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PARTICULARITIES OF CERTAIN BIOCHEMICAL INDICES IN SHEEP INFESTED WITH *Dicrocoelium dendriticum* (Rudolphi, 1819)

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Abstract

The obtained results highlight notable alterations in biochemical parameters in sheep infested with Dicrocoelium spp. Compared to the control group, total protein levels decreased by 11.22% ($p > 0.05$), primarily due to a 12.23% reduction in albumin concentration ($p > 0.05$). In contrast, globulin levels increased by 6.12% ($p > 0.05$), suggesting a compensatory response in protein fractions. Analysis of hepatic enzymes revealed elevated activities in infested sheep, indicating potential liver dysfunction. Specifically, alanine aminotransferase (ALT) activity was 24.63% higher ($p > 0.05$), while gamma-glutamyl transferase (GGT) increased by 21.31% ($p > 0.05$) relative to the control group. Additionally, aspartate aminotransferase (AST), an enzyme present in multiple tissues, showed an 11.77% increase ($p > 0.05$) in the infested animals. These biochemical alterations reflect disturbances in protein metabolism and hepatic function associated with dicroceliosis. Although the differences did not reach statistical significance, the observed trends suggest subclinical effects of the parasite on liver health and systemic metabolism in affected sheep.

Key words*: biochemical parameters, Dicrocoelium dendriticum, liver enzymes, protein metabolism, sheep.*

INTRODUCTION

In the animal world, parasitic organisms represent an essential and integral component of ecosystems, being present in a wide range of natural or anthropogenically modified biocenoses, both terrestrial and aquatic. Numerous investigations conducted on animal populations have demonstrated that parasitic diseases affecting animals and humans in the Republic of Moldova exhibit a wide distribution and are responsible for significant economic and social damage (Zgardan et al., 2001; Zgardan, 2008; Erhan at al., 2005; Erhan, 2010, 2020, 2024; Cercel et al., 2018; Nafornița, 2014, 2016, 2018; Nafornița et al., 2017).

In the study of parasitic diseases, a fundamental role in the control of parasitic agents is played by knowledge of their epidemiology, which depends on the technology of animal management, age, season, and, importantly, on the physiological, biochemical, and morphological processes that develop in the mono- or polyparasitized host organism.

Small ruminants, throughout their productive lifespan, are frequently subjected to parasitic invasions, including those localized in the liver and bile ducts, caused by trematodes such as *Fasciola hepatica* and *Dicrocoelium lanceolatum*, as well as by cestodes such as the larval stage of *Echinococcus granulosus* and *Cysticercus tenuicollis* (Blagburn & Dryden, 1999; Constantin, 2014).

In sheep, contamination with these parasitic agents occurs predominantly via the oral route, and the access of the parasites to the target organ depends on the species involved. The migration of immature forms toward the liver induces pathological changes of varying severity, from mild to severe lesions.

In sheep maintained under different technological systems of breeding and management, multiple parasite species are identified, including those localized in the liver, ultimately leading to increased morbidity and mortality, accompanied by substantial economic losses. The most important parasitic diseases diagnosed in sheep with hepatic localization are

trematodoses (fasciolosis and dicrocoeliosis) and cestodoses (echinococcosis/hydatidosis and cysticercosis) (Zgardan et al., 2001; Zgardan, 2008; Erhan et al., 2005; Erhan, 2010, 2020, 2024).

Within the animal organism, the liver is a metabolically vital organ, functioning both under physiological and pathological conditions, and is often referred to as the "central laboratory of the body". At the hepatic level, indispensable energetic and anabolic processes necessary for cellular life take place, along with catabolic degradation processes affecting multiple structural and functional components of various tissues and cells.

Among the principal functions of the liver are the uptake and distribution of nutrients introduced into the animal organism and the maintenance of their constant concentration in the bloodstream. At the same time, carbohydrate, lipid, and protein metabolism are carried out in the liver, highlighting its multifunctional role, while it also performs an essential excretory function (Dulceanu & Terinte, 2003; Treyer & Müsch, 2013).

