International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2025; 8(2): 80-85
Received: 09-04-2025
Accepted: 12-05-2025

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Histopathological and biochemical changes relating with lesion severity of coccidiosis in laying hens

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DOI: https://www.doi.org/10.33545/pathol.2025.v8.i2b.2081

Abstract
Due to the paucity of reliable data on the pathogenic effects of coccidiosis on poultry in Waist province, which is in the middle of Iraq. Thus, the purpose of the current investigation was to identify the biochemical and histological alterations in laying hens infected with coccidiosis. This study was carried out on 120 laying hens during the period January to October 2020 in Waist province, canter of Iraq. The current study's findings showed that the concentrations of albumin, globulin, total cholesterol, and glucose gradually decreased in laying hens infected with coccidiosis. On the other hand, when compared to healthy controls, the active blood enzymes ALP, ALT and AST significantly increased. Besides, the current study found that the duodenum and jejunum cavities of hens afflicted with coccidiosis were clogged and filled with yellowish or greenish faeces that were very soft to the touch and had a lot of mucus exudate and mucosa became thicker and showed haemorrhagic patches in cases of severe disease. Additionally, the examination's histopathological results revealed a variety of pathological alterations, including desquamation, villi mucous membrane destruction and loss, villous epithelium necrosis, severe haemorrhages, and inflammatory cell infiltration in the lamina propria and submucosa.

Keywords: Biochemical parameter, histopathological, coccidiosis, and laying hens

Introduction
Chicken coccidiosis is a global parasitic disease that seriously threatens the development of animal husbandry and causes huge economic losses every year (Abbas *et al*., 2012; Adamu *et al.*, 2013; Abd El-Maksoud *et al.*, 2014) [1, 3, 2]. Researchers both locally and globally have conducted a thorough investigation into chicken coccidiosis in an effort to stop its spread and reduce financial losses (Bílková *et al.*, 2017; Arczewska-Włosek *et al.*, 2018) [7, 6]. Coccidiosis is a significant and highly prevalent infectious disease in animal husbandry production, caused by the parasitism of *Eimeria* in chickens, which is widespread and has a high epidemic intensity. It can roughly be said that wherever there are chickens, there is coccidiosis, the infection rate is 50%-70%, the death rate is 20%-30%, and it can reach 80% in severe cases (Conway and McKenzie, 2007; Patra *et al.*, 2010) [9, 20]. Furthermore, the economic loss that frequently results in chicken deaths is higher and poses a severe danger to the development of aquaculture since chicken coccidiosis slows down flock growth and decreases egg production (Da Silva *et al.*,2009; Mondal *et al.*,2011) [11, 19]. Coccidiosis is a parasite illness that affects many birds (Alani *et al.*, 2022) [5] Coccidiosis is caused by nine *Eimeria* species, the most prevalent of which are *Eimeria tenella*, *E. maxima*, *E. acervulina*, and *E pecatrix*. (Dalloul and Lillehoj, 2006; Kawashima *et al.*, 2010) [10, 15]. Morphologically, Eimeria species differ according to the characteristics of ovule size and form, membrane structure, micropyle presence or absence, covering, polar granules, residual bodies in the oocyst, and spores within the oocyst (Shirley *et al.*, 2007) [27]. In addition to being dangerous in and of itself, coccidiosis is linked to other illnesses that are extremely dangerous for the poultry industry. Malnutrition, bacterial and viral infections, and even a minor case of coccidiosis (Allen, 1997; Rodgers *et al.*, 2015) [4, 22]. According to the clinical symptoms and the results of the microscopic examination, together with the results of the laboratory tests, the outbreak was diagnosed as chicken coccidiosis infection (Ruff *et al.*, 1974; Rose and Long, 1980) [24, 23]. The analysis found that the main risk factors for coccidiosis in chickens were a history of coccidiosis, failure to quarantine imported chickens, unscientific deworming procedures, and early cleaning of feces.

