

## HISTOPATHOLOGICAL CHANGES OF USING RAW LENTIL SEEDS AS A PARTIAL SUBSTITUTE FOR THE FISH MEAL IN DIETS OF THE COMMON CARP *CYPRINUS CARPIO* L.

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### ABSTRACT

This experiment was carried out at College of Veterinary Medicine, University of Sulaimani. Microscopically, no adverse histopathological changes were seen in the kidneys, liver, gills and intestines of *Cyprinus carpio* for T2(replacing fishmeal with 5% lentil) and T3 (replacing fishmeal with 10% lentil) in comparison with the control (T1). However, various levels of adverse histopathological changes were seen in the T4(replacing fish meal with 15% lentil) and T5 (replacing fishmeal with 20% lentil). In the kidneys, dilation of the Bowman's spaces was evidently associated with a decrease in the mesangial cellularity of glomerular tuft. In the liver, congestion of the central vein was apparent together with centrilobular infiltration of inflammatory cells. In the gills, hyperplasia of primary and secondary lamellae was evident. In the intestine, vacuolar degeneration of the lining epithelial cells was apparent together with extravasation of RBCs associated with marked infiltration of inflammatory cells in the lamina propria. In conclusion, the partial replacement of the fishmeal in diets of *C. carpio* by crude lentil seeds was safe at the 5% and 10% levels as indicated by various levels of adverse histopathological effects on the kidneys, liver, gills, and intestine.

**Keywords:** fish, replacement, kidney, liver, gill

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التغيرات النسيجية المرضية لاستخدام بذور العدس كبديل جزئي لمسحوق السمك في اسماك الكارب الشائع

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المستخلص

تم إجراء الدراسة الحالية في كلية الطب البيطري، جامعة السليمانية، العراق. على المستوى المايكروسكوبي لم يلاحظ تغييرات نسيجية مرضية في الكلى و الكبد والغلاصم وامعاء اسماك الكارب الشائع في كل من المعاملة الثانية (استبدال مسحوق الاسماك بالعدس بنسبة 5%) والمعاملة الثالثة (استبدال مسحوق الاسماك بالعدس بنسبة 10%) مقارنة باسماك معاملة السيطرة. ولكن ظهرت مستويات مختلفة من تغييرات نسيجية مرضية في كل من المعاملة الرابعة (استبدال مسحوق الاسماك بالعدس بنسبة 15%) و المعاملة الخامسة (استبدال مسحوق الاسماك بالعدس بنسبة 20%). لوحظ في الكلية تخفيفا لفراغات بون التي ارتبطت مع انخفاض الخلايا الميسينكية للخصل الكلوية. لوحظ التحام الوريد المركزي مع بعض ومع الخلايا الالتهابية المترشحة. وفي الغلاصم لوحظ تضخم الصفائح الاولية و الثانوية مع بعض. في الامعاء لوحظ تحلل فجوى للخلايا الطلائية المبطنة مع ظهور للخلايا الدمية الحمر بالتصامن مع الخلايا الالتهابية المترشحة. ونسنتج من هذه النتائج ان استبدال مسحوق السمك بنسب 5% و 10% تكون امنة لاسماك الكارب الشائع كما لوحظ في التغييرات النسيجية للكلى و الكبد و الغلاصم و الامعاء.

الكلمات المفتاحية: أسماك، احلال، الكلى، الكبد، الغلاصم

## INTRODUCTION

By the middle of the twenty-first century, human civilizations will be faced with the huge problem of feeding and sustaining a population of well over 9 billion people while also dealing with the disproportionate consequences of climate change and environmental degradation on the resource base. The 2030 Agenda for Sustainable Development and its 17 Sustainable Development Goals (SDGs) provide a unique, transformational, and integrated strategy to moving the world toward a sustainable and resilient future that leaves no one behind (8). Fishmeal (FM) is a highly digested protein with a high content of amino acids, vitamins, and minerals. Proteins in high grade fishmeal are tasty, highly digestible (90 percent), and anti-nutritional factors (ANFs) are minimal (10). Common carp is very much favored for cultivation in ponds in Iraq, Asia, Near East, mono or polyculture with other fishes, because of its excellent growth and feed efficiency rate, omnivorous habit, hardy culture and easy adaptation to artificial feeds. Consequently, this fish was introduced into many countries throughout the world, including Europe, Australia and North America with very different ecological conditions and variable growth rates, so that probably genetic varieties of the GH gene might be of adaptive importance. Al-Hassani, S.T. and Mustafa, S.A. 2022 The global demand for safe food has provoked the search for natural and alternative growth promoters to use in fish feeds (16). One of the costliest components of aquaculture diets is protein. Animal protein sources, particularly fishmeal, are expensive, restricted in quantity, and of variable quality. Because of its high protein content and balanced essential amino acid (EAA) profile, FM has historically been used as the major protein source in commercial aquatic feeds. Essential fatty acids, digestible energy, minerals, and vitamins are all abundant in FM. FM is the costliest protein source in livestock and aquaculture diets, which comes as no surprise (23). Lentil *Lentilla culinaris* is an ancient crop that originated in the Near East near the Fertile Crescent and is now a significant grain legume in the agriculture of many nations across the world. Its seed is

