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Histological Effect of Saccharomyces Cerevisiae in the Ration on Duodenum of Lambs

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Abstract

In this study, sixteen male Awassi lambs were allocated into four experimental groups. The control group (T1) received no yeast, while the treatment groups (T2, T3, and T4) were administered 3g, 5g, and 7g of Saccharomyces cerevisiae (yeast) per lamb, respectively. All lambs were fed a diet consisting of barley grain, yellow corn, soybean meal, salt, and a mixture of vitamins and minerals, with the yeast supplement added accordingly. The feeding trial lasted for 75 days, after which all lambs were slaughtered, and tissue samples were collected from the first, second, and third sections of the duodenum. These samples were fixed in 10% formalin and processed using standard histological techniques to create slides stained with Hematoxylin and Eosin. The slides were then photographed using a digital camera mounted on an Olympus microscope. The findings revealed that groups T1, T2, and T3 exhibited longer villi lined with simple columnar epithelium, with goblet cells present between the epithelial cells. The lamina propria contained numerous mucus glands, along with lymphocytic infiltration and plasma cells. In contrast, group T4 showed necrotic cells from the simple columnar epithelium sloughing off into the duodenal lumen, while other components of the duodenum remained intact.

Keywords: Saccharomyces cerevisiae, Histological effect, Duodenum

Introduction

The gastrointestinal tract cannot be independently colonized by yeast (Sprague et al., 2022). However, yeast can adhere to the intestinal epithelium, competing with pathogenic bacteria and enhancing the host's immune response (Takiishi et al., 2017). Since the 1950s, there has been extensive research on the application of dietary yeast cultures to influence rumen fermentation (Amin & Mao, 2021).

This research has gained renewed significance following the European Union's ban on antibiotics. The effectiveness of Saccharomyces cerevisiae products varies widely, largely due to differences in yeast strains and cell viability. Various models have been developed to elucidate the impact of yeast on rumen fermentation (Pang et al., 2021). Supplementing ruminant diets with yeast has the potential to enhance feed intake, although results have varied significantly depending on the diet's composition. Additionally, improvements in weight gain and digestion have been reported (Amin & Mao, 2021). Regional or local dairy products are of significant importance, and breeding programs for dairy ewes adapted to the Mediterranean region of Croatia have commenced (Carta et al., 2009). Saccharomyces cerevisiae has been extensively studied as a model eukaryote, and an international initiative is currently underway to elucidate its nucleotide sequences (Vanderwaeren et al., 2022).

Recently, numerous new food products and components have entered the food and pharmaceutical markets, capturing consumer interest due to their purported health benefits. Nutraceutical, functional, probiotic, and synbiotic products are well- documented in clinical and scientific literature, and are recommended for improving human health, preventing diseases, and providing essential metabolites with dietary and therapeutic properties (Latif et al., 2023). Products containing probiotic micro- organisms play a primary role, especially given recent developments in knowledge of the complex intestinal microflora (Fijan, 2014). The term "probiotic" refers to live microorganisms used as dietary or medicinal supplements that enhance health by improving one or more of the key functions of the normal intestinal microflora, including resistance to pathogen colonization, modulation of the immune system, and nutritional support (Maftei et al., 2024). Despite their established role in the dominant or distinctive microflora of various fermented foods, the use of yeast as active dietary supplements remains relatively limited (Dimidi et al., 2019).

Yeast's potential as probiotics has garnered interest due to their ability to withstand passage through the gastrointestinal tract, endure low pH levels, and tolerate bile salts. However, their use in ewes remains limited (Alkalbani et al., 2023), and research on identifying or developing new probiotic yeast strains has also been limited (Tullio, 2024). This study aims to assess the impact of varying concentrations of Saccharomyces cerevisiae yeast supplements in the feed on the duodenal mucosal layer in lambs.

Methods

Sixteen local Awassi male lambs, aged 6 to 6.5 months with an average initial weight of 36 ± 0.34 kg, were assigned to four treatment groups, each consisting of four lambs. The treatments were as follows: T1 (control), T2 (3 g Saccharomyces cerevisiae per lamb per day), T3 (5 g Saccharomyces cerevisiae per lamb per day), and T4 (7 g Saccharomyces cerevisiae per lamb per day).

The lambs were housed individually in cages measuring 1.75 x 1.25 meters, each equipped with two portable plastic feeders for concentrate and roughage feed, as well as a water pail and a mineral salt block. An adaptation period of two weeks preceded the study to acclimate the lambs to their new environment and feeding regimen. During this period, routine veterinary care was administered to all lambs.

Throughout the study, all lambs had ad libitum access to wheat straw as a roughage source, while their concentrate diet is detailed in Table 1.

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Table 1. Formula and chemical composition of concentrate diet

Ingredient	Percentage %
Barley grain	49
Yellow corn	39
Soybean meal	10
Salt	1
Min. and vit. mixture	1
	100

Each treatment lamb was provided with concentrate feed at a rate of 2.5% of their weekly live body weight throughout the 75-day study period.

Histological Technique

After euthanizing the animals, duodenal specimens (0.5 cm^3) from the first, second, and third portions were collected and immediately fixed in 10% formalin following washing with tap water. The histological processing involved dehydration through a graded series of alcohol, clearing with xylene, and embedding in paraffin wax at 58°C. Dehydration was performed using a descending series of alcohol concentrations, followed by staining with hematoxylin and eosin. The specimens were then examined under a light microscope and photographed using a digital camera attached to an Olympus microscope (Troiano et al., 2009).

Results and Discussion

The duodenum in control

1st portion

The mucosa consisted of numerous finger-like villi lined with simple columnar epithelium and topped with microvilli. The lamina propria was densely populated with crypts of Lieberkühn, which were also lined by simple columnar cells, with lymphocytes interspersed among them. The submucosa contained Brunner's glands (Figure. 1).

The muscularis mucosa appeared as a thin layer composed of smooth muscle fibers. The submucosa was abundant with duodenal mucus glands. The tunica muscularis consisted of an inner layer of circular muscle fibers and an outer layer of longitudinal muscle fibers.

The tunica serosa was composed of delicate connective tissue, covered by mesothelial cells. This layer also contained blood vessels and nerve plexuses.

Duodenum mid portion

The tunica mucosa in this section contained duodenal villi that were shorter compared to those in the first portion (Figure. 2).