It is recommended to strengthen the quarantine when introducing chickens and regular deworming chickens, and to strengthen the fecal cleaning and harmless treatment, in order to lower the prevalence of coccidiosis in hens (Stringfellow *et al.*, 2011) [29]. Coccidiosis is diagnosed based on the macroscopic intestinal signs and lesions characteristic of each species, which are associated with the microscopic observation of a large number of oocysts. Coccidiosis can sometimes be caused by immunosuppressive diseases such as Gumboro disease, Marek's disease, infectious anemia of chickens, and others. In general, the characteristics of the lesions and their location in the intestine indicate which species of Eimeria cause coccidian (Saleh *et al*., 2011) [26]. Coccidiosis must be differentiated from other avian diseases that cause enteritis and diarrhea: necrotic enteritis, ulcerative enteritis, dysbacteriosis, gastrointestinal mycosis, cecal salmonellosis, vesicular histoplasmosis, and enterocolliosis. *E. acervulina* is a fairly dangerous pathogen that can go unnoticed in the subclinical form of coccidian. It affects the crypts of the duodenal epithelium, initially, it causes white lesions in the form of points, plaques, or cracks that later develop into ladders. Upon coalescence, these white lesions erode the mucous membrane and, if enlarged, subsequently lead to the disintegration of the mucosal tissue *E. tenella* and *E. necatrix* are the species that are most harmful. E. Brunetti is rare but harmful when it does appear; E. acervulina, E. maxima, and E. mivati are common and mildly to moderately pathogenic. Generally speaking, E. mitis, E. praecox, and E. hagani are not harmful species (Dalloul and Lillehoj 2006) [10]. The current study was conducted to identify the histopathological and biochemical changes in laying hens infected with coccidiosis since there is a lack of reliable information on the pathogenic impact of the disease on poultry in the Waist province.

Materials and Methods

Study locations and sample processing

In order to ascertain the histological and biochemical alterations in coccidiosis-infected laying hens in Waist province, central Iraq, 120 laying hens were used in this study, which was conducted between January and October 2020. The research was divided into different points: experimental design, blood samples, biochemical analysis, and, finally, statistical analysis. The samples were divided into two groups: 100 infected and the comparison group. Samples have been placed in a cold box and brought to the Medicine College/University of Waist parasitology lab for analysis.

Biochemical examination

Each examined bird had 5ml of blood drawn from its brachial wing vein using a disposable syringe (5ml with an EDTA tube and5ml without an EDTA tube). Following the previously outlined procedure (Singh *et al*., 2011) [28], serum was extracted and kept in Eppendorf tubes at -20 °C for use in ensuing biochemical tests, such as albumin, globulin, Total cholesterol, Glucose, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT). Using the Tehnicon RA-1000 automatic analyzer, total cholesterol and glucose were measured in accordance with normal protocols using the method Jasim *et al*., (2023) [13].

Histopathological examinations

Following the documentation of the macroscopic alterations, fragments of the infected chickens' small intestines were gathered and preserved using 10% formalin. There was a histology investigation (Jasim and Al-Amery, 2023) [14].

Statistical Analysis.

The SPSS statistical software (SPSS for Windows ver.17.00) was used to analyze the study's data in accordance with the program of analysis variance.

Results

The present study revealed significant biochemical differences between coccidiosis-infected hens and healthy controls. Data indicated that the levels of albumin, globulin, total cholesterol, and glucose were notably lower in infected hens compared to healthy chickens. Conversely, the activity of blood enzymes ALP, ALT, and AST was significantly higher in infected hens, as detailed in table and figure (1). Gross examination of coccidiosis-infected chickens was limited to the small intestine. The study observed that the duodenum and jejunum of infected hens were obstructed and contained soft, greenish or yellowish fecal matter with substantial mucus exudate. In severe cases, the intestinal mucosa thickened and displayed hemorrhagic patches. On the other hand, Histopathological analysis of the small intestine in infected chickens revealed varying degrees of degenerative changes, including destruction and shedding of the mucosal epithelial layer in heavily infected cases, as depicted in Figure 2. Further pathological alterations, such as desquamation, disintegration, and loss of the villi mucous membrane, were also observed, as illustrated in Figure 3. Additionally, the lamina propria and submucosa exhibited inflammatory cell infiltration, severe bleeding, and necrosis of the villous epithelium, as shown in Figure 4. Finally, the inflammatory response was characterized by the widespread infiltration of mononuclear cells, primarily lymphocytes and eosinophils, throughout the mucosa, particularly in the lamina propria, as demonstrated in Figure 4.

