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Effect of Replacing Fishmeal with Dietary Pumpkin Meal on the Intestinal Histology of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Twelve ponds were used in this experiment to study the effects of replacing 0, 50, 75, and 100% of fishmeal with pumpkin meal in the diet on certain intestinal histological parameters of the Nile tilapia. Each treatment was replicated in three ponds, and the experiment was conducted over 60 days. The Nile tilapia fingerlings (average weight 2.41g) were transferred to the experimental ponds at a rate of 50 fingerlings per pond.

At the end of the experimental period, there were no statistically significant differences in survival rates among the fish fed the different experimental diets. Examination of the intestinal tissue revealed that the control group had mature villi, a well-developed muscle layer, and normal goblet cells. The group receiving 50% pumpkin meal showed very long and branched villi with abundant goblet cells, while the 75% pumpkin group exhibited mature villi and a high number of goblet cells. These findings indicate an increase in absorption, which was reflected in weight gain for this group. The 100% pumpkin meal group also displayed mature villi, a thick muscle layer, and abundant goblet cells on the surface of the mucous membrane.

INTRODUCTION

Aquaculture contributes substantially to global food and nutrition security, and it is a major producer of aquatic foods **(Edwards *et al.*, 2019)**. Global freshwater aquaculture has benefited from the 70% growth in the Nile tilapia (*Oreochromis niloticus*) productivity during the past eight years **(FAO, 2020)**. One of the most widely farmed fish species is the Nile tilapia (*O. niloticus*) and contributes to food security, providing affordable fish for the resource poor. Due to its high fertility, rapid growth, good performance under suboptimal nutritional conditions, and ability to withstand poor water quality, the Nile tilapia is considered an ideal fish species for aquaculture **(Hassanien *et al.*, 2011; Opiyo *et al.*, 2018; Tibihika *et al.*, 2020)**.

Access to affordable and sustainable feed ingredients that maintain fish growth and well-being is a requirement for improving profits in fish farming **(Allam *et al*., 2020; Khalil *et al*., 2022)**. Reviews on the future of aquaculture production and the development of fish diets **(Hardy *et al.*, 1996; Naylor *et al.*, 2000)** have focused on replacing fish meal, which forms the basis of most fish diets, with economically viable and environmentally friendly plant protein alternatives. Pumpkin is a significant vegetable crop widely grown in South Asia, Africa, India, Latin America, and the United States. While pumpkins have long been a staple food in rural and some urban areas, recent horticultural, commercial, industrial, and scientific studies have explored their properties in greater depth **(Ahmad & Khan, 2019)**.

The combination of various protein sources may help meet dietary requirements similar to those of fish meal. Consequently, the interaction between aqua diets and gut health significantly influences physiological processes such as immunity and digestion **(Butt & Volkoff, 2019)**. This study aimed to examine how different combinations of plant meals affect certain intestinal histological parameters.

MATERIALS AND METHODS

Experimental fish and diet preparation

The Nile tilapia fingerlings were obtained from the General Authority for Fish Resources Development (GAFRD) - Sahari region, Aswan City.

Four isonitrogenous diets were formulated from commercial ingredients as indicated in the first published part of the current study **(Khattab *et al*., 2023)**. The dry ingredients were passed through a sieve (aperture of 0.3mm in diameter) before being mixed into the diet. Emulsified oil was added with an equal quantity of water with 0.7% phosphatidylcholine (lecithin) according to **El-Dahhar and El-Shazly (1993)**, to the experimental rations. Subsequently, boiling water was then mixed with the ingredients at a ratio of 50% to facilitate pelleting.

Twelve ponds were used in this experiment to study the effect of replacing (0, 50, 75, and 100) fishmeal by pumpkin meal in the diet on some intestinal histological parameters of the Nile tilapia. Each treatment was replicated in three ponds. The Nile tilapia fingers (2.41g) were transferred to the experimental ponds at a rate of 50 fingerlings/hap. Each pond was supported by continuous artificial ventilation. The replacement ratios in the experimental diets were as follows:

C: zero Pumpkin (control diet).

T1: 50 % Pumpkin.

T2: 75 % Pumpkin.

T3: 100 % Pumpkin.

Histopathological examination

Three fish from each group (C, T1, T2, and T3) were taken at the end of the experiment (60 days) for embedding paraffin sections. The fish were immediately washed

with distilled water, dissected, and eviscerated. The intestines were collected and immediately fixed in 10% neutral buffered formalin. Histological experimentation was conducted following the methods outlined by **Abdelhakeem *et al.* (2022)** and **Bancroft and Gamble (2002)**. Three sections of the intestine were excised and subjected to ascending grades of alcohol (70, 80, 90, and 100%) for dehydration. After dehydration, the samples were cleared in methyl benzoate and embedded in paraffin wax.

