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EFFECT OF ADDING BIOACTIVE COMPOUNDS FROM BROWN ALGAE (*SARGASSUM* SP.) IN FEED ON INTESTINE AND LIVER HISTOLOGY OF SIAM PATIN FISH (*PANGASIUS HYPOPTHALMUS*) SEEDS

Pengaruh Penambahan Senyawa Bioaktif Dari Alga Cokelat (*Sargassum* Sp.) Pada Pakan Terhadap Histologi Usus Dan Hati Benih Ikan Patin Siam (*Pangasius Hypopthalmus*)

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ABSTRACT

Addition of Sargassum sp. into feed as a feed supplement is one effort that can be done to increase fish growth and immunity. This research aims to determine the effect of adding bioactive compounds from Sargassum sp. extract in feed on the health picture of Siamese catfish through structural changes that occur in the intestines and liver. Fish observations were carried out for 40 days. The results obtained regarding the survival rate (SR) value of Siamese catfish seeds show that there is no difference or has the same percentage, namely 100%. The results of observations on the histopathology of intestinal organs analyzed in a comparative descriptive manner showed that Siamese catfish fry were fed with Sargassum sp. extract experienced damage than fish seeds fed without the addition of Sargassum sp. extract (control). The damage that occurs includes leukocyte infiltration (IfL), necrosis (Ne), goblet cell proliferation (Psg), hyperplasia (Hpl), and hemorrhage, with a damage score analyzed using the semiquantitative scoring method, namely 1. Damage to the liver, namely experiencing congestion in the blood vessels.

Keywords: Brown Algae (Sargassum sp.), Siam Patin, Histopagology, Intestine, Liver

ABSTRAK

Penambahan *Sargassum* sp. ke dalam pakan sebagai *feed supplement* menjadi salah satu upaya yang dapat dilakukan demi meningkatkan pertumbuhan dan imunitas ikan. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan senyawa bioaktif dari ekstrak *Sargassum* sp. pada pakan terhadap gambaran kesehatan ikan patin siam melalui perubahan struktur yang terjadi pada organ usus dan hati. Pengamatan ikan dilakukan selama 40 hari. Hasil yang diperoleh terhadap nilai tingkat kelangsungan hidup (SR) benih ikan patin siam

menunjukan tidak adanya perbedaan atau memiliki persentase yang sama yakni 100%. Hasil pengamatan terhadap Histopatologi organ usus yang dianalisis secara deskriptif komparatif menunjukan benih ikan patin siam yang diberi pakan dengan ekstrak *Sargassum* sp. mengalami kerusakan daripada benih ikan yang diberikan pakan tanpa penambahan ekstrak *Sargassum* sp. (kontrol). Kerusakan yang terjadi diantaranya yakni Infiltrasi leukosit (IfL), Nekrosis (Ne), Proliferasi sel goblet (Psg), Hiperplasia (Hpl), dan Hemoragi, dengan skor kerusakan yang dianalisis menggunakan metode *semiquantitative* scoring yakni sebesar 1. Kerusakan pada organ hati yakni mengalami kongesti di pembuluh darah.

Kata Kunci : Alga Cokelat (*Sargassum* sp.), Patin Siam, Histopagologi, Usus, Hati

INTRODUCTION

Patin fish (Pangasius sp.) is a type of freshwater fish that is very popular with the public and has important economic value in the world of aquaculture. In Indonesia there are 14 species of catfish (Pangasius sp.), but the one that is widely cultivated is the catfish from Thailand, namely the Siamese catfish (Pangasius hypothalamus) (Iskandar et al., 2022). The addition of natural ingredients as supplements to feed is usually done to support the cultivation of Siamese catfish to be more optimal, because in cultivating this fish it cannot be separated from the problem of poor environmental conditions which in the end can cause the fish to experience stress, their growth is hampered, their immune system decreases, susceptible to disease and often die.

