# Original Article

# Exploratory Study on the Relationship Between Age, Reproductive Stage, Body Condition Score, and Liver Biochemical Profiles in Rembi Breed Ewes

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# ABSTRACT

**Background:** The Rembi sheep breed is renowned for its exceptional meat quality and significant economic profit. Nevertheless, there is a gap in research on the hepatic parameters of this breed in Algeria despite the considerable value of the liver in the Algerian population's diet.

**Objectives:** The research aimed to determine the impact of physiological factors (age, physiological stage, and body condition score) on biochemical sheep liver parameters.

**Methods:** Sixty clinically healthy female Rembi breed sheep, aged between 3 months and 7 years, raised at the Technical Institute of Livestock (ITELV) Ksar Chellala Farm in Tiaret Province, Algeria, were selected for biochemical analysis and assessing aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB), and total protein (TP).

**Results:** The concentrations of AST, ALP, and GGT increased significantly ($P \leq 0.001$) in young sheep, while ALT and TP levels decreased ($P \leq 0.05$). However, a high level of GGT ($P < 0.001$) was also observed in lactating ewes, and during pregnancy, females have a low level of ALP ($P < 0.001$). Furthermore, the body condition score does not affect any of the measurements.

**Conclusion:** Considering the animal's age and physiological stage is critical before interpreting the results.

**Keywords:** Age, Body condition score, Liver biochemical parameters, Physiological state, Rembi breed sheep

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## Introduction

Algeria has multiple sheep breeds, including the Rembi breed, which comprises around 2 million heads (Laoun et al., 2015). The breed is predominantly raised in the Tiaret area and nearby environs (Fekhreddine et al., 2015). Unfortunately, due to uncontrolled crossbreeding, some breeds, including the Rembi, have undergone genetic modification (Gaouar et al., 2015). The Rembi breed is tough and has adapted to mountainous terrain, cold, and drought conditions (Harkat et al., 2015). To preserve its original genetic heritage, initiatives like the Technical Institute of Livestock (ITELV) Ksar Chellala program have been implemented.

Optimal metabolism depends on healthy liver function to preserve animal health (Antunović et al., 2009). Therefore, checking the biochemical indicators of liver function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB), and total protein (TP) proves helpful in identifying early liver problems (Braun et al., 2010). However, blood biochemical values published from other countries may not apply to our country due to variations in breed, age, environment, and analytical processes adopted by researchers (Allison et al., 2015; Mohri et al., 2007). To correctly interpret the results, we must create our liver reference values.

This research aimed to examine the influence of age, physiological stage, and body condition on hepatic parameters of Rembi breed sheep raised in Ksar Chellala.

## Materials and Methods

Over 3 months (from December 2022 to February 2023), the blood samples were collected from 60 clinically healthy sheep raised in the ITELV Ksar Chellala in the Tiaret Province, Algeria. These sheep were separated into groups based on their age (group 1: 3 months, group 2: 1.5 to 3 years, group 3: older than 3 years), physiological state (pregnant, lactating non-pregnant, non-pregnant), and the body condition score (BCS) in adult animals over one year old (group 1: BCS <2.5; group 2: 2.5≤ BCS <3; group 3: BCS ≥3) following the protocol of Russel et al. (1969).

### Animal's diet

The livestock's diet at the farm is typically managed, considering the animals' weight and physiological stages (pregnancy, lactation, fighting, and growth) and food type availability. The diet comprises of coarse feeds (barbey/oat hay, alfalfa hay, and straw), as well as concentrated feed (barley, soybean meal, limestone, salt, dicalcium phosphate, trace element) and vitamin A, E, D3 supplements, with unrestricted access to water (Table 1).

The diet is distributed across two meals: Morning and evening.

### Blood collection

Blood (4 mL) was collected from the jugular vein using a heparin-labeled tube via sterile needles. The obtained samples were transferred to the Veterinary Biochemical Laboratory situated at the Veterinary Institute of Tiaret in a cooler for centrifugation at 3000 xg for 10 minutes to extract the plasma, transfer it to Eppendorf tubes, and maintained at -20°C for further analysis.

