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ORIGINAL ARTICLE

Changes in Hematobiochemical Parameters with Aging in Afshari Sheep

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Abstract

The Afshari sheep, valued for their adaptability and productivity, are vital to Iran's livestock sector, especially in the Mashhad region. This study examines the effects of age on biochemical and hematological parameters in eighteen clinically healthy non-pregnant Afshari sheep divided into three age-based groups of six each: 1-2 years, 3-4 years, and over 4 years. Following thorough health assessments, blood samples were collected and analyzed for markers including total protein, albumin (Alb), globulin (Glo), aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and hematological parameters such as white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Results showed that total protein and albumin levels were significantly higher in the sheep aged between 1-2 years compared to older ones ($p < 0.001$). ALP concentrations were elevated in younger sheep, while GGT levels increased significantly in sheep older than 4 years ($p < 0.001$). Hematological analyses indicated that RBC counts, HCT, and Hb concentrations were also greater in the youngest group ($p < 0.001$). WBC was lower in the oldest group, but differential leukocyte counts for neutrophils ($p = 0.910$) and lymphocytes ($p = 0.720$) did not differ significantly across ages. These findings underline the significant impact of age on the hematobiochemical profiles of Afshari sheep, providing critical insights for health management practices. This research enhances understanding of age-related physiological changes, aiding farmers and veterinarians in optimizing strategies for the welfare and productivity of this essential livestock breed.

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Introduction

The Afshari sheep, recognized for their adaptability and productivity under various environmental conditions (1), are an integral part of the livestock population in Iran, particularly in the Mashhad region. This breed is valued for its high-quality meat and wool, as well as its important role in local agricultural economies (2, 3). Understanding the physiological and biochemical characteristics of Afshari sheep at different ages is crucial for optimizing their health management, breeding strategies, and overall productivity. Age is a significant factor influencing various physiological parameters in livestock, including biochemical and hematological profiles (4). These profiles reflect the metabolic and immunological status of animals, providing insights into their health and nutritional needs (5). As sheep mature, their metabolic requirements change, resulting in alterations to their biochemical markers and blood parameters (6). For instance, variations in serum biochemical indices such as total protein, albumin, and liver enzymes may indicate developmental changes or stress responses associated with growth and aging (7). Similarly, hematological parameters, including red blood cell count, hemoglobin concentration, and white blood cell profiles, indicate an animal's health status, nutritional adequacy, and immune competence.

Previous studies on sheep have demonstrated that age-related changes in blood biochemistry and hematology can significantly influence productivity (8). Moreover, understanding these changes is vital for informing the timing of interventions, such as vaccinations and nutritional supplements, to enhance the health and productivity of sheep throughout their lifespan (9). Although considerable research has been conducted on the physiology and health management of sheep in Iran, there remains a gap in the comprehensive understanding of how age affects hematobiochemical parameters, specifically in Afshari sheep. This gap is particularly relevant given the breed's unique characteristics and its importance to local agriculture. Investigating these age-related dynamics is essential for developing targeted health management strategies, optimizing feed formulations, and improving breeding practices (10). We hypothesize that aging alters hematobiochemical factors in Afshari sheep; therefore, the present study aims to investigate the effects of age on various biochemical and hematological parameters in Afshari sheep located in the Mashhad region of Iran.

Materials and Methods

Study Area

The study was conducted in Mashhad, located in the northeast of Iran at 36°19'35"N 59°32'36"E, with an elevation of 985 meters above sea level. The semi-arid climate of the study area features cold winters and hot summers, with an annual rainfall of 250–300 mm, primarily occurring during winter and spring. Distinct seasonal variations, influenced by the region's specific climate, affect agricultural practices and livestock management.

Selection and Management of Animals

Eighteen clinically healthy, non-pregnant Afshari sheep were selected for the study. Prior to inclusion, each sheep underwent a thorough health assessment to ensure the absence of diseases or disorders that could affect the study outcomes. Parameters such as rectal temperature, heart rate, and respiratory rate were measured and recorded. The sheep were subsequently categorized into three age groups of six animals each, based on the dental formula: 1-2 years (20-30 kg), 3-4 years (30-40 kg), and over 4 years (40-45 kg). This classification is commonly used in sheep to determine age-related physiological and metabolic changes. All sheep were managed under similar feeding and housing conditions to minimize variations in dietary intake. They were provided with a balanced diet including dry alfalfa, wheat straw, and barley flour, ensuring consistent nutritional support throughout the study period.