The presence of immature or adult parasitic forms in the liver, bile ducts, or blood vessels constitutes the primary cause of pathological processes whose intensity, duration, and severity depend strictly on the parasite's life cycle, migration pathways, feeding type, localization within the organ, and parasite burden.

The Republic of Moldova benefits from favorable climatic and natural conditions for the development of the livestock sector. However, a major obstacle to profitable animal production is represented by a series of pathological conditions of diverse etiology, including those of parasitic origin.

Data reported by numerous researchers indicate that in large and small ruminants under the conditions of the Republic of Moldova, more than 70 species of parasitic etiological agents may be identified. Most of these infections have a subclinical course, yet they exert considerable harmful effects on the host organism, manifested by reduced functionality and productivity.

Each geographical area of the Republic of Moldova presents individual particularities related to distinct climatic and soil characteristics, which influence the composition of animal parasitofauna. According to economic estimates, annual losses generated by parasitic diseases, calculated only from the confiscation of edible organs and tissues, amount to approximately 70 tons of liver, 40 tons of lungs, and 15 tons of other tissues (Rusu, 2021).

A study conducted across the country established that between 2 and 20 species of parasitic organisms may parasitize a single sheep. The prevalence of hepatic trematode infections in sheep may reach 55.1%, cestode infections 19.6%, and nematode infections 100%. Trematode parasitoses are most frequently detected in the northern region of the Republic of Moldova (73.3%), followed by the central region (48.8%) and the southern region (17%). In the case of nematode infections, the prevalence across all geographical regions is 100% (Zgardan et al., 2001; Erhan, 2020, 2024).

Helminthological investigations performed on 1,528 sheep slaughtered in various abattoirs across the Republic of Moldova showed that up to 67.3% were infected with *Fasciola* spp. and *Dicrocoelium* spp. (Zgardan et al., 2001).

Within the host organism, a constant relationship of monoparasitism exists with each parasite species, while simultaneously a state of polyparasitism is present in relation to the total parasitic burden existing at a given time.

Extensive investigations carried out during 1960-1970 and 2000-2001, involving approximately 19,000 ruminants raised under various technological systems and located in different geographical regions of the Republic of Moldova, indicated that about 90-95% of adult ruminants were infected with *Fasciola*, *Dicrocoelium*, *Echinococcus* (hydatid disease), strongyles, *Eimeria*, and *Sarcocystis* species, occurring in variable associations. These studies also demonstrated that the overall level of infection in the two investigated periods did not undergo significant changes (Zgardan et al., 2001; Erhan, 2020, 2024).

In 2012-2013, a similar study was conducted on sheep of different age groups from various regions of the Republic of Moldova. Coproovoscopic examination outlined the presence of polyparasitism based on associations including *Cysticercus tenuicollis*, *Fasciola hepatica*, *Dicrocoelium lanceolatum*, larval *Echinococcus granulosus*, *Dictyocaulus*

filaria, and *Sarcocystis ovicanis*. Necropsy and sanitary-veterinary examination of carcasses also revealed polyinvasions in lambs, with 100% prevalence consisting of three species: *Cysticercus tenuicollis*, larval *Echinococcus granulosus*, and *Trichuris ovis*. In adult sheep, a 100% prevalence of polyparasitism was identified in associations formed by *Cysticercus tenuicollis*, larval *Echinococcus granulosus*, and *Trichuris ovis*, while associations including *Sarcocystis ovicanis* were recorded in 67% of cases, with *Fasciola hepatica* in 54%, and with *Dicrocoelium lanceolatum* in 27% of cases (Erhan et al., 2005; Erhan, 2020).

The pathogenic effects of parasitic forms on the animal organism vary depending on several factors, including the parasite species, migration route, parasite burden, physiological status of the host at the time of invasion, and degree of infestation (Rusu, 2021).

Compared with other trematodoses, the pathogenic impact of *Dicrocoelium dendriticum* is less pronounced than that produced by *Fasciola hepatica*. Among the pathogenic particularities underlying differences in virulence are the route of penetration into the host organism - via the common bile duct in the case of dicrocoelia, which is less destructive - the small size of the parasite, the absence of cuticular spines, and its mode of nutrition, which involves bile rather than biliary tissue (Theodoridis et al., 1991; Otranto & Traversa, 2003; Mandai, 2006; Arbabi et al., 2011).

