



Scientific Events Gate

The International Innovations Journal of Applied Science

Journal homepage: <https://ijas.eventsgate.org/ijas>

ISSN: 3009-1853 (Online)



Evaluation of the Effects of the Al-Jazeera Poultry Station's Routine Use of Tetracycline and Robenidine on the Bursa of Fabricius and Duodenum in Broiler Chickens in Libya

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ARTICLE INFO

Article history:

Received 12 Dec. 2025,
Revised 3 Jan. 2026,
Accepted 7 Jan. 2026,
Available online 15 Mar. 2026

Keywords:

Broiler chickens
Tetracycline
Robenidine
Histology
Poultry management

ABSTRACT

The widespread use of antimicrobial agents in poultry production raises concerns regarding their effects on intestinal integrity and immune function. Tetracycline is a commonly used broad-spectrum antibiotic, while robenidine is a widely applied anticoccidial in broiler management. Understanding their combined impact on key immunological and digestive organs is essential for flock health and productivity. This study evaluated the effects of the routine management protocol at Al-Jazeera Poultry Station in Libya, which involves tetracycline in drinking water and robenidine in feed, on the bursa of Fabricius and the duodenum in Ross broiler chickens. A total of 180 birds were assigned to three groups: Group 1 received water free of tetracycline and feed without anticoccidials; Group 2 received water free of tetracycline and feed containing robenidine (0.005 g/kg); Group 3 received water containing tetracycline (500 mg/0.5 kg body weight) and feed with robenidine according to the station's standard program. Birds were sampled at 35, 41, and 48 days for macroscopic and histological assessment. Histological analysis revealed variations in tissue morphology across groups, reflecting the effects of the station's existing management practices. These results provide insights into the impact of routine antimicrobial and anticoccidial use on critical immunological and digestive organs in broilers.

1. Introduction

The introduction of penicillin, streptomycin, and chlortetracycline during the 1940s initiated a transformative era in poultry production, fundamentally changing approaches to disease management and growth optimization. These early antibiotics not only provided an effective means of controlling infectious outbreaks, but they were also discovered to enhance gastrointestinal health and promote improved weight gain without increasing feed consumption (Cully, 2014). Subsequent research demonstrated that antimicrobial agents could modulate the intestinal microbiota, strengthen immune function, and improve nutrient utilization, thereby increasing feed efficiency in intensively reared poultry (Masoda *et al.*, 2005;

Ali *et al.*, 2009; Rushton, 2015). As a result, antibiotics rapidly became integral components of poultry feed formulations, widely used as growth promoters and prophylactic agents. Their use expanded parallel to the intensification of aquaculture and livestock systems, where high-density rearing heightened susceptibility to disease challenges and increased dependence on medicated diets to maintain productivity (Jerbi *et al.*, 2011).

One of the most concerning trends in global animal production is that a substantial proportion of manufactured antimicrobials are employed not for therapeutic purposes but instead as growth promoters or routine prophylaxis (Bush *et al.*, 2011; Cabello *et al.*, 2013). Overreliance on these compounds has contributed to the emergence of numerous

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adverse outcomes, including toxicity, teratogenic and carcinogenic effects, the persistence of antimicrobial residues in meat and eggs, and, of greatest public health significance, the rapid development of antimicrobial resistance in animals and humans (Manyi-Loh *et al.*, 2018). In response to mounting scientific evidence, the European Union implemented a region-wide ban on antibiotic growth promoters in animal feed beginning January 1, 2006, marking a major regulatory shift intended to protect human and animal health (Alabi *et al.*, 2024).

The poultry industry itself has undergone remarkable growth, with broiler production surging from approximately 5 billion pounds to more than 40 billion pounds over the past five decades (Fairchild *et al.*, 2005). This expansion was facilitated by vertical integration, where a limited number of companies acquired the majority of processing and production facilities, enabling standardized management strategies and improved production efficiency. Despite these advancements, the routine use of antimicrobials within such systems has exacerbated the selection and dissemination of antibiotic-resistant bacteria, some of which possess zoonotic potential and pose direct risks to human health (Aarestrup *et al.*, 2001; Casewell *et al.*, 2003). Tetracycline resistance is among the most frequently reported patterns of resistance in *Campylobacter* isolates within the National Antimicrobial Resistance Monitoring System (NARMS), attributed largely to the widespread use of oxytetracycline and chlortetracycline in food-animal production systems (Fairchild *et al.*, 2005).