Table 1: The Changes of some biochemical parameters due to Coccidiosis in laying hens

Biochemical parameter	Infection group	Control group
Albumin	1.33±0.44	2.55±0.55
Globulin	2.22± 0.453	3.32±0.47
ALP	230.4±10.2	153.1±1.2
Total cholesterol	140±1.2	220.3±1.77
AST	134.7±1.99	88±0.67
ALT	88.5±2.6	24.7±0.88
Glucose	180±2.5	222±1.44

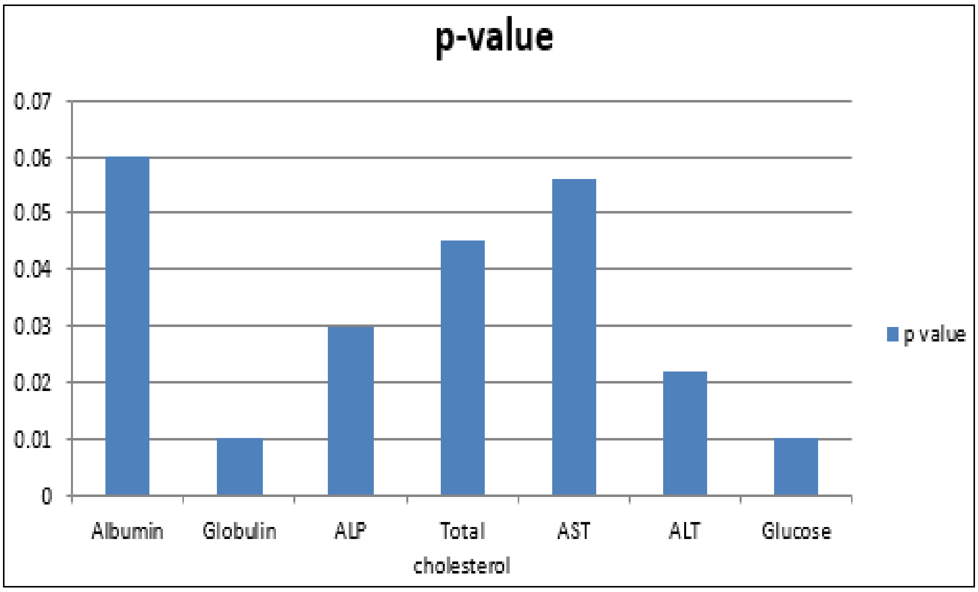

Fig 1: Show the percentage of biochemical parameter change of infected laying hens by coccidiosis

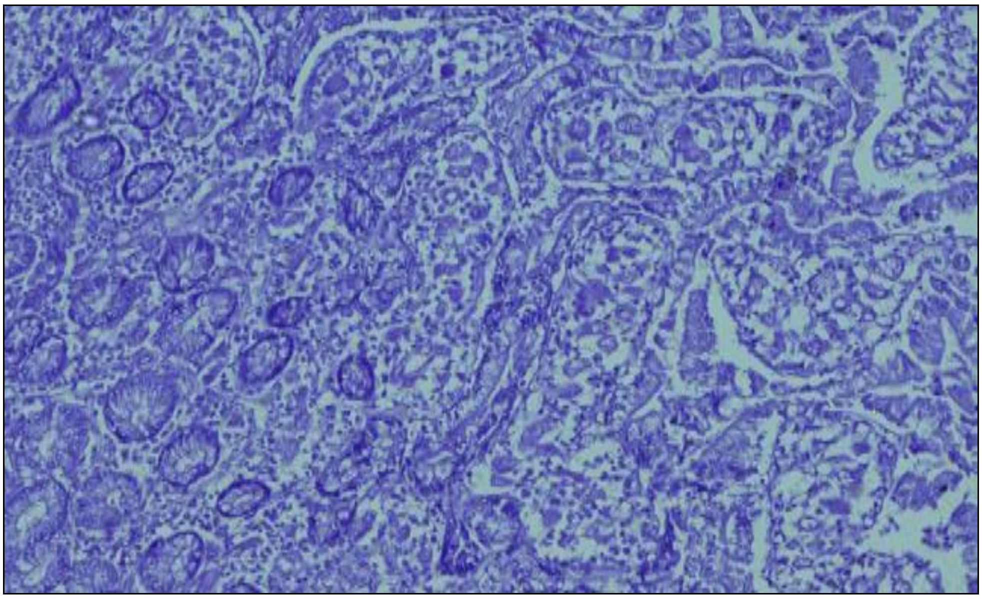

Fig 2: Showed disruption of submucosal tissue with and sloughing of epithelial layer of intestinal mucosa accompanied with diffuse MNCs infiltration (10X)