End portion (Terminal portion)

The mucosa featured tall villi lined with simple columnar epithelium, which contained a higher number of goblet cells compared to the middle and first portions of the duodenum (Figure. 5).

The lamina propria was characterized by a high density of tubular mucus glands, located both on the mucosal surface and at the base of the villi. The submucosa was composed of connective tissue and contained blood vessels.

The muscular layer was very thin, consisting of inner circular and outer longitudinal muscle fibers. The serosa was similar to that found in the first and middle portions (Figure. 7).

Duodenum 3%

1*st portion*

The duodenal mucosa featured numerous villi, many of which were branched, and were covered with simple columnar epithelial cells. There was a high density of goblet cells interspersed among the epithelial cells (Figure. 8).

The core of the villi contained a significant number of lymphocytes and other white blood cells, which extended into the lamina propria. This layer was densely populated with mucus glands that also extended into the submucosa (Figure. 9).

2nd portion (mid portion)

The mucosa displayed villi-like projections extending into the duodenal lumen. The interspersed columnar epithelium contained numerous goblet cells, and lymphocytes were distributed among the epithelial cells (Figure. 10).

The lamina propria was densely populated with tubular mucus glands. Similarly, the submucosa contained numerous mucus glands forming glandular masses, accompanied by infiltration of white blood cells (Figures. 11, 12).

Terminal portion of duodenum 3 %

The mucosa featured large, closely spaced villi that coalesced into broad projections, covered by simple columnar epithelium with a high density of goblet cells.

The lamina propria was characterized by numerous long, tubular mucus glands extending toward the mucosal surface, which were filled with mucus droplets. Additionally, there was a significant aggregation of lymphocytes throughout the area (Figure. 13). The submucosa was a thin layer containing blood vessels and nerve plexuses. The lamina propria housed mucus glands, specifically the crypts of Lieberkühn, located at the bases of the villi.

The submucosa was heavily populated with duodenal mucus glands, surpassing the density found in the first portion. Additionally, there was a significant presence of white blood cells (Figure. 14).

Duodenum 5%

1st portion

The mucosa featured villi with substantial interstitial spaces populated by a high concentration of white blood cells. These cells infiltrated both the outer regions and the core of the villi, extending into the deepest layers of the lamina propria. This area was associated with the crypts of Lieberkühn and was connected with the mucous glands of the lamina propria (Figures. 15, 16).

Mid portion

The villi were broad and branched, interspersed with goblet cells within the simple columnar epithelium (Figure. 17).

The lamina propria contained numerous tubular mucus glands extending to the bases of the villi, with lymphocytic infiltration present between these glands. In the submucosa, Brunner's (duodenal) glands were organized into groups, featuring branched structures and a modest presence of lymphocytic aggregation (Figure. 18).

Terminal portion of duodenum 5%

The villi exhibited high, broad projections and were interspersed with goblet cells among the columnar epithelial cells.The lamina propria displayed significant aggregation of white blood cells between the tubular mucus glands (Figure. 19).

The submucosa consisted of delicate connective tissue with blood vessels and lacked any glands or lymphocytic infiltration (Figures. 20, 21).

Duodenum 7%

1st portion

The mucosal villi were narrow and widely spaced, with hypertrophied simple columnar epithelium. Some of these epithelial cells were desquamated into the lumen.

The lamina propria contained mucus glands that were distinct from one another, and the aggregation of lymphocytes was less pronounced compared to other groups (Figure. 22).

The Brunner's glands in the duodenum were fewer in number, and their cells exhibited signs of degeneration.

The duodenal villi were poorly defined due to extensive degeneration of their cells, although numerous mucus-secreting goblet cells were present. The lamina propria contained degenerated cells within the mucus tubular glands and was populated with many lymphocytes. Notably, there were nodular aggregations of white blood cells, predominantly lymphocytes, in the deepest layers of the lamina propria (Figure. 23).

No Brunner's glands were observed in the submucosa.

The terminal portion

The villi were slender and appeared fragmented, with most of the simple columnar epithelium showing degeneration. The goblet cells also appeared dispersed (Figure. 26).

The lamina propria contained the mucus glands of Lieberkühn, which had degenerated mucus cells within their lumens (Figures. 27, 28).

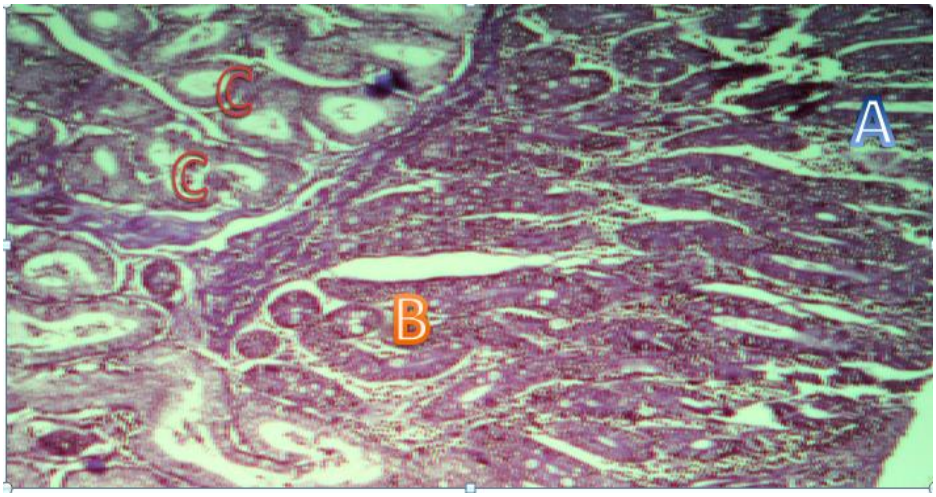

Figure 1. Control, demonstrating a finger like villi of mucosa (A), luberkuhn glands of lamina properia (B), ------- (C) (H&E X10**)**