Sections were then cut using a semi-automated sliding microtome at a thickness of 3 to 5µm. The paraffin sections were mounted on glass slides, dried, deparaffinized in xylene, rehydrated in a graded alcohol series (100, 90, 80, and 70%), and stained with Harris's Haematoxylin and Eosin. The stained sections were visualized and examined using a DMLS light microscope (Leica, Germany) equipped with an MC120 HD camera (Leica).

RESULTS AND DISCUSSION

Histological changes in the intestine of the Nile tilapia were observed in T1, T2, and T3 groups. The intestine of the control group displayed a typical histological architecture (Fig. 1), characterized by mature villi, a well-defined muscular layer, and mucosal epithelium interspersed with goblet cells.

In the T1 group (Fig. 2), the intestinal villi were very tall and branched, with a clearly recognizable intestinal lumen and abundant goblet cells.

For the T2 group (Fig. 3), there was some similarity to T1. Most intestinal villi were mature, extremely tall, and occupied the intestinal lumen, which was partially occluded. The surface mucosal epithelium contained fewer goblet cells, and the muscular layer was present.

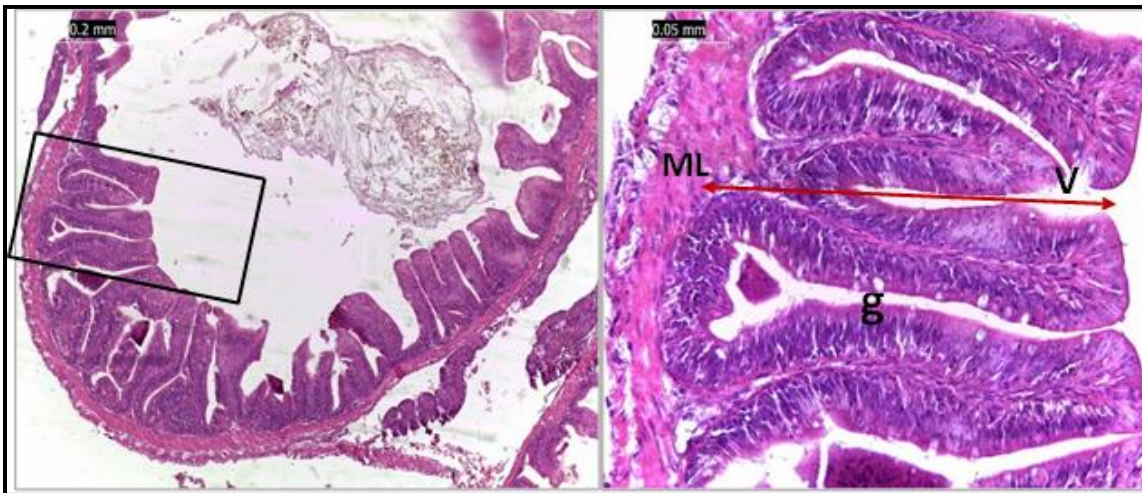

Fig. 1. Intestinal histology of the control group fed 100% fish meal showing mature villi (V), muscular layer (ML), and mucosal epithelium interspersed with goblet cells (g)