### Biochemical analyses

The blood parameters were analyzed using a biochemistry auto-analyzer (Roche, Cobas, Germany). The measurements comprised TP, ALB, ALT, AST, TBIL, DBIL, GGT, and ALP using commercial kits provided by Roche Diagnostics.

### Statistical analysis

SPSS software, version 22 was used for data processing. The effects of age, physiological state, and BCS on the investigated parameters were analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey test for multiple comparisons when a significant ANOVA result was obtained ($P<0.05$).

**Table 1.** Diet composition of sheep

| Animal | Concentrated Feed (kg/animal/d) | Coarse Feed (kg/animal/d) |
|---|---|---|
| Lactating | 0.6 | 0.8 (alfalfa hay) |
| Pregnant and non-pregnant | 0.6 | 0.8 (wheat/barley straw) |
| Lambs | 0.2 | 0.4 (wheat/barley straw) |

Outliers were eliminated, and certain variables were converted using the natural logarithm to meet the ANOVA criteria (homogeneity of variances and normality of residuals). The mean of transformed variables can be substituted with the median.

## Results

The analyses of liver parameters revealed differences regarding age (Table 2) and physiological stage (Table 3) among sheep, not BCS (Table 4). In terms of age, significant differences (P<0.05) were seen between young and adult groups (groups 2 and 3) in all parameters except bilirubin (total and direct). Furthermore, the examination based on the physiological stage demonstrated a significant difference (P<0.001) only in GGT and PAL activities, which increased in lactating and non-pregnant ewes.

## Discussion

### Age and liver parameters

The results demonstrated significant differences among the age groups in the levels of AST and ALT in Rembi sheep; the elevated enzymes in young animals might indicate stress (Abdel-Fattah et al., 2013) and high metabolic rate. However, Devrim et al. (2015) observed a reduced level of AST and ALT in young Honamlı and Native Hair goats. Additionally, in the study conducted by Azimzadeh and Javadi (2020), it was observed that transaminase levels were elevated in Iranian Red sheep aged over two years. Runa et al. (2022) showed no effect of age on transaminase activity in Black Bengal goats. This discrepancy in results could be attributed to the variations in the breed (Hajinejad-Bamroud et al., 2020), surroundings, dietary intake, and overall health condition of the animals (Beura et al., 2014).

Protein levels elevate as sheep get older. These results were in accordance with the data of Barbosa et al. (2022), Rattana et al. (2011) in goats (southern Thailand), Islamov et al. (2021) in Merino Sheep (Kazakhstan), and Bonsmara cattle (Brazil). An increase in total protein concentration in adults could be attributed to a rise in the concentration of albumin and globulins (Chaudhary et al., 2003) synthesized in the liver (Taheri Mirghaed et al., 2023). While Alberghina et al. (2010) mentioned that age has little effect on the physiological concentrations of total protein and protein fractions (albumin and globulins) in mature animals. It could also be attributed to the modification of diet between the juvenile and adult, which rises in mature animals.

The level of GGT and ALP decline in adult sheep, which were consistent with the findings of Borges et al. (2011), and Santo Da Cruz et al. (2017), who revealed higher level in younger compared to adult sheep; however, in the study of Daramola et al. (2005), ALP lev-

**Table 2.** The influence of age on hepatic parameters in Rembi sheep

| Parameter | Age | | | | | | P |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | Group 1 | | Group 2 | | Group 3 | | |
| | No. | Mean±SD | No. | Mean±SD | No. | Mean±SD | |
| AST (IU/L) | 8 | 107±5.5[a] | 25 | 84.3±14.1[b] | 25 | 92.1±15.1[b] | 0.001 |
| ALT (IU/L) | 9 | 14.4±2.6[a] | 25 | 19.5±4.3[b] | 24 | 21.4± 3.9[b] | <0.001 |
| TP (g/L) | 10 | 58.3±2.7[a] | 25 | 61.2±6.8[a, b] | 25 | 65.8±9.5[b] | 0.02 |
| ALB (g/L) | 10 | 32.6±2[a] | 25 | 30.4±3.4[a] | 25 | 32.2±3.3[a] | 0.08 |
| ALP (U/L) | 10 | 517±63.3[a] | 20 | 80.7±22[b] | 24 | 93.8±45.2[b] | <0.001 |
| GGT (IU/L) | 10 | 84.4±11[a] | 25 | 41.7±7.9[b] | 25 | 45.4±11.3[b] | <0.001 |
| TBIL (mg/L) | 10 | 2 | 25 | 2 | 25 | 2 | >0.05 |
| DBIL (mg/L) | 10 | 1 | 10 | 1 | 10 | 1 | >0.05 |

