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Histopathological Alteration of the Internal Organs in the Fingerlings Carp Fed with Variable Proportion of Blue Algae

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ABSTRACT

Microphytes are considered as eco-friendly and alternative for antibiotic, making them a highly important, valuable substitute and recommended in aquaculture. *Spirulina* spp. is one of microphytes that is introduced recently to the fish diet as a source of protein. Therefore, the study aimed to evaluate the histopathological effects of spirulina added to fish diet at variable levels (0, 1.45, 2.9, 3.54 and 6.1%) over a 56- day feeding trail. The samples of liver, intestine and kidney were collected for histological technique after the experimental period. Microscopic examination revealed morphological alteration in the apex of the villi in intestine with sloughing in fish diet with spirulina at 1.45%. Other histopathological changes varied in severity according to the supplemented spirulina percentage. These include cellular infiltration, cell growth disturbances, villi adhesion with narrowing villi crypt and necrosis. In the liver, lesions were severe in the fish treated with 6.1% of spirulina which is characterized by circulatory disturbances as thrombus formation in the central vein and dilatation of sinusoid with infiltration of inflammatory cell, fibrosis, and vacuolar degeneration. The end product metabolism of high protein component in spirulina lead to kidney architecture disturbances in all fish treatment. It is histologically characterized by dilatation in the Bowman's space with edema and hyperaplasia of the epithelial cells lining renal tubule, necrosis in some tubule with edema and interstitial nephritis. Although microalgae as spirulina is beneficial and widely used in fish aquaculture, however this study highlight potential adverse effects and tissue disorder, particularly at high percentages. Future study is recommended to study the histopathilogical effects of spirulina to better understand optimal dosage in fish diets.

INTRODUCTION

The traditional sources of fodder used for fish production are basically made up of the animal protein concentrate (fish trituration and the animal protein concentrate) and plant concentrated proteins, especially soybean meal. In addition, non-traditional proteins

concentrates such as cotton seeds or cake **(Mohammad & Salaei, 2008)**, sunflower and turnip meals **(Mohammad *et al.*, 2015)** are also used. Furtherore, some forulations include probiotics or antioxidants from medical herbs to enhance the fish growth and health **(Mohammad, 2017)**.

Soybean crop *Glycine max* L. is considered one of the summer oil crops that belongs to the Leguminasae family. It is widely used in the animal and fish production sector. The soybean meal is characterized by a high content of raw protein that might reach up to 45%, and as much as 70% in soybean protein concentrate **(Hardy, 2000)**. This makes soybean a cost-effective and nutritionally benfenficial constituent in aquafeeds. Its use in fish farming supports optimal growth performance, reduces production costs, increases profitability, and helps in decreasing environmental waste which is typically associated with intensive aquaculture production.

New trend emerged recently in terms of making use of the natural sources of feed that exist naturally in the water ecosystems including the blue – green algae (*Arthospira platensis*). It is regarded as a biological product since it is a protein product with high value, containing specific compounds that play an important role in enhancing the animal body immunity **(Al-Koye, 2013)**. *Spirulina* is a filamentous, spiral-shaped blue-green alga, uniquely classified as an autotrophic, prokaryotic organism. It is similar to bacteria rather than being of true plant origin. It is contains high content of B-complex vitamins (particularly B1 and B2) and provitamin A (β-carotene) **(Tadros, 1988)**.

The chemical structure of the spirulina mainly consists of 55-70% of raw protein, 15-25% of carbohydrates, 18% of basic fatty acids and minerals and pigments like the carotenes and the chlorophyll **(Kapoor & Mehta, 1993; Ali & Salih, 2012)**. They also include all the essential amino acids and these nutrition properties play an important role in imroving the growth of fish **(El-Motty *et al.*, 2010)**. The properties of this biological product make it qualified to be a competitor of the traditional feeds. Moreover, it is commercially produced in several countries such as Bangladesh, India and the United States of America **(FAO, 2008)**. This study was conducted to evaluate the histological effects of *Spirulina* spp. used as a fish partial soybean replacement in practical diets for common carp, *Cyprinus carpio* L.

