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Article

Effect of different levels of Moringa oleifera seed powder to the diet on some immunological and histological parameters of common carp fish Cyprinus carpio L.

Jaber Bander Zghair Al-Rawashi*, Ali Hussain Salman, Ahmed Jawad Al-Yasiri, and Jassim Kassim Al-Gharawi
Animal Production Department, Agriculture College, Al-Muthanna University, Iraq.
*Correspondence: Email: jabersamawa13@gmail.com
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Abstract
75 common carp fish with an average weight of 65.08±0.42 g were used. The fish were fed on experimental diets, which were divided into five equal treatments in terms of protein percentages, different in the proportions of adding Moringa seed powder, was 0, 0.5, 1, 1.5 and 2%, respectively; the fish were fed on the experimental diets at 5% of the live weight, divided into 4 meals a day. The liver histological examinations in T2 and T3-treated fish showed a significant improvement in the histological characteristics of hepatocytes and the contents of the liver tissue parenchyma. At the same time, it was observed that the liver tissue was slightly affected in the fish of the fourth and fifth treatments without affecting the health status of the fish. Histological examinations also showed the improvement and superiority of T2, T3, T4 and T5 treatment fish compared to control treatment fish on histological parameters of small intestine and gill tissue. This indicates that adding Moringa seed powder to fish diets at low rates stimulated the immune system and raised the concentration of IgM and IgG immunoglobulins to improve the fish's tissue health status.

Keywords: Moringa oleifera, immunological, histological, common carp fish Cyprinus carpio L.

Introduction

Despite the role and importance of fish farming as a food source for most of the world's population, it faces many challenges that hinder its sustainability and development. Diseases affecting fish are one of the main factors that limiting aquaculture development and culture[1]. The expansion of world trade and increased demand for fish led to the expansion of fish farming according to intensive systems, facilitated the spread and development of the most deadly pathogens and the spread of infectious diseases, exposed animals to stress and deteriorated the performance of their immune system[2]. The breeders resort to antibiotics and chemical veterinary drugs, substances that have adverse side effects, which weaken the fish's immune system and pollute the aquatic environment. Drugs and vaccines were very expensive[3].

Researchers and specialists motivate the use of nutritional supplements in diets, like medicinal plants, that caught the attention of scientists because they contain potent plant compounds with different biological activities[4,5]. Reverter et al.[6] indicated that medicinal plants could be used as functional supplements and as successful and effective feed alternatives, lead to the improvement of physiological

and biochemical parameters in the blood, enhance immune standards and disease resistance, and increase survival rates, contribute to improving fish farming resources, without leaving any collateral damage to the animal or the aquatic environment, as the use of the Moringa plant, which is the focus of our current study.
The Moringa tree, scientific name Moringa oleifera Lam, is one of the most widely used and cultivated species of the family Moringaceae; this family includes 13 species[7]. It is native to South Asia. It grows on the Himalayan foothills in India, Pakistan, Bangladesh and Afghanistan[8]. Moringa seeds contain bioactive plant compounds, such as phenols, glycosides, alkaloids, steroids, tannins and saponins. However, these substances and compounds are not considered nutrients. In addition to being non-essential to the body, in order to perform its primary functions of maintenance, protects the body from many diseases, shows a large number of medicinal potential and health benefits, they act as antimicrobial, antibacterial, antioxidant, and anti-inflammatory, ridding the body of toxins and stimulating the enzymes of the immune system[9]. Kamble et al.[10] indicated that Moringa seeds possess many properties, which enabled them to have several medical and therapeutic applications in enhancing the health status of fish and improving immunity against pathogenic microbes and as fertility stimulants.
Because of the review on the importance of this plant, its medicinal applications and its many health benefits, there was a need to study the effects of using Moringa seeds in the diets of common carp Cyprinus Carpio L. on some immunological and histological parameters of some internal organs of the fish body.

