INSIGHTS-JOURNAL OF
LIFE AND SOCIAL SCIENCES

INSIGHTS-JLSS
Insights-Journal of Life and Social Sciences

INFLUENCE OF AGE AND SEX ON BLOOD AND BIOCHEMICAL MARKERS IN BALOCHI SHEEP BREED

Original Article

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Conflict of Interest: None

Grant Support & Financial Support: None

Acknowledgment: The authors gratefully acknowledge the support of the local livestock department and participating farmers in Quetta, Balochistan.

ABSTRACT

Background: Sheep are among the most valuable livestock species globally and play a significant role in the rural economy of Pakistan, particularly in Balochistan, where the Balochi breed is well adapted to the region's arid environment. Assessing hematological and biochemical health indicators in these animals is essential for improving disease diagnosis, health management, and productivity, with age and sex being critical variables that influence physiological responses and metabolic efficiency.

Objective: To evaluate the influence of age and sex on hematological and biochemical parameters in the native Balochi sheep breed.

Methods: A total of 40 clinically healthy Balochi sheep of both sexes and different age groups (<12 months and >12 to <24 months) were selected from Quetta, Balochistan. Blood samples were collected aseptically and analyzed for red and white blood cell indices, liver enzymes, and serum biochemistry using commercially available diagnostic kits. Statistical analysis was performed using one-way ANOVA with a significance level of $p<0.05$.

Results: Adult sheep showed the highest values for RBCs (7.24×10^6/µL), HCT (39.75%), MCV (84.04 fL), MCH (29.75 pg), neutrophils (13.65×10^3/µL), urea (9.01 mg/dL), creatinine (1.14 mg/dL), total protein (8.67 g/dL), glucose (72.37 mg/dL), and AST (123.95 U/L). In contrast, younger sheep had lower values across most markers, including RBCs (6.69×10^6/µL) and total protein (5.44 g/dL). Female sheep recorded higher values in WBCs (7.04×10^3/µL), Hb (11.41 g/dL), cholesterol (55.69 mg/dL), and ALT (33.90 U/L), whereas males exhibited lower values in nearly all parameters.

Conclusion: The study confirms that age and sex significantly impact the hematological and biochemical profiles of Balochi sheep, underscoring the need for age and gender specific reference ranges in health evaluations.

Keywords: Age Factors, Animal Health, Biochemical Markers, Hematological Parameters, Liver Enzymes, Sex Characteristics, Sheep Physiology.

INTRODUCTION

Sheep farming holds a vital role in global agriculture, not only for its historical roots but also for its enduring contribution to food systems and various industrial sectors. For thousands of years, sheep have been domesticated for their meat, wool, and by-products, forming the backbone of rural economies across continents. Today, countries such as China, Australia, New Zealand, the United States, Pakistan, and several European nations have developed robust sheep farming industries, adapted to their unique environmental conditions and consumer demands (1). Among its many contributions, sheep farming plays a key role in meeting global dietary needs by providing lamb and mutton, which are widely consumed for their taste and nutritional value. Beyond meat, the industry also supports the production of lanolin and wool, which are critical inputs in the cosmetics, pharmaceutical, and textile industries (2,3). In managing these livestock systems, the health and productivity of sheep are closely monitored using haematological and biochemical parameters. These metrics serve as fundamental indicators of physiological, nutritional, and pathological status, guiding both preventive and therapeutic interventions in veterinary practice. In older animals, immune responses may also decline with age, requiring more frequent vaccinations to maintain protection; some studies have shown that males may be more susceptible to FMD (3,4). Haematological profiles—including red blood cells (RBCs), white blood cells (WBCs), haemoglobin (Hb), haematocrit (HCT), and platelet counts—offer insights into oxygen-carrying capacity, immune function, and potential infectious or inflammatory conditions. RBC metrics like mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) are routinely used to detect anemia and other blood disorders, while WBC differentials help identify infections and immune responses (5).