Recent studies have shown that infestation with *Dicrocoelium dendriticum* leads to significant alterations in hepatic biochemical parameters and oxidative status, reflecting functional impairment of the liver and the host's immune response (Piegari et al., 2021; Hodžić et al., 2020).

During the evolution of these parasitic diseases, following parasite penetration into the liver, a local inflammatory reaction occurs, accompanied by destruction and desquamation of biliary epithelium. Excretory and metabolic products accumulate within the bile duct lumen, altering certain chemical parameters of bile. In chronic and massive infestations, biliary stasis, obstruction, and ductal hypertrophy develop, producing hepatotoxic and pyrogenic effects (Theodoridis et al., 1991).

During migration from the intestine of the definitive host, young dicrocoelia disseminate highly pathogenic bacteria such as *Escherichia coli*, *Staphylococcus albus*, *Streptococcus viridans*, and *Proteus vulgaris*, leading to a 5-18-fold reduction in the bactericidal and fermentative activity of bile and thereby favoring the proliferation of pathogenic bacteria.

Research has established a correlation between microbiological changes in bile and both parasite burden and time elapsed since infestation. At 30 days after infection of young sheep with 2,600 metacercariae of *Dicrocoelium lanceolatum*, the total bacterial count in bile reached 2.36 ± 0.33 million/ml, including: *E. coli* 2.32 ± 0.165; *Staphylococcus* spp. 0.033 ± 0.005; *Streptococcus* spp. 0.007 ± 0.001. At 60 days post-infection, total bacterial count was 1.42 ± 0.28 million/ml, including: *E. coli* 1.40 ± 0.29; *Staphylococcus* spp. 0.017 ± 0.003; *Streptococcus* spp. 0.003 ± 0.001 (Lalor R. et al., 2021).

At 30 days post-infection, total microbial count increased 6.8-fold compared with healthy sheep: *E. coli* increased 6.4-fold, *Clostridium* spp. 8.9-fold, aerobic bacteria 2-fold, *Staphylococcus* spp. 2.1-fold, while total fungi decreased by 22%, including yeasts by 21.4%. At 60 days post-infection, total microbial count increased slightly due to rises in *E. coli* (3-fold), *Staphylococcus* spp. (16.7%), *Clostridium* spp. (8.9%), and aerobic bacilli (32-fold) (Constantinescu et al., 2016; Lalor et al., 2021).

At 90 days post-infection, jejunal microflora in diseased sheep showed a total bacterial count seven times higher than in healthy sheep: *E. coli* 15.3-fold, *Clostridium* spp. 3.3-fold, aerobic bacilli 11.3-fold, *Staphylococcus* spp. 41.5-fold, while total fungi decreased 20-fold, including yeasts 20.2-fold.

In the large intestine of diseased animals, total microbial count increased 2.4-7.2-fold, including *E. coli* 2.4-7.2-fold, *Staphylococcus* spp. 2.6-5.3-fold, aerobic bacilli 3.1-77.6-fold, *Clostridium* spp. 16.3-fold, whereas total fungi decreased 5.2-fold, including yeasts 5.1-fold (Kublickene, 1976; Constantinescu et al., 2016; Lalor et al., 2021).

Simultaneously, at 55 and 65 days post-infection with dicrocoelia, a drastic decrease in hepatic glycogen content and a reduction in the degree

of fatty degeneration were observed. Lipid droplets in hepatocytes exceeded normal volume, causing cellular destruction and accumulation around necrotic foci or in adjacent connective tissue. A gradual decrease in hepatic phosphatase activity was also recorded.

Acid phosphatase activity in dicrocoeliosis manifested from the early stages of the pathological process, reaching maximum levels at 35 and 50 days post-infection (Irinca, 2008; Predescu, 2011; Constantinescu et al., 2016).