Materials and Methods

Experiment place and cages

The experiment was conducted at the first agricultural research and experiment station, Umm Al-Akf area, Al-Muthanna Governorate, from 1/10/2120 to 20/12/2021, in dugout ponds, 45 m long, 35 m wide and 1.5 m deep, it was about 570 m away from the Euphrates River, Atshan river. The experiment used fish farming cages consisting of two rectangular pieces of wood, 244 cm long and 122 cm wide. 8 circles were drilled in each piece with a diameter of 45 cm in two parallel rows. The circular holes in the wooden cages were filled by installing 15 clip-on plastic cylindrical troughs with a diameter of 45 cm and a depth of 65 cm.

Experiment fish

Seventy-five Common carp Cyprinus carpio L., with an average weight of 65.08±0.42 g, were used, distributed randomly and evenly to the experimental cages (5 fish in each tank).

Feed manufactured

Moringa seeds were bought from local markets. After drying well by the sun, it was ground using a home grinding machine. Then, it was added to other experimental diet components distributed to treatments at rates 0, 0.5, 1, 1.5 and 2%, respectively (Table 1).

Items	T1 Control	T2 0.5% Moringa seeds	T3 1.0% Moringa seeds	T4 1.5% Moringa seeds	T5 2.0% Moringa seeds
Concentrated protein	20.00	20.00	20.00	20.00	20.00
Soybean	35.00	35.00	35.00	35.00	35.00

Wheat bran		15.00	15.00	15.00	15.00	15.00
Maize		15.00	15.50	15.00	15.00	15.00
Barley		10.00	10.00	10.00	10.00	10.00
Flour		3.00	3.00	3.00	3.00	3.00
Oil		1.00	1.00	1.00	1.00	1.00
Premix		1.00	1.00	1.00	1.00	1.00
Moringa seeds powder*		0	0.50	1.00	1.50	2.00
Total		100.00	100.00	100.00	100.00	100.00
Chemical analysis						
Humidity (%)	**Protein (%)**	**Ether extract (%)**	**Ash (%)**	**Fiber (%)**	**NFE (%)**	**Energy (kcal)**
7.94	28.00	5.66	8.53	4.69	45.18	390.73

* Moringa seed powder was added to the rations in 5, 10, 15 and 20 g per 1 kg of the ration, after raising an amount of the mixture equal to the amount added and according to each treatment.

Table 1. The composition of the diets in the experiment.

Blood tests

At the end of the experiment, blood was drawn from a group of experimental fish via the caudal vein using a 3ml plastic syringe. The amount of blood withdrawn ranged between 1-2 ml. The blood samples were placed in special tubes. IgG and IgM immunofluorescence assays were performed using the German Genrui PA54 device.

Preparation and examination of tissue sections

After the end of the experiment, two samples of fish for each treatment were randomly drawn for autopsy. Samples were taken from each organ (liver, small intestine, gills) with a length of 2 cm and placed in small plastic containers containing formalin at a concentration of 10% to preserve samples until the histological technique was performed, which went through stages to manufacture glass slides carrying the tissue samples to be studied according to the method[11,12]. The prepared tissue sections were examined and photographed using a compound microscope, Leica KARL KOLB, of German origin, an eyepiece with a magnification of 400 X, equipped with a digital camera connected directly to a computer screen, to take a digital picture, save it, and then read it.

Statistical analysis

Randomized Complete Design (CRD) was used to study treatments' effect on the traits studied. Significant differences between means were tested using Duncan's[13] multiple range test, at a significance level 0.05. The ready-made statistical program SPSS[14] was used to analyze the data.

Results

Table 2 shows a significant difference ($P \leq 0.05$) in the concentration of IgM in the blood serum for each of the treated fish T2, T3 and T4, with values of 1.70 mg/100 ml compared with T1 and T5. In contrast, T3-treated fish outperformed the IgG concentration in the other treatments' blood, with a value of 0.070 mg/100 ml. A recorded increase may be considered as an indication of a significant effect of Moringa seeds to enhance the innate immunity and the specific acquired adaptive immunity of fish during its effect on enhancing the secretion of immune globulins, raising the rate of phagocytosis and increasing lymphocyte proliferation[15], contains

a rich mixture of active and biologically active compounds, such as phenolic compounds, tannins and alkaloids, in addition to its properties as antioxidants and resistance to viruses and pathogenic bacteria[16,17].