Similarly, serum biochemical markers provide vital information about organ function and metabolic status. Liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) indicate hepatic health, though their values may fluctuate due to non-hepatic causes. Sometimes these enzymes value are increase or decrease due to some other factors, not due to the abnormal function of liver (6,7). Renal function is assessed through creatinine and blood urea nitrogen (BUN) levels, while electrolytes such as sodium, potassium, chloride, and bicarbonate reflect fluid balance and neuromuscular health. Glucose concentrations, total protein, albumin, and globulin levels further help evaluate energy status, immunity, and nutritional balance. Additionally, trace elements like calcium, magnesium, iron, and zinc, along with lipid profiles including cholesterol, reveal bone integrity, enzymatic activity, and endocrine function (3,8,9). Several factors influence these parameters, including breed, age, and sex. Breed-specific genetic variability has been associated with differences in metabolic and immune responses, as seen among Merino, Dorper, and Suffolk sheep. Environmental circumstances, gender, age, breed, diet, and other internal and external variables can all affect biochemical and blood metrics in animals (9,10,11). Sex hormones contribute to notable differences in haematological and biochemical values; testosterone promotes erythropoiesis and muscle mass in males, while estrogen in females affects calcium and lipid metabolism. Similarly, autoimmune diseases can affect animals at any age, but some may have a higher prevalence in certain age groups (12). Age also plays a pivotal role, with younger animals often displaying higher WBC counts due to developing immunity and lower RBC indices due to immature haematopoietic systems. In contrast, older sheep may exhibit declining organ function, affecting parameters like Hb and HCT (8,13,14).

Furthermore, variations related to age and sex may influence the prevalence and severity of diseases. Older male animals, for instance, are more prone to infections like tuberculosis and may exhibit altered immune responses, necessitating tailored veterinary care (10,13). Gender differences in immune response, reproductive status, and metabolic demand also shape haematological profiles, particularly during gestation and lactation in females (14,15). These physiological variations can result in overlapping or divergent clinical presentations, underscoring the need for individualized interpretation of diagnostic tests. Despite the recognized utility of haematological and biochemical assessments, a standardized understanding of how age and gender influence these parameters in healthy sheep remains limited. While prior studies have highlighted these associations, data remain fragmented, particularly across different breeds and environmental contexts (10,16,17). A comprehensive evaluation of these variables is essential for accurate diagnosis, effective disease monitoring, and optimized herd management strategies. The objective of this study is to investigate the impact of age and gender on haematological and biochemical parameters in healthy sheep, aiming to generate breed-relevant reference values and enhance diagnostic precision in veterinary practice.

METHODS

This study was designed as a cross-sectional comparative investigation involving a total of 40 clinically healthy native Balochi sheep selected from the region of Quetta, Balochistan. The sheep were grouped based on age and gender into four distinct cohorts: young

males (<12 months), young females (<12 months), adult males (>12 to <24 months), and adult females (>12 to <24 months), with ten animals in each subgroup (n=10 per subgroup), resulting in an equal distribution across age and sex (n=20 males; n=20 females). All animals included in the study were apparently healthy, free from clinical signs of disease, and had not received any medication at least four weeks prior to sampling. Animals with signs of systemic illness, recent disease history, or those undergoing treatment were excluded. All sheep were housed under semi-intensive management conditions, having access to open pasture during the day with free access to canal water. Standardized feeding and housing protocols were followed across all groups. Ambient environmental conditions, vaccination status, and general animal health management were thoroughly recorded. Ethical approval for the study was obtained from the Institutional Animal Care and Use Committee (IACUC) of the local veterinary institution and informed consent was obtained from the livestock owners prior to sample collection.

Blood samples were collected in the early morning hours via jugular venipuncture using 10 ml vacuum tubes—both with and without anticoagulant—to minimize circadian variation in hematological and biochemical parameters. The sample collection was performed using sterile techniques to reduce physical stress and avoid sample contamination. Immediately after collection, the samples were stored in an ice box maintained at 4°C for two to three hours to preserve sample integrity during transportation. In the laboratory, the blood samples were centrifuged at 3000 rpm for 15 minutes. The resulting serum was carefully pipetted into 1.5 ml Eppendorf tubes and preserved at −20°C until further biochemical analysis. All hematological parameters including red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count were assessed. Biochemical parameters such as total cholesterol, total protein, albumin, creatinine, bilirubin (BIT), blood urea nitrogen (urea), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alkaline phosphatase (ALK) were measured using commercially available diagnostic kits following the manufacturers' instructions. Statistical analysis was performed using ExcelStat software. Descriptive statistics were expressed as mean ± standard error of the mean (SEM). The independent samples t-test was used to assess the statistical differences between adult and young sheep, while one-way ANOVA was applied to determine gender-based differences across the variables. A p-value <0.05 was considered statistically significant.