Adding brown algae (Sargassum sp.) to feed as a feed supplement is one effort that can be made to increase fish growth and immunity. The main component of the nutritional content of Sargassum sp. namely 64.67% carbohydrates and other components 30.4% water, 2.08% ash, 2.08% protein and 0.81% fat (Sumarni, 2022). Sargassum sp. Extract. can stimulate the immune system and organ function due to the bioactive compounds it contains. Bioactive content of Sargassum sp. shows a role as an antioxidant. Antioxidants are able to protect body cells from the oxidation process so that free radicals do not cause cell damage (Udlhi et al., 2023).

It is necessary to research the effect of adding bioactive compounds from Sargassum sp extract. on feed to determine the health of Siamese catfish through structural changes that occur in the intestines and liver. The intestine is an organ that is often exposed to pathogenic agents and parasites. The liver is the largest and most metabolically complex organ in the body. This organ is involved in the metabolism of food substances as well as most drugs and toxicants. Histopathological analysis can be used to determine the health of fish through structural changes that occur in organs which are the main targets of materials entering the fish's body (Dutta, 1996).

METHODS

The research was carried out in November-December 2023, in the Aquaculture Laboratory Building 4, Faculty of Fisheries and Marine Sciences, Padajajaran University. The samples used were Siamese catfish seeds (Pangasius hypopthalmus) measuring 11 ± 13 cm long and weighing around 8 ± 15 g. The materials used include commercial feed PF-1000, binder in the form of progol and 100 g of brown algae extract (Sargassum sp.) which has been previously made in the Aquaculture Laboratory Building 2, Faculty of Fisheries and Marine Sciences, Padajajaran University, and has been tested for content. bioactive compounds at the Chemistry Laboratory, Padjadjaran University.

Fish rearing lasts for 40 days, in a container in the form of an aquarium measuring 40x25x25 cm3, containing 15 fish/aquarium with a density of 1 fish/l. During maintenance, the

feed given is test feed in the form of commercial feed with Sargassum sp extract added. The research scheme is depicted in Figure 1, below.

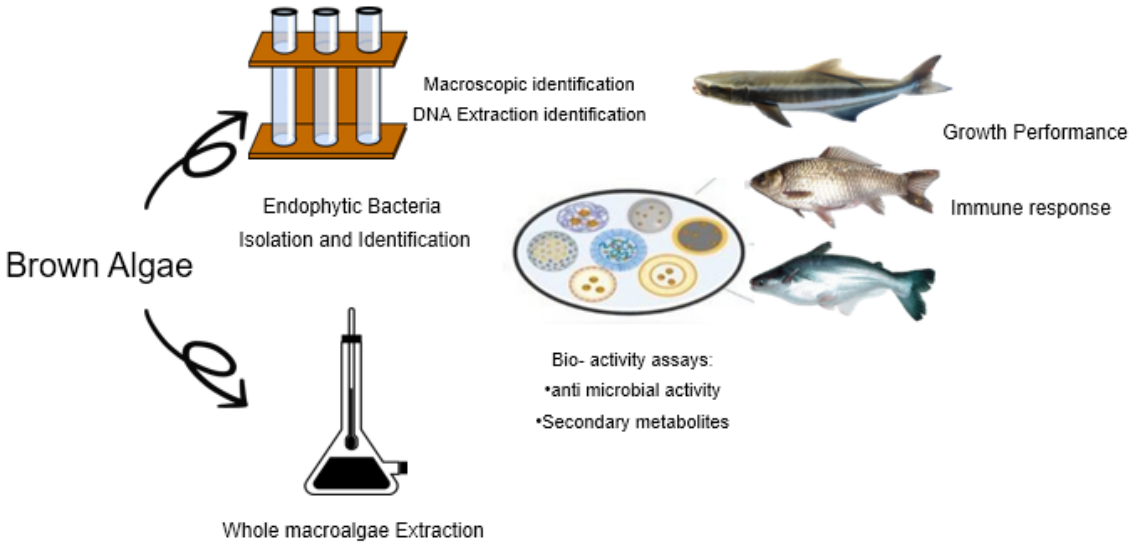


Fig 1. Testing on fish
Source: (Personal)