MATERIALS AND METHODS

Fish

Cyprinus carpio L. fingerlings, with an average weight 50 ± 5g, were transmitted from a fish Hatchery in Erbil Province to the fish laboratory in the College of Agriculture and Forestry/University of Mosul. They were kept in a glass aquarium for at least 10 days for acclimation and fed a commercial pellet diet (Table 1). Water quality parameters were maintained at pH 7.7-7.8, temperature 24- 26°C and oxygen 5.5- 6.0mg/ l.

Table 1. Ingredients components of *C. carpio* diet

Ingredients	Percentage
Animal protein	10
Soybean	30
Local barley	20
Yellow corn	18.5
Wheat bran	19
Food salt	1
Vitamin & Mineral Mix	0.5
Lime stone	0.5
binder (Bentonite)	0.5

Diet regime

Fifty fish were divided into five groups according to basel diet ingredient with spirulina and soybean

Group (1): Fish fed with commercial diet as a control group.

Group (2): Fish fed with commercial diet supplemented with 27.5% of soybean meal and 1.45% spirulina from the overall diet.

Group (3): Fish fed with commercial diet supplemented with 25% of soybean meal and 2.9% spirulina from the overall diet.

Group (4): Fish fed with commercial diet supplemented with 22.5% of soybean meal and 3.54% spirulina from the overall diet.

Group (5): Fish fed with commercial diet supplemented with 20% of soybean meal and 6.1% spirulina from the overall.

The experimental fish were fed at a twice daily rate of 3% body weight throughout the experiment, which lasted for at least eight week. The crude protein and metabolic energy in the basal diet was 27% and of 13.48 MJ/kg respectively. Tables (2 and 3) show the chemical composition and component of the experimental diets.

Table 2. Dietary ingredients composition of the experimental diets containing different percentages of *Spirulina Arthospira platensis*

Diet / Ingredients	Control (1)	Spirulina (2) 1.45%	Spirulina (3) 2.9%	Spirulina (4) 3.54%	Spirulina (5) 6.1%
Soybean meal	30	27. 5	25	22.5	20
Spirulina	----	1.45	2.9	3.54	6.1
Yellow corn	18.5	19	19.5	19	20.2
Animal protein	10	10	10	10	10
Food salt	1	1	1	1	1
Local barley	20	19.45	21	22.46	21
Vita. & Miner. Mix.	0.5	0.5	0.5	0.5	0.5
binder (Bentonite**)**	0.5	0.5	0.5	0.5	0.5
Wheat bran	19	20.1	19.1	20	20
Lime stone	0.5	0.5	0.5	0.5	0.5

Table 3. The chemical composition (%DM) of experimental diets

Diet / Nutrients	Control (1)	Spirulina (2) 1.45%	Spirulina (3) 2.9%	Spirulina (4) 3.54%	Spirulina (5) 6.1%	Spirulina (determine)
Crude protein	29.0	28.3	28.75	28.79	28.99	63.0
Ether extract	2.90	2.95	2.90	2.99	2.90	3.34
Dry matter	89.25	89.26	89.26	89.24	89.24	95.9
Ash	6.97	6.85	6.97	6.89	6.89	11.67
Nitrogen free extract	55.18	55.95	55.70	55.70	55.34	11.99
Crude fiber	5.95	5.95	5.68	5.63	5.88	10.0
ME (MJ/Kg)	13.43	13.46	13.51	13.51	13.53	14.31

At the end of experimental period (56 days), the fish were exposed to general anesthesia using MS-222 **(AL-Taee *et al*., 2021)**. Samples from liver, intestine and kidney were dissected using the standard technique of histopathological examination **(Bancrofti *et al*., 2013)**. The histopathological alteration in all examined organs were classified according to the criteria presnted in Table (4) **(Al-Taee & Al-Hamdani, 2022)**.