RESULTS

The results of the study revealed significant age- and gender-based variations in the hematological and biochemical profiles of healthy Balochi sheep. White blood cell (WBC) counts were highest in young sheep (<12 months) and female sheep, with mean values of $6.99\times10^3/\mu L$ and $7.07\times10^3/\mu L$, respectively. Adult sheep (>12 to <24 months) and male sheep demonstrated the highest red blood cell (RBC) counts, at $7.24\times10^6/\mu L$ and $7.04\times10^6/\mu L$. Hemoglobin concentration was higher in young (10.22 g/dL) and female sheep (11.41 g/dL), while mean corpuscular volume (MCV) was elevated in adult (84.01 fL) and female groups (76.51 fL). Mean corpuscular hemoglobin (MCH) peaked in adult (29.75 pg) and female sheep (27.15 pg), whereas mean corpuscular hemoglobin concentration (MCHC) was greatest in young (33.50 g/dL) and female animals (34.46 g/dL). Platelet counts were substantially higher in young sheep ($194.22\times10^3/\mu L$) and females ($181.61\times10^3/\mu L$). Lymphocytes were more prominent in adult ($8.47\times10^3/\mu L$) and female sheep ($8.04\times10^3/\mu L$), while monocyte counts reached their maximum in young ($4.86\times10^3/\mu L$) and female sheep ($4.34\times10^3/\mu L$). Eosinophil levels were markedly increased in young and female sheep ($5.64\times10^3/\mu L$), and basophils were highest in adult ($0.99\times10^3/\mu L$) and female sheep ($1.24\times10^3/\mu L$). Neutrophil values were elevated in adult ($13.65\times10^3/\mu L$) and female sheep ($12.36\times10^3/\mu L$).

Hematocrit values varied less distinctly, though they were highest in young (39.75%) and male sheep (40.96%). Among biochemical parameters, adult sheep showed elevated levels of urea (9.01 mg/dL), creatinine (1.14 mg/dL), total proteins (8.67 g/dL), albumin (39.01 g/dL), cholesterol (60.96 mg/dL), bilirubin (1.32 mg/dL), and glucose (72.37 mg/dL). Female sheep also showed high values in these same parameters: urea (8.77 mg/dL), creatinine (1.21 mg/dL), total proteins (7.83 g/dL), albumin (21.67 g/dL), cholesterol (55.69 mg/dL), bilirubin (1.16 mg/dL), and glucose (66.61 mg/dL). Liver enzyme levels followed a similar trend. Adult sheep exhibited the highest levels of ALK (123.93 u/L), ALT (38.44 u/L), gamma-glutamyl transferase (36.54 u/L), and AST (123.95 u/L), while female sheep also demonstrated elevated levels of ALK (111.38 u/L), ALT (33.90 u/L), gamma (33.84 u/L), and AST (111.92 u/L). All comparisons showed statistically significant differences between age groups and genders using LSD post-hoc analysis. These findings highlight consistent patterns indicating that age and sex exert substantial influence over the hematological and biochemical indices in sheep.

An evaluation of interaction effects between age and gender on hematological and biochemical parameters revealed noteworthy trends across several indices. Parameters such as white blood cell (WBC) count demonstrated a cross-over interaction, where young male sheep exhibited higher values ($7.34\times10^3/\mu L$) than young females ($6.65\times10^3/\mu L$), whereas in the adult group, females showed higher counts ($7.42\times10^3/\mu L$) compared to males ($6.31\times10^3/\mu L$). A similar pattern was noted for red blood cells (RBC), where young males had elevated values ($7.40\times10^6/\mu L$) compared to young females ($5.99\times10^6/\mu L$), yet adult females showed a pronounced increase ($7.79\times10^6/\mu L$) over adult males ($6.68\times10^6/\mu L$), indicating a significant interaction between age and gender. Hemoglobin concentrations followed this trend; although young males had slightly higher levels (10.49 g/dL) than young females (9.96 g/dL), adult females recorded markedly higher hemoglobin (12.86 g/dL) relative to adult males (6.12 g/dL), suggesting that hemoglobin values in females increase more with age than in males. This interaction effect was further supported by mean corpuscular volume (MCV), where adult females reached the highest values (85.37 fL) while young males recorded the lowest (65.54 fL). Mean corpuscular hemoglobin concentration (MCHC) also showed a differential pattern, particularly in females, who displayed a sharp rise from young (36.30 g/dL) to adult (32.63 g/dL) stages, whereas males showed a modest change (30.71 g/dL in young and 31.33 g/dL in adults). Platelet counts demonstrated a pronounced age-related rise in females ($254.13\times10^3/\mu L$ in young vs. $109.09\times10^3/\mu L$ in adults), while in males, values were consistently lower across ages. Biochemical variables such as urea, creatinine, and liver enzymes (ALT, AST) followed similar interaction patterns, with adult females consistently showing elevated levels compared to other subgroups, while young females showed only modest or intermediate values.