Parallel to concerns regarding antimicrobial resistance, avian coccidiosis remains one of the most economically devastating parasitic diseases in the global poultry sector. Caused by protozoa of the genus *Eimeria*, coccidiosis leads to impaired nutrient absorption, reduced growth performance, and increased mortality, resulting in substantial economic losses due to both decreased productivity and the high costs of prevention and treatment (Williams, 1999). Bera *et al.*

(2010) estimated that coccidiosis accounted for 95.61% of total economic losses in commercial broiler operations, underscoring its significance. The environmental resilience of *Eimeria* oocysts further complicates control efforts, as these oocysts can survive for extended periods under harsh environmental conditions.

Although vaccination is considered an effective method for managing coccidiosis, especially in layers and breeders, its application in broiler production remains limited due to factors such as short production cycles, high costs, and logistical challenges. Consequently, anticoccidial drugs continue to play a crucial role in disease control programs (Huczynski *et al.*, 2012; Zhou *et al.*, 2013). Robenidine, a synthetic anticoccidial compound, has been widely utilized in poultry and rabbit production to prevent *Eimeria* infections (Gerhold *et al.*, 2011). Decoquinate, the primary active substance in robenidine-based products, disrupts mitochondrial energy metabolism in coccidia and is particularly effective during the early stages of parasite development, making it a valuable tool for broiler disease management (Guo *et al.*, 2007; Kaewthamasorn *et al.*, 2015). Despite advances in both antibiotic and anticoccidial drug use, there remains a notable lack of research exploring the combined effects of these agents on avian immune and digestive tissues, particularly under intensive production systems where birds are exposed to multiple stressors (Abreu *et al.*, 2023; Martins *et al.*, 2022). The interaction between antimicrobial agents and immune organs such as the bursa of Fabricius (BF), as well as intestinal structures like the duodenum, is not fully understood, and existing studies often report inconsistent or incomplete outcomes when antimicrobials and anticoccidials are used together (Lanckriet *et al.*, 2010; Lee *et al.*, 2012). Moreover, the potential of combined therapy to influence antimicrobial resistance dynamics or to exacerbate tissue-level changes warrants further investigation (Abreu *et al.*, 2023).

The BF is the primary lymphoid organ responsible for B-lymphocyte differentiation in birds, playing a pivotal role in shaping adaptive

immunity (Glick, 1991). Meanwhile, the duodenum constitutes the first segment of the small intestine and serves as a major site for nutrient absorption as well as early immunological interaction with ingested compounds (Scanes, 2015).

Given the essential roles of the BF in immune cell maturation and the duodenum in nutrient assimilation and mucosal defense, any antimicrobial-induced alterations in these tissues may compromise immune functionality and gut homeostasis. Accordingly, the present study aims to rigorously assess the impact of combined tetracycline and robenidine administration on the structural integrity of both organs in broiler chickens. By focusing on detailed histological evaluation, this work seeks to clarify the nature of tissue responses to dual-drug exposure and to provide deeper insight into the broader implications of antimicrobial interactions within modern, high-intensity poultry production systems.

2. Methodology

2.1. Experimental design

The study was conducted at Al-Jazeera Poultry Station for broiler production in Libya, following the station's routine management and preventive programs. A total of 180 Ross broiler chickens (*Gallus gallus domesticus*) were divided into three groups of 60 birds each, reared in a closed, air-conditioned system. A random sample of farms was selected, cleaned, and disinfected according to Begum *et al.* (2025) before the arrival of the birds. Temperature was maintained at 22°C and relative humidity at 50% using a thermal reduction system, with 24-hour lighting and feed provided ad libitum (Lampman *et al.*, 1997, 1983; Ali *et al.*, 2009). The groups received water and feed according to the station's standard protocol:

Group 1: (control) received drinking water free of tetracycline and feed without anticoccidials.