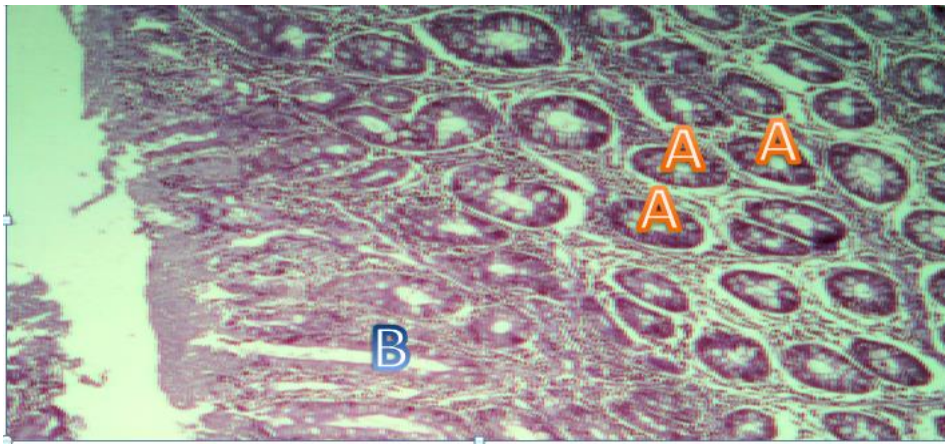

Figure 2. Duodenal mucus gland in the lamina properia (A), crypts of luberkuhun (B) (H&E x 10).

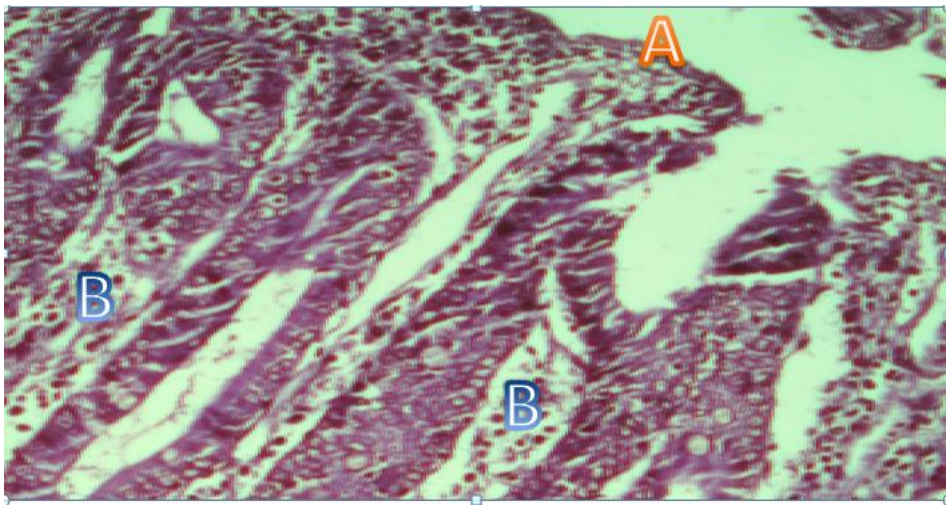

Figure 3. Duodenal villi covered with simple columnar epithelium (A), the core of villi infiltrated with WBC (B) (H&E X10).

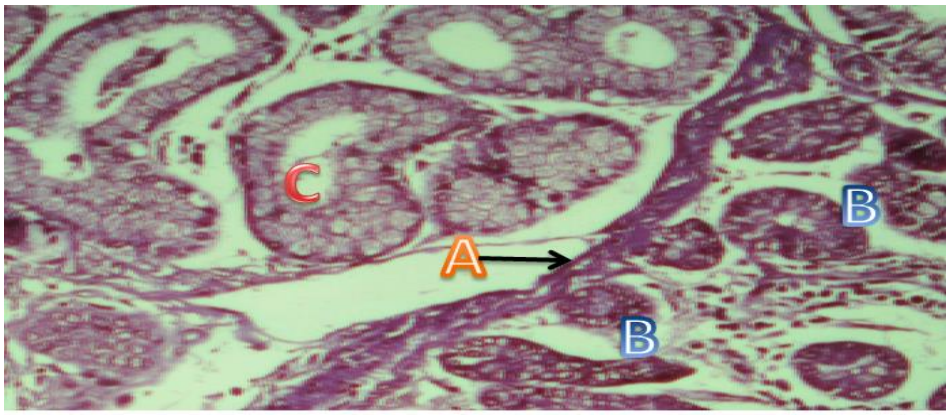

Figure 4. Muscularis mucosa(A), crypt of luberkuhn in the lamina properia (B), Brunner glands (C) (H&E X10).

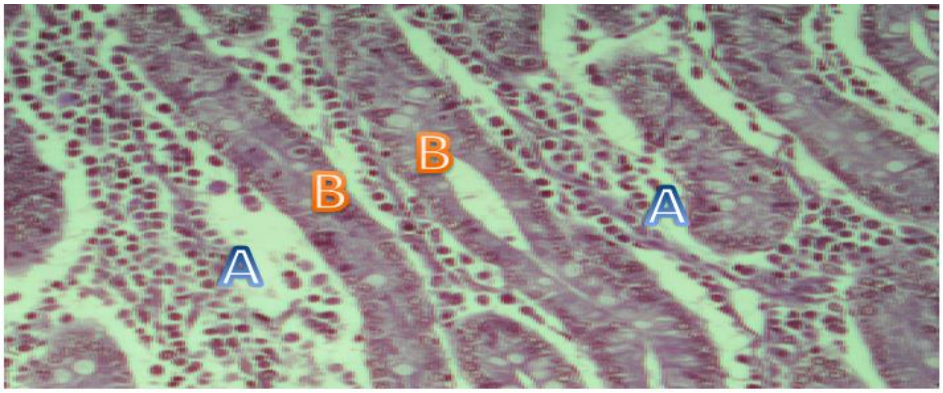

Figure 5. First portion 3%. A great number of lymphocyte in the lamina properia of duodenum (A), crypt of luberkuhn (B) (H&E X10).