abundant in proteins, minerals, vitamins, dietary fiber, and natural antioxidants, all of which are beneficial to human nutrition. It also plays an important role in animal nutrition and soil improvement. According to the USDA National Nutrient Database, 100 g of raw lentils (variety unspecified) provide 353 calories; the same weight of cooked lentils provides 116 calories (4, 19). It is evident from the results of Jimoh (14) study that dietary replacement of soybean meal by differently toasted sunflower seedmeal in *Clarias gariepinus* diet can be done up to 30% level using survival, growth, carcass composition, digestibility, haematology and histology of the liver as indices. At higher replacement level of 45% for the differently toasted sunflower meal, the fish physiological functioning was becoming compromised though survival was not affected, there was reduced growth and changes to the blood and histopathological parameters of the liver. To minimize the present reliance on fishmeal and soybean meal as the major protein sources for aquatic animal diets, alternative plant protein sources are required (20). As a result, several researches have been conducted to investigate the effects of substituting fish meal in rainbow trout diets with other sources of protein such as plant proteins or animal by-products (5; 6; 11; 15; 24). As a result, several researches have been conducted to investigate the effects of substituting fish meal in rainbow trout diets with other sources of protein such as plant proteins or animal by-products (12). Red lentil meal is one of the most often used grain basic ingredients. In the human diet, red lentil meal is an essential and affordable source of carbohydrate and protein (9). The study of Gurkan *et al.* (13) showed the effects of thyme, rosemary and fenugreek powders on disease resistance and histopathological changes in intestine and liver tissues of *O. mossambicus* before and after exposure to *Streptococcus iniae*. Histopathological effects of ethanol extract of *Adenium obesum* stem bark was investigated in the gills and skin of African sharp tooth catfish, *Clarias gariepinus* over a 96-h exposure period as an endpoint of toxicity. The extract caused some histopathological lesions in the gills and skin of the exposed fish. However, the severity but

not the type of the lesions observed in the gills and skin of the exposed fish was concentration-dependent. Although the degree of tissue changes (DTC) grading indicated moderate damage in the gills of the exposed fish, there were no significant ( $p > 0.05$ ) differences between gills DTC of the exposed and unexposed fish. However, lesions in the skin did not affect the normal functioning of the tissue but significant ( $p < 0.05$ ) differences were recorded in the DTC between the skin of the exposed and the unexposed fish. The extract was toxic to the exposed fish and therefore, *A. obesum* can be used as a potent organic pesticide for effective fishpond management (1).

### MATERIALS AND METHODS

The experiment was conducted out at the Fish Disease Laboratory of the University of Sulaimani's College of Veterinary Medicine. In this investigation. A total of 165 common carp (*C. carpio* L.) were employed from a commercial fish farm in Peramagrun, Iraq's Sulaimaniya region. The average weight of the fish was 98g. The fish were acclimated in the laboratory for around 15 days before the actual feeding trial, and they were fed commercial pellets for 84 days. The experiment used a completely randomized design consisting of five treatment groups and three replicates with eleven fish per replicate. Treatments were as follows: Control (T1) group: No fishmeal replacement (0%) by lentil seed. T2 group: Replacement of 5% fishmeal by lentil seed. T3 group: Replacement of 10% fishmeal by lentil seed. T4 group: Replacement of 15% fishmeal by lentil seed. T5 group: Replacement of 20% fishmeal by lentil seed. At the end of the feeding period, two fishes were removed from each treatment and anesthetized using clove powder for few minutes and then killed to remove the liver, gill, kidney and intestines for histological analysis. The organs were carefully removed from the body of the fish to avoid damage and preserved in 10% formalin solution. Then, the organs were fixed in Bouin's solutions and then routinely processed for histology including dehydration in graded ethanol and clearing in xylene. Following that, the organs samples were impregnated and embedded paraffin and five  $\mu\text{m}$  thick tissue sections were obtained using a rotary