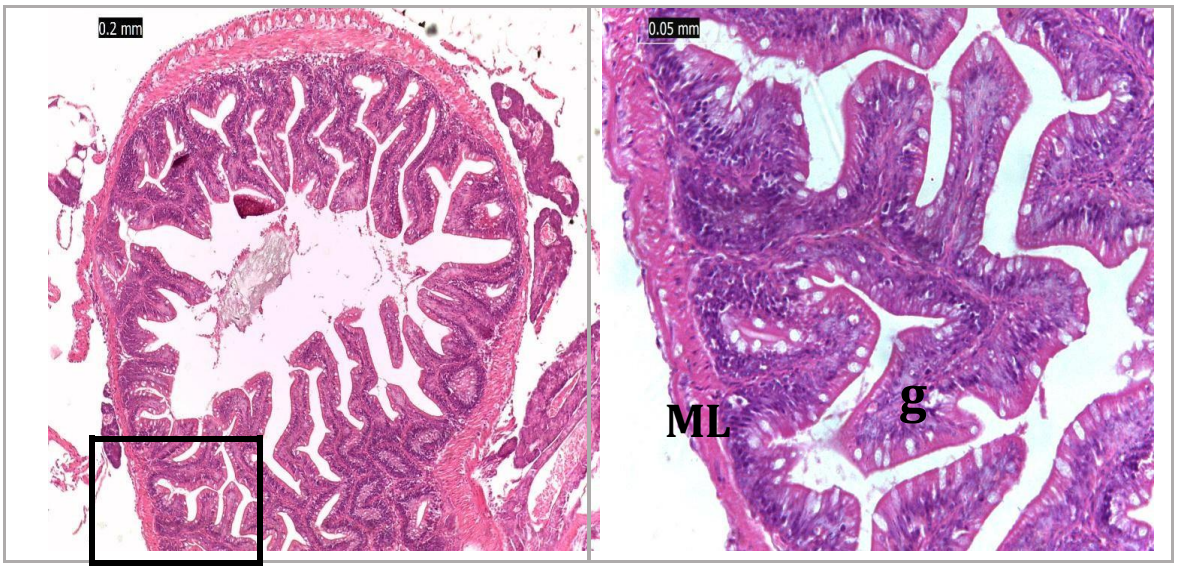

Fig. 2. Intestinal histology of the group fed 50% pumpkin showing very tall and branched mature villi (V), a well-defined muscular layer (ML), and mucosal epithelium with abundant goblet cells (g)

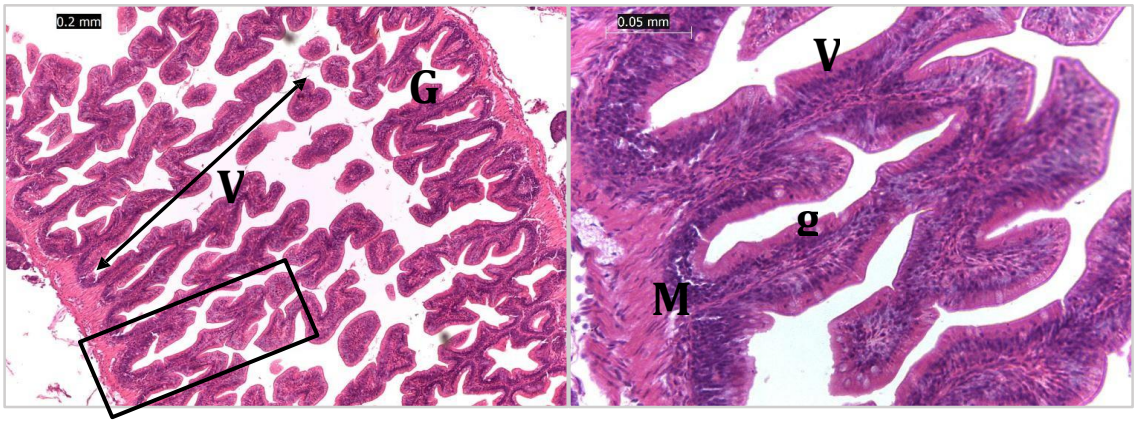

Fig. 3. Intestinal histology of the group fed 75% pumpkin and 25% fish meal showing mostly mature villi (V) that are extremely tall, partially occluding the intestinal lumen, along with shorter growing villi (GV). The surface mucosal epithelium has fewer goblet cells (g), and the muscular layer (ML) is also visible

These results agree with **Toutou *et al*. (2024)**, who found that the histological examination revealed an increase in the length of villi and the number of goblet cells in the intestine of tilapia zillii fed on different combinations of dietary plant meal protein treatments (lupine, sesame, and jojoba meal) compared to the control group.

Furthermore, **Halimatussakdiah *et al*. (2021)** denoted that feeding *Osphronemus gourami Lac*. on a combination of avocado (*Persea americana M*.) and pumpkin seed (*Cucurbita moschata Duch*.) affected the villi height and goblet cell number of goblet fish. The results indicated that feeding 20 grams of avocado seeds, 60 grams of pumpkin seeds, and 20 grams of mixed substances improved both the height of the villi and the quantity of goblet cells in the intestines of the gourami fish. The study concluded that the combination

of avocado seeds and pumpkin seeds has a significant impact on the histological appearance of the gourami's digestive organs.