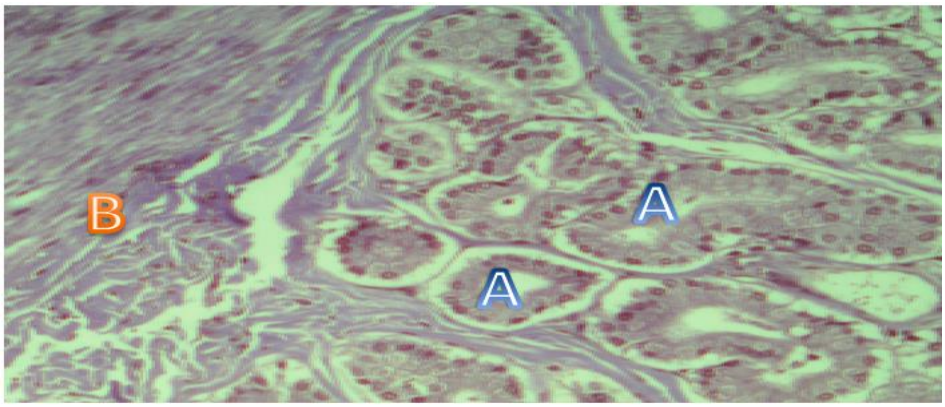

Figure 6. First portion 3%. The submucosa is embedded with a great number of duodenal mucus glands (Brunner glands) (A), muscular coat (B) (H&E X10).

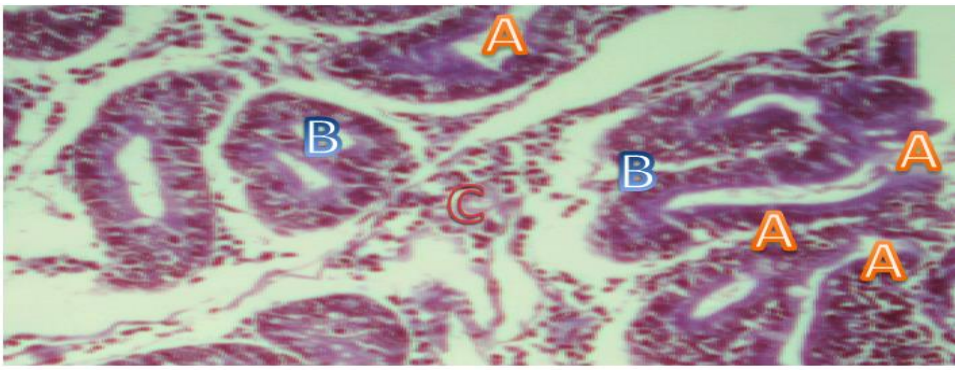

Figure 7. second portion 3%. Simple columnar epithelium of villi with goblet cells (A), crypts of luberkuhn (B), lymphocytic diffusion (C) in the lamina properia (H &E X10).

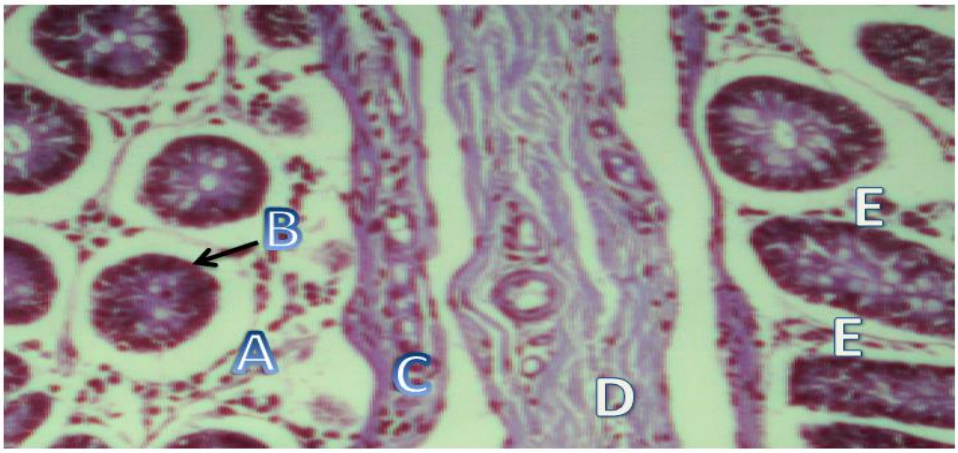

Figure 8. Lamina properia of duodenum (2nd portion) with lymphocytic infiltration (A), surrounded by mucus acini(B), muscularis mucosa (C), submucosa (D) and Brunner glans (E) (H&E X10).

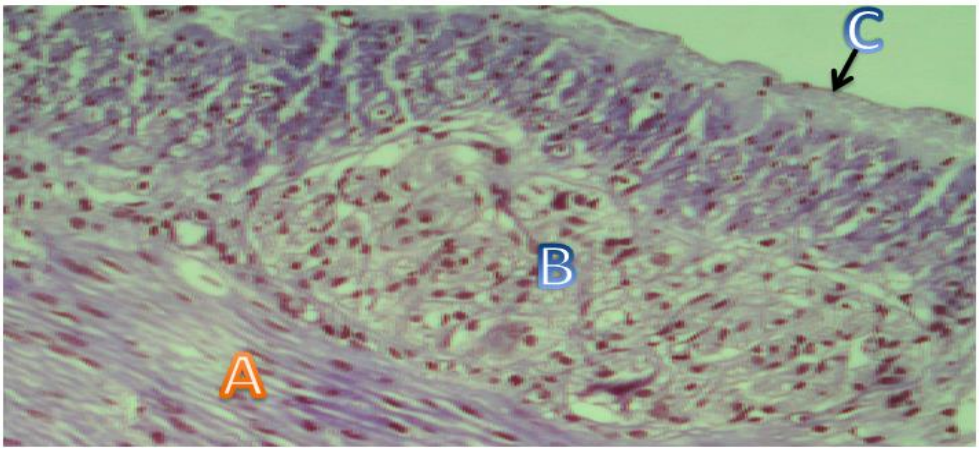

Figure 9. Inner circular smooth muscles of duodenum (A), outer longitudinal muscles fiber (B), Tunica serosa (C) (H&E X10).