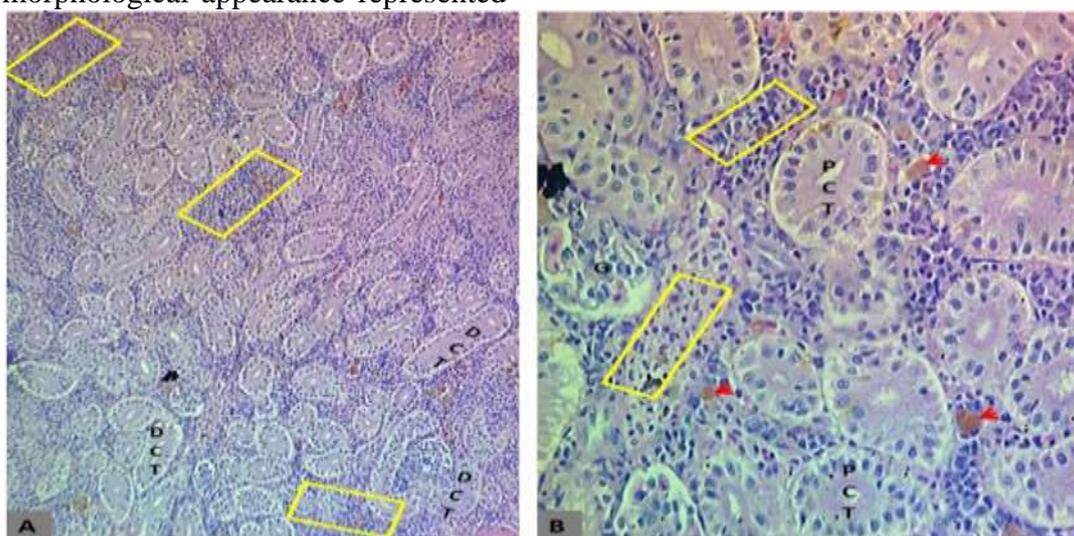
microtome. The sections were stained with Hematoxylin and Eosin and examined under a light microscope (17). Finally, digital imaging of the sections was carried out using a camera mounted on an Olympus BX51 light microscope and analyzed using DP2-BSW software.

### RESULTS AND DISCUSSION

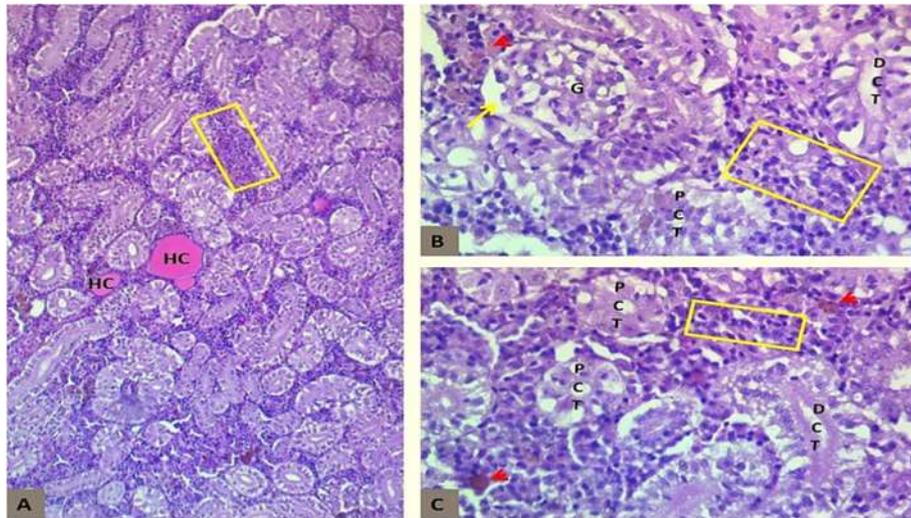
The microscopical examination of the renal parenchyma sections of the T2 and T3 fish groups revealed no adverse histopathological changes in comparison with fish of the control (T1) group. It shows normal histological morphology of the proximal and distal convoluted tubules associated with plentiful melanomacrophage and normal-looking hematopoietic cells among the interstitial connective tissue (Fig. 1). On the other hand, the microscopical sections of the renal parenchyma of the T4 revealed mild dilation of Bowman's space, mild decrease in mesangial cellularity of glomeruli, moderate degeneration of proximal and distal convoluted tubules, hyaline (protein) cast in the renal tubules lumina associated with normal looking hematopoietic tissue and existence of melanomacrophage (Fig. 2). In the T5, the renal parenchyma sections showed marked dilation of the Bowman's space, severe decrease in mesangial cellularity of the glomerular tuft, moderate-marked degeneration of proximal and distal convoluted tubules associated with normal-looking hematopoietic cells (Fig. 3). Compared to fish of the control (T1) group, microscopical sections of the liver parenchyma of the T2 and T3 revealed no adverse histopathological changes. They showed normal morphology represented by rows of polygonal hepatocytes around the central vein, sinusoidal capillaries, kupffer cells and few melanomacrophage (Fig. 4). However, the liver parenchymal sections of the T4, fish showed congestion of central vein, centrilobular infiltration of inflammatory cells and marked vacuolar degeneration of the hepatocytes (Fig. 4). liver parenchyma in T4 exhibit congestion of central vein (CV), centrilobular infiltration of inflammatory cells, marked vacuolar degeneration of hepatocytes and presence of leukocytes within sinusoidal capillaries as shown in (Fig. 5). Sections of the