Treatments	Immunity tests	
	IgM (mg/ 100 ml)	IgG (mg/ 100 ml)
T1	0.10±1.20 b	0.02±0.030 ab
T2	0.10±1.70 a	0.005±0.015 b
T3	0.00±1.70 a	0.010±0.070 a
T4	0.10±1.70 a	0.00±0.010 b
T5	0.10±1.30 b	0.015±0.045 ab
Sig.	*	*

*Different letters indicate the presence of significant differences within the same column at the level of significance (P<0.05).
Table 2. Immunological Tests (Mean ± Standard Error).

Histological characteristics of the liver, small intestine and gills

The results of the histological examination of the liver showed the superiority of T2 and T3-treated fish over the control-treated fish during the proliferation of-hepatocytes in the parenchyma of the liver tissue, diffusion of pancreatic tissue, vessels, sinusoids, and areas of connective tissue surrounding the parenchyma of the liver tissue, an increase in the density of hepatocytes and an increase in fat-droplets were also observed., cytodegeneration of intrinsic hepato-pancreatic tissue, which indicates the activity of liver tissue due to the average concentrations of Moringa seeds, which stimulates the liver tissue to increase activity, excrete fatty substances through the bile ducts, and store carbohydrates optimally Figure 1, 2.

The liver tissue is also one of the tissues with a high blood supply, represented by the abundance of blood sinusoids and their spread within the liver cells and affected by any high concentration, it may be positive for the body but it shows some slight effects on the tissue, represented by expansion of the blood sinusoids and congestion of blood vessels and slight cellular degeneration in the liver tissue, in addition, there was a slight enlargement of the cells and a state of disorder and loss of hepatocyte membranes with shrinkage and necrosis of some liver cells, with the occurrence of hypertrophy of the exocrine pancreatic cells with a slight infiltration of inflammatory cells without affecting the health status of the fish Fig. 3. These results are in agreement with [20] who indicated that negative histological changes in the liver of tilapia fish, caused by the effect of the moringa seeds added to the diets, it also agreed with the results of the study conducted by [21], which indicated that the use of Moringa leaves in the diets of Pangasius bocourti fish led to liver deterioration by causing bleeding in the liver tissue.

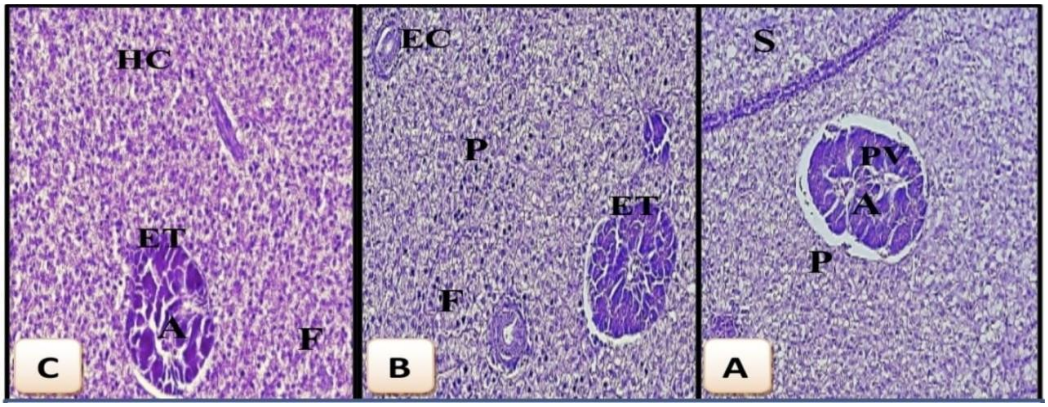

Figure 1. A cross-section of liver tissue in T1 control group fish. (PV) shows hepatic portal vein, (P) parenchyma of liver tissue, (S) Sinusoids, (HC) hepatocytes, (ET) internal pancreatic tissue, (A) exocrine vegetation of pancreatic tissue, (F) lipid droplets, (B) blood vessels, (EC) epithelial cells (H&E), (A) 100X, (B) 100X, (C) 100X.