Table 1: Grouping on The Basis of Age and Gender of Healthy Balochi Sheep Breed

Breed of sheep	**Age and gender groups**					
	Young		Adult		Total	
	≤12 months		≥12 months to ≤24 Months		Total	
	Male	Female	Male	Female	Male	Female
Balochi sheep	n=10	n=10	n=10	n=10	n=20	n=20
Total						**40**

Table 2: White Blood Cells, Red Blood Cells, and Hemoglobin Levels (Mean ± SE) in Balochi Sheep by Age and Gender

Parameter	**Age Group**	**Male**	**Female**	**Mean**	**SE± (Group)**	**SE± (Gender)**	**SE± (G × G)**	**LSD 0.05 (Group)**	**LSD 0.05 (Gender)**	**LSD 0.05 (G × G)**
WBC ($\times10^3/\mu L$)	Adult (>12 to <24 mo)	6.31	7.42	6.87 A	0.0865	0.0865	0.1223	0.1754	0.1754	0.2480
	Young (<12 months)	7.34	6.65	6.99 A						
	Mean	6.82 B	7.04 A							
RBC ($\times10^6/\mu L$)	Adult (>12 to <24 mo)	6.68	7.79	7.24 A	0.1199	0.1199	0.1695	0.2431	0.2431	0.3438
	Young (<12 months)	7.40	5.99	6.69 B						
	Mean	7.04 A	6.89 A							
Hemoglobin (g/dL)	Adult (>12 to <24 mo)	6.12	12.86	9.49 B	0.0785	0.0785	0.1110	0.1592	0.1592	0.2252
	Young (<12 months)	10.49	9.96	10.22 A						
	Mean	8.30 B	11.41 A							

Table 3: Erythrocyte Indices (MCV, MCH, MCHC) in Balochi Sheep by Age and Gender

Parameter	Age Group	Male	Female	Mean	SE± (Group)	SE± (Gender)	SE± (G × G)	LSD 0.05 (Group)	LSD 0.05 (Gender)	LSD 0.05 (G × G)
MCV (fL)	Adult (>12 to <24 mo)	82.64	85.37	84.01 A	1.0228	1.0228	1.4464	2.0743	2.0743	2.9335
	Young (<12 months)	65.54	67.65	66.59 B						
	Mean	74.09 B	76.51 A							
MCH (pg)	Adult (>12 to <24 mo)	29.07	30.43	29.75 A	0.2220	0.2220	0.3140	0.4503	0.4503	0.6368
	Young (<12 months)	22.98	23.87	23.42 B						
	Mean	26.02 B	27.15 A							
MCHC (g/dL)	Adult (>12 to <24 mo)	31.33	32.63	31.98 B	0.4380	0.4380	0.6194	0.8882	0.8882	1.2561
	Young (<12 months)	30.71	36.30	33.50 A						
	Mean	31.02 B	34.46 A							

Table 4: Leukocyte Subpopulations, Platelet Count, and Hematocrit Levels in Balochi Sheep by Age and Gender

Parameter	Age Group	Male	Female	Mean	SE± (Group)	SE± (Gender)	SE± (G × G)	LSD 0.05 (Group)	LSD 0.05 (Gender)	LSD 0.05 (G × G)
Platelets (×10³/µL)	Adult (>12 to <24 mo)	107.22	109.09	108.15 B	6.1183	6.1183	8.6525	12.408	12.408	17.548
	Young (<12 months)	134.32	254.13	194.22 A						
	Mean	120.77 B	181.61 A							
Lymphocytes (×10³/µL)	Adult (>12 to <24 mo)	8.08	8.86	8.47 A	0.1066	0.1066	0.1507	0.2161	0.2161	0.3056
	Young (<12 months)	6.52	7.22	6.87 B						
	Mean	7.30 B	8.04 A							
Monocytes (×10³/µL)	Adult (>12 to <24 mo)	2.74	3.63	3.18 B	0.1173	0.1173	0.1659	0.2379	0.2379	0.3365
	Young (<12 months)	4.67	5.05	4.86 A						
	Mean	3.70 B	4.34 A							

