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Histological digestive tract of milkfish's (*Chanos chanos* Forskal.) from District Ujung Pangkah, Gresik

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Abstract:
Milkfish is a commodity with a high production rate. From the milkfish commodity, Gresik is the center of fishery production in East Java. Milkfish belongs to the herbivore group with the type of food from the plant group. Milkfish cultivation in traditional ponds still uses natural food as the main food for the cultivated commodities. Fish food affects the structure of the digestive tract, especially the intestines of fish. herbivorous fish have very long intestines even many times the body length, compared to omnivorous and carnivorous fish. Thus, this study aims to find out more clearly about the structure of the milkfish gut tissue. The digestive tract is related to the process of digestion, absorption and disposal of food waste, so it has an important role in the growth of fish. The research method used was descriptive experimental by making preparations of fish intestinal tissue and staining with Hematoxylin Eosin. The intestines of the fish observed were the front, middle and back. Observation of the preparations using a microscope with a dot slide scan photo. From the results of the study obtained an overview of the intestinal tissue structure of the upper (proximal), middle (middle) and lower (distal) fish. The structure of the front, middle and back of the milkfish gut tissue is similar, the difference lies in the number and thickness of the villi.

Keywords: Hematoxylin Eosin, Histological, Intestinal Tissue, Milkfish, Tradisional Ponds.

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Introduction

Gresik is a district with a fairly high aquaculture production center. Fishery potential in Gresik is supported by the vast land for inland fisheries, especially ponds. The potential for milkfish cultivation in Gresik also deserves attention. Data from the Fisheries Service of Gresik Regency states that the area of ponds in Gresik reaches 40% of the total area of ponds in East Java. Center for Statistics and Information from the Ministry of Maritime Affairs and Fisheries website in 2020, milkfish production in East Java Province has increased. In 2011, milkfish production was more than 80 thousand tons and continued to increase to more than 182 thousand tons in 2019. This enormous fishery potential certainly should not stop our collective efforts not to carry out various technological innovations with the aim of further development and improvement. fisheries production, especially milkfish. Problems that are still encountered in milkfish cultivation, especially in Gresik Regency, include the lack of knowledge of most cultivators about good and correct cultivation methods, including the development of cultivation technology which is still minimal. In addition, the mortality rate from fish/shrimp disease is still high, both caused by viruses and bacteria, and water quality management is still not optimal. The problem becomes complex which can affect the growth and even cause of fish death.

Milkfish cultivation in ponds with a polyculture system is widely applied in Gresik Regency. Milkfish is usually polycultured with tilapia, vaname shrimp and even seaweed. Polyculture is carried out with the aim of increasing land use efficiency and increasing productivity by utilizing different types of food between cultivated commodities. This is in accordance with research conducted by Permana, et al. (2021) that milkfish cultured with a polyculture system together with white vaname shrimp and seaweed can increase the

productivity of ponds. Each cultured organismmust have a mutually beneficial relationship in the pond ecosystem.

The type of food affects the structure of the digestive tract, especially the intestines of fish. Herbivorous plant-eating fish have longerintestines than omnivores and carnivores. The structure of intestinal tissue has an important role because it relates to the process of digestion of food and absorption of nutrients in the fish body. Fish growth is also directly affected by the processof digestion and absorption of nutrients that occurin the intestines of fish. From the results of this study, it can contribute to knowledge about the intestinal tissue structure of milkfish, especially those that live in ponds with the characteristics of the waters of Gresik Regency. Knowing thecondition of the fish structure can also be used to determine the health level of the fish. It is hoped thatin the future we can determine the type of food thatis in accordance with the structure of the fish intestinal tissue.

Methods

Fish sampling and collection of milkfish digestive tract

Milkfish were taken from 2 plots of traditional ponds with a weight of about 65 g as many as 3 fisheach. The cervical dislocation method is used to killfish. The digestive tract of fish is removed from the stomach aseptically and cut into 3 parts, namely the upper, middle and lower parts. Samples of the digestive tract that had been cut were preserved in bottles containing 10% formaldehyde solution.

Tissue preparations

The digestive tract organs that have been soaked ina 10% formaldehyde solution were then washed with PBS 3 times and followed by immersion in 70%, 95%, 100% and xylene ethanol solutions. Thetissue was glued with paraffin and stored at 4oC in a water bath so that the tissue moisture was maintained before cutting. The tissue was cut usinga microtome with a thickness of 4-10 m.