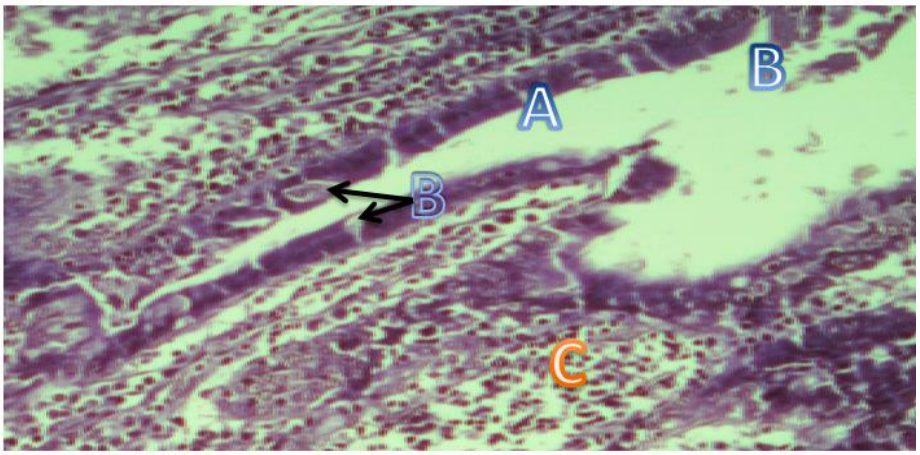

Figure 10. Duodenal villi with simple columnar epithelium (A), with goblet cells inbetween (B), lamina properia with a great number of lymphocyte (C) (H & E X 10).

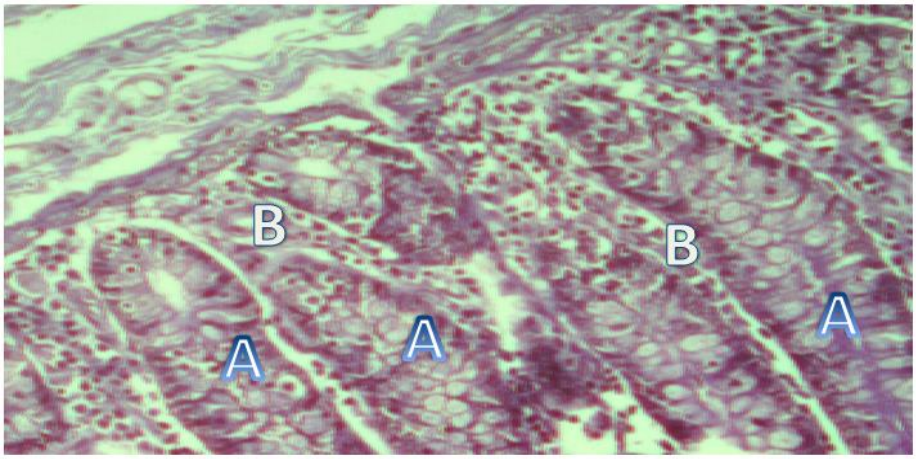

Figure 11. Lamina properia with great number of intestinal glands (A), lymphocytic diffusion in between glands (B) (H& E X 10).

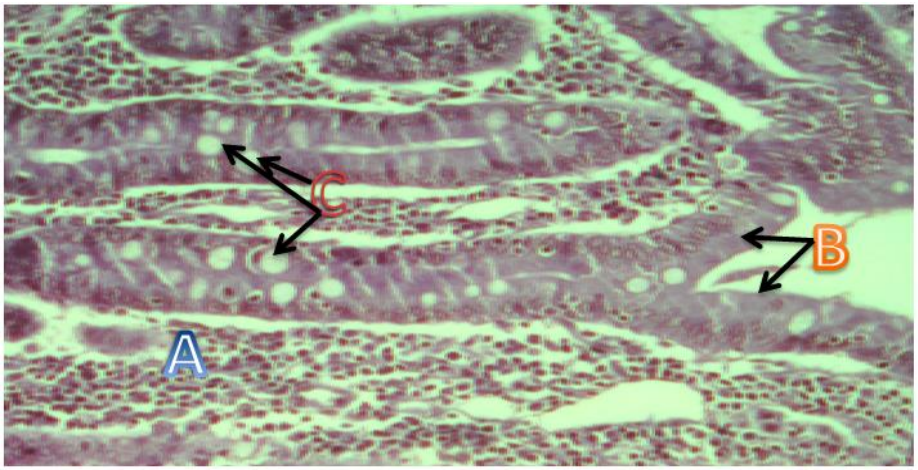

Figure 12. Lymphocytic diffusion of lamina properia (A), columnar epithelium of duodenal villi (B), goblet cells (C) (H & E X 10).

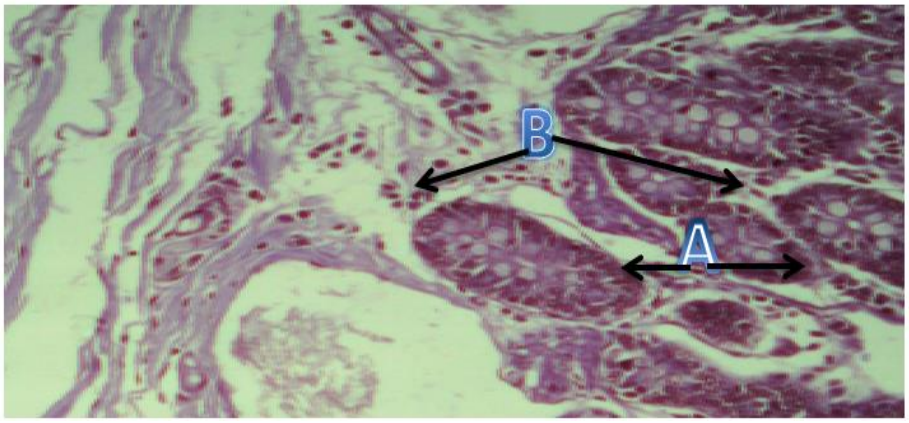

Figure 13. Brunner glands of 2nd portion of duodenum (A), with few lymphocytic aggregation (B), in the submucosa (H & E X10).