Group 2: (Rob) received drinking water free of tetracycline and feed containing

robenidine (0.005 g/kg) (Ali *et al.*, 2009; Dorne *et al.*, 2013).

Group 3: (TCs + Rob) received drinking water containing tetracycline (500 mg/0.5 kg body weight) (Masoda *et al.*, 2005) and feed containing robenidine (0.005 g/kg) (Ali *et al.*, 2009) following the station's preventive therapeutic program.

These doses and protocols reflect the routine management practices at the station and were not assigned experimentally by the researchers. Sampling and analyses were performed to evaluate the impact of the station's standard management on bird health, organ morphology, and tissue histology. Samples were collected at 35, 41, and 48 days of age, with five replicates per group and three birds per replicate (Masoda *et al.*, 2005). The anticoccidial agent was withdrawn from the feed of Groups 2 and 3 one week before slaughter (48 days). This study assesses the effects of the station's existing management protocol on broiler health and tissue morphology.

2.2. Laboratory study

2.2.1. Examination of fecal samples

Random fecal samples were collected from birds in all three groups throughout the study period. The samples were examined for the presence of parasites using the flotation technique (Lagrue & Presswell, 2016) to confirm that they were free from pathogenic parasites.

2.2.2. Examination of internal organs

Birds were weighed and subsequently dissected. Internal organs, including the BF and intestines, were weighed and examined to assess macroscopic changes in organ morphology (Cazaban *et al.*, 2015).

2.2.3. Histological examination

Tissue samples were collected from the BF and the duodenum. Samples were fixed in 10% formalin for 48 hours, dehydrated through a graded alcohol series, cleared in xylene, and embedded in paraffin blocks. Sections of 5 µm thickness were prepared using a microtome

(Leica RM-2125), stained with hematoxylin and eosin, and examined microscopically. Photomicrographs were taken according to the method described by Lilie (1970).

2.3. Statistical examination

Data were subjected to a two-way analysis of variance (ANOVA) for a factorial

3. Results and discussion

3.1. Health of the birds

No clinical signs were recorded. No gross lesions were found at post-mortem examination.

3.2. Changes in body weight

As illustrated in Figure 1, body weight increased progressively with age, measured at 35, 41, and 48 days. At 35 days, weights ranged from 757.8 ± 99.25 to 1479.5 ± 42.5 g, showing significant differences among groups. By 41 days, body weights rose to 1414.3 ± 43.7 – 1578.56 ± 70.3 g, and at 48 days, the highest values were observed, ranging from 1662.8 ± 95.4 to 2026.5 ± 68.2 g, with Group 3 being significantly heavier. These observations suggest that, alongside the natural age-related growth, treatment exerted a notable influence on body weight. This is consistent with the findings of Nitsan *et al.* (1991), who emphasized that broiler growth is predominantly age-dependent and is accompanied by increases in internal organ weights. Moreover, some studies have also reported that anticoccidial treatments can affect body weight, though not necessarily through growth stimulation (Lee *et al.*, 2012; Abo-Aziza *et al.*, 2022). On the other hand, Taylor *et al.* (2022) and de Freitas *et al.* (2023) observed that certain anticoccidials may reduce body weight due to their parasitic-control effects rather than direct anabolic action. The variations in body weight observed among treatments in this study can therefore be attributed to the efficacy of anticoccidial agents in lowering parasitic burden and improving nutrient absorption efficiency in the intestine. Supporting this, recent meta-analyses indicate that effective control of coccidial infection

experiment conducted under a completely randomized design (CRD) (Petrie & Watson, 1999). All statistical analyses were performed using SPSS software. When a significant effect was observed at the ($p \leq 0.05$), the Least Significant Difference (LSD) test was applied to separate the means.

correlates with significant weight gain and enhanced production performance, primarily through reduced intestinal tissue damage and improved nutrient utilization (Taylor *et al.*, 2022).