Sargassum sp. Extract. previously dissolved in 60ml of distilled water then given a binder and put into a spray, shaken until homogeneous then mixed evenly over the entire surface of the feed using the spraying method (Yuliana et al., 2021). After being evenly distributed, the test feed was air-dried for ± 1 hour at room temperature. Then, store it and close it tightly in a ziplock bag, add silica gel to keep the food from getting damp or moldy. Feed is given 3 times a day, namely at 07.00 WIB, 12.00 WIB and 17.00 WIB. The amount of feed given is 5% of the fish biomass. The treatment given is as follows:
Treatment (A) : 100% commercial feed (without the addition of Sargassum sp. extract.)
Treatment (B) : Commercial feed + 10 g/kg Sargassum sp extract.
Treatment (C) : Commercial feed + 15 g/kg Sargassum sp extract.
Treatment (D) : Commercial feed + 20 g/kg Sargassum sp extract.

Making Histological Incisions

Histological gill incisions were made at the Biology Laboratory, Padjadjan University. Before histopathological preparations were carried out in the laboratory, the fish was dissected directly to remove the intestines and liver. The fish sample used was 1 fish from each treatment. Before surgery, the fish samples were measured for body length and weight, then stunned. Shortly after the organ is dissected, preservation is carried out by soaking the organ using Bouins' solution. Histopathological preparations of the intestine and liver were made using the paraffin method and Haematoxylin-Eosin staining (Suntoro, 1983).

Histopathological preparation starts from the trimming process (organ cutting), tissue processing which includes Fixation, Dehydration, Clearing, Infiltration, Embedding (organ implantation), Sectioning (cutting), placing on a glass object, Affixing, Deparaffinization, Staining (Coloring), Mounting (attaching the cover glass to the object glass), and labeling. Specimen staining using Hematoxylin–Eosin (HE).

Observation Parameters

Survival rate (SR)

Survival Parameters (SR) are test parameters for fish endurance during maintenance. According to Fadhillah et al. (2023) can be calculated using the following formula:

$$SR = \frac{Nt}{No} x\ 100\%$$

Information :
SR = Survival rate (%)
Nt = Final fish count (ekor)
No = Initial number of fish (ekor)

Bioactive Compound Testing (Phytochemical Test)

Senyawa bioaktif yang diuji fitokimia pada ekstrak alga coklat A dan B adalah senyawa flavonoid, triterpenoid, alkaloid dan fenlolik. Adapun prosedur pengujian senyawa bioaktif adalah sebagai berikut:

1. Phenolic Compounds

As much as 2g of algae ethanol extract, several drops of 5% FeCl3 were added and the sample was positive for containing phenolics if the color changed to blackish blue (Karina, 2020).

2. Flavonoid Compounds

A total of 2g of algae extract was added with H2SO4 and chloroform in a 1:1 ratio, filtered with cotton wool and transferred to another test tube. Dragendorff reagent is positive if there is a red precipitate and for Wagner reagent it is positive if there is a brownish precipitate (Karina, 2020).

3. Triterpenoid Compounds

As much as 2g of algae extract, chloroform and water were added to form a 1:1 ratio, then 1 drop of concentrated H2SO4 was added and 1 drop of anhydrous acetic acid was added. A positive sample if it changes color to red or brown contains terpenoids (triterpenoids) Blue, purple or green contains steroids (Karina, 2020).

4. Alkaloid Compounds

A total of 2g of algae extract was added with H2SO4 and chloroform in a 1:1 ratio, filtered with cotton wool and transferred to another test tube. Dragendorff reagent is positive if there is a red precipitate and for Wagner reagent it is positive if there is a brownish precipitate (Karina, 2020).

Histopathological Testing

Parameters observed in the intestinal organs include leukocyte infiltration, congestion, hyperplasia, hemorrhage, goblet cell proliferation, necrosis, and epithelial abrasion. Meanwhile, parameters observed in the liver include fibrosis and congestion in blood vessels, leukocyte infiltration, cloudy swelling or edema and necrosis.