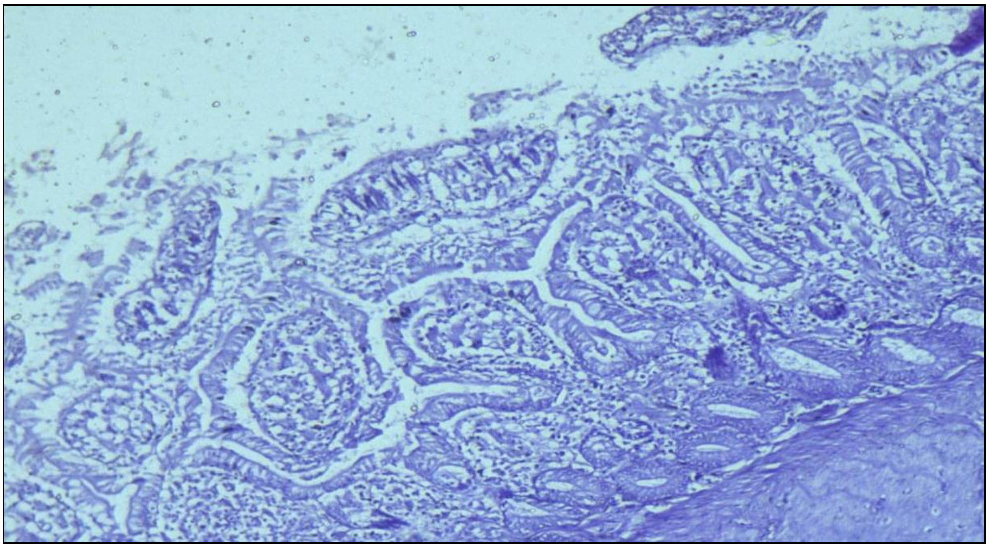

Fig 3: Showed severe necrotic of intestinal crypts and loss of mucous membrane of villi and accompanied with diffuse MNCs infiltration (40X)

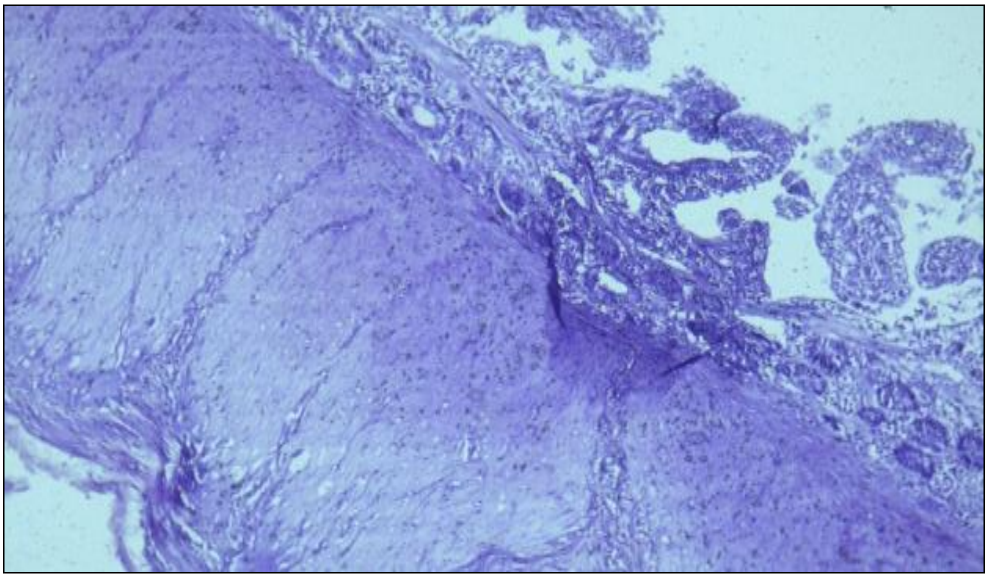

Fig 4: Showed necrosis of villous epithelium, massive hemorrhages and infiltration of inflammatory cells in the lamina propria and sub mucosa (40X).