Table 4. Categories of pathological lesions with descriptions

Pathological lesions categories	Definition and description	Lesions
Inflammation phase	Changes in the permeability of blood vessels and blood flow	Dilatation of sinusoid Edema Hemorrhage Congestion and thrombus formation
	Cellularity	Infiltration of inflammatory cells and proliferation of fibroblast
Cell Injury	Regressive alteration	Nucleus condensation and pyknosis Vacuolar degeneration Necrosis
Cell growth adaptation	Progressive alteration	Increase cells size(hypertrophy) Increase cells number(hyperplasia) Increase nucleus mitosis and division with uncontrolled cell growth (hyperplastic)

RESULTS

Plant-based diets can have varying impacts on fish gut and liver architecture, and in kidneys of fish, depending on the species, dietary component and additional levels. There were no morphological alterations in fish liver among the experimental group (1.45% spirulina) in contrast to control group, as shown in Fig. (1-A). Additionally, there were dilatation of sinusoid in the liver of fish treated with spirulina at levels of 2.9% for 8 weeks (Fig. 1-B). Further histopathalogical changes were noted in fish treated with spirulina at levels of 3.54%, inclding vacuolar degeneration, hepatic cell hypertrophy and pyknotic nucleous of hepatic cells (Fig. 1-C). The present study revealed that there were significant histological alteration in the liver of fish supplemented with high level of spirulina (6.1%), with lesions characterized by atrophy of pancreas, and circulatory disturbances represented by hemorrhage and edema (Fig. 1-D). Additional findings include congestion, inflammation exudate and proliferation of fibroblast (Fig. 2-E and F), in addition to thrombus formation and cuffing of inflammatory cells around the central vein with edema (Fig. 2-G) .