Moreover, Ahmad *et al.*'s (2023) review highlighted that the response to different anticoccidials, including Rob, may vary depending on dosage, administration regimen, and the intensity of the parasitic challenge, which helps explain the differential growth performance across treatments. The substantial weight gain observed in the TCs + Rob group aligns with findings by Kaewthamasorn *et al.* (2015), who reported that combining potent anticoccidial agents can reduce intestinal lesions and improve growth efficiency compared with single-drug treatments. In contrast, the comparatively lower body weight in the Rob-only group is consistent with EFSA (2020), which notes that Robenidine is not a growth-promoting agent and that observed weight variations are typically a physiological response to the drug and experimental conditions rather than an intrinsic growth-stimulating effect.

Table 1: Averages of the body weight changes in the experimental groups (g).

Age	Experimental groups		
	Control	Rob	TCs + Rob
35 days	1118.44±58.7 d	757.8±99.25 e	1479.5±42.5 cb
41 days	1525.7±99.91 cb	1414.3±43.7 c	1578.56±70.3 cb
48 days	1662.8±95.4 b	1676.3±41.5 b	2026.5±68.2 a

3.3. Changes in the weight and length of the small intestine

The statistical analysis presented in Table 2 revealed no significant differences among treatments regarding small intestine weight, and no interactions with age were observed.

Minor variations are likely attributable to the natural growth processes of the birds. In terms of small intestine length (Table 3), the observed differences reflect normal developmental changes associated with aging. Previous studies have demonstrated that small intestine length changes during growth and significantly influence nutrient absorption efficiency (Uni *et al.*, 1996; Sedgh-Gooya *et al.*, 2022). These results are consistent with Nitsan *et al.* (1991), who reported that body weight increases in broiler chickens are generally accompanied by increases in internal organ weights, which largely depend on age and physiological growth. Moreover, the consistently small intestine weight across treatments suggests that the digestive system can adapt to dietary or experimental variations without significantly affecting intestinal structural growth. Recent evidence indicates that dietary supplements or experimental interventions may influence intestinal length without altering weight significantly, implying functional rather than structural modifications (Wu *et al.*, 2013; Novotný *et al.*, 2023).

Table 2: Averages of the weight of the small intestine changes in the experimental groups (g).

Age	Experimental groups		
	Control	Rob	TCs + Rob
35 days	87.4±12.4 a	69.0±7.2 a	93.95±1.4 a
41 days	87.2±6.2 a	83.7±6.5 a	97.0±5.7 a
48 days	106.6±4.5 a	81.4±2.4 a	95.97±9.2 a

Table 3: Averages of the length of the small intestine changes in the experimental groups (cm).

Age	Experimental groups		
	Control	Rob	TCs + Rob
35 days	134.2±8.04 a	135.4±7.5 a	136.3±5.5 a
41 days	141.6±6.6 a	139.0±4.3 a	143.0±3.0 a
48 days	148.4±5.3 a	146.0±3.3 a	149.0±0.6 a

3.4. Changes in the weight of the BF:

Table 4 summarizes the statistical analysis of bursa weight, showing significant differences among treatments and interactions with age. At 48 days of age, one treatment

group (TCs + Rob) exhibited a slight increase in bursa weight (0.8 g) compared to other groups, likely reflecting normal physiological development. These findings align with the observations of Udoumoh *et al.* (2022); Cheng *et al.* (2023), who reported that bursa size decreases gradually with age in poultry, reflecting the maturation of the immune system. Contemporary studies have further confirmed that bursa weight may be influenced by dietary supplements, immunomodulatory agents, or exposure to certain treatments (Mohamed *et al.*, 2022; Ravi *et al.*, 2025). Furthermore, the slight increase in bursa weight in certain treatments may represent an initial immune response or a transient effect of experimental factors (Qin *et al.*, 2024). Conversely, the age-related decline reflects the natural regression of lymphoid activity within the gland, which is a key indicator for assessing the immune status and health of poultry (Abdel-Fattah *et al.*, 2025).

Table 4: Averages of the weight of the BF changes in the experimental groups (g).