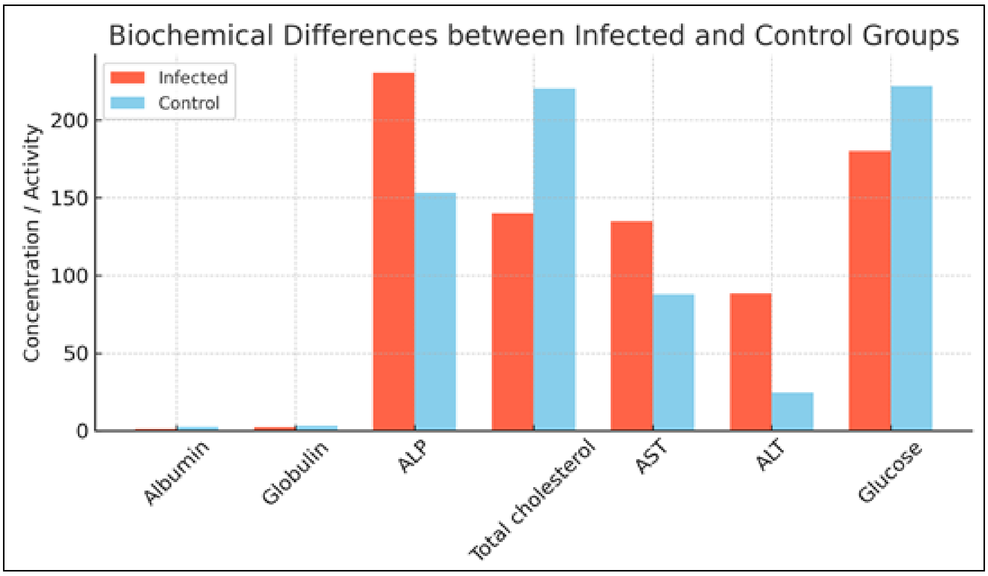

Fig 5: Serum concentrations.

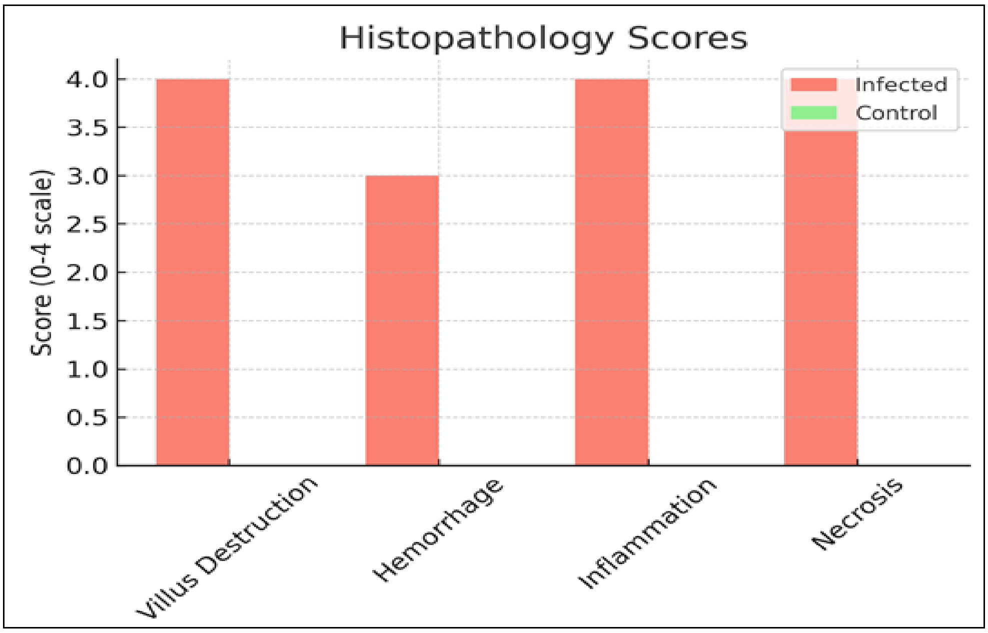

Fig 6: Histopathological lesion translation severity scoring (scale 0-4).