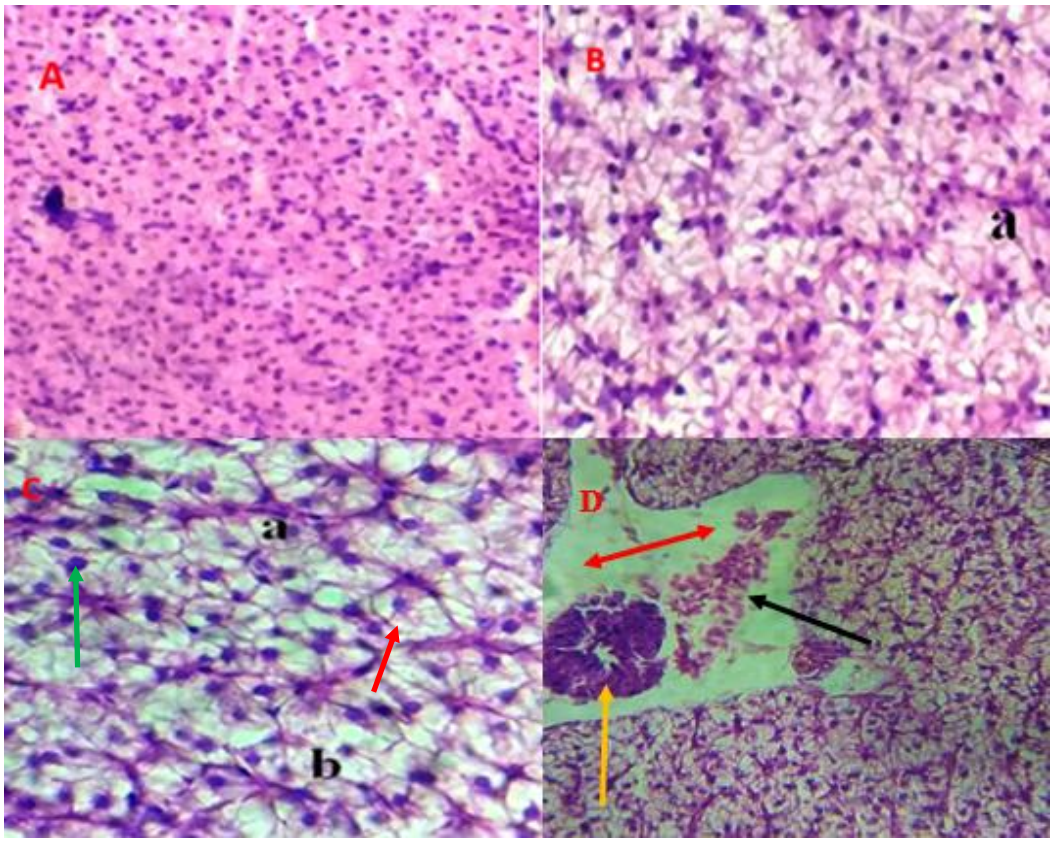

Fig. 1. Microscopical examination of liver in *C. carpio* fed with spirulina at variable levels for 8 weeks: (A) fish have been fed at 1.45% revealed normal architecture 10*4, (B) fish have been fed at 2.9% revealed dilatation of sinusoids(a) 10*4, (C) fish have been fed at 3.54% addition to dilatation of sinusoids (a) there was vacuolar degeneration (b) hepatic cell hyper trophy (red row) and pyknosis (green row) 10*1.4, (D) with atrophy of pancreas (yellow row), hemorrhage (black row) and edema (two head red row) 10*1.3, H&E

