

Exploratory Study on the Relationship among 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 impact. Nevertheless, there is a noticeable absence of dedicated research on hepatic parameters of this breed in Algeria, despite the considerable value of liver in Algerian population's diet.

Objectives: The purpose of this research was to determine the impact of physiological factors (age, physiological stage, and body condition score) on biochemical liver parameters,

Methods: Sixty clinically healthy female Rembi breed sheep, aged between 3 months and 7 years old, and located at the technical institute of livestock (ITELV) ksar Chellala Farm in Tiaret province, Algeria, were selected for biochemical analysis, 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).

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, high level of GGT ($P < 0,001$) was also observed in lactating ewes, and during pregnancy, females have low level of ALP ($P < 0,001$). Furthermore, body condition score has no effect on any of the measurements.

Conclusion: it is critical to consider both the animal's age and physiological stage before interpreting the results.

Key words: Age; body condition score; liver biochemical parameters; physiological state; Rembi breed sheep.

1. Introduction

Algeria has a multiple variety of sheep breeds, including the Rembi breed, which comprise around 2 million head (Laoun *et al.*, 2015) and is raised predominantly in the Tiaret area and nearby environs (Chellig., 1992). Unfortunately, due to uncontrolled crossbreeding, some breeds, including the Rembi, have undergone genetic modification (Gaouar *et al.*, 2015). The Rembi breed is hardy, adapted to mountainous terrain, cold, and drought conditions (Harkat *et al.*, 2015). To preserve its original genetic heritage, initiatives like the ITELV Ksar Chellala program have been implemented.

Optimal metabolism depends on healthy liver function to preserve animal health (Antunović *et al.*, 2009). Therefore, examining the biochemical indicators of the liver, 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) is useful for identifying early liver problems (Braun *et al.*, 2010). However, blood biochemical values published from other countries may not be appropriate to our circumstances due to variations in breed, age, environment, and analytical processes adopted by researchers (Trall *et al.*, 2015, Mohri *et al.*, 2007). To correctly interpret the results, it is essential to create our own liver reference values.

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

2. Materials and Methods

Over a 3 months period (December 2022 to February 2023) the farm ITELV Ksar Chellala located in the Tiaret province, Algeria, played an important role as the site for blood collection in a total of 60 clinically healthy sheep. these sheep were separated into groups based on their age (group1: 3 months, group 2: 1,5 to 3 years, group: older than 3 years), physiological state (pregnant, lactating non pregnant, non pregnant) and the body condition score (BCS) were taken in adult animals over one year old: group 1 ($BCS < 2.5$) , group 2 ($2.5 \leq BCS < 3$), group 3 ($BCS \geq 3$) following the protocol of Russel *et al.* (1969).

Animal's diet:

The livestock's diet at the farm is typically managed, taking into consideration the animals' weight and physiological stages (pregnancy, lactation, fighting and growth) and availability of food type. This is achieved through a combination of (table1) 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.

The diet is distributed across two meals: morning and evening

Table (1): Diet composition of sheep

Animal	Concentrated feed (kg/anim/d)	Coarse feeds (kg/anim/d)
Lactating	0.6 kg	0 ,8Kg (alfalfa hay)
Pregnant and non-pregnant	0.6 kg	0.8 kg (wheat/barley straw)
Lambs	0,2 kg	0,4 kg (wheat/barley straw)

kg: kilogram, anim: animal, d: day



Blood collection

Blood was collected (4ml) from the jugular vein using a heparin-labeled tube using 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 g for 10 minutes in order 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), including measurements of total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) using commercial kits provided by Roche Diagnostics®.

Statistical analysis

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

Certain 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.

3. Results

The analyses of liver parameters revealed differences dependent on both age (table 2) and physiological stage (table 3) among sheep, while based on BCS (table 4), there is no significant difference in all parameters. In terms of age, a significant variation ($P < 0.05$) in all parameters except bilirubin (total and direct) between young and adult groups (group 2 and

3). Furthermore, the examination based on physiological stage demonstrated a significant difference ($P < 0,001$) only in GGT and PAL activities, which increased in lactating and non pregnant ewes.

4. Discussion

Regarding to the influence of age in liver parameters

The analysis 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 elevated of metabolic rate, while 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 old. Runa *et al.* (2022) Showed no effect of age on transaminase activity in Black Bengal goats. This difference of results could be attributed to the variations in breed (Bamroud-Hajinej *et al.*, 2020), surroundings, dietary intake, and overall health condition of the animals (Beura *et al.*, 2014).