Age	Experimental groups		
	Control	Rob	TCs + Rob
35 days	1.3±0.3 a	0.8±0.07 a	1.3±0.08 a
41 days	1.15±0.2 a	1.0±1.0 a	1.5±0.1 a
48 days	0.65±0.1 a	0.85±0.13 a	0.8±0.1 a

3.5. Histological examination

Histological assessment of the BF revealed marked deviations from the normal architecture observed in the control group, which consistently exhibited well-defined cortical and medullary regions within the bursal follicles, intact interfollicular epithelium, and a normal connective tissue stroma across days 35, 41, and 48 (Figs. 1, 4, and 7). At day 35, birds treated with Rob showed a noticeable reduction in follicular size while retaining a relatively normal interfollicular epithelium and stromal organization (Fig. 2). By day 41, more advanced lesions appeared, including shrinkage of the follicles, mild lymphoid depletion, focal necrotic areas, epithelial hyperplasia, and thickening of the subepithelial stroma (Fig. 5).

These alterations progressed further by day 48, when the bursa displayed small follicles with moderate lymphoid depletion, epithelial cyst formation, and stromal oedema (Fig. 8). A similar but more pronounced pattern was observed in the combined treatment group (TCs + Rob). At 35 days, birds exhibited reduced follicular size associated with mild lymphoid loss, degeneration of the interfollicular epithelial lining, and increased thickness of the subepithelial connective tissue (Fig. 3). By day 41, the lesions became severe, characterized by extensive depletion and lysis of bursal follicles, development of multiple large epithelial cysts, and marked subepithelial oedema (Fig. 6). At 48 days, the bursa showed extensive architectural destruction, with only a few residual follicles, degenerative changes in the interfollicular epithelium, prominent oedema, and accumulation of necrotic debris within the stroma (Fig. 9).

The present study provides preliminary evidence that exposure to Robenidine, either alone or in combination with Tetracycline, is associated with notable histological alterations in the BF, including reduced follicular diameter, epithelial thinning, cyst formation, and lymphoid depletion. These changes suggest a potential decline in B-cell proliferation and maturation, which could compromise humoral immune capacity, given the bursa's central role in avian B-cell development (Kaspers & Schat, 2013; Debbarma *et al.*, 2025). Such effects are consistent with the literature, which reports that chemical stressors and oxidative imbalance increase reactive oxygen species (ROS) generation and trigger apoptotic pathways in lymphoid tissues, leading to reduced lymphocyte density and disrupted tissue architecture (Debbarma *et al.*, 2025). Notably, the Rob + TCs group exhibited more severe lesions, suggesting a potential additive or synergistic effect. However, as direct evidence of this interaction in the context of poultry is lacking, this interpretation remains a hypothesis warranting further investigation.

Histological evaluation of the duodenum demonstrated notable alterations in the treated groups compared with the control, which

consistently exhibited normal villus morphology and intact epithelial layers at days 35, 41, and 48 (Figs. 10, 13, and 16). At day 35, both the Rob and Rob + TCs groups maintained normal villus and epithelial architecture (Figs. 11 and 12). However, by days 41 and 48, all treated groups displayed a significant reduction in villus height and epithelial thickness, indicating progressive structural impairment associated with the administered treatments (Figs. 14, 15, 17, and 18). This pattern likely reflects impaired epithelial turnover and diminished structural integrity of the intestinal mucosa, which could compromise nutrient absorption and barrier function. Similar morphological alterations have been reported in broilers under conditions of oxidative stress and chronic intestinal inflammation (Awad *et al.*, 2017; Tellez *et al.*, 2023). It should be noted, however, that these interpretations are extrapolated from general oxidative stress models, as direct evidence regarding the specific effects of Rob ± TCs on duodenal architecture remains limited.

The concurrent degeneration of bursal and duodenal tissues highlights the integrative nature of the gut-immune axis. Reduced bursal function may impair mucosal immune responses, while intestinal injury could limit the availability of nutrients necessary for lymphoid cell proliferation, potentially exacerbating tissue damage in both systems (Li *et al.*, 2024; Mishra & Jha, 2019; Tellez-Isaias *et al.*, 2023). This observation aligns with prior literature demonstrating systemic consequences of oxidative stress and chronic inflammation on lymphoid organs and intestinal health in poultry (Ducatelle *et al.*, 2023).