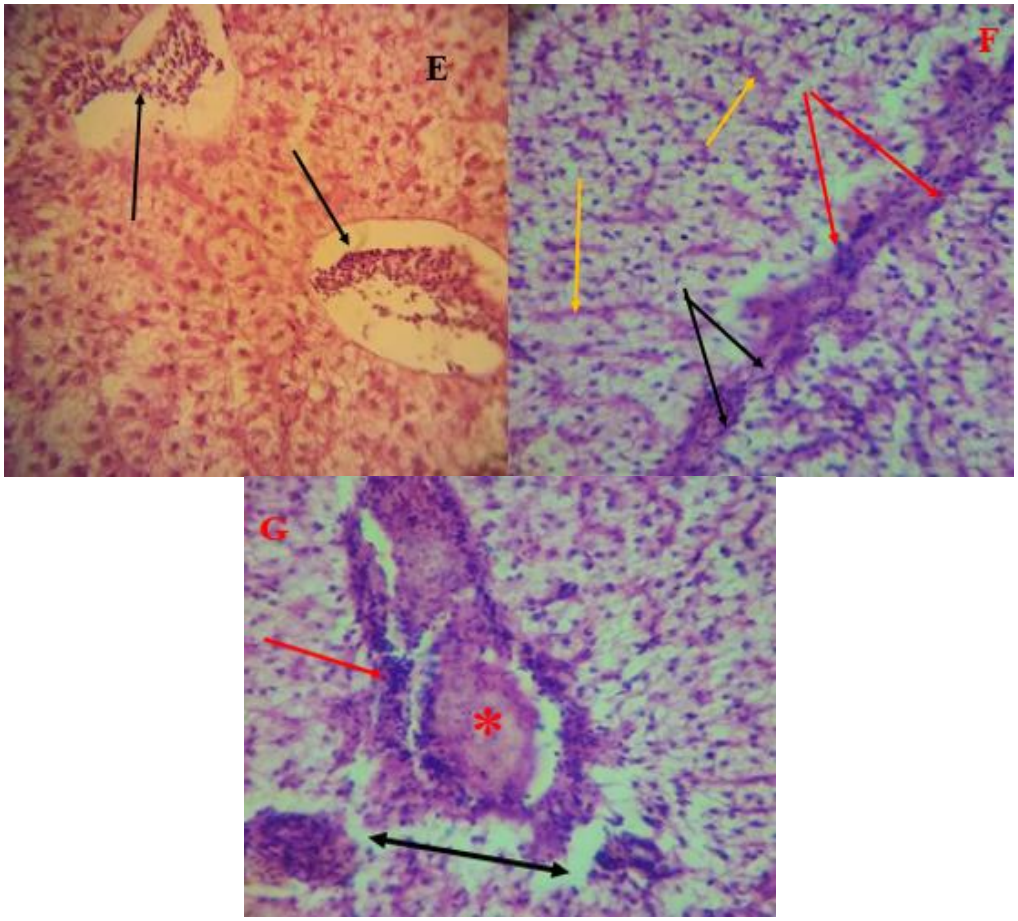

Fig. 2. Microscopical examination of liver in *C. carpio* fed with spirulina at 6.1% for 8 weeks showing: (E) congestion and inflammation exudate (black row) 40*1, (F) proliferation of fibroblast (black row), infiltration of inflammatory cells (red row) and dilatation of sinusoids (yellow row) 10*1.3, (G) thrombus formation (red star), infiltration of inflammatory cells around central vein (red row) with edema (two head black row) ,10*1.4, H&E

Microscopical examination showed significant histopathological alteration in the architecture of the intestine and varying severity according to the percentage of spirulina added to the fish diet. There was only morphological alteration in the apex of the villi in intestine with sloughing of fish diet with spirulina at 1.45% (Fig. 3-A). On the other hand, there were severe lesion in the intestine of fish feed with spirulina at levels of 2.9% represented by thickening of the muscular layer with hyperplasia in enterocyte with infiltration of inflammatory cells (Fig. 3-B). In addition to these lesions, there were narrowing of the villi crypt with abnormal folded in the villi of intestine of fish feed with spirulina at levels of 3.54% (Fig. 3-C). Moreover, there was mucoid degeneration with sloughing in the lumen (Fig. 3-D). In fish fed a diet comprising 6.1% *Spirulina*, further intestinal alterations were noted, including necrotic villi, epithelial sloughing, villi

adhesion, and necrosis in the muscular layer (Zenker's necrosis). Hyperplasia of enterocytes was also evident in these fish (Fig. 3E).