Blood Collection and Sample Preparation

Approximately 10 ml of blood was collected from the jugular vein of each sheep for biochemical and hematological evaluations. Of the collected blood, 2 ml was immediately transferred into a tube containing EDTA anticoagulant for the assessment of hematological parameters. The remaining 8 ml of blood was placed in a lab tube and allowed to clot at room temperature. Once clotting was complete, the samples were centrifuged at 3500 rpm for 5 minutes to separate the serum, which was then stored at -20°C until further analysis.

Hematological and Biochemical Analyses

The hematological parameters including the red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC), were evaluated using an automated cell counter device, which operates by drawing a predetermined volume of blood into a chamber where it passes through a laser beam. The device measures changes

in light scattering and determines the concentration of red blood cells, white blood cells, and platelets based on their size and opacity. Biochemical parameters, including aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total protein, albumin (Alb), and globulin (Glo), were measured using specific assay kits from Pars Azmoon Company. The analyses were performed according to the manufacturer's instructions, ensuring the accuracy and reliability of the biochemical data. General hematobiochemical parameters in Afshari sheep were have been presented in Table 1 (2).

Table 1. General hematobiochemical parameters in Afshari sheep

Factors	Min - Max	Mean \pm SD
Total protein (g/dL)	6.4 - 7.7	6.95 \pm 0.34
Alb (g/dL)	2.6 - 4.1	3.57 \pm 0.42
Glo (g/dL)	2.9 - 4.0	3.38 \pm 0.25
ALP (U/L)	122 - 581	272.6 \pm 120.4
GGT (U/L)	31 - 67	42.78 \pm 9.60
ALT (U/L)	17 - 38	28.22 \pm 5.72
AST (U/L)	96 - 201	131.83 \pm 23.6
RBC ($\times 10^6/\mu\text{L}$)	7.6 - 9.9	8.68 \pm 0.67
HCT (%)	25.9 - 35.8	30.72 \pm 2.81
Hb (g/dL)	8.7 - 11.4	10.37 \pm 0.89
MCH (pg)	10.8 - 13.4	11.63 \pm 0.63
MCHC (g/dL)	11.6 - 35.5	29.68 \pm 7.66
MCV (fL)	31.1 - 37.7	33.57 \pm 1.78
WBC ($\times 10^3/\mu\text{L}$)	9.4 - 11.2	10.13 \pm 1.35
Neu (%)	36 - 59	49.00 \pm 6.29
Lym (%)	35 - 59	46.56 \pm 6.24
Mono (%)	0 - 5	2.00 \pm 1.41
Eos (%)	0 - 4	2.35 \pm 1.22

Statistical Analysis

Statistical analyses were conducted using SPSS version 24. The data were subjected to a one-way ANOVA, followed by Tukey's post-hoc test to identify significant differences among the age groups. A *p-value* of less than 0.05 was considered statistically significant, indicating that any observed differences in hematobiochemical parameters among age groups were meaningful and not due to random variation. This systematic approach provided valuable insights into the effects of age on the health indicators of Afshari sheep, contributing to the body of knowledge regarding this important livestock breed.

Results

Hematological Parameters

The effect of age on hematological factors is presented in Table 2. RBC counts were significantly higher in sheep aged between 1-2 years compared to older age groups, with the 3-4 years old sheep also displaying higher counts than those over 4 years old. Moreover, HCT levels followed a similar pattern, indicating significant differences between the youngest and oldest groups, as well as between the 3-4 years old and over 4 years old sheep. Additionally, the youngest age group exhibited significantly higher Hb concentrations compared to the older cohorts, with the 3-4 years old sheep also displaying higher levels than sheep over 4 years. Also, the MCV was significantly elevated in sheep aged 1-2 years relative to the sheep over 4 years. There was no significant age effect on MCH and MCHC levels in the studied sheep. Furthermore, the WBC counts showed significant differences, with younger ewes having higher counts than those over 4 years old. In contrast, differential leukocyte counts for neutrophils and lymphocytes did not exhibit significant variations across age groups. Notably, monocyte levels in sheep aged 1-2 years were significantly higher than those in sheep aged 3-4 years, while eosinophil counts did not differ significantly with age.