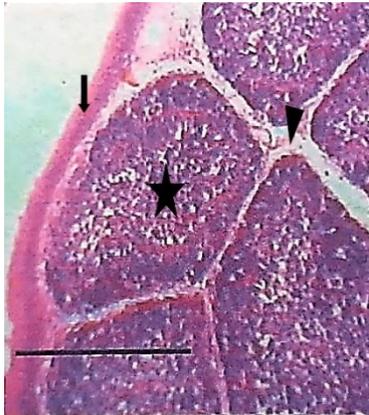


Fig. 1. Cross-section in the BF of control chickens (35 days) showing that normal bursal follicles consist of the cortex of the follicle, medulla (star) of the follicle, with inter-follicular surface epithelium (arrow), and normal connective tissue stroma (head arrow). Bar=175 μ m. 400X.

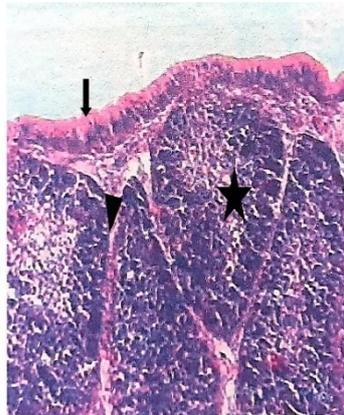


Fig. 2. Cross-section in the BF of Rob chickens (35 days) showing the small size of bursal follicles (star), with normal inter-follicular surface epithelium (arrow), and normal connective tissue stroma (head arrow). Bar=175 μ m. 400X.

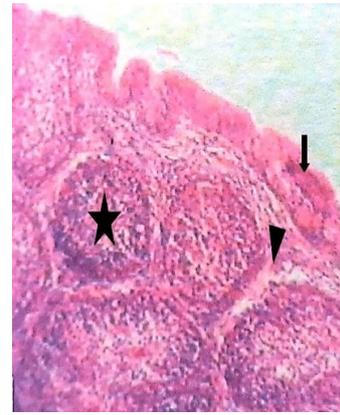


Fig. 3. Cross-section in the BF of (TCs + Rob) chickens (35 days) showing the small size of bursal follicles with mild depletion of lymphoid follicles (star), degeneration of inter-follicular surface epithelium (arrow), and thickening of subepithelial connective tissue stroma (head arrow). Bar=175 μ m. 400X.



Fig. 4. Cross-section in the BF of control chickens (41 days) showing that normal bursal follicles consist of the cortex of the follicle, medulla (star) of the follicle, with inter-follicular surface epithelium (arrow), and normal connective tissue stroma (head arrow). Bar=225 μ m. 400X.

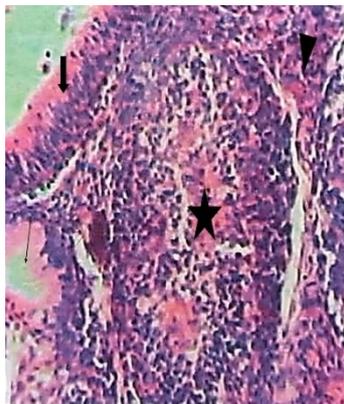


Fig. 5. Cross-section in the BF of Rob chickens (41 days) showing the small size of bursal follicles with mild depletion of lymphoid follicles (star), with necrosis area (thin arrow), hyperplasia of epithelium (arrow), and thickening of subepithelial connective tissue stroma (head arrow). Bar=225 μ m. 400X.

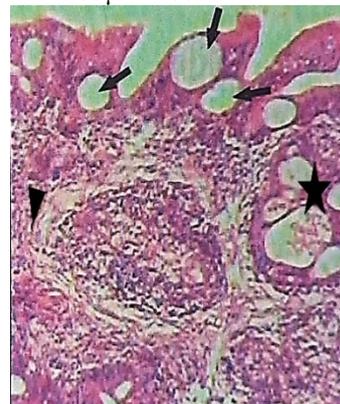


Fig. 6. Cross-section in the BF of (TCs + Rob) chickens (41 days) showing severe depletion and lysis of bursal follicles (star), formation of multiple bigger epithelial cysts (arrow), and subepithelial oedema in connective tissue stroma (head arrow). Bar=225 μ m. 400X.