liver parenchyma of the T5, fish displayed marked congestion of central vein associated with severe vacuolar degeneration of the hepatocytes represented by appearance clear cytoplasmic vacuoles pushing the nucleus into an eccentric location (Fig. 6). The microscopical examination of the gill sections of the T2 and T3 fish group revealed no adverse histopathological changes in comparison with control (T1). They show normal primary lamellae or gill filament which were lined by stratified epithelium and contain pavement and chloride cells, normal-looking secondary lamellae lined by simple squamous epithelium and spooled cells or pillar cells, and normal supportive cartilaginous skeleton part (Fig. 7). In the T4, the gills show hyperplasia of primary and secondary lamellae associated with moderate fusion between them and hypertrophy of chondrocytes in the supportive cartilaginous skeleton (Fig. 8). The gill sections in T5 showed marked, diffuse hyperplasia of primary and secondary lamellae associated with severe fusion between them, hypertrophy of chondrocytes in the supportive cartilaginous skeleton and infiltration of inflammatory cells, mainly lymphocytes and plasma cells (Fig. 9). Microscopical examination of intestinal sections in fish of the T2 and T3 showed no adverse histopathological changes in comparison with the control (T1) fish group. They showed normal morphological appearance represented

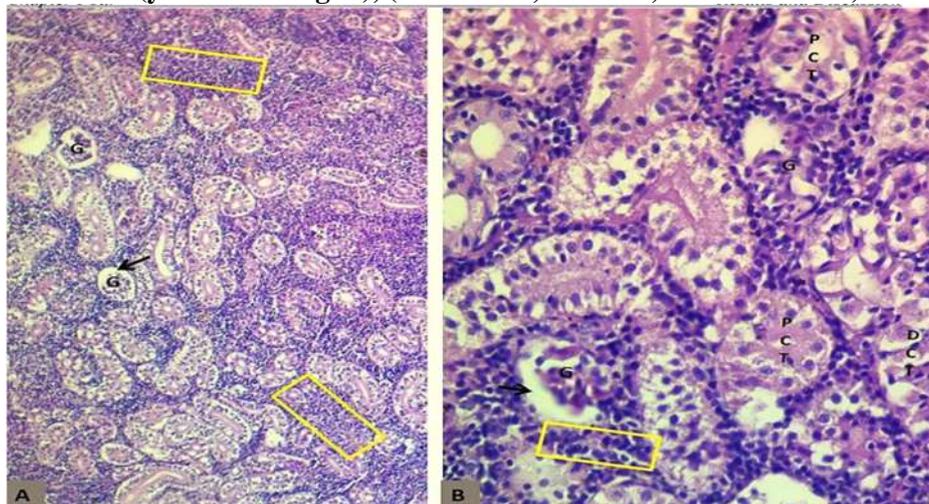
by numerous finger-like villi lined by simple columnar cells or enterocytes and mucous cells or goblet cells supported by a lamina propria with gut-associated lymphoid tissue (GALT), and normal-looking submucosa, muscularis externa, and serosa (Fig. 10, 11). However, in the T4 fish group, the intestinal sections exhibited mild-moderate vacuolation of the enterocytes, extravasation of RBC in the lamina propria, and normal histological arrangement of submucosa, muscularis externa and serosal layers (Fig. 12). In the T5 fish group, the intestinal sections revealed marked vacuolation of the lining epithelial cells, focal extravasation of RBCs associated with marked infiltration of inflammatory cells in the lamina propria, mild-moderate vacuolation of the smooth muscle fibers in the muscular layer and infiltration of inflammatory cells in the serosal layer (Fig. 13). The histopathological findings revealed that the partial replacement of the fishmeal in diets of the common carp fish *C. carpio* L. with 5% and 10% crude lentil seeds was safe and not associated with any adverse histopathological changes in the kidneys, liver, gills and intestine of the fish. On the other hand, the partial replacement of the fishmeal by 15 and 20% crude lentil seeds was not safe and it was associated various levels of adverse histopathological changes in in the kidneys, liver, gills and intestine of the fish.



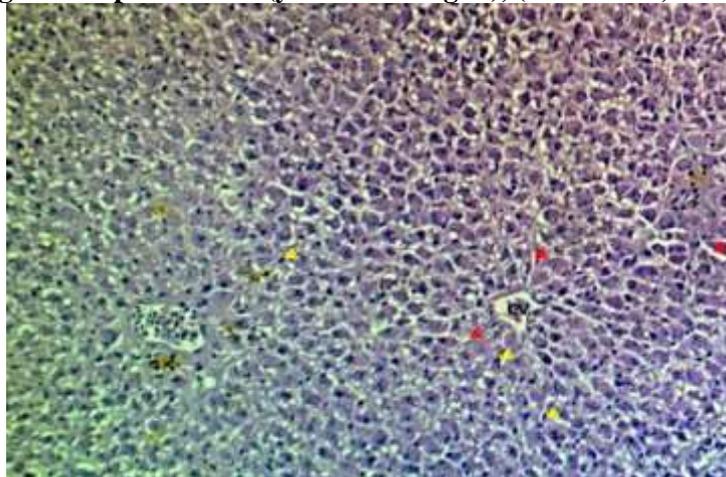
**Figure 1. Microscopical sections of the renal parenchyma in T2. They showed normal morphology represented by normal-looking glomeruli (G), proximal and distal convoluted tubules (PCT and DCT), melanomacrophage (red head arrows) and hematopoietic cells (yellow rectangles), (H&E stain, A:100X, B: 400X).**