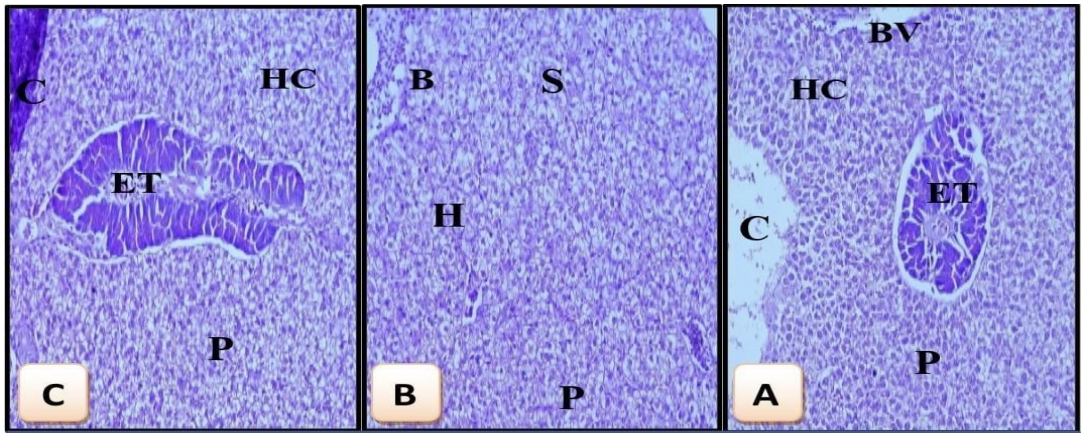

Figure 2. Cross-section of liver tissue in T2 and T3 fish. (C) shows hepatocyte capsule, (P) parenchyma of liver tissue, (S) Sinusoids, ((HC) hepatocytes, (ET) internal pancreatic tissue, (BV) engorged blood vessels, (H) hepatocyte hyperdensity (H) H&E), (B) 100 X (A) 100 X 100 X (C) 100 X.

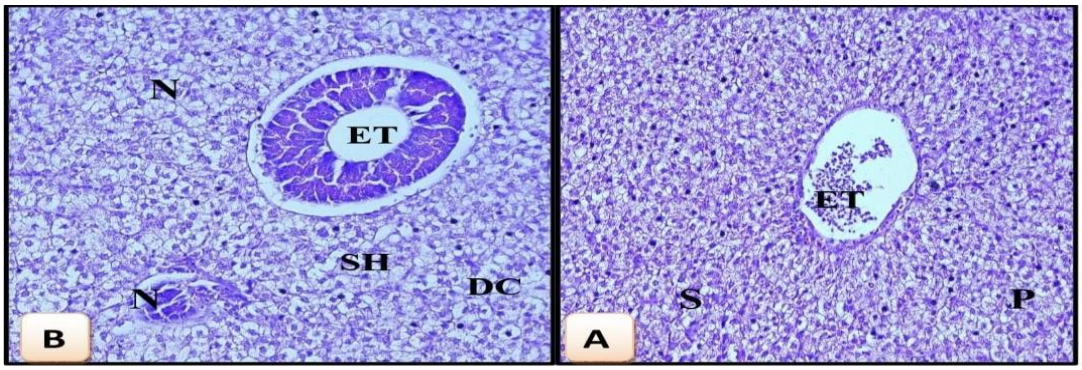

Figure 3. Cross-section of liver tissue in 4T and T5 fish. (P) shows hepatocyte parenchyma, (S) Sinusoids dilatation, (SH) Shrinkage of Hepatic cells, (ET) cellular degeneration of hepato-pancreatic internal tissue, (DC) loss of hepatocyte membranes, (N) hepatocyte necrosis and in Hepato-Pancreatic Necrosis (H&E), (A) 100X, (B) 400X.