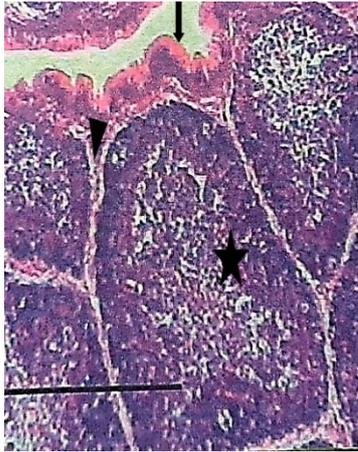


Fig. 7. Cross-section in the BF of control chickens (48 days) showing that normal bursal follicles consist of the cortex of the follicle, medulla (star) of the follicle, with inter-follicular surface epithelium (arrow), and normal connective tissue stroma (head arrow). Bar=250µm. 400X.



Fig. 8. Cross-section in the BF of Rob chickens (48 days) showing the small size of bursal follicles with moderate depletion of lymphoid cells of bursal follicles (star), the formation of epithelial cysts (arrow), and oedema in connective tissue stroma (head arrow). Bar=250µm. 400X.

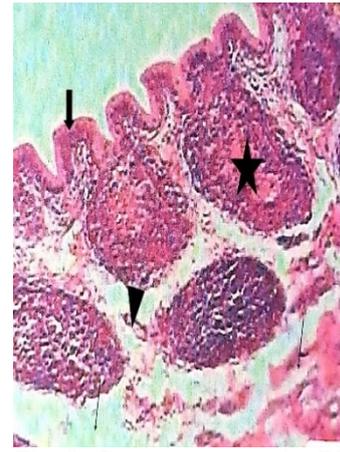


Fig. 9. Cross-section in the BF of (TCs + Rob) \ chickens (41 days) showing severe depletion and a few bursal follicles (star), degeneration of inter-follicular surface epithelium (arrow), and subepithelial oedema in connective tissue stroma (head arrow) with necrotic debris (thin arrows). Bar=250µm. 400X.

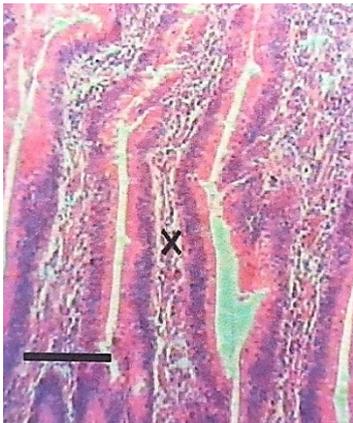


Fig. 10. Cross-section in the duodenum of control chickens (35 days) showing the normal structure in villi (x). Bar=125µm. 400X.

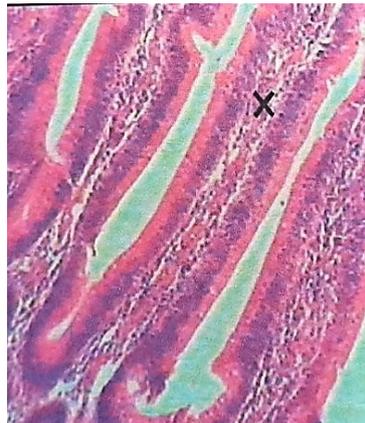


Fig. 11. Cross-section in the duodenum of Rob chickens (41 days) showing the normal structure in villi (x). Bar=125µm. 400X.

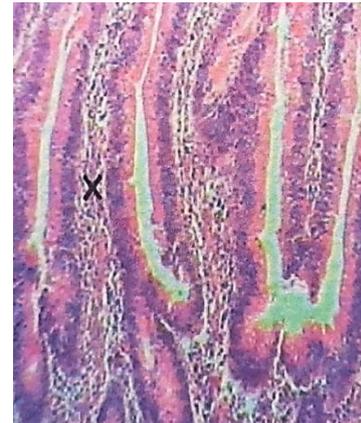


Fig. 12. Cross-section in the duodenum of (TCs + Rob) chickens (48 days) showing the normal structure in villi (x). Bar=125µm. 400X.