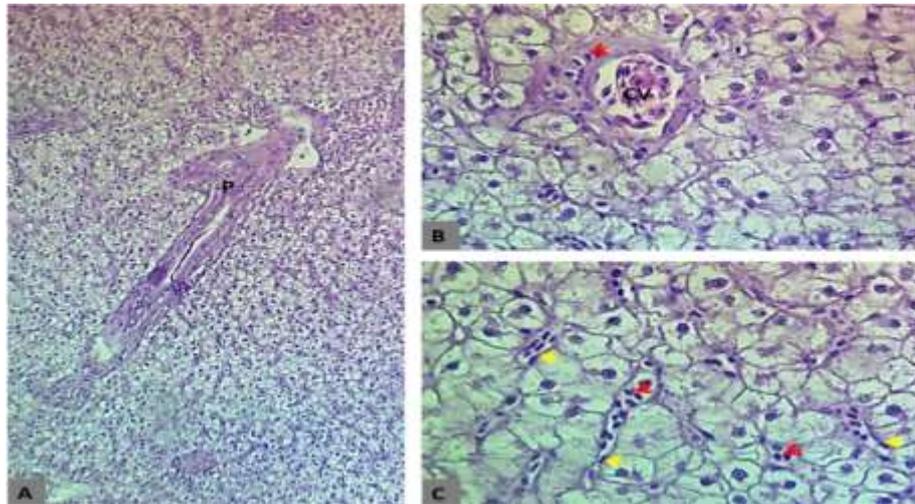
**Figure 2.** Microscopical sections of the renal parenchyma in T4. A-C: Mild dilation of Bowman's space (yellow arrow), mild decrease in the mesangial cellularity of glomeruli (G), moderate degeneration of the proximal and distal convoluted tubules (PCT and DCT), hyaline cast (HC) in some of the convoluted tubules lumina, melanomacrophage (red head arrows) and normal-looking hematopoietic cells (yellow rectangles), (H&E stain, A: 100X, B and C: 400X).



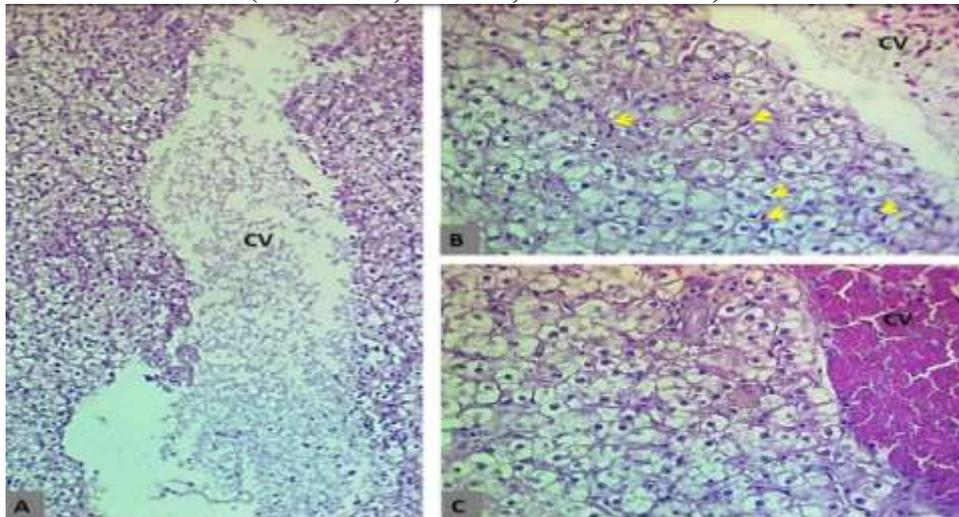
**Figure 3.** Microscopical sections of the renal parenchyma in T5. It reveal marked dilation of the Bowman's space (black arrow), severe decrease in mesangial cellularity of the glomerular tuft (G), moderate-to-marked degeneration of proximal and distal convoluted tubules (PCT and DCT) and normal-looking hematopoietic cells (yellow rectangles), (H&E stain, A: 100X, B: 400X).



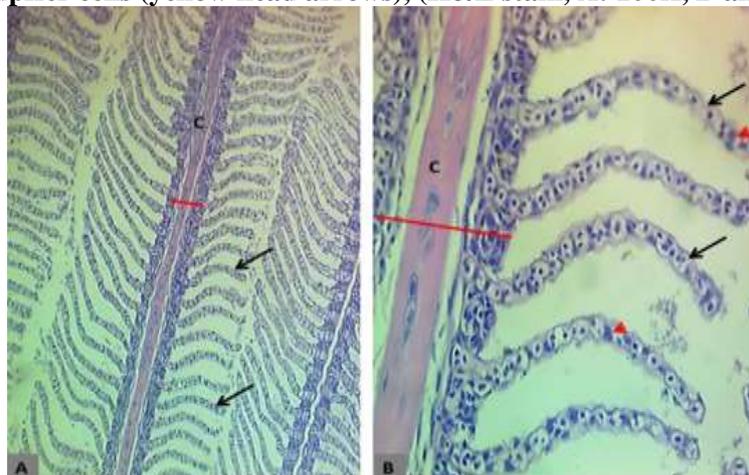
**Figure 4.** Microscopical section of the liver parenchyma in T3. It shows rows of normal looking polygonal hepatocytes surrounding a central vein (CV), sinusoidal capillaries (red head arrows) and kupffer cells (yellow head arrows), (H&E stain, 200X).