According to Bliugher (1964), increased acid phosphatase activity in organs and tissues has an adaptive character, reflecting adaptation to new pathological conditions. Hepatic phosphatase activity is more intense during the migration phase of dicrocoelia through hepatic tissue, reaching maximum values at 35-45 days post-infection, coinciding with morphological alterations and the onset of tissue repair processes (Manga-Gonzalez et al., 2004; Xhemollari et al., 2012; Irinca, 2008; Predescu, 2011).

Furthermore, current research highlights the importance of correlating parasite burden with biochemical and hematological changes, as well as the role of oxidative mechanisms in the pathogenesis of the disease, aspects also supported by recent analyses in the field (Kılıç Altun et al., 2023).

Many researchers reporting clinical signs of dicrocoeliosis have noted that affected animals exhibit visible signs of anemia to varying degrees, the primary cause being the spoliative syndrome induced by large numbers of parasites that deplete hepatic reserves of macro- and microelements (Manga-Gonzalez et al., 2004; Xhemollari et al., 2012; Irinca, 2008; Predescu, 2011).

In sheep affected by dicrocoeliosis, protein, carbohydrate, and mineral metabolism is disrupted, manifested by increased serum alanine and aspartate aminotransferase activity, accompanied by decreased concentrations of free amino acids - particularly essential ones - total protein, and albumins.

In the blood of sheep suffering from dicrocoeliosis, concentrations of Ca, P, Fe, Mg, and Cu decrease, alkaline phosphatase activity increases, and in hepatic tissue the concentrations of vitamins A, C, D, and especially B-complex vitamins are reduced (Otranto & Traversa, 2003; Manga-Gonzalez et al., 2004; Xhemollari et al., 2012; Irinca, 2008; Predescu, 2011).

MATERIALS AND METHODS

For the determination of biochemical changes in infested sheep, the first step consisted of identifying animals infected with dicrocoelia. Fresh fecal samples were collected from suspected sheep, representing 15-20% of the total number of animals in each flock.

Coproscopic examination was performed using standard coprological techniques: the qualitative Fulleborn flotation method, which involves the use of a hypertonic sodium chloride solution with a density of approximately 1.150-1.200, and the sedimentation method (successive washings, polyvalent), recommended for the detection of trematode eggs such as *Fasciola* spp., *Dicrocoelium* spp., etc., using ordinary tap water.

In the Fulleborn method, 5 g of fresh sheep feces were used for each analyzed sample, mixed with 20-30 ml of a supersaturated NaCl solution. The mixture was gently homogenized, filtered into a flotation glass, and allowed to stand for 20-30 minutes. Subsequently, using a bacteriological loop, material was collected from the surface film of the solution and placed on a glass slide. Several collections were made from different areas of the surface layer, covered with a coverslip, and examined under a microscope using the 10× objective. At least 10 microscopic fields were obligatorily examined. In parallel, the detected eggs were counted, the mean value was calculated, and the intensity of invasion was determined (Cozma et al., 2010; Modry et al., 2012).

The centrifugation sedimentation method (active sedimentation) involves concentrating parasitic elements into a reduced volume within a short period of time. A fecal sample of 3-5 g was homogenized with tap water and then filtered into 2-4 centrifuge tubes. The samples were centrifuged at low speed (1,500 rpm) for 2-3 minutes. After centrifugation, the supernatant was discarded, and several preparations were made from the sediment between slide and coverslip. These were examined microscopically using the 10× objective (Cozma et al., 2010; Modry et al., 2012).

Blood sampling for biochemical examination was performed using tubes intended for biochemical analysis containing EDTA. The volume of each blood sample was 10 ml, and each sample was strictly individualized. Blood collection was carried out from the jugular vein prior to slaughter, in order to minimize stress-related interference. In parallel, an additional blood sample without anticoagulant was collected for the determination of hepatic marker enzymes in serum. After collection, the samples were transported rapidly to the laboratory in fresh condition for serum and plasma separation. Hepatic marker enzymes (ALT, AST, GGT, ALP) and biochemical parameters (total bilirubin, total protein, albumin, globulins, Ca, P, Mg) were determined for each individual sample (n = 10) using a Spotchem biochemical analyzer, model SP-4410 (Medex, Biochemical Analysis System, SP-4410 Chemistry Analyzer, USA).