Parameter	Age Group	Male	Female	Mean	SE± (Group)	SE± (Gender)	SE± (G × G)	LSD 0.05 (Group)	LSD 0.05 (Gender)	LSD 0.05 (G × G)
Eosinophils (×10³/µL)	Adult (>12 to <24 mo)	1.64	2.65	2.15 B	0.3897	0.3897	0.5511	0.7903	0.7903	1.1177
	Young (<12 months)	3.01	8.28	5.64 A						
	Mean	2.32 B	5.46 A							
Basophils (×10³/µL)	Adult (>12 to <24 mo)	0.49	1.48	0.99 A	0.0754	0.0754	0.1066	0.1529	0.1529	0.2162
	Young (<12 months)	0.97	1.00	0.98 A						
	Mean	0.73 B	1.24 A							
Neutrophils (×10³/µL)	Adult (>12 to <24 mo)	12.39	14.90	13.65 A	0.2536	0.2536	0.3586	0.5143	0.5143	0.7274
	Young (<12 months)	9.81	7.60	8.71 B						
	Mean	10.00 B	12.36 A							
Hematocrit (%)	Adult (>12 to <24 mo)	39.19	40.30	39.75 A	0.6962	0.6962	0.9846	1.4119	1.4119	1.9968
	Young (<12 months)	36.29	42.74	39.51 A						
	Mean	40.96 A	38.29 B							

Table 5: Serum Biochemical and Liver Enzyme Profiles of Balochi Sheep by Age and Gender

Parameter	Age Group	Male	Female	Mean	SE± (Group)	SE± (Gender)	SE± (G × G)	LSD 0.05 (Group)	LSD 0.05 (Gender)	LSD 0.05 (G × G)
Urea (mg/dL)	Adult (>12 to <24 mo)	8.45	9.56	9.01 A	0.2392	0.2392	0.3382	0.4850	0.4850	0.6860
	Young (<12 months)	4.03	7.99	6.01 B						
	Mean	6.24 B	8.77 A							
Creatinine (mg/dL)	Adult (>12 to <24 mo)	0.58	1.69	1.14 A	0.0937	0.0937	0.1325	0.1900	0.1900	0.2687
	Young (<12 months)	0.71	0.74	0.72 B						
	Mean	0.65 B	1.21 A							

Parameter	Age Group	Male	Female	Mean	SE± (Group)	SE± (Gender)	SE± (G × G)	LSD 0.05 (Group)	LSD 0.05 (Gender)	LSD 0.05 (G × G)
Total Protein (g/dL)	Adult (>12 to <24 mo)	7.83	9.50	8.67 A	0.1736	0.1736	0.2455	0.3520	0.3520	0.4978
	Young (<12 months)	4.72	6.17	5.44 B						
	Mean	6.27 B	7.83 A							
Albumin (g/dL)	Adult (>12 to <24 mo)	38.45	39.56	39.01 A	0.0937	0.0937	0.1326	0.1901	0.1901	0.2688
	Young (<12 months)	3.63	3.78	3.71 B						
	Mean	21.04 B	21.67 A							
Cholesterol (mg/dL)	Adult (>12 to <24 mo)	34.18	45.07	60.96 A	1.0808	1.0808	1.5285	2.1920	2.1920	3.0999
	Young (<12 months)	55.61	66.31	39.63 B						
	Mean	44.90 B	55.69 A							
Bilirubin (mg/dL)	Adult (>12 to <24 mo)	0.76	1.87	1.32 A	0.0765	0.0765	0.1081	0.1551	0.1551	0.2193
	Young (<12 months)	0.33	0.45	0.39 B						
	Mean	0.55 B	1.16 A							
Glucose (mg/dL)	Adult (>12 to <24 mo)	66.75	78.00	72.37 A	1.1024	1.1024	1.5590	2.2358	2.2358	3.1618
	Young (<12 months)	44.33	55.23	49.78 B						
	Mean	55.54 B	66.61 A							
ALK (U/L)	Adult (>12 to <24 mo)	111.33	136.53	123.93 A	2.3990	2.3990	3.3927	4.8654	4.8654	6.8807
	Young (<12 months)	61.32	86.23	73.77 B						
	Mean	86.32 B	111.38 A							
ALT (U/L)	Adult (>12 to <24 mo)	33.31	43.56	38.44 A	1.1165	1.1165	1.5789	2.2643	2.2643	3.2023
	Young (<12 months)	14.87	24.25	19.56 B						
	Mean	24.09 B	33.90 A							
Gamma (U/L)	Adult (>12 to <24 mo)	35.98	37.09	36.54 A	1.2236	1.2236	1.7304	2.4816	2.4816	3.5094
	Young (<12 months)	25.50	30.60	28.05 B						
	Mean	30.74 B	33.84 A							