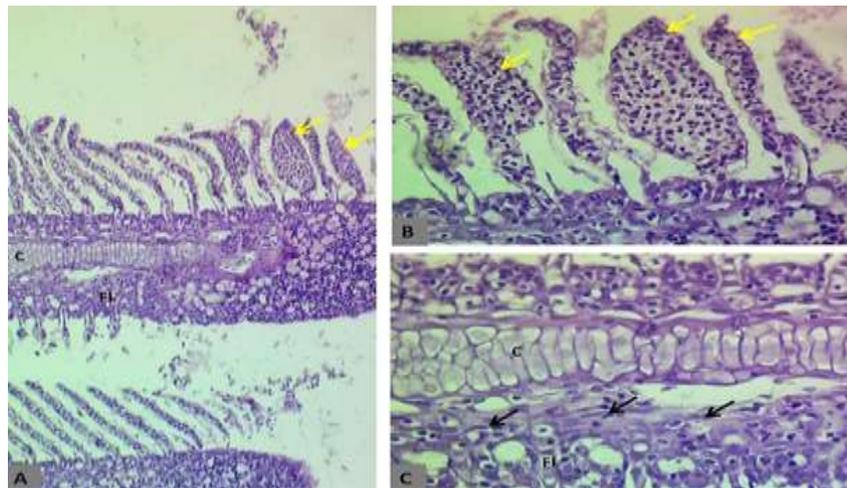
**Figure 5.** Microscopical sections of the liver parenchyma in T4. They exhibit congestion of central vein (CV), centrilobular infiltration of inflammatory cells (red head arrows), marked vacuolar degeneration of hepatocytes and presence of leukocytes within sinusoidal capillaries (red head arrows), (H&E stain, A: 100X, B and C: 400X).



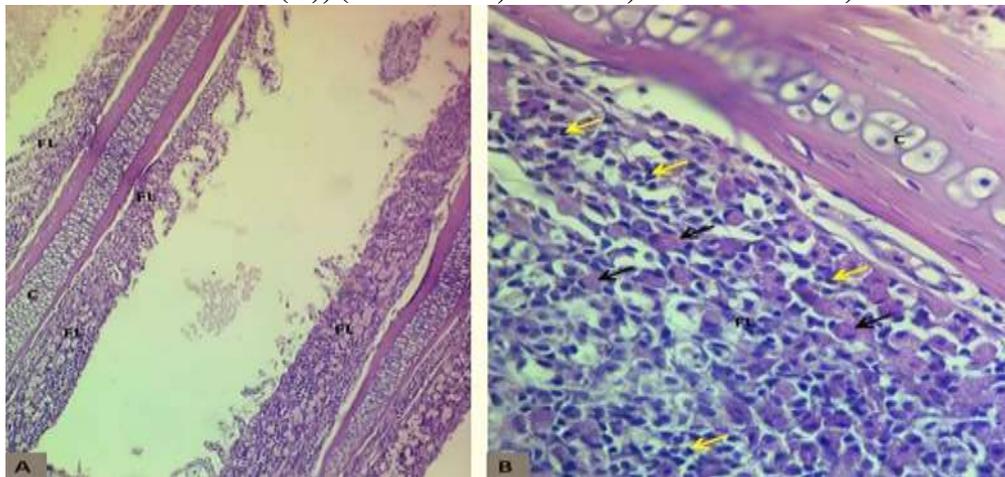
**Figure 6.** Microscopical sections of the liver parenchyma in the T5 showing marked congestion of the central vein (CV) associated with severe vacuolar degeneration of hepatocytes represented by appearance of clear cytoplasmic vacuoles pushing the nucleus into an eccentric location, normal-looking Kupffer cells (yellow head arrows), (H&E stain, A: 100X, B and C: 400X).