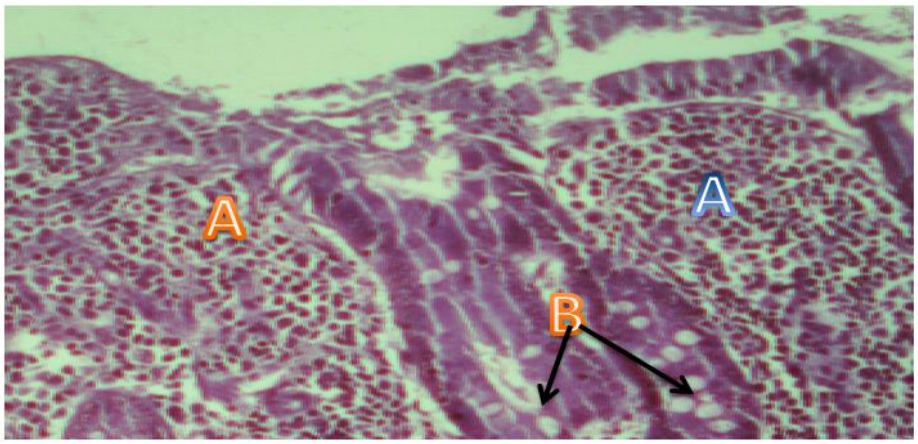

Figure 14. A huge number of lymphocyte in the lamina properia (A), epithelium of villi with goblet cells (B) (H& EX10).

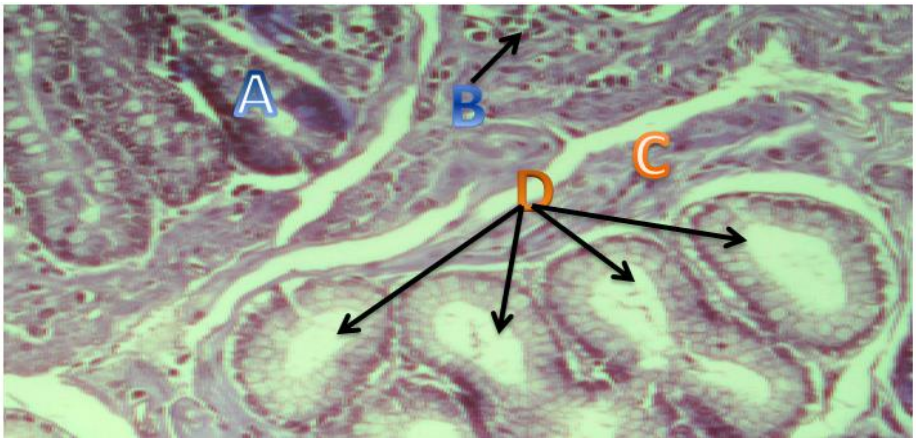

Figure 15. Crypts of luberkuhn in the lamina properia (A), few lymphocytes in the lamina properia (B), Brunner glands (C) in the submucosa (D) (H& E X10).

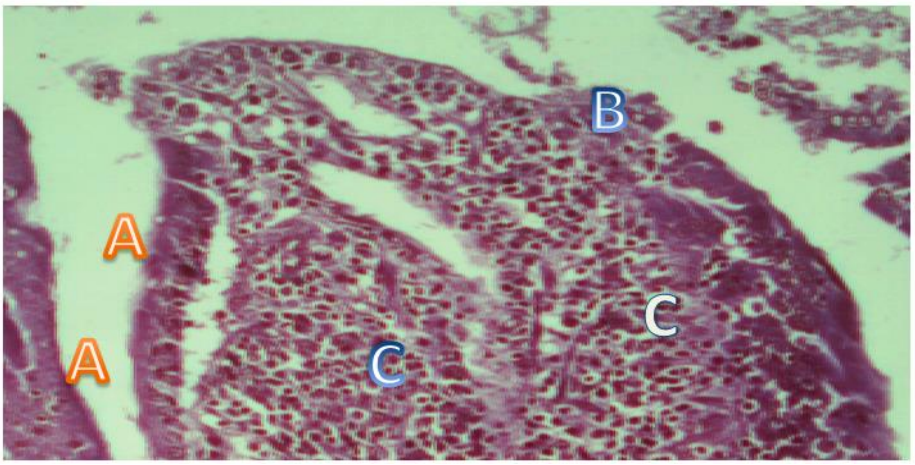

Figure 16. Hypertrophy of simple columnar epithelium of villi (A), desquamation of epithelium (B), lymphocytic infiltration of lamina properia (C) (H& E X10).

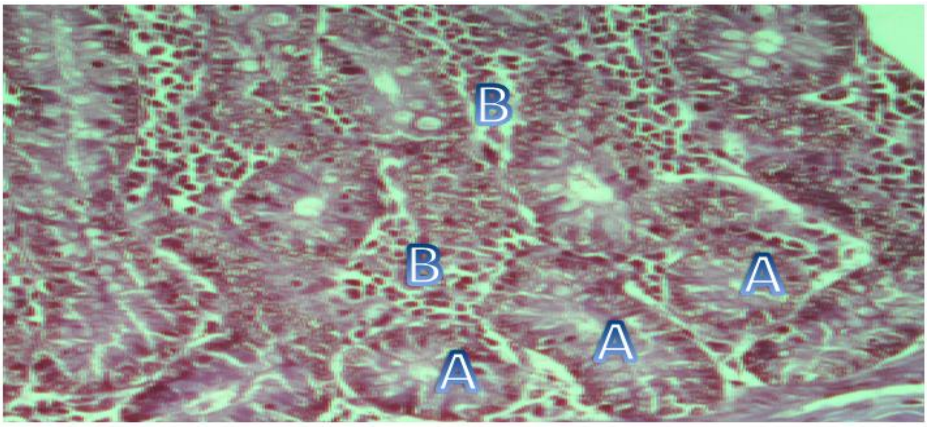

Figure 17. Submucosa of duodenum with Brunner glands (A), lymphocytic infiltration (B) (H&E X10).

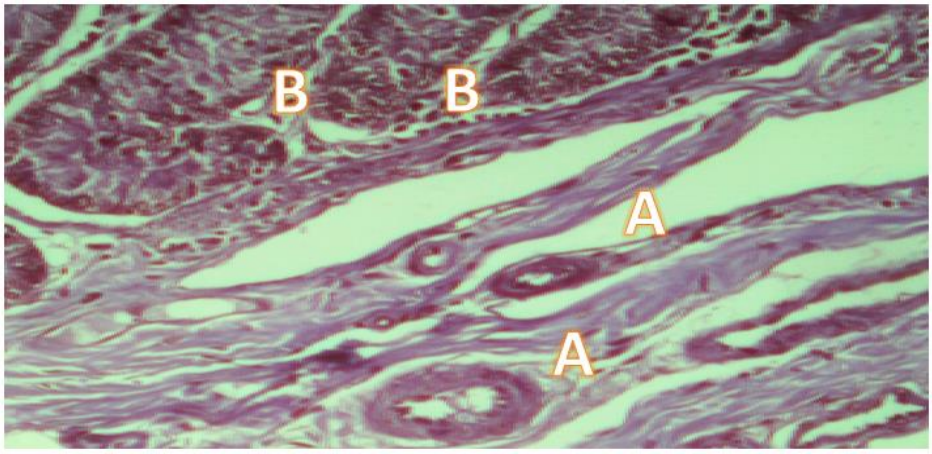

Figure 18. Submucosa with delicate C.T with B.V(A), crypts of luberkuhn of lamina properia (B) (H &E X10)