The histological results of the small intestine showed that the experimental treatments T2, T3, T4 and T5 were superior to the control treatment by increasing the number of micro villi scattered over the surface of the intestinal epithelium that become pseudo-applied to provide a larger surface area for absorption, increased

acid granulocytes and an increase in the thickness of the longitudinal smooth muscle layer as clear and positive indicators of an increase in the tissue activity of the intestine, its motility and its histological structure, due to the effect of the active substances in the composition of Moringa seeds, it stimulated the secretory cells and the substances involved in the tissue composition of the intestine. It increased their activity and effectiveness (Figures 4 and 5).

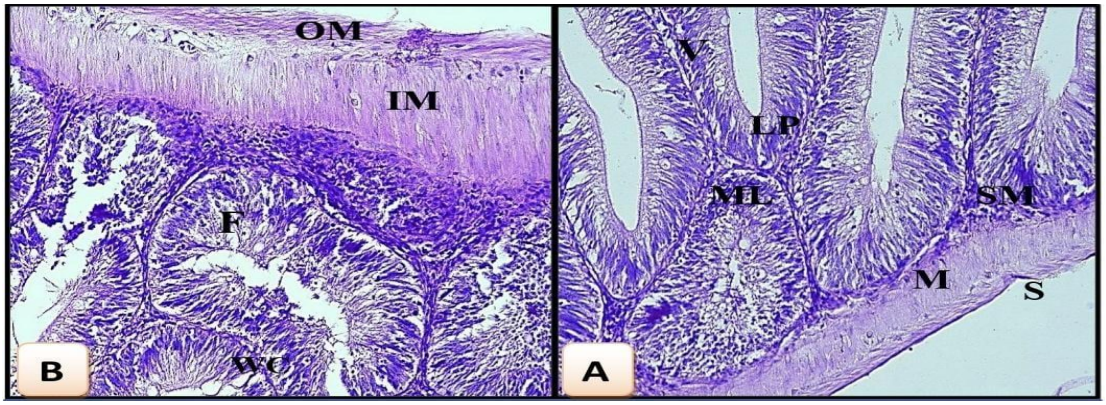

Figure 4. Longitudinal section of small intestine tissue in control fish T1. (M) Intestinal muscular layer, (S) Intestinal serous layer, (SM) Intestinal submucosal layer, (ML) Intestinal mucosa, (IM) Inner circular muscle of the muscularis layer, (OM) outer longitudinal muscle of the muscularis layer (V) villi, (WC) roving eosinophils, (F) mucosal folds, (LP) submucosal fraction (H&E), (A) 100X, (B)) 400X.