Table 2: the influence of age on hepatic parameters in Rembi sheep

Parameters	Age						
	Group1		Group2		Group3		<i>P</i> value
	N	M ± SD	N	M ± SD	N	M ± SD	
Aspartate aminotransferase AST (IU/L)	8	107±5,5 ^a	25	84,3±14,1 ^b	25	92,1±15,1 ^b	0,001
Alanine aminotransferase ALT (IU/L)	9	14,4±2,6 ^a	25	19,5±4,3 ^b	24	21,4± 3,9 ^b	<0,001
Total Protein TP (g/L)	10	58,3±2,7 ^a	25	61,2±6,8 ^{a,b}	25	65,8±9,5 ^b	0,02
Albumin ALB (g/L)	10	32,6±2 ^a	25	30,4±3,4 ^a	25	32,2±3,3 ^a	0.08
Alkaline phosphatase ALP U/L	10	517±63,3 ^a	20	80,7±22 ^b	24	93,8±45,2 ^b	<0,001
Gamma glutamyl transferase GGT (IU/L)	10	84,4±11 ^a	25	41,7±7,9 ^b	25	45,4±11,3 ^b	<0,001
Total bilirubin (mg/L)	10	2	25	2	25	2	>0.05

Direct bilirubin (mg/L)	10	1	10	1	10	1	>0.05
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N: Number of observations; M: Mean; SD: Standard deviation. **Means values within the same row with different superscripts differ at ($p<0.05$)**

Group 1: 3 months, Group2: 1,5 to 3 years, Group 3: older than 3 years.

Table 3: The effect of physiological state on hepatic parameters in Rembi ewes sheep

Parameters	Physiological state						<i>P</i> value
	Pregnant		Non pregnant		Lactating		
	N	M ± SD	N	M ± SD	N	M ± SD	
Aspartate aminotransferase (AST) U/L	23	86,9±12	18	87,5±17,2	9	93±17,7	0,58
Alanine aminotransferase (ALT) U/L	23	20,2±4	18	21±4,3	9	21,3±6,3	0,78
Total Protein (TP) g/l	23	61,2±5,9	18	65,6±9,5	9	65±11,4	0,23
Albumin (ALB) g/l	23	31,3±3,4	18	31,1±3	9	31,7±4,5	0,89
Alkaline phosphatase (ALP) U/L	22	68,7±23,7 ^a (63)	18	127±58,5 ^b (117)	9	124 ±57 ^b (113)	<0,001
Gamma glutamyl transferase (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
Total bilirubin	23	2	18	2	9	2	>0.05
Direct bilirubin	23	1	18	1	9	1	>0.05

N: Number of observations; M: Mean; SD: Standard deviation. **Means values within the same row with different superscripts differ at ($p<0.05$)**. The value in parentheses is the median of the transformed variable.

Protein levels elevate as individuals age, These results were in accordance with the data of Barbosa *et al.* (2022), and 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 an increase in the concentration of albumin and globulins (Chaudhary *et al.*, 2003) synthesized in the liver (Mirghaed *et al.*, 2023). While Alberghina *et al.* (2010) mentioned that age has very 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 rise in mature animals.

Table 4 : impact of body condition score on liver parameters in Rembi ewes sheep

Parameters	Body condition score			<i>P</i> value
	BCS < 2,5	2,5 ≤ BCS < 3	BCS ≥ 3	
	M ± SD	M ± SD	M ± SD	
	N : 12	N : 22	N : 16	
Aspartate aminotransferase (AST) U/L	84,6 ±17,4	87,2±13,6	92,3±14,9	0,38
Alanine aminotransferase (ALT) U/L	19,2±5	21±3,9	21,3±4,8	0,42
Total Protein (TP) g/l	60,3±7,7	65,2±9,4	63,5±7,6	0,28
Albumin (ALB) g/l	30,5±4,1	31,6±2,9	31,4±3,7	0,69
Alkaline phosphatase (ALP) U/L	121±69,6	103±53	90,6±50,3	0,36
Gamma glutamyl transferase (GGT) U/L	39,5±8,4	45,4±10,9	44,1±8,8	0,24
Total bilirubin	2	2	2	>0,05
Direct bilirubin	1	1	1	>0,05

N: Number of observations; M: Mean; SD: Standard deviation; BCS: Body condition score

The level of GGT and ALP decline in adults, 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, however, in the study of Daramola *et al.* (2005), alkaline phosphatase

(ALP) levels 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 and Boesze-Battaglia., 2007). While Aliyo *et al.* (2022), and Gwaze *et al.* (2012) reported that age had no effect on ALP and GGT levels ($P > 0.05$).