Biochemical Parameters

The effect of age on biochemical factors is shown in Table 3. Significant differences were observed in total protein levels, with 1-2 years old sheep exhibiting higher levels than the other two groups. Additionally, Alb levels were also highest in sheep aged between 1-2 years compared to the other age categories, with significant differences noted between the 3-4 years old sheep and those over 4 years old. In contrast, Glo levels did not vary significantly among the age groups. Furthermore, ALP showed significant differences, with the youngest age group having higher levels compared to older age groups, while significant elevations were also found in the 3-4 years old sheep in comparison to those over 4 years old. The GGT levels were significantly higher in sheep over 4 years compared to both younger age groups. The youngest age group showed lower ALT levels compared to the older age groups, while significant differences in AST levels were noted, with older sheep presenting higher concentrations than the younger cohorts.

Discussion

Hematological and serum biochemical tests are routinely employed to identify serious health issues in animals that result in economic losses for livestock, such as reduced growth in fur, fiber, and milk production (11). This study examined significant differences in biochemical and hematological parameters among Afshari ewes, highlighting the crucial role of age in sheep physiology. The biochemical and hematological values observed in the Afshari sheep in this study were largely consistent with the reference ranges established by previous research (12) (Table 1). In comparison with other breeds of sheep in Iran, the mean RBC count and Hb concentration observed in this study are notably higher than the values reported in previous studies (13). This elevation suggests a superior capacity for erythropoiesis in Afshari ewes, potentially attributable to better nutritional management or genetic factors, resulting in improved physiological conditions that may enhance oxygen-carrying capacity. Furthermore, the MCV and MCH values observed were consistently lower compared to

the findings of previous studies examining various sheep breeds (14). Additionally, the observed WBC count of 17,138.89 cells/ μ L in Afshari sheep in the current study significantly surpasses those reported in Balouchi sheep (13) and Mazandaran Zel sheep (14), which may imply an enhanced immune response or a physiological reaction to environmental stressors. Elevated WBC levels may indicate increased immunological activity, warranting further exploration into the health and management practices of these sheep. Moreover, the Afshari sheep in the current study showed higher levels of the Alb and AST values, and lower levels of total protein and ALP compared to the study on Sangsari sheep (15), suggesting increased metabolic turnover or bone remodeling, particularly in growing populations. Also, the mean Glo level in the present study was markedly lower than those reported by (13). The elevation of Glo levels is often associated with immune responses, and higher values might reflect a greater burden of pathogens or stressors. The relatively moderate levels of Glo in this study suggest a balanced immune status.

Table 2. Effect of age on hematological parameters in Afshari sheep

Parameters	1-2 years	3-4 years	> 4 years	p-value
RBC ($\times 10^6$ /mL)	9.45 \pm 0.25 ^a	8.58 \pm 0.39 ^b	8.03 \pm 0.33 ^c	<0.001
HCT (%)	33.40 \pm 1.45 ^a	31.35 \pm 0.87 ^b	27.42 \pm 1.38 ^c	<0.001
Hb (g/dL)	11.25 \pm 0.13 ^a	10.60 \pm 0.15 ^b	9.26 \pm 0.46 ^c	<0.001
MCH (pg)	11.48 \pm 0.33 ^a	11.92 \pm 0.92 ^a	11.50 \pm 0.51 ^a	0.436
MCHC (g/dL)	33.41 \pm 1.29 ^a	26.28 \pm 9.42 ^a	29.35 \pm 8.85 ^a	0.285
MCV (fL)	35.10 \pm 1.34 ^a	33.45 \pm 1.85 ^{ab}	32.16 \pm 0.66 ^b	0.008
WBC ($\times 10^3$ /mL)	13.23 \pm 1.45 ^a	12.05 \pm 4.073 ^b	10.13 \pm 4.501 ^c	0.393
Neu (%)	49.00 \pm 5.69 ^a	48.17 \pm 6.17 ^a	49.83 \pm 7.91 ^a	0.911
Lym (%)	48.33 \pm 4.59 ^a	45.83 \pm 6.30 ^a	45.50 \pm 8.11 ^a	0.717
Mono (%)	1.00 \pm 1.26 ^a	3.33 \pm 1.03 ^b	1.67 \pm 0.81 ^{ab}	0.005
Eos (%)	1.67 \pm 1.03 ^a	2.40 \pm 1.51 ^a	3.00 \pm 0.89 ^a	0.169