Parameter	Age Group	Male	Female	Mean	SE± (Group)	SE± (Gender)	SE± (G × G)	LSD 0.05 (Group)	LSD 0.05 (Gender)	LSD 0.05 (G × G)
AST (U/L)	Adult (>12 to <24 mo)	111.90	136.01	123.95 A	2.3625	2.3625	3.3411	4.7913	4.7913	6.7760
	Young (<12 months)	63.96	87.84	75.90 B						
	Mean	87.93 B	111.92 A							

Note: ALK = Alkaline Phosphatase, ALT = Alanine Transaminase, AST = Aspartate Aminotransferase, SE = Standard Error, LSD = Least Significant Difference.

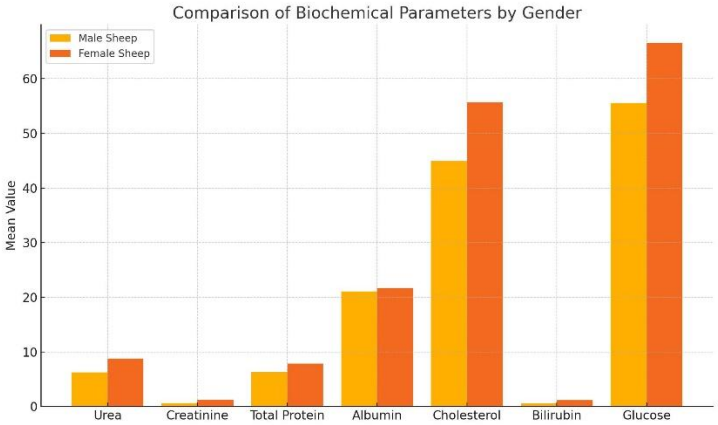

Figure 1 Comparison of Biochemical Parameters by Gender

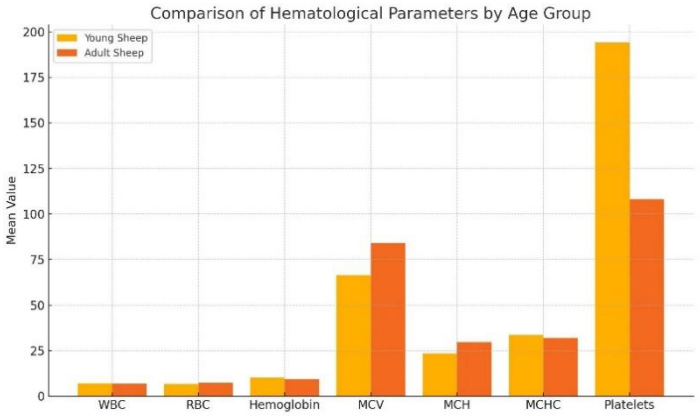

Figure 2 Comparison of Hematological Parameters by Age Group

DISCUSSION

The present study revealed distinct hematological and biochemical patterns across age and gender in healthy Balochi sheep, aligning with and expanding upon existing reference values. Increased WBC counts in the female group may suggest enhanced immune function, potentially driven by better nutrition, environmental adaptability, or hormonal regulation. Prior studies have associated elevated WBC levels with superior health status and disease resistance, while reduced WBC counts in males may point toward physiological stress or suboptimal health conditions (15). RBC values in adult males and females remained within the normal reference range for sheep (6.0–8.5 $\times 10^6/\mu L$), which supports the interpretation that mature animals may have a more developed and stable circulatory system (16). In contrast, the lower RBC values in younger females, though still within range, may indicate developmental or nutritional influences that warrant attention. Hemoglobin levels showed gender-based variation, with female sheep, particularly adults, demonstrating higher concentrations. These values fell within the expected range of 8.5 to 12 g/dL, suggesting adequate oxygen transport capacity in adults and well-maintained erythropoiesis (17). However, the notably low hemoglobin observed in adult males might reflect iron deficiency, underlying anemia, or metabolic stress, necessitating further evaluation. Similarly, creatinine levels remained within normal physiological ranges (0.5–1.5 mg/dL) in all groups, although slightly elevated values in adult females may indicate early renal strain or protein catabolism, which are often linked to dietary protein intake or dehydration (18).

Urea concentrations in adult sheep hovered around the upper normal limit of 10–30 mg/dL, a finding that could imply heightened protein metabolism or mild dehydration, both common in animals with higher metabolic demand (19). Total protein and albumin levels in adults, particularly females, remained within the expected physiological ranges (6.0–8.0 g/dL), reflecting adequate hepatic function and nutritional status (20). In contrast, the significantly reduced protein levels observed in young animals raise concerns regarding nutritional adequacy, protein absorption, or weaning-associated stress. The comparatively low albumin values in younger sheep further corroborate the likelihood of nutritional shortfalls during early development. Cholesterol levels in adult sheep were markedly elevated, nearing or exceeding the upper normal limit of 100 mg/dL. This may indicate a dietary influence, possibly high in fat content, or altered lipid