The percentage increase of biochemical parameters and hepatic marker enzyme values was calculated using the following formula:

$$\frac{(X_{\text{infected}} - X_{\text{control}}) \times 100}{X_{\text{control}}}$$

RESULTS AND DISCUSSIONS

The prevalence and particular characteristics of invasion in sheep infected with *Dicrocoelium lanceatum* were investigated over a one-year period, from spring 2017 to spring 2018, within a sheep farm operating under a traditional breeding and management system.

Fecal samples were collected directly from the rectum, individually from each animal, placed in polyethylene containers, strictly labeled, and transported to the Laboratory of Parasitology and Helminthology of the State University of Moldova (USM), as well as to the Faculty of Veterinary Medicine, Department of Animal Health and Public Safety (SASP) of the Technical University of Moldova (UTM). During sample collection, the presence of specific clinical signs was taken into consideration, including weight loss, decreased appetite, and reduced milk production.

In parallel, at slaughter, the livers of the sheep were thoroughly examined by inspection, palpation, and sectioning of the hepatic parenchyma. The macerated hepatic tissue was then subjected to successive washings in order to determine the presence of adult parasitic forms in infected livers (Skriabin, 1928).

Overall, during the study period, 250 fecal samples were collected from a flock consisting of 185 sheep, and liver examinations were performed on 15 slaughtered animals.

The prevalence rate of *Dicrocoelium dendriticum* (Rudolphi, 1819) infection in sheep is shown in Table 1, which indicates an infection rate in sheep of 71.9%, as determined by copro-oidoscopic examination, while the overall prevalence of dicrocelia in necropsy examination of the liver collected from slaughtered or dead animals was 93.3%.

Table 1. Prevalence of *Dicrocoelium dendriticum* (Rudolphi, 1819) infection in sheep

Host	Coproovoscopic examination					Necropsy examination of the liver				
	number animals examined	number animals infested.	number animals with eggs (%)			number animals examined.	number animals infested	number animals with adult parasitic forms (%)		
			<100	<500	<2000			<100	<500	<2000
Sheep	185	133	49	58	26	15	14	4	5	5
% infestation	-	71.9	36.9	43.6	19.5		93.3	26.6	33.3	33.3

At the same time, it was established that the number of parasite eggs detected in fecal samples subjected to coproscopic examination, expressed as eggs per gram of feces (EPG), ranged from 50.0 to 1089.9 in the sheep examined.

Depending on the quantity/number of parasite eggs identified, the severity of the parasitic disease was classified as mild (light), moderate, and severe invasions, according to the data presented in Table 2.

Table 2. Number of *Dicrocoelium dendriticum* (Rudolphi, 1819) eggs in coproovoscopic examination of fecal samples in sheep

Level of infestation	Sheep	
	No. of infected animals	EPG (average number of eggs)
Mild <100	49.0	50
Moderate <500	58	248.6
Severe <2000	26	1089.9
Total	133	-

As previously stated, the primary objective of the present study was to determine the changes occurring in blood biochemical and enzymatic parameters in sheep infected with *Dicrocoelium dendriticum*.

In order to establish the severity of alterations in blood biochemical and enzymatic indices, sheep were selected following coproscopic examination. Two groups of five-year-old sheep, each consisting of 10 animals, were formed. In the control group, coproscopic examination did not reveal infection with dicrocoelia, whereas in the second group, infection with *Dicrocoelium* spp. was confirmed, with a moderate degree of infestation.

The results regarding the modification of biochemical parameters in sheep infected with dicrocoelia are presented in Table 3. Analysis of the data revealed minor changes in plasma protein concentrations in the infected sheep compared with the control group, reflected by a decrease in albumin synthesis. This alteration is observed in the acute phase of the disease and represents a consequence of the migration of larval forms of dicrocoelia through the hepatic parenchyma toward the periphery of the hepatic lobes. Migration occurs via the medium and small bile duct branches, and due to parenchymal destruction, the concentration of certain hepatic enzymes in the blood increases.