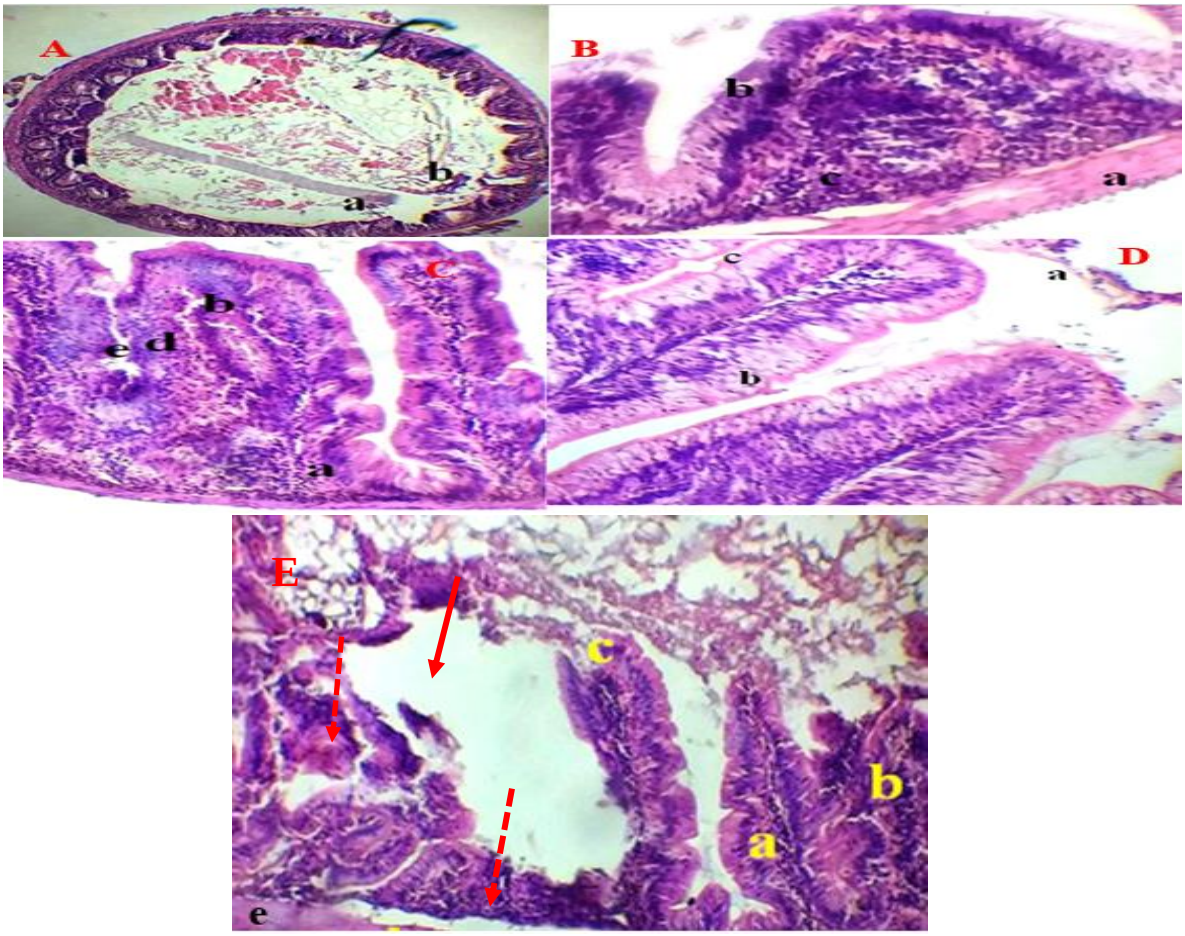

Fig. 3. Microscopical examination of intestine in *C. carpio* fed with spirulina at variable levels for 8 weeks: (A) fish have been fed at 1.45% revealed morphological alteration in the apex of the villi in intestine (a) with sloughing (b) 4*1 ,(B) fish have been fed at 2.9% revealed thickening of the muscular layer (a) with hyperplasia in enterocyte (b) and infiltration of inflammatory cells (c) 10*3.5, (C) fish have been fed at 3.54% exhibit infiltration of inflammatory cells (a), hyperplasia in enterocyte (b) with abnormal folded of villi (d) and narrowing villi crypt(e) 10* 2.4,(D) in fish feed with spirulina at 6.1% the lesions were represented in addition to sloughing to the lumen (a), mucoid degeneration (b) there was narrowing in the villi crypt(c) 10*2.3, (E) exhibit hyperplasia of enterocyte (a) adhesion between villi (b) with sloughing to the lumen (c) Zenker's necrosis (e) and necrotic villi (red row) with edema (red dot row), H&E

Renal lesions were evident in all treated groups, including the control group. These were characterized by dilation of Bowman's space, edema, hyperplasia of the epithelial cells lining the renal tubules, and tubular necrosis (Fig. 4F). The severity of the lesions increased in fish fed diets containing 3.54 and 6.1% *Spirulina*, where further pathological

changes comprised vascular congestion, interstitial nephritis, and luminal stenosis of renal tubules, often exhibiting a star-shaped appearance indicative of cloudy swelling (Fig. 4G).

