erythrocyte reference values of the Philippine sheep which is important in monitoring their health and productivity.

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NOVELTY STATEMENT

This retrospective study provides the first locally reported red blood cell distribution width (RDW) values for Philippine sheep and is, to our knowledge, the first to report erythrocytic parameters of clinically healthy Philippine sheep of varied ages measured with an automated hematology analyzer. Using health records from a semi-intensively managed flock (n = 41), we document age-associated differences in HCT, MCV and MCHC and demonstrate no sex-related differences under the farm's management conditions. These results fill a methodological and population gap left by earlier manual-method and smaller studies, offering preliminary, instrument-based baseline data to inform future establishment of reference intervals and routine herd-health screening in Philippine sheep.

AUTHOR'S CONTRIBUTION

Conceptualization- ICBP; Methodology- PMT, ICBP, and JLC; Formal analysis- PMT, ICBP, and JLC; Writing (Original Draft)- PMT: Writing (Review and Editing)- PMT, ICBP, and JLC; and Supervision- ICBP and JLC.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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