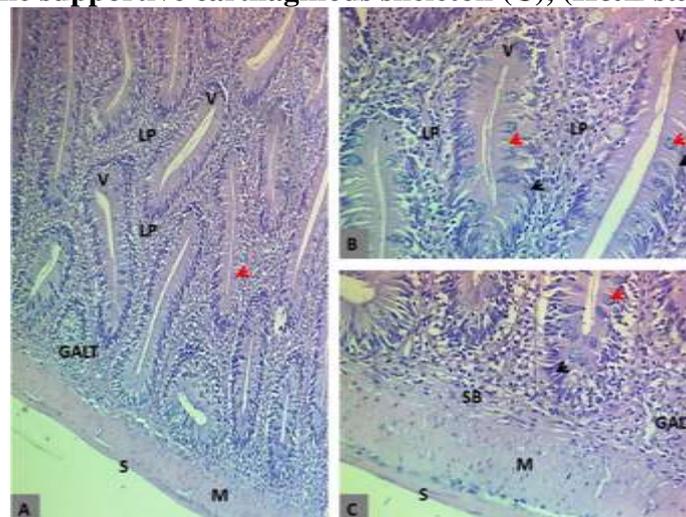
**Figure 7.** Microscopical gill sections of the T2 showing normal-looking primary lamellae or gill filament lined by stratified epithelium and containing pavement and chloride cells (red lines), secondary lamellae lined by simple squamous epithelium (black arrows) and spooled cells or pillar cells (red head arrows), and normal supportive cartilaginous skeleton (C), (H&E stain, A: 100X, B: 400X).



**Figure 8. Microscopical gill sections of the T4. A: Moderate fusion of primary and secondary lamellae (FL). B and C: Marked hyperplasia of primary (black arrows) and secondary lamellae (yellow arrows) and hypertrophy of chondrocytes in the supportive cartilaginous skeleton (C), (H&E stain, A: 100X, B and C: 400X).**



**Figure 9. Microscopical gill sections of the T5 revealing severe fusion of primary and secondary lamellae (FL), diffuse-marked hyperplasia of primary and secondary lamellae, infiltration of lymphocytes (yellow arrows) and plasma cells (black arrows) and hypertrophy of chondrocytes in the supportive cartilaginous skeleton (C), (H&E stain, A:100X, B: 400X)**



**Figure 10. Microscopical sections of the intestine in T2 showing normal finger-like villi (V) lined by simple columnar cells or enterocytes (black head arrows) and mucous cells or goblet cells (red head arrows), supported by a lamina propria (LP) with gut-associated lymphoid tissue (GALT), submucosa (SB), muscularis externa (M), and serosa (S), (H&E stain, A: 100X, B and C: 400X).**

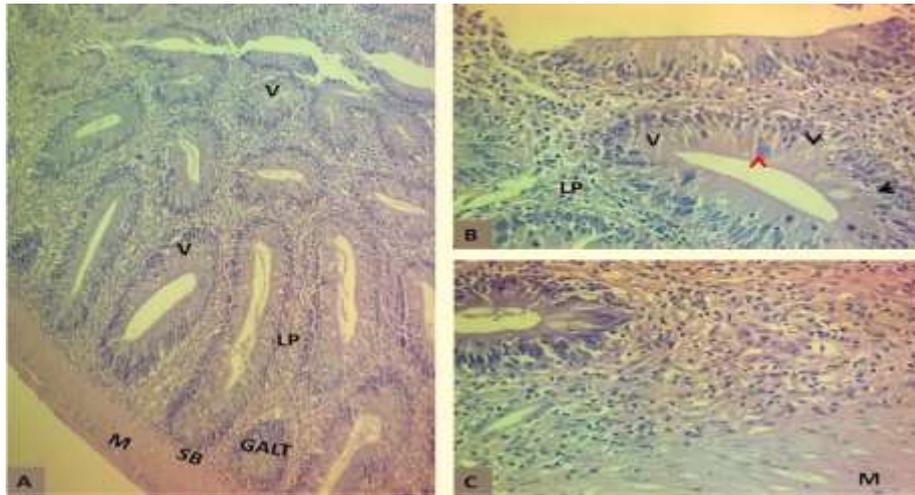


Figure 11. Microscopical sections of the intestine in the T3 exhibiting normal finger-like villi (V) lined by simple columnar cells or enterocytes (black head arrows) and mucous cells or goblet cells (red head arrows), supported by a lamina propria (LP) with lymphoid tissue (GALT), submucosa (SB), muscularis externa (M), and serosa (S), (H&E stain, A: 100X, B and C: 400X).

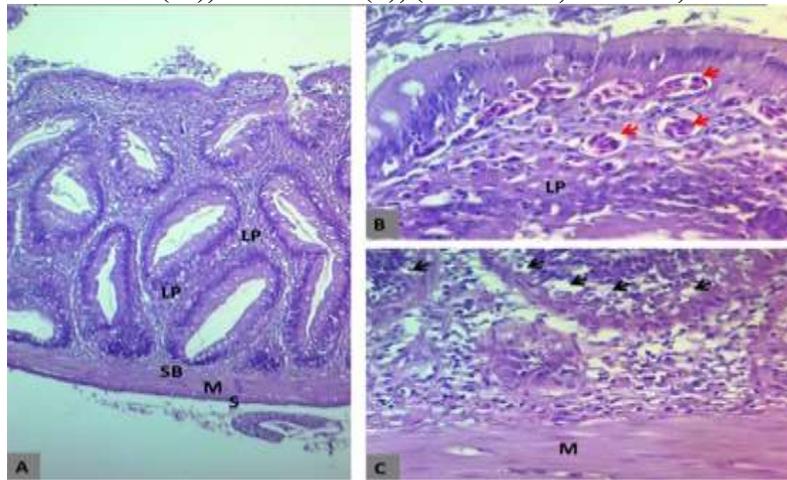


Figure 12. Microscopical sections of intestine in the T4 displaying mild-moderate vacuolation of enterocytes (black head arrows), extravasation of RBC (red head arrows) in the lamina propria (LP), and normal-looking submucosa (SB), muscularis externa (M), and serosa (S) layers, (H&E stain, A: 100X, B and C: 400X).