Mean values within the same row with different superscripts differ at P<0.05.

Group 1: 3 months, Group 2: 1.5 to 3 years, Group 3: Older than 3 years.

[a, b]Significant difference (P<0.05).

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**Table 3.** The effect of physiological state on hepatic parameters in Rembi Ewes sheep

| Parameter | Physiological State | | | | | | P |
|---|---|---|---|---|---|---|---|
| | Pregnant | | Non-pregnant | | Lactating | | |
| | No. | Mean±SD | No. | Mean±SD | No. | Mean±SD | |
| AST (U/L) | 23 | 86.9±12 | 18 | 87.5±17.2 | 9 | 93±17.7 | 0.58 |
| ALT (U/L) | 23 | 20.2±4 | 18 | 21±4.3 | 9 | 21.3±6.3 | 0.78 |
| TP (g/L) | 23 | 61.2±5.9 | 18 | 65.6±9.5 | 9 | 65±11.4 | 0.23 |
| ALB (g/L) | 23 | 31.3±3.4 | 18 | 31.1±3 | 9 | 31.7±4.5 | 0.89 |
| ALP (U/L) | 22 | 68.7±23.7[a] (63) | 18 | 127±58.5[b] (117) | 9 | 124±57[b] (113) | <0.001 |
| GGT (U/L) | 22 | 39±5.2[a] (40) | 15 | 48.5±8.1[b] (48) | 9 | 50.2±11.2[b] (55) | <0.001 |
| TBIL (mg/L) | 23 | 2 | 18 | 2 | 9 | 2 | >0.05 |
| DBIL (mg/L) | 23 | 1 | 18 | 1 | 9 | 1 | >0.05 |

Mean values within the same row with different superscripts differ at P<0.05. The value in parentheses is the median of the transformed variable.

[a, b]Significant difference (P<0.05).

els were higher in adult compared to young animals (P<0.05). The consumption of colostrum may have contributed to the elevated GGT value reported in younger, non-weaning animals (Gokçe et al., 2021), and the higher value of ALP in the young may be associated to bone development, where they execute the function of mineralization during osteogenesis (Golub & Boesze-Battaglia., 2007). While Aliyu et al. (2022), and Gwaze et al. (2012) reported that age did not affect ALP and GGT levels (P>0.05).

In this research, the mean albumin and bilirubin (TBIL and DBIL) activities of Rembi sheep demonstrated no significant differences between the age groups. Identical findings were reported by Mamun et al. (2014) on albumin concentration and by Barini (2007) on bilirubin levels. Nevertheless, Ramesh et al. (2019) observed a significant effect of age on bilirubin value. Shamohamadi et al. (2021) noted that serum levels of DBIL and TBIL serve as markers for the liver function in the disintegration and excretion of substances.

**Table 4.** Impact of body condition score on liver parameters in Rembi Ewes sheep

| Parameter | Body Condition Score | | | P |
|---|---|---|---|---|
| | Mean±SD | | | |
| | BCS <2.5 (n=12) | 2.5≤ BCS <3 (n=22) | BCS ≥3 (n=16) | |
| AST (U/L) | 84.6 ±17.4 | 87.2±13.6 | 92.3±14.9 | 0.38 |
| ALT (U/L) | 19.2±5 | 21±3.9 | 21.3±4.8 | 0.42 |
| TP (g/L) | 60.3±7.7 | 65.2±9.4 | 63.5±7.6 | 0.28 |
| ALB (g/L) | 30.5±4.1 | 31.6±2.9 | 31.4±3.7 | 0.69 |
| ALP (U/L) | 121±69.6 | 103±53 | 90.6±50.3 | 0.36 |
| GGT (U/L) | 39.5±8.4 | 45.4±10.9 | 44.1±8.8 | 0.24 |
| TBIL (mg/L) | 2 | 2 | 2 | >0.05 |
| DBIL (mg/L) | 1 | 1 | 1 | >0.05 |

BCS: Body condition score.