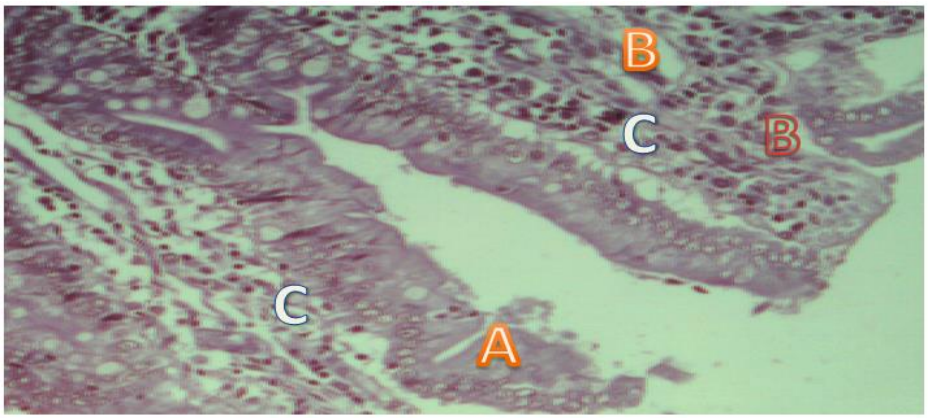

Figure 19. Hypertrophy of epithelium (A), desquamation of epithelium (B), lymphocytic diffusion (C) (H&E X10).

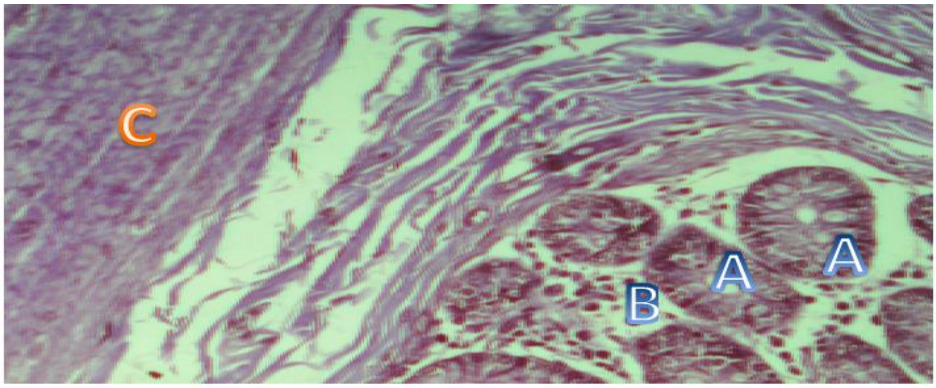

Figure 20. Few Brunner glands (A), lymphocytic diffusion (B), Tunica muscularis (C) (H&E X10).

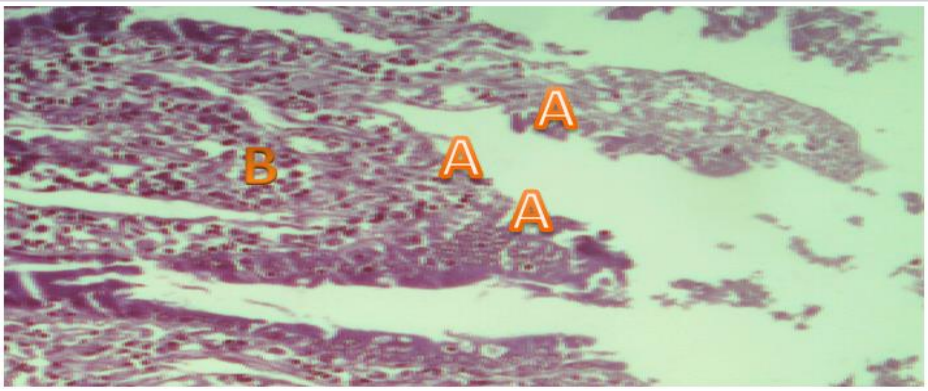

Figure 21. Desquamation of epithelium from villi (A), lymphocytic diffusion (B), (H&E X 10).

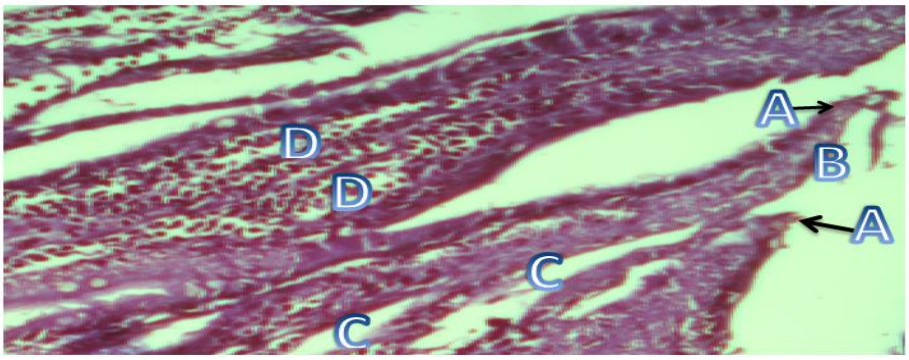

Figure 22. cylinder shape of intestinal villi (A), with desquamation of epithelium (B), loosning of core of villi (C), lymphocytic diffusion (D) (H&E X 10).