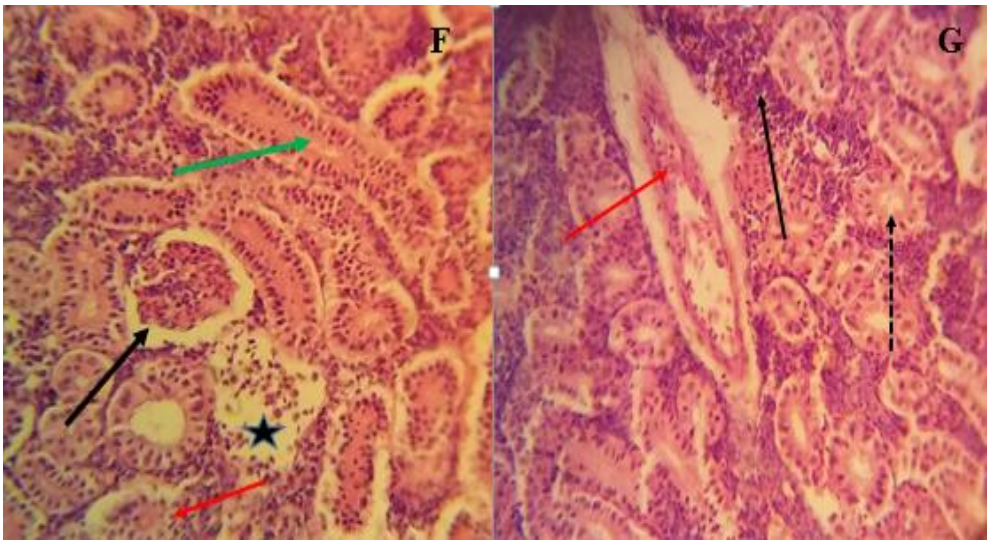

Fig. 4. Microscopical examination of kidney in *C. carpio* fed with spirulina at variable levels for 8 weeks showing: (F) Dilatation in the Bowman's space (black row), edema (black star) and necrosis in some tubule (red row) with hyperaplasia of the epithelial cells lining renal tubule (green row), 40*1, (G) in fish feed with spirulina at 6.1%, the lesions were represented by congestion (red row), interstitial nephritis (black row) and stenosis of lumen of tubule with star shape appearance 40*1.3, H&E

DISCUSSION

Spirulina is one of the main plants used in improving the health and growth in humans and animals **(Dawood *et al*., 2018; Mohammad *et al*., 2022)**. This is due to the metabolic compounds such as vitamins, amino acid, phenols and minerals that improves the immunity and tolerance to stress **(Soni *et al*., 2017)**. The liver and intestines are among the most important organs of the body for the digestion and absorption of nutrients for feed. Hence, they have an important role in evaluating the effectiveness and impact of dietary alternatives and nutritional supplements on the digestive system **(Rašković *et al*., 2011)**.

Although most alternatives or nutritional supplements as probiotic, *Saccharomyces cerevisiae* **(Said *et al.*, 2023)**, and herbal plants, have good nutritional specifications due to their components of protein, amino acids, carbohydrates, fats, and so on, partial or complete protein replacement in fish can cause stress and metabolic disturbances. This can lead to pathological changes in a variety of organs, mainly in the digestive system and cause economic problem **(Caballero *et al*., 2003; Rosenau *et al*., 2021; AL-Taee, 2022; AL-Taee *et al*., 2022)**.

Histopathological alteration in the intestines vary depending on the type of fish, its nutrition as well as the percentages of food supplements added to the diet **(Baeverfjord &**

Krogdahl, 1996). Therefore, it was observed through this study that when spirulina was replaced in carp ration at different rates of 1.45, 2.9, 3.54 and 6.1%, with the replacement rates of soybean 9.6, 22.5, 20.3 and 27.5%, it led to histopathological changes that varied in severity according to the percentages of the addidative food. Therefore, the lesions showed more severety in the intestines of fish fed spirulina at 6.1% represented by the occurrence of necrosis and shedding of the tops of the villi, hyperplasia of epithelial cells and mucosal degeneration in addition to thickening of the muscular layer and the occurrence of edema. This result agrees with the data reported by the **Urán *et al*. (2008)**, who stated that mild enteritis would occur in carp feed with spirulina after a short period.