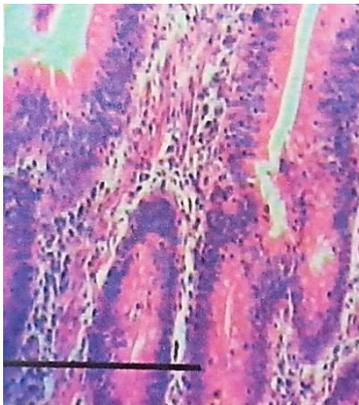


Fig. 13. Cross-section in the



Fig. 14. Cross-section in the

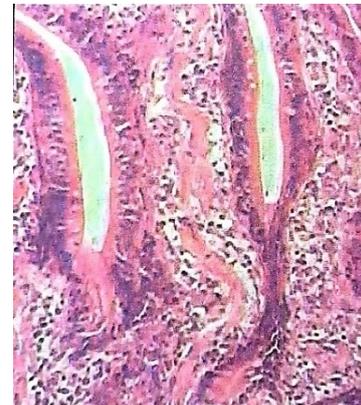


Fig. 15. Cross-section in the

duodenum of control chickens (35 days) the normal structure in villi (x). Bar=125µm. 400X.

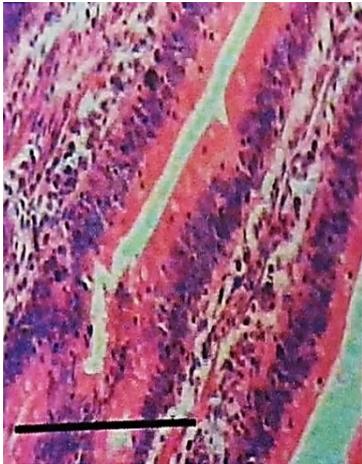


Fig. 16. Cross-section in the duodenum of control chickens (35 days) showing that normal bursal follicles consist of the cortex of the follicle, medulla (star) of the follicle, with inter-follicular surface epithelium (arrow), and normal connective tissue stroma (head arrow). Bar=125µm. 400X.

duodenum of Rob chickens (41 days) showing the reduced width of the villi and epithelial layer. Bar=125µm. 400X.



Fig. 17. Cross-section in the duodenum of Rob chickens (41 days) showing the reduced width of the villi and epithelial layer with damage in it (arrow). Bar=125µm. 400X.

duodenum of (TCs + Rob) chickens (48 days) showing the reduced width of the villi and epithelial layer. Bar=125µm. 400X.

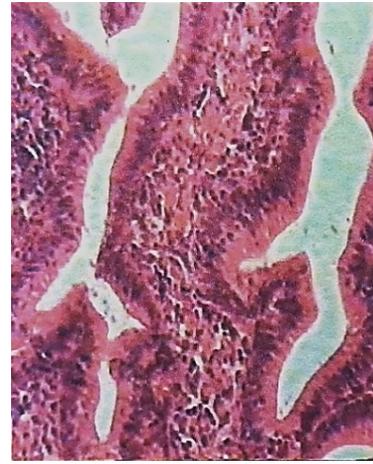


Fig. 18. Cross-section in the duodenum of (TCs + Rob) chickens (48 days) showing the reduced width of the villi and epithelial layer. Bar=125µm. 400X.

4. Conclusions

This study demonstrates that the routine combined use of TCs and Rob in broiler management leads to significant histological alterations in the BF and the duodenum, including follicular atrophy, lymphoid depletion, and epithelial degeneration. These changes indicate potential impairment of humoral immunity and compromised nutrient absorption, which could ultimately affect flock health and growth performance. These findings suggest that routine administration of tetracycline in combination with robenidine should be carefully re-evaluated. Possible management adjustments include optimizing dosing schedules, limiting the duration of combined exposure, or implementing alternative coccidiosis control strategies such as rotation of anticoccidial drugs, targeted therapeutic administration rather than continuous prophylaxis, or incorporating vaccination programs where feasible. By tailoring antimicrobial and anticoccidial use

according to flock needs, poultry producers can reduce adverse tissue effects, support immune competence, and maintain efficient growth performance.

Further studies are warranted to explore safer alternatives, determine the minimum effective doses, and evaluate the long-term impacts on immunity, intestinal health, and resistance development.

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