In this research, the mean of 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's function in the disintegration and excretion of substances.

Concerning 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 lactating group, whereas the ALB levels were found to be significantly higher in 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) reported also significantly high concentrations of total protein and albumin in pregnant ewes compared to non-pregnant.

Several investigations also showed that physiological stage had no influence on transaminase activities in Fat-Tailed and Wonosobo sheep (Sarmin *et al.*, 2021, and Sarmin *et al.*, 2022).

Serum ALP levels is an indirect asset for assessing liver status and it represent 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, which may be related with increased milk production. These findings were contrary to those reported by Ismaeel *et al.* (2023) and Yokus *et al.* (2006), who discovered a considerable rise in ALP during pregnancy. Furthermore, result of Sarmin *et al.* (2021) revealed no influence of 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 linked to the elevated 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 indicates 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 and Abou-Khali, 2017).

This study aligned with the findings of Nicolae *et al.* (2021) in Turcană mixed-breed ewes, demonstrating no significant impact of physiological state on bilirubin (total and direct) levels. Whereas, 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, and contradicted with the conclusions of Caldeira *et al.* (2007), who reported a significant effect of body condition score in albumin and total protein, and Aiche *et al.* (2023) who demonstrated a significant impact of body condition score on GGT levels in Rembi breed sheep.

5. Conclusion

In conclusion, the examination of 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 takes center stage as a prominent influencer, affecting the majority of parameters, reflecting the physiological modifications produced during life. In contrast, the restricted influence of physiological state on only ALP and GGT, demonstrating its particular impact. However, there is no correlation between BCS and liver tests. Therefore, the determination of these liver parameters aided 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 breed. To improve the validity of the results, more research should involve a larger sample of Rembi breed maintained in diverse farms around the country, ensuring a more precise comprehension of the breed's characteristics.

Ethical Considerations

Compliance with ethical guidelines

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Authors' contributions

This article was prepared with equal contributions from all authors.

Conflict of Interest

The authors declare that there is no conflict of interest related to this research.

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Uncorrected Proof

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بررسی آلودگی تک یاخته های میکروسپوریدیا، بلاستوسیستیس، ژیاردیا و کریپتوسپوریدیوم در پرنده‌گان حانگی ارجاع شده به درمانگاه‌های دامپزشکی شهر تهران به روش انگل شناسی و مولکولی

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زمینه مطالعه: میکرووارگانیسم‌های فرست طلب دستگاه گوارش از جمله *Giardia* spp., *Cryptosporidium* spp., *Blastocystis* sp. و *microsporidia* به طور چشمگیری مسئول اختلالات بالینی در گونه‌های مختلف میزبان از جمله انسان هستند.

هدف: در مطالعه حاضر حضور ارگانیسم‌های فوق در میزبان پرندگان خانگی بررسی شد.
روش کار: نمونه‌های دفع شده از 150 پرنده خانگی از کلینیک‌های دامپزشکی شهر تهران جمع‌آوری و به صورت میکروسکوپی و مولکولی بررسی شد.

نتایج: سه مورد از مدفعهای مورد بررسی حاوی ژنوتیپ (B1) (٪2) انسفالیتوزوون هلم با روش PCR و تعیین توالی بود. ارگانیسم‌های میکروسپوریدیایی از مدفع یک طوطی گرین چیک، یک طوطی خاکستری آفریقایی و یک طوطی برزیلی جدا شدند. سایر انگل‌ها در نمونه‌های بررسی شده یافت نشد.

نتیجه‌گیری نهایی: مطالعه حاضر ثابت کرد که پرندگان خانگی نگهداری شده در قفس منبع احتمالی عفونت میکروسپوریدیایی هستند. ماهیت بسیار مقاوم اسپورهای میکروسپوریدیا، علاوه بر این واقعیت که انسفالیتوزوونوزیس عمدتاً در پرندگان تحت بالینی است، می‌تواند صاحبان آن‌ها را در معرض افزایش خطر ابتلا به بیماری از طریق استنشاق یا بلعیدن اسپور قرار دهد. اگرچه منبع و ماهیت عفونت ناشناخته باقی مانده است، ممکن است مطالعات بیشتری برای روشن کردن نقش پرندگان خانگی در اپیدمیولوژی پاتوژن‌های فرست طلب مشترک بین انسان و دام توصیه شود.

کلمات کلیدی: بالاستوسیستیس، بیماریهای مشترک، ژیاردیا، کریپتوسپوریدیوم، میکروسپوریدیا

Uncorrected Proof