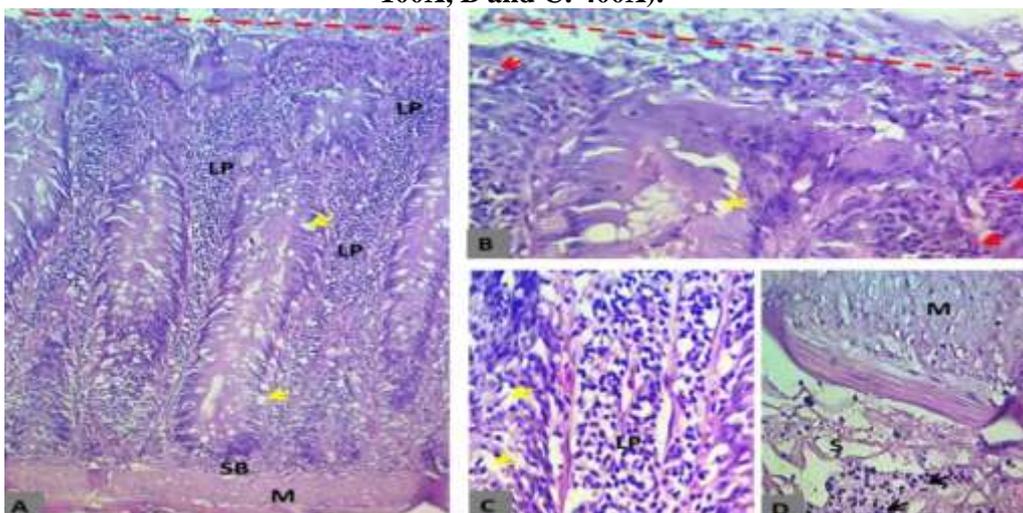


Figure 13. Microscopical sections of the intestine in the T5. A-C: Focal sloughing of the lining epithelium (red dash lines), marked vacuolation of the lining epithelial cells, focal extravasation of RBCs (red head arrows) and marked infiltration of inflammatory cells in the lamina propria. D: Mild-moderate vacuolation of the smooth muscle fibers in the muscular layer (M) and infiltration of inflammatory cells in the serosal layer (S), (H&E stain, A: 100X, B, C and D: 400X).

Results of hepatic histopathology of fishmeal replacement in diets of Nile tilapia *O. niloticus* juveniles showed absence of tumors, lesions, and parenchymal inflammation in all treatments. However, mild cell membrane lysis, mild, and mild to moderate apoptosis were evident in liver samples. Based on the results, KFM can partially and completely replace dietary protein from fishmeal. Moreover, D4 (75% KFM) is considered the optimal KFM replacement level for Nile tilapia juveniles (21). In the study of Alwash et al (3) *C. carpio* from the control group shows the normal structural organization of gills while an infected and treated group shows histopathological alterations namely (epithelial lifting, hyperplasia, fusion of lamellae, hemorrhage, and telangiectasis). The epithelial lifting could possibly be examples of general defense mechanisms since the gap through the bloodstream is increased by separating the epithelium of the lamellae. One of the main histological changes found in fish exposed to stress is cell proliferation, which results in hyperplasia and contributes to lamellar fusion, as seen in the current research and this seen in the gill sections of the present study. The result of the study of Mustafa et al., (18) indicated that the histology could be an effective tool in describing the alterations in the selective tissue of common carp having fungal infection and also the results of the present study in using histopathological study to detect the cellular and histological changes in target organs. In study of Elabda *et al.*, (7) Yellow perch (*Perca flavescens*) was exposed to common forms of physical stressors and anti-oxidative effects of dietary incorporated *Astragalus membranaceus* (AM) and *Glycyrrhiza glabra* (liquorice) were assessed. Immunological, biochemical and histopathological profiles were evaluated; and fish were redistributed to be exposed to heat, cold, hypoxia and capture stressors. The current findings demonstrated that *A. membranaceus* and *G. glabra* dietary incorporation remarkably enhanced antioxidative and biochemical parameters. In addition, the study showed markedly up-regulation of related genes expression; and revealed better liver histology in supplemented groups over the control. The study of Sohrabnezhad *et al.* (22) aimed to evaluate the

effect of soybean meal and multienzyme supplementation on intestinal histopathology of beluga sturgeon (*Huso huso*). From histological analyses, intact villus with normal mucosa was found in the FM group, representing the presence of intact epithelium. Supplementation of enzyme at both levels and soybean meal (alone) in the diet resulted in histopathological alterations in the intestine.

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