Results and discussions

The morphology of the milkfish samples is presentedin Figure 1 and the structure of the internal organs in the stomach is presented in Figure 2 below. The shape of the milkfish digestive tract and its length is presented in Figure 3.

Figure 1 shows the morphology of the milkfish, namelythe body color is silver and the whole body is covered with scales. The body shape of the milkfish is flat and elongated, bilateral, has a curved tail fin divided into two with a pointed tip, dorsal fin, pelvic fin and anal fin. The mouth is pointed forward, with large round eyes. Moyle and Joseph (2000) characterize the morphology of milkfish, namely a slender body shape with a terminal mouth, cycloid scales with soft fin rays.

Figure 1. Morphology of the milkfish

Tissue is placed on a glass object that has been coated with gelatin and put in a hot plate at 37oCfor 30 minutes, then in an oven at 40^{O}C for 2-3 hours.

Staining using Hematoxylin and eosin is divided into 2 stages, namely staining and mounting. Staining begins with the deparaffination process (removing paraffin on the tissue surface) by immersing the preparations in xylol I and II solutions for 5 minutes each. The process of withdrawing fluid from the cell by immersing the preparation in an alcohol solution graded from 96%, 90%, 80% and 70%. The preparations werewashed in running water for 5 minutes and stained with Hematoxylin for 5 minutes (staining the cellnuclei). The preparations were washed again in running water for 5 minutes, then stained with Eosin (staining the cytoplasm) for 5 seconds and washed again in running water for 4 minutes. The preparations were immersed in a graded alcohol solution of 70%, 80%, 90%, 96%, absolute alcohol and finally xylol solution. Mounting preparations using enthelan as much as 1-2 drops.

Observation of the tissue on the preparations wascarried out using a dot slide scan method using an Olympus brand binocular microscope. The

software used for observing network scan results is Olivia. Number of dorsal fins between 13-17, anal fins 9- 11, pelvic fins 11-12, caudal fin long and forked, the number of scales on the lateral line there are 75-80 pieces.

Figure 2. Arrangement of organs in the stomach of milkfish

In Figure 2 above, you can see the structure of the organs in the dissected fish's stomach. Visible liver, intestines, heart and fat that fills the abdominal cavity. In Figure 2 below shows the intestines of milkfish that have been described lengthwise beside the ruler. It canbe seen that the intestines of milkfish weighing about 65 g have an intestine length of > 200 cm. Some of theparts that make up the digestive tract of fish are stomach, cardiac stomach, pyloric stomach, intestine and rectum. The esophagus and mouth, which are also digestive tract in this image, are missing because their position is not possible to take when cutting the digestive tract. This is supported by research by Fujaya (2014) which states that the digestive tract of fish consistsof the mouth, oral cavity, pharynx, esophagus, stomach, intestines, and anus. Each organ of the digestive tract has aspecial function, all of which are related to the process of digestion of food, absorption of nutrients into the body andthe disposal of indigestible food waste

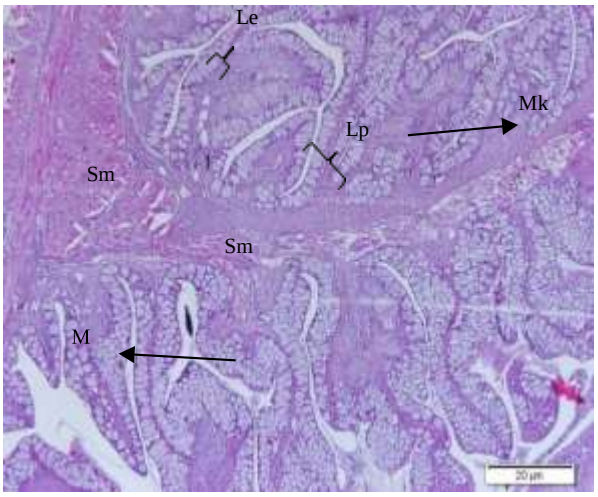

Figure 3. The shape and length of the milkfish intestine.CS (Cardiac Stomach); PS (Pyloric Stomach); I (intestines); R (Rectum)