^{a, b, c}: Different letters in the same row indicate statistically significant differences ($p < 0.05$)

Table 3. Effect of age on biochemical parameters in Afshari sheep

Parameters	1-2 years	3-4 years	> 4 years	p-value
Pro (g/dL)	7.33 \pm 0.24 ^a	6.88 \pm 0.14 ^{a, b}	6.65 \pm 0.18 ^b	<0.000
Alb (g/dL)	3.97 \pm 0.12 ^a	3.65 \pm 0.10 ^b	3.10 \pm 0.34 ^c	<0.000
Glo (g/dL)	3.37 \pm 0.18 ^a	3.23 \pm 0.20 ^a	3.55 \pm 0.29 ^a	0.095
ALP (u/l)	408.33 \pm 90.00 ^a	259.17 \pm 21.14 ^b	150.33 \pm 22.39 ^c	<0.000
GGT (u/l)	34.33 \pm 3.44 ^a	47.17 \pm 5.45 ^B	52.83 \pm 7.88 ^c	<0.000
ALT (u/l)	22.00 \pm 2.82 ^a	28.17 \pm 1.47 ^b	34.50 \pm 2.73 ^c	<0.000
AST (u/l)	112.33 \pm 10.82 ^a	127.67 \pm 3.67 ^b	155.50 \pm 24.82 ^c	0.001

^{a, b, c}: Different letters in the same row indicate statistically significant differences ($p < 0.05$)

The results of the present study demonstrated significant variations in the biochemical and hematological parameters of Afshari sheep across different age groups, underscoring the influence of age on physiological and metabolic functions in sheep. This finding is critical for understanding the health, management, and nutritional requirements of sheep at various life stages. Our results revealed that the youngest group (1-2 years old) had significantly higher total protein and Alb levels compared to the older groups. This observation aligns with the findings of (16), who noted that younger sheep often exhibit higher serum protein levels due to their active growth phase. The elevated Alb levels may reflect better nutritional status and liver function in younger sheep, as they are typically in a phase of rapid growth and tissue development (17). In contrast, these findings deviate from those reported by (18) and (19), who observed that total protein and albumin levels in sheep increase with age. The reasons for these discrepancies are not straightforward and may be associated with factors such as the breed of the animals studied, management practices, climatic conditions, and nutritional status (16).

In addition, the Glo levels were found to be higher in the older group (over 4 years) compared to the younger groups, which is in parallel with the data from (20) who documented a higher level of Glo in sheep aged over 3 years. The decrease in serum Glo concentration is primarily associated with the lack of passive immunity transfer in young animals (21). Conversely, in pathological conditions, an increase in serum Glo may occur due to heightened production, particularly during inflammatory processes (22, 23).

The average serum concentration of ALP was significantly influenced by age, with higher levels observed in the youngest group (1-2 years). This finding is consistent with the study by (24) which reported higher ALP levels in Iranian red sheep under 2 years than over 2 years, affirming its role as a marker of bone growth and metabolic activity, particularly in growing animals. In young growing animals, the osseous ALP is the predominant form of serum ALP, which tends to decrease as maturation occurs and the epiphyseal plates close (25).

The results of the present study revealed significantly higher GGT levels in sheep over 4 years old compared to younger groups, reflecting hepatic changes associated with aging. The trend in serum GGT activity observed in this study supports the findings of (26), who reported higher GGT activity in older sheep compared to younger ones. However, it contradicts the observations of (19), who noted a decrease in serum GGT activity with advancing age. Elevated GGT levels have been documented as indicators of oxidative stress or liver dysfunction (27). Aging often

leads to compromised liver function, as noted in the study by (28).