Data Analysis

Survival parameters were tested by comparing the number of fish that were alive at the beginning of the rearing period with the number of fish that were alive at the end of the rearing. Histopathological analysis was carried out by observing histopathological images microscopically using a light microscope with a magnification of 400x (10x40). The histology image was photographed with an Optilab digital microscope camera integrated with the microscope, then the histology image was taken using the ImageView application to create a photo micrograph. Observational data were analyzed in a comparative descriptive manner where the results of histopathological testing of the intestines and liver of Siamese catfish seeds

given Sargassum sp extract. on feed compared with the histopathological condition of the intestines and liver of Siamese catfish fry on feed without the addition of Sargassum sp extract. (control). as a comparison. The quantitative data results were analyzed using the semiquantitative scoring method to determine the level of intestinal and liver histology pathology which ranges from 0 – 3 depending on the width of the field of view observed.

RESULT

Analysis of Brown Algae Bioactive Compounds

No	Secondary Metabolite	Methods	Result *Sargassum* sp.
1.	Phenolic	FeCI3 5%	0.09 ± 0.01
2.	Flavonoids	a. HCI + Mg	0.15 ± 0.01
		b. NaOH 10%	0.12 ± 0.02
3.	Triterpenoids	Lieberman-Burchard	N/A
4.	Alkaloids	Dregendorf	+

Survival Rate

The survival rate (SR) values obtained for Siamese catfish seeds are as follows:

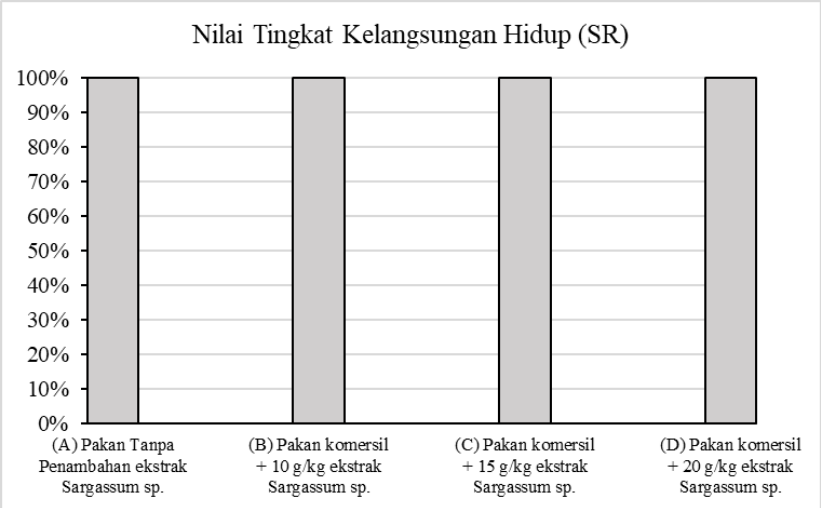


Fig 2. Survival rate (SR)

Intestinal Histologyi

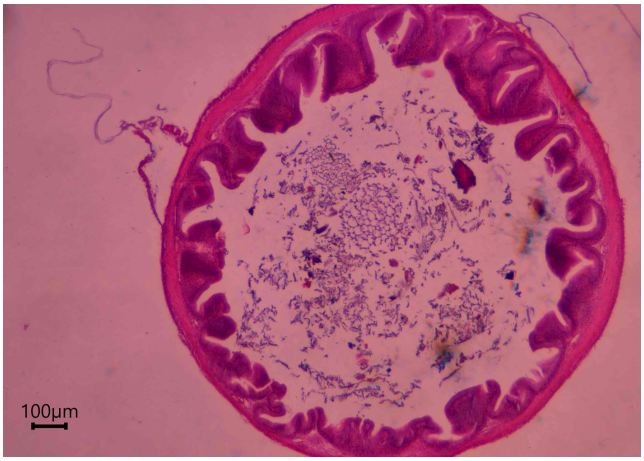
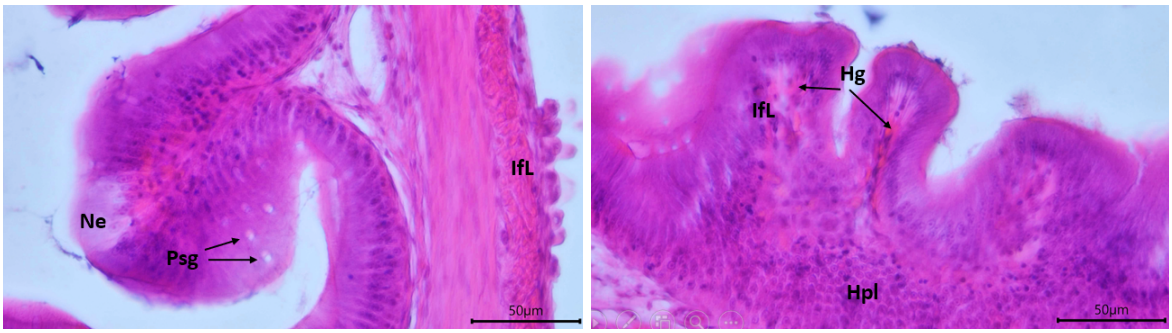