metabolism—conditions commonly linked to hepatic or endocrine function (21,22). Gender emerged as a critical biological variable in this study. Females consistently showed higher values in WBCs, RBCs, hemoglobin, total proteins, and albumin, suggesting overall superior metabolic and hematological profiles. This could reflect hormonal advantages or better adaptation to feeding and environmental conditions. Literature has also suggested that hormonal regulation, such as estrogen's role in lipid metabolism and immune modulation, may contribute to the observed differences (23,24). Moreover, the interaction between age and gender was evident in several parameters, highlighting the necessity for stratified reference ranges in clinical evaluations.

The hematological ranges for WBCs, RBCs, and hemoglobin in this study were largely consistent with previously published data for Balochi sheep and other indigenous breeds, strengthening the reliability of the findings (18,25). The biochemical data similarly aligned with established values, although the markedly low total protein and cholesterol values in younger animals require cautious interpretation and raise the possibility of transient nutritional deficiencies or management-related factors (26). The inclusion of both genders and defined age categories adds robustness to the dataset and provides a comprehensive view of physiological norms in this breed. A key strength of the study lies in its detailed subgroup analysis and its consideration of age and gender as biological variables, which are often overlooked in veterinary profiling. However, the study's limitations must be acknowledged. The sample size, though evenly distributed, remained relatively small (n=40), which may limit generalizability to broader populations. Additionally, the lack of dietary history and exact environmental stressor data limits the ability to fully contextualize the findings. No longitudinal follow-up was conducted, which could have provided insights into seasonal or developmental fluctuations in the measured parameters. Future studies should consider incorporating larger and more geographically diverse populations of Balochi sheep to enhance external validity. Moreover, integrating hormonal profiling and nutritional biomarkers would help in elucidating the physiological mechanisms behind the observed variations. Establishing breed-specific, age-stratified, and gender-specific reference intervals should also be a priority to improve diagnostic accuracy and animal health management in regional farming systems.

CONCLUSION

This study concludes that both age and gender significantly influence the haematological and biochemical profiles of healthy Balochi sheep. Adult animals demonstrated more favorable markers of circulatory and metabolic function compared to younger counterparts, while females consistently exhibited superior health indicators than males. These findings emphasize the need to consider age- and sex-specific reference values when evaluating the physiological status of Balochi sheep. The results offer practical insights for veterinarians and livestock managers, supporting more precise health assessments, improved disease monitoring, and tailored nutritional strategies for enhancing flock productivity and welfare.

AUTHOR CONTRIBUTION

Author	Contribution
Shakeel Khan	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Allah Bux Kachiwal	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Jahanzaib Khaliq*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Haleema Noor	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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Nisar Ahmad	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Tayyab Ahmad	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Fayaz Ahmed	Writing - Review & Editing, Assistance with Data Curation

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