The steadily increasing trend of serum AST activity with increasing age of the sheep is consistent with the results obtained by (29). However, some authors, such as (30), found higher concentrations of this enzyme in younger animals than older ones. In sheep, AST is poorly specific to the liver and is used as a marker of liver damage only when other possible causes of variation have been excluded (22). The increasing serum AST activity may be attributed to increasing muscle mass during the intense growth phase or, alternatively, to liver and skeletal muscle damage (25), as well as to increasing physical activity (31).

Furthermore, in the current study, ALT levels were notably lower in younger sheep, suggesting reduced hepatic stress or damage in younger animals, which aligns with findings from (16), who reported similar trends in sheep in Brazil. The higher ALT levels in older sheep may indicate liver function decline, consistent with the work of (8), which emphasizes the need for careful monitoring of these parameters in aging populations. In terms of hematological findings, the higher RBC counts, Hb, and HCT levels observed in younger sheep are in parallel with reports from (32). This reflects the elevated metabolic rate exhibited by younger animals during their growth phase (33). Similarly, previous studies demonstrated that the age of animals affects RBC count, with younger animals showing greater RBC values (29, 34). The higher HCT values observed in younger animals may be attributed to functional changes in hematopoiesis or possibly to hemoconcentration resulting from dehydration (35). In contrast, some studies showed a higher RBC (36) and Hb (37) content in older animals compared to younger ones. Various factors such as animal strains, gender, geographical distribution, parasitic infestations, and health conditions can influence RBC and Hb levels (38).

In the present study, no significant differences were observed in MCH and MCHC levels based on the age of the animals, which is in agreement with the results of (24) and (39). The MCH level was reported to be lower in the youngest group (1-2 years) than the older ones (3-4 years), which agrees with the findings of previous studies (30, 40). This suggests that adult sheep have a more developed immune system than their younger counterparts.

Additionally, a decrease in MCV values with increasing age is in parallel with data from (19), further supporting the notion that younger sheep possess a more dynamic hematopoietic response to growth and increased metabolic demands (41). In contrast, older sheep may show reduced red blood cell size due to factors such as iron deficiency or the effects of chronic diseases. The significant difference in

WBC counts in the current study, particularly the lower levels observed in sheep over 4 years old, underscores an important aspect of immunosenescence, where the immune response may weaken with age (42). This finding is consistent with previous research by (24), who recorded higher WBC values in younger sheep (under 2 years) compared to older ones (over 2 years). It emphasizes the importance of monitoring WBC counts as a potential indicator of general health and immune function in aging sheep. Moreover, no significant differences were found in the differential leukocyte counts for neutrophils, lymphocytes, and eosinophils across age groups, which is in agreement with data from previous studies (29), but diverges from the findings of (43), who reported age-related variations in leukocyte populations. It has been reported that the WBC and differential leukocyte counts in sheep can vary based on age, physiological stage, and parasitic infections (44).

Conclusion

The substantial variations observed in biochemical and hematological parameters among different age groups of Afshari sheep underscore the critical role of age as a factor in sheep management and care. Our findings are consistent with existing literature while also providing new insights into the specific health implications for this breed. Future research avenues could benefit from longitudinal studies exploring these parameters over time in controlled environments to better understand the factors driving the observed age-related changes. Such studies could provide deeper insights into the nutritional, health, and management needs of sheep transitioning through various life stages, ultimately contributing to improved welfare and productivity in sheep farming.

Limitations of the study

This study is limited by its relatively small sample size and by cross-sectional design. Future studies with larger population and longitudinal data are recommended to validate and expand upon these findings.

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Author contributions

Kiana Karbasi: Methodology, sampling, writing the original draft. **Mohammad Mazrouei Sebdani:** Conceptualization, formal analysis, investigation, supervision, **Amin Ahmadi:** Sample analysis, investigation, review & editing.

Data Availability

All data analyzed in this study are included in this published article.

Ethical Approval

The authors declare that all ethical standards have been respected in the preparation of the submitted paper.

Conflict of Interest

The authors affirm that there are no competing interests with the publication of this work.

Consent for Publication

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