The results of this study also agreed with the results of the study of the **Baeverfjord and Krogdahl (1996)**, who reported that the addition of vegetable protein to the salmon ration led to the non-infectious subacute enteritis, and the lesion was represented by short villi and degeneration, intestinal cell vacuolation, infiltration of inflammatory cells and hyperplasia of enterocyte, which have an important role in the absorption process. The pathologic changes noted in the intestines of fish could occur due to the long-term feeding of *Spirulina* for 8 weeks, where it might act as an external stimulus. Additionally, the naturally extended intestinal tract of carp could increase the time available to digest and absorb nutrients **(Fadl *et al.*, 2013)**, thus increasing the time intestinal tissue would come into contact with dietary components. This extended exposure could promote enterocyte proliferation to maintain balance within the intestine in response to stimuli **(McGavin & Zachary, 2007)**. Additionally, the efficiency of protein digestion in fish can differ among species, and the form of plant-based protein can contribute to protein digestion efficiency. For example, certain Cyprinids such as silver carp (*Hypophthalmichthys molitrix*) have been shown to have lower digestibility of *Spirulina* **(Ekpo & Bender, 1989)**, which could be responsible for contributing to non-infectious enteritis and liver injury. In the current study, the addition of *Spirulina* in varying amounts caused different tissue changes. No pathological changes were seen in the liver of fish consuming 1.45% or 2.9% *Spirulina*; however, increased *Spirulina* inclusion (3.54% and 6.1%) indicated the presence of histopathological lesions, which were most severe at the highest inclusion amount.

Spirulina is generally a balanced nutritional supplement which contains important elements (nutrients, minerals, nucleic acids), but it does lack some important substances such as tryptophan and phosphorus based acids (phospholipids). These aspects of *Spirulina*, seem to disrupt protein formation with amino acids from tryptophan in the liver, forming niacin (B3), which will cause a disruption in energy metabolism and glycogenesis that has the potential to affect the pancreas histopathology. Tryptophan deficiency may also prevent maintenance of cell membrane fluidity and ionic homeostasis that can cause vacuolar degeneration. The organic matter digestibility of *Spirulina* is low. This can further lead to increased oxidative stress and production of reactive oxygen species (ROS) damaging the cell membrane and disruption of ionic homeostasis. This mode of action has been indicated as the mode of action for hepatic vacuolar degeneration **(Olvera-Novoa *et***

***al.*, 1998; McGavin & Zachary, 2007; Maradonna *et al.*, 2015)**. These findings are consistent with tissue results from fishes being fed *Spirulina* diets indicating hepatic cell vacuolation and vascular congestion was observed in *Oreochromis niloticus* fed 50% *Spirulina* **(Dohaish *et al.*, 2018)**. Microalgae, especially *Spirulina*, are generally high in lipids and low in fiber, which may facilitate lipid accumulation and fatty infiltration in hepatic cells **(Suganya *et al.*, 2016)**, however, they appear to be pivotal in lipid metabolism as they have shown to elevate saturated and polyunsaturated fatty acid levels. Spirulina intake has parallels with hepatic steatosis development or with evidence contributed toward hepatic excessive lipogenesis **(Rosenau *et al.*, 2021)**.

The kidneys are vital organs that specialize in detoxification and excretion of metabolic wastes and work closely with the liver and intestines. Thus, liver or intestinal pathophysiology could indirectly affect renal tissue. Kidney damage may also result from dietary components, such as soybean-derived protease inhibitors, contributing to the inability to digest protein properly, increased urea, and altered renal architecture **(Schrier *et al.*, 2004)**. Some studies showed limited or no positive effects of *Spirulina* on species, *Cyprinus carpio* **(Nandeesha *et al.*, 1998)**, *Catla catla* **(Nandeesha *et al.*, 2001)**, and the Nile tilapia (*Oreochromis niloticus*) **(Lu & Takeuchi, 2002)**. **Roohani *et al.* (2020)** hypothesized that even if *Spirulina* is fed to fish with lower protein requirements, target aquatic organisms like *Spirulina*/vegetation would ultimately benefit a carnivorous fish species vs. an omnivorous species like carp.

CONCLUSION

This study concluded that spirulina is an important nutrient that has an impact on human health and may have an effect on fish since it has a high percentage of protein, nutrients, and minerals. However, it's lack in some amino acids make it hard and difficult for digestion, therefore it may have a detrimental effect. Depending on the species, dosege of algae used, the type and age of the fish, it can cause histopathological alterations in the internal organs of carp fish, which may lead to economic losses.

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