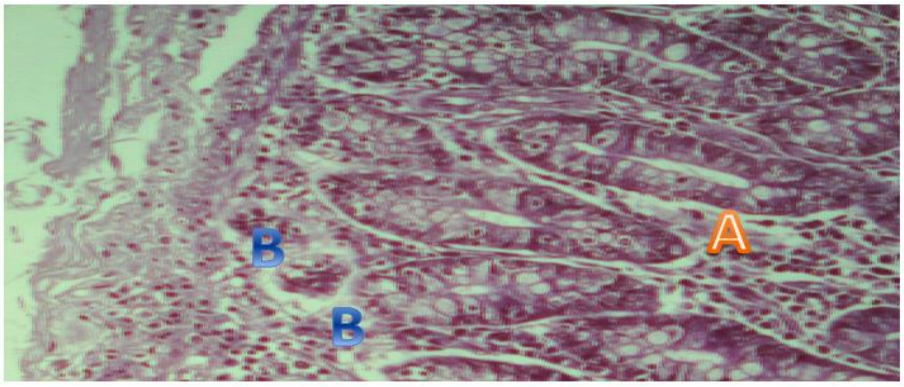

Figure 23. Lamina properia with crypts of luberkuhn, surrounded by lymphocytes (A), focal lymphocytic aggregation (B) (H&E X 10).

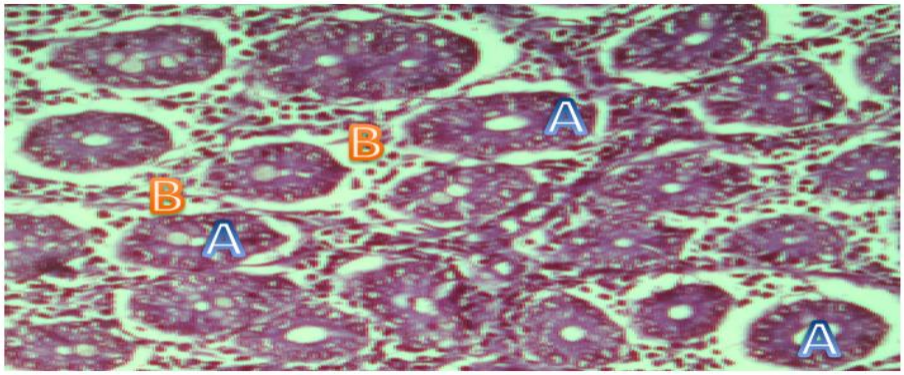

Figure 24. Degeneration mucus cells of the intestinal glands of lamina properia (A), lymphocytes diffusion (B) (H&E X 10).

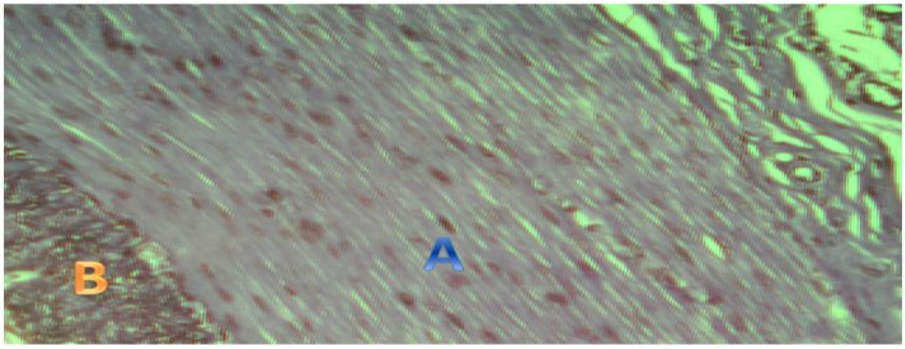

Figure 25. Tunica muscularis of duodenum (3rd portion). outer longitudinal (A), inner circular (B) (H&E X 10).

The duodenum which was the target organ for this study containing a huge number of villi in its mucosa which are essential for the absorption of food materials by the aid of duodenal Brunner glands (Gelberg, 2018).

The current study demonstrated that supplementing the diet with yeast enhanced food intake, as evidenced by an increase in the number and height of villi, a greater number of mucus glands in the lamina propria, and a higher density of Brunner's glands in the submucosa, particularly in the first portion of the duodenum.

In all treatment groups, the duodenum exhibited branched villi covered with simple columnar epithelium and numerous interspersed goblet cells, consistent with previous findings (Serra & Jani, 2006). The presence of substantial lymphocytic infiltration in the core of the villi and the lamina propria suggests that yeast supplementation supports the localized immune system within the gastrointestinal tract, particularly in the duodenum. According to (Takiishi et al., 2017), an increase in white blood cells in the mucosa of the small intestine is considered a key indicator of intestinal health, as ruminants may consume contaminated feed. The presence of numerous white blood cells, including lymphocytes and plasma cells responsible for antibody production, reflects enhanced localized immunity, aligning with (Sanchez, 2018), who noted that immune cell elements in the gastrointestinal tract are derived from systemic blood circulation and bone marrow. Thus, the observed effects in the duodenum across the different yeast supplementation levels (T1 control, T2 3 g, T3 5 g, and T4 7 g) can be attributed to the inclusion of yeast in the diet (Duan et al., 2019).

The administration of 7 grams of yeast per lamb resulted in the desquamation of some epithelial cells from the villi and an increase in white blood cells in both the lamina propria and the core

of the villi. These findings suggest that a higher concentration of yeast in the diet may inhibit epithelial cell activity and nutrient absorption. This observation contrasts with (Powell et al., 2011), who reported that yeast could have an inhibitory effect on the gastrointestinal mucosa. In conclusion, the muscular coat and blood vessels of the duodenal wall remained unaffected across all treatment groups

Conclusion

Supplementation of lambs with yeast at concentrations of 3, 5, and 7 grams per head improved food absorption, as evidenced by an increase in the number of branched villi. Enhanced lymphocytic aggregation in the lamina propria and submucosa, along with an increased presence of Brunner's glands, indicated improved metabolic capacity for nutrient processing. However, the highest dosage of 7 grams resulted in reduced absorption efficiency due to the desquamation of some epithelial cells on the villi surface.

Acknowledgment

Faculty of veterinary medicine of Tikrit university.

Authors' Contribution

Conceptualization, bader khatlan ,hadeel Mahdi and firas Hussein. methodology and the write-up of the manuscript hadeel mahdi.; anatomical part BKH, Histological part ;firas Hussein.

Ethical Approval

This study proposal approved by the faculty of veterinary medicine Tikrit university, Iraq.

Competing Interests

The authors have not declared any conflict of interest.

Ethical Consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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