**The effect of physiological state on liver parameters**

The results indicated no effect of reproductive status on AST, ALT, TP, ALB, TBIL, and DBIL. Similar outcomes were observed by Sarmin et al. (2022) on TP and ALB in Wonosobo sheep, while in Nellore sheep, the TP was significantly higher in the lactating group, and the ALB levels were significantly higher in the pregnant group (Chandra et al., 2023). In Saidi ewes, blood total protein reduced considerably throughout late pregnancy and postpartum compared to pre-mating (Teleb et al., 2019). Antunovic et al. (2011) also reported significantly higher total protein and albumin concentrations in pregnant ewes compared to non-pregnant ones.

Several investigations also showed that the physiological stage did not influence transaminase activities in fat-tailed and Wonosobo sheep (Sarmin et al., 2021; Sarmin et al., 2022). Serum ALP level is an indirect asset for assessing liver status. It represents a sensitive indicator of cholestasis (Rafiee et al., 2021). In this study, lactating and non-pregnant sheep had much higher ALP levels than pregnant ewes, possibly related to increased milk production. These findings contradict those reported by Ismaeel et al. (2023) and Yokus et al. (2006), who discovered a considerable rise in ALP during pregnancy. Furthermore, the result of Sarmin et al. (2021) study revealed no influence of the physiological stage on this parameter. Differences between the findings of this study and previous research might be attributed to environmental factors such as seasonal fluctuations and feed quality.

The elevated level of GGT in lactating and non-pregnant sheep could be related to the high GGT activity in the mammary gland (Ramos et al., 1994). Additionally, the colostrum is noted to possess a significant quantity of GGT (Kaneko et al., 1997) and can also indicate an increase in metabolism related to high productivity (Nicolae et al., 2021). This study was similar to the data of Antunovic et al. (2011) in Tsigai ewes, While in Saidi ewes, an increase in GGT levels is observed during the first trimester of pregnancy (Mohamed & Abou-Khali, 2017).

This study aligned with the findings of Nicolae et al. (2021) in Țurcană mixed-breed ewes, demonstrating no significant impact of physiological state on bilirubin (total and direct) levels. Meanwhile, in Ouled Djellal breed sheep, the highest bilirubin levels were observed in lactating ewes (Deghnouche et al., 2011).

This study found no noticeable influence of body condition score on the examined parameters, which were in agreement with the findings of Ferreira et al. (2021) in tropical ewes, Titaouine et al. (2022), and Boudebza et al. (2020) in Ouled Djellal ewes. However, Caldeira et al. (2007) reported a significant effect of body condition score in albumin and total protein, and Aiche et al. (2023) demonstrated a significant impact of body condition score on GGT levels in Rembi breed sheep.

## Conclusion

In conclusion, the effect of age, physiological state, and body condition score on liver biochemical parameters (AST, ALT, GGT, ALP, TP, ALB, TBIL, and DBIL) demonstrates a complex system of relationships. Age is a prominent influencer, affecting most parameters and reflecting the physiological modifications produced during life. In contrast, the restricted influence of the physiological state on ALP and GGT demonstrates its particular impact. However, there is no correlation between BCS and liver tests. Therefore, the determination of these liver parameters helps in the diagnosis and improvement of animal health. A potential limitation of this study was that it concentrated on one farm, which might not accurately reflect all Rembi breeds. To improve the validity of the results, more research should involve a larger sample of the Rembi breed maintained in diverse farms around the country, ensuring a more precise comprehension of the breed's characteristics.

## Ethical Considerations

**Authors' contributions**

All authors equally contributed to preparing this work.

**Conflict of interest**

The authors declared no conflict of interest.

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