The esophagus is the connecting tube between the mouth and the stomach. The stomach of milkfish servesas a temporary shelter for food and the process of breaking down food into smaller particles making it easier for digestion and absorption. In addition to the mechanical digestion process, in the stomach there is also a chemical digestion of food using digestive enzymes. The intestine has a function as a place of digestion and absorption of nutrients that have been digested. In the intestine there are villi as a place of absorption, with the form of thick folds that function toexpand the absorption area. In accordance with the statement of Ikpegbu, et al. (2014) that the intestine functions as an organ of digestion and absorption of nutrients. Furthermore, it is said that the best nutrient absorption process occurs in the proximal intestine because it has villous height, villi width and a large number of villi, thereby increasing nutrient absorption into the blood. The villi are covered by mucosa on the surface. The function of the mucosa is to prevent the entry of bacteria and kill bacteria because there are mucins that contain antibacterial substances.

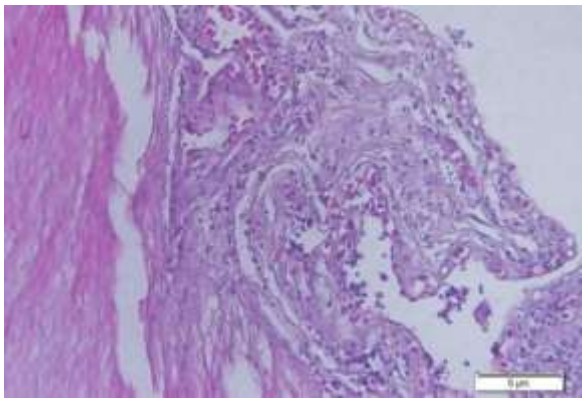

Figure 4. Histological structure of the middle milkfish intestine

Histological structure of the esophagus, stomach, and intestines of fish in general is composed of four main layers, namely mucosa, submucosa, muscularis, and serosa (Tambayong, 1995). The mucosal layer consists of the lamina epithelium, the lamina propria, and the muscularis mucosa. The submucosa layer consists of dense irregular connective tissue, blood vessels, lymph and nerves. The muscularis layer is composed of longitudinal (longitudinal) and circular (circular) muscles. The serous layer consists of loose connective tissue, blood vessels and adipose cells (Junquira and Carneiro, 2007).

Figure 5 shows the structure of the lower (distal) intestine of the fish. Mumford et al. (2007) stated that the anterior part of the intestine functions to 1) transportfood material from the stomach to the posterior intestine, for complete digestion by secreting enzymes from the walls and accessory glands, 3) to absorb the end products of digestion into the blood and lymph vessels in the intestine. its walls, and 4) to secrete certain hormones (namely Secretin, stimulates pancreatic secretion). Posterior gut functions include absorption fluids, mucus secretions (more goblet cells) and some digestion is accomplished by enzymes present in foodstuffs, and excretion.

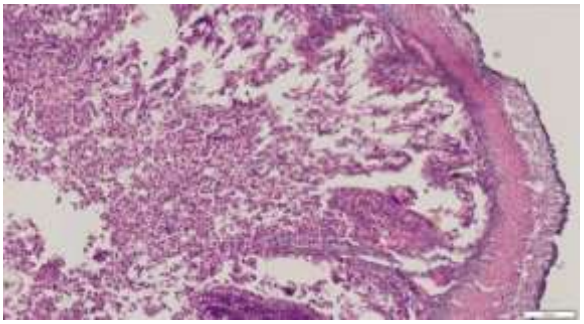

Figure 5. Histological structure of the lower (distal) intestine of milkfish.

Villi are composed of mucosa, lamina propria, goblet cells and submucosa with a thick layer that supports mechanical digestion that changes the size of food particles into very small ones. Nazlic et al. (2014) stated that the thick and strong circular muscle in the stomach plays a role in gastric peristalsis and allows certain food to pass into the intestine through the pyloric valve. lamina propria. Small food particles will facilitate further processing in the middle intestine where food will mix with digestive enzymes. Furthermore, the food goes to the bottom / distal whichallows food nutrients to be absorbed through the intestinal villi and into the blood circulation in the intestinal capillaries.

Conclusion

The structure of the fish intestine tissue in this study was observed in the proximal, middle and distal sections. The intestinal tissue structure of the three parts has the same structure, starting from the outer layer of the mucosa, lamina propria, submucosa, tunica muscularis and serosa respectively. The difference in structure between the three parts of the intestine is in the thickness of each structure of the intestinal lining, especially the lamina propria.

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