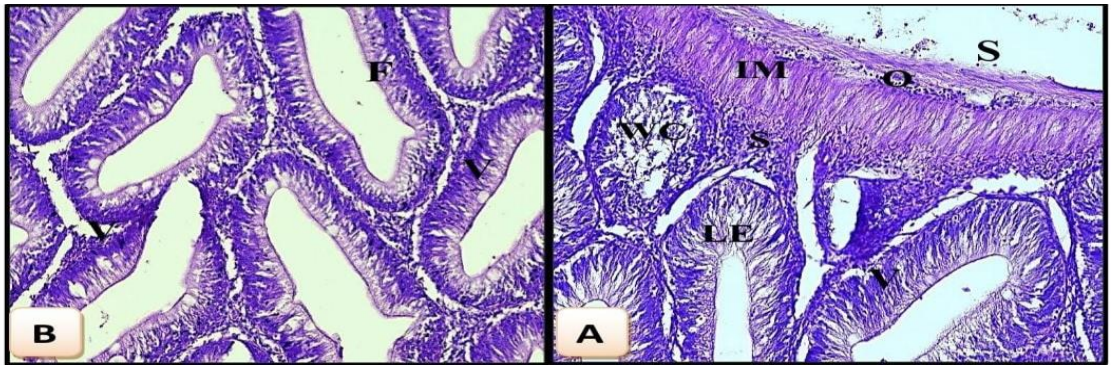

Figure 5. Longitudinal section of small intestine tissue in T2, T3, T4 and T5 fish. (M) shows the thickness of the muscular layer of the intestine, (S) the serous layer of the intestine, (SM) the submucosal layer of the intestine, (IM) the thickness of the inner circular muscle layer, (OM) the outer longitudinal muscle of the muscle layer, (V) an increase in Villus count, (WC) increased number of circulating eosinophils, (F) increased mucosal folds, (LE) fraction of pseudo-applied epithelium (H&E), (A) 100X, (B) 400X.

The results of histological sections of the gills of experimental T2, T3, T4 and T5 fish showed an increase in the number of paired rows for pairs of elementary plates, as well as an increase in the number of secondary platelets, an increase in the thickness of the epidermal tissue covering the gill arch, increased mucous cells and lymphoid tissue surrounding the skin. These cells play a prominent role in resisting pathogens that may penetrate the body through the gills and protect the fish from toxic substances[23]. An increase in the number of chloride salt-secreting cells was also observed. In addition to the increase in the number of overlapping squamous epithelial cell layers and the number of piler cells with a defensive function, the number of microvilli and goblet cells was also observed on the epithelial surface of the primary and secondary plates. All of these work to increase contractility and

motor activity and increase the vital capacity of these plates, to increase the capacity for gas exchange and increase the protection of platelets and the synthesis of the gill epithelium from diseases, which may be attributed to the effect of the active substances involved in the composition of Moringa seeds, stimulate and activate the cells that make up the structure of the gills (Fig. 6 and 7).

Discussion

The increase in the blood serum may be considered as an indication of a significant effect of Moringa seeds to enhance the innate immunity and the specific acquired adaptive immunity of fish during its effect on enhancing the secretion of immune globulins, raising the rate of phagocytosis and increasing lymphocyte proliferation [15], contains a rich mixture of active and biologically active compounds, such as phenolic compounds, tannins and alkaloids, in addition to its properties as antioxidants and resistance to viruses and pathogenic bacteria [16]; [17]. The results were similar to those found by [15 ‘ 18] in the two studies they conducted on sea bream. They concluded that including Moringa leaf powder at a level of 5% in the experimental diets enhances humoral and mucosal immunity by stimulating immune activity and raising the levels of lysozyme and IgM in the blood. T4 and T5 fish treatments were fed on diets with higher concentrations of Moringa seeds; histological sections showed that the liver tissue was affected. This effect may be due to the active and active substances in high concentrations in these seeds and the accumulation of lipids and glycogen in hepatocytes [19]. These results are in agreement with [21 ، 22], who showed in his study that the use of Moringa seeds in the diets of Pangasius bocourti basa fish resulted in a significant improvement in the tissue composition of the intestine due to the seeds of the Moringa plant. The results of our current study also agreed with the findings of [20 ‘ 16] when studying some histological changes resulting from the use of aqueous extract of Moringa seeds in the diets of Nile tilapia fish, which indicated an increase in the number of villi and glands in the intestinal tissue, while it was found that there are signs of disease resulting from the toxic effect of Moringa seeds on the liver tissue of fish. Moreover, these results agree with [24] and [25], indicating an improvement in the histological composition of the gills of Nile tilapia fed on rations containing aqueous extract of Moringa seeds.

Conclusion

The results obtained from this study showed that using Moringa seeds in the diets of common carp fish at low rates has a positive and moral effect on improving the acquired immunity in fish by increasing the concentration of IgM and IgG immunoglobulins. It also positively improved the histological characteristics and parameters of the experimental fish's liver, small intestine and gills.

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