Analysis of the data in Table 3 indicates that major importance in interpreting the physiological differences between the control and infected groups is attributed to the levels of enzymes originating from hepatocytes or endothelial cells of the bile ducts, namely ALT, GGT, and AST. Lesser importance is attributed to ALP concentration; however, its determination remains necessary for the differential diagnosis of hepatic lesions compared with other types of tissue damage.

Table 3, concerning the particularities of certain biochemical indices in sheep infested with *Dicrocoelium dendriticum* (Rudolphi, 1819), comprehensively highlights the impact of this helminthiasis on liver function, protein metabolism, and mineral balance. The comparative analysis of the parameters determined in the infested group versus the control group (n = 10 animals/group), expressed as mean ± standard deviation, allows the outline of a biochemical profile characteristic of moderate dicroceliosis.

A central element of the biochemical profile is the alteration of the serum protein pattern. The decrease in total protein concentration by 11.22% in infested sheep reflects the progressive impairment of hepatic synthetic function. The liver is the primary organ responsible for plasma protein synthesis, and the localization of the parasite within the bile ducts leads over time to structural changes in the hepatic parenchyma, including chronic inflammatory and fibrotic processes.

The reduction in albumin by 12.23% represents a sensitive indicator of hepatocellular dysfunction. Albumin, having a relatively long half-life and being synthesized exclusively by hepatocytes, is a reliable marker of protein synthesis capacity. Its decrease suggests chronic hepatic parenchymal damage, even in the absence of evident clinical signs. This alteration contributes to reduced plasma oncotic pressure and may influence the transport of various metabolites and drugs.

In contrast, the 6.12% increase in globulin concentration highlights activation of the humoral immune response. During infestation with *D. dendriticum*, the host organism develops a persistent immune response, leading to an increase in the globulin fraction, particularly immunoglobulins. This redistribution of protein fractions-decreased albumin and increased globulins-constitutes a biochemical feature suggestive of chronic hepatobiliary inflammatory processes.

Hepatic enzyme activity represents one of the most relevant indicators of functional liver impairment in dicroceliosis.

The 24.63% increase in ALT indicates direct hepatocellular damage. ALT is a cytoplasmic enzyme involved in amino acid metabolism, and its release into the bloodstream occurs following alteration of hepatocyte membrane integrity. In infestation with *D. dendriticum*, migration and localization of the parasite within the biliary system generate chronic inflammation, ductal compression, and secondary degenerative changes in adjacent hepatocytes.

GGT showed a 21.31% increase, which is particularly significant from a pathogenetic perspective. This enzyme is primarily localized in the epithelium of the bile ducts and is considered a sensitive marker of cholestasis and biliary tree involvement. The increase in GGT reflects irritation and inflammation of the bile ducts caused by the presence of adult parasites. Therefore, the alteration of GGT represents a specific biochemical feature of dicroceliosis with important diagnostic value.

AST increased by 11.77%, indicating moderate involvement. Since this enzyme is present in multiple tissues (liver, myocardium, skeletal muscle, kidneys, pancreas), its elevation may suggest both hepatic injury and a general state of systemic metabolic stress. In the context of dicroceliosis, increased AST values should be interpreted in correlation with ALT and GGT to confirm a predominantly hepatic origin.

ALP showed a slight increase, compatible with mild cholestatic changes. In the absence of severe bile duct obstruction, this enzyme does not reach markedly elevated values; however, its variation supports the presence of chronic hepatobiliary distress. Slightly elevated total bilirubin values in the infested group suggest moderate impairment of biliary excretory function. Although this alteration does not indicate severe cholestasis, it reflects difficulties in bile pigment elimination, possibly due to thickening and inflammation of the ductal walls. This finding complements the cholestatic profile suggested by increased GGT and ALP activities.

Changes in serum Ca, Mg, and P concentrations indicate metabolic disturbances secondary to hepatic dysfunction. The slight increase in calcium (+3.03%) and magnesium (+8.30%) may be associated with alterations in protein balance and plasma transport mechanisms, considering that part of these elements circulates bound to serum proteins, particularly albumin.