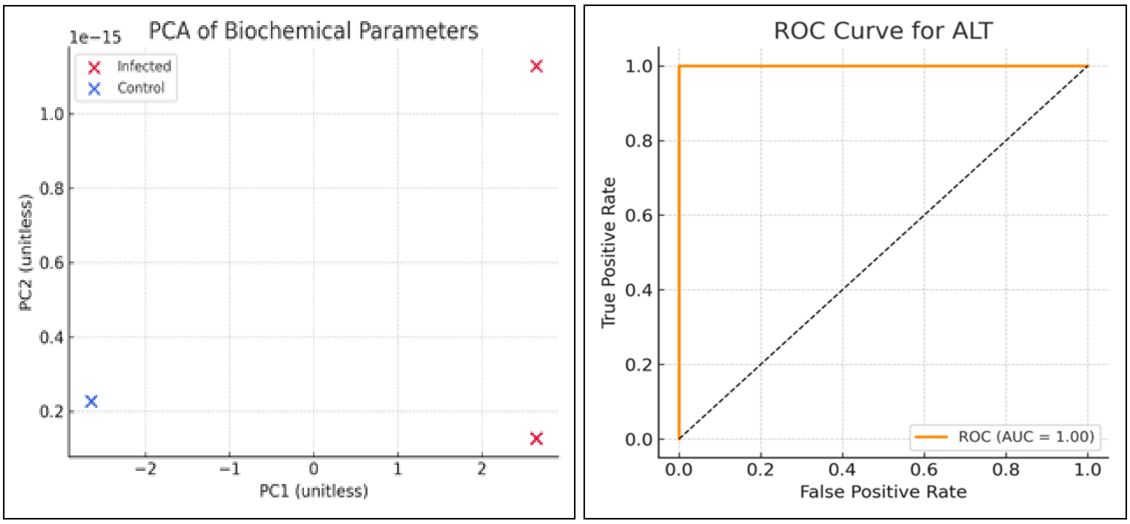

Fig 7: PCA based on biochemical analyses, ROC Curve for ALT for diagnostic successfulness

Discussion

Because of the high morbidity, mortality, and expense of treatment and control measures, Coccidiosis is an important health problem in terms of both prevalence and pathogenicity. Poultry, especially chickens frequently experience a loss in productivity (Adamu *et al.*, 2013) [3]. Coccidia infection causes parasitic enteritis in multiple parts of the digestive tract, causing poor functioning, loss of pigmentation, diarrhea, and in severe cases, death of the animal (Ogbe *et al.*, 2010) [25]. Coccidiosis is transmitted by the fecal-oral route through the ingestion of contaminated food or water. After being ingested, the parasite completes its complex life cycle inside the intestine, during which it multiplies inside the intestinal cells, causing them to rupture Infected birds will excrete the eggs through their feces, thus infecting other birds; After several cycles, the birds develop long-term, but species-specific, immunity (Alani *et al.*, 2022) [5]. Challenges with other coccidia species will cause new infestations and possible infection, depending on the number of eggs consumed. (Razzaq *et al.*, 2003) [21]. Additionally, the current study demonstrated elevated ALT, ALP, and AST enzyme activity in laying hens infected with coccidiosis. These findings were at odds with research by Biu *et al.*, (2006) [8], who found that mixed coccidian-infected chickens had higher ALT levels and lower ALP activity. Mondal *et al.*, (2011) [19] showed that infected chickens receiving a lower dose of *E. tenella* had higher plasma AST activity. This increase is explained by the fact that ALT and AST are enzymes present in erythrocytes; the drop in serum ALT and AST activities observed in this study could be linked to the significant decrease in erythrocytes as a result of blood loss into the gastrointestinal tract or because of the notably elevated serum activities of ALP observed in this study may be linked to bone marrow damage and metabolic alteration as a result of compensating for blood losses; the bone marrow may be compelled to produce an excessive amount of blood cellular components (Kerr, 2002; Adamu *et al.*, 2013) [16, 3]. On the other hand, the presence of numerous oocysts, schizonts, and significant tissue destruction in the caeca revealed the severity of the illness, according to the results of the histological investigation in hens infected with coccidiosis. These findings were consistent with those of McDougald and Fitz-Coy (2008) [17], who identified the second generation schizont as the most harmful stage of *E. tenella*, causing severe tissue damage, hemorrhage, caecal gland disruption, and the breakdown of the mucosa and muscularis layer. On days 6 and 7 following infection, schizont microgametes and macrogametes are observed in the tissue, and large quantities of mature oocysts are discharged into the lumen. The current findings were comparable to those reported by Getachew (2004) [12] and Mersha *et al*. (2009) [18], who investigated broiler chickens infected with E. tenella and E. acervulina, respectively, as well as local hens.

Conclusion

The data showed gradual decrease Albumin, Globulin, Total cholesterol and Glucose concentration of chickens infected with coccidiosis compared to the control group. ALP, ALT, and AST showed significant increases compared to healthy control, in contrast ALT, AST, and a significant increase in coccidiosis infection were found in the chicken group compared to the control group. Besides, Histopathological and Biochemical Changes to Coccidian infection all showed extremely high disease severity.

Competing interests

The authors declare that they have no competing interest.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article
Fadhil SR. Histopathological and biochemical changes relating with lesion severity of coccidiosis in laying hens. International Journal of Clinical and Diagnostic Pathology. 2025;8(2):80-85.