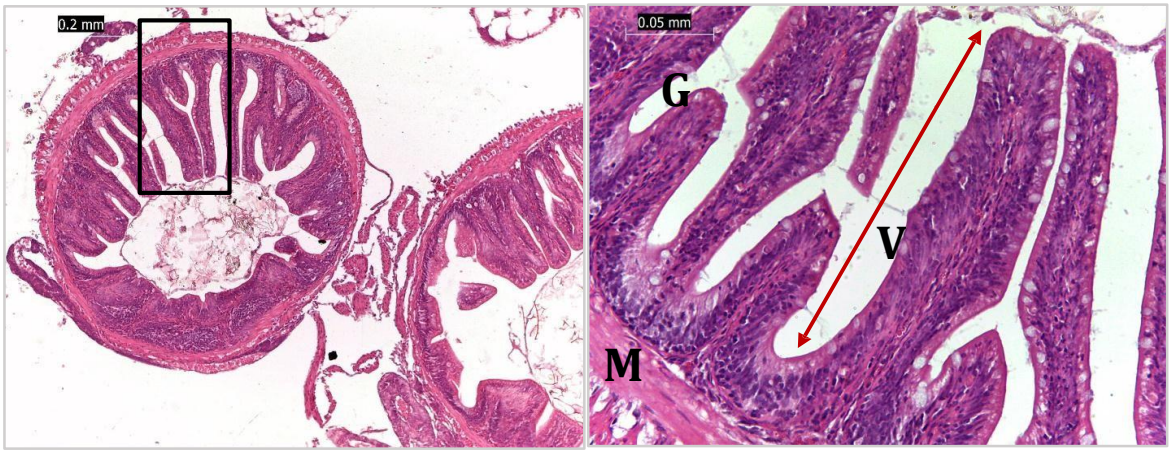

Fig. 4. Intestinal histology of the group fed 100% pumpkin showing very tall mature villi (V), numerous growing villi (GV), a thick muscular layer (ML), and mucosal epithelium with abundant goblet cells (g)

Villi are finger-like projections that increase the surface area of the intestinal walls, enhancing nutrient absorption and producing digestive secretions. Histological changes in the intestines of the Nile tilapia were observed in the T1, T2, and T3 treatments. The intestine of the control group exhibited typical histological architecture (Fig. 1), with mature villi, a well-defined muscular layer, and mucosal epithelium interspersed with goblet cells.

Jiang *et al.* (2016) and **Adeniyi *et al.* (2018, 2021, 2022)** reported that herbal supplements can stimulate feed digestion in cultured fish. **Ekanem *et al.* (2010)** elucidated that the wistar rats treated with dried leaves of *T. occidentalis* (a type of pumpkin) developed nephrotoxicity, which included enlarged tubules and glomeruli, deformed Bowman's capsules, and contracture of the proximal and distal convoluted tubules. Fish fed a graded level of 100% dried leaves of *T. occidentalis* exhibited moderate atrophy of the intestinal villi, indicating reduced size due to poor absorption and circulation of nutrients from their diet. **Reed (1995)** noticed that indigestion in grazing animals is caused by tannins and saponins found in *T. occidentalis* leaves.

Conversely, pumpkin pomace has been used as a feed ingredient in some monogastric species, such as pigs **(Medina *et al.*, 2019; Ribadeneira *et al.*, 2020)**. Pumpkin pulp is rich in starch and soluble fiber, primarily pectin, which enhances digestibility **(Bai *et al.*, 2020; Hosseini *et al.*, 2020)**. Pectin also acts as a growth promoter in fish by regulating intestinal microbiota and influencing cellular activity **(Hosseini, 2020)**.

Interestingly, the recent findings from the first part of this study regarding the effect of replacing fishmeal with pumpkin on the productive performance of the Nile tilapia **(Khattab *et al.*, 2023)** align well with the observed intestinal histological parameters. **Khattab *et al.* (2023)** reported that the 75% pumpkin group recorded the highest values for final body weight (FBW), weight gain (WG), specific growth rate (SGR), protein productive value (PPV), protein efficiency ratio (PER), and the best feed conversion ratio (FCR). Specifically, the values were as follows: 6.22, 8.86, 10.15, and 6.18 g/fish (FBW); 3.81, 6.45, 7.74, and 4.77 g/fish (WG); 2.22, 3.10, 3.40, and 2.59 (SGR); 1.39, 1.95, 2.65, and 2.32 (PER); and 100.34, 111.98, 160.39, and 153.18 (PPV); and 2.39, 2.12, 1.28, and 1.65 (FCR) for the 0, 50, 75, and 100% pumpkin groups, respectively.

The best growth performance in the 75% pumpkin group correlates positively with the favorable intestinal histological parameters observed in this group (Fig. 3). Therefore, the growth-promoting effects seen in the 75% pumpkin group may be attributed to the phytochemicals present in the supplement. These phytochemicals likely enhanced the activities of digestive enzymes, improved gut health (with intestinal villi mostly mature and extremely tall, occupying the intestinal lumen) (Fig. 3), and increased nutrient absorption, resulting in better nutrient utilization and ultimately improved growth performance.

CONCLUSION

Histological interpretations of the intestines of the group of fish that were fed with 75% pumpkin showed an increase in the length of the villi in the intestinal lumen and the presence of abundant goblet cells.

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