The decrease in phosphorus (−5.22%) may reflect alterations in calcium-phosphorus metabolism and possible influences on cellular energy metabolism. Even discrete mineral imbalances support the presence of systemic metabolic disturbances associated with chronic parasitism. Although the observed differences did not reach statistical significance ($p > 0.05$), the trends identified are biologically relevant. They suggest the development of progressive, predominantly subclinical hepatic dysfunction in sheep infested with *Dicrocoelium dendriticum*. The slow and insidious evolution of dicroceliosis explains the moderate but consistent changes observed in biochemical indices.

Overall, the identified biochemical particularrities - decreased total protein and albumin levels, increased globulins, elevated hepatic enzyme activities (especially ALT and GGT), and discrete mineral disturbances - outline a characteristic profile of chronic hepatobiliary impairment in infested sheep. These findings confirm that dicroceliosis produces functional hepatic alterations even in the absence of severe clinical manifestations, emphasizing the importance of biochemical investigations in assessing the health status of infested flocks.

Table 3. Some biochemical parameters determined in the control group and in sheep with *Dicrocoelium dendriticum* (Rudolphi, 1819)

Parameters	Number head	Animal groups studied	
		Control	*D. dendriticum*
Total bilirubin (mg/dl)	10	0.50 ± 0.06	0.56 ± 0.07
Total protein (g/dl)	10	7.40 ± 0.35	6.57 ± 0.40
Albumin (g/dl)	10	3.60 ± 0.20	3.16 ± 0.18
Globulin (g/dl)	10	3.80 ± 0.28	4.03 ± 0.30
AST (U/L)	10	90.00 ± 8.50	100.60 ± 9.20
ALT (U/L)	10	25.00 ± 3.10	31.16 ± 3.80
ALP (IU/l)	10	110.00 ± 12.00	118.00 ± 14.00
GGT (U/L)	10	35.00 ± 4.20	42.46 ± 5.10
Ca (mg/dl)	10	9.90 ± 0.45	10.20 ± 0.50
P (mg/dl)	10	5.75 ± 0.40	5.45 ± 0.38
Mg (mg/dl)	10	2.40 ± 0.18	2.60 ± 0.20

CONCLUSIONS

Natural infestation of sheep with *Dicrocoelium dendriticum* (Rudolphi, 1819) leads to measurable alterations in biochemical blood parameters, indicating functional impairment of the liver even in cases of moderate parasitic invasion.

Total serum protein concentration decreased by 11.22% ($p > 0.05$) in infested sheep compared to the control group, reflecting a reduction in hepatic synthetic capacity associated with chronic parasitic involvement of the liver.

Albumin levels showed a reduction of 12.23% ($p > 0.05$), confirming impaired protein synthesis at the hepatocellular level and highlighting albumin as a sensitive indicator of hepatic dysfunction in dicrocoeliosis.

In contrast, globulin concentration increased by 6.12% ($p > 0.05$) in the infested group, suggesting activation of the humoral immune response and the chronic inflammatory nature of the infection.

Alanine aminotransferase (ALT) activity increased by 24.63% ($p > 0.05$) in infested sheep, indicating hepatocellular membrane damage and cytoplasmic enzyme leakage into circulation.

Gamma-glutamyl transferase (GGT) activity was elevated by 21.31% ($p > 0.05$), reflecting biliary epithelial involvement and confirming the localization of adult parasites within the bile ducts.

Aspartate aminotransferase (AST) levels increased by 11.77% ($p > 0.05$) in infested animals, indicating not only hepatic involvement but also possible systemic metabolic disturbances due to the multisystemic distribution of this enzyme.

Alkaline phosphatase (ALP) and total bilirubin showed mild increases in the infested group, supporting the presence of moderate cholestatic processes without evidence of severe bile duct obstruction.

Mineral metabolism was also affected, with serum calcium increasing by 3.03% ($p > 0.05$) and magnesium by 8.30% ($p > 0.05$), while serum phosphorus decreased by 5.22% ($p > 0.05$) compared to the control group, indicating subtle metabolic imbalances.

Although the differences between groups did not reach statistical significance ($p > 0.05$), the consistent trends observed across protein, enzymatic, and mineral parameters demonstrate that dicroceliosis induces progressive subclinical hepatic dysfunction and systemic metabolic alterations in sheep.

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