Fig 3. Histological picture of the intestine (fish fed without the addition of Sargassum sp. extract), (100x magnification).
(Source: Personal 2024)



Information: Treatment A (Feed without the addition of Sargassum sp. extract), (Magnification 400x), Damage: Leukocyte infiltration (IfL), Necrosis (Ne), Goblet cell proliferation (Psg), Hyperplasia (Hpl), Hemorrhage, Damage Score: 1.

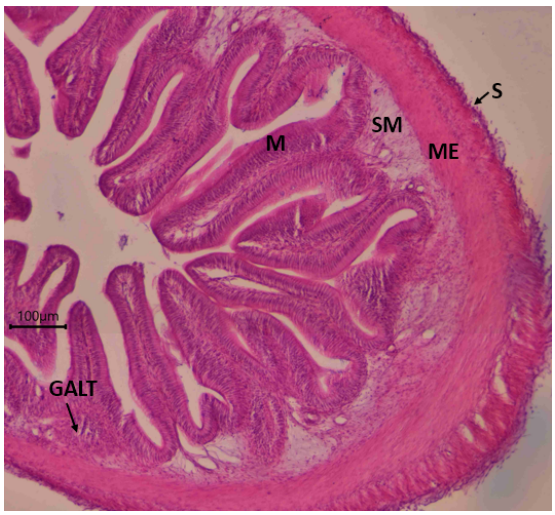
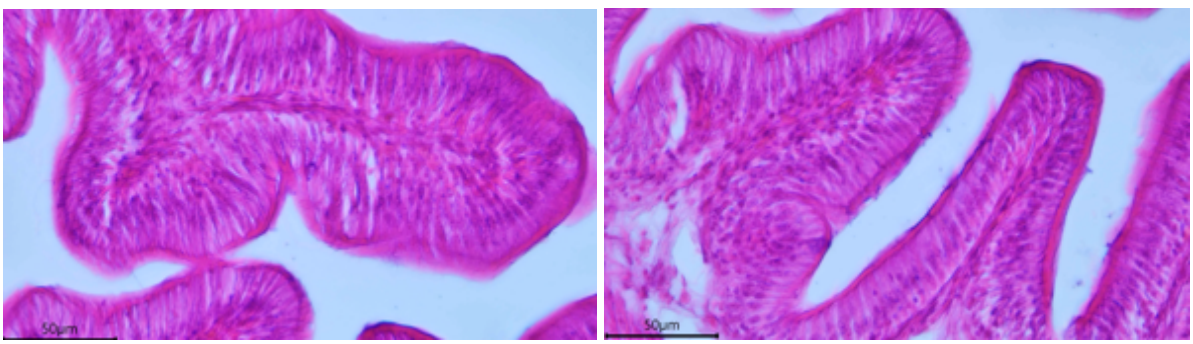


Fig 4. Histological picture of the intestine (fish fed with the addition of Sargassum sp. extract), (GALT) gut-associated lymphoid tissue, (ME) Muscularis external, (SM) submucosa, (S) Serosa, (M) mucosa.
(100x magnification).
(Source: Personal 2024)



Information: Treatment D (Commercial feed + 20 g/kg Sargassum sp. extract), (400x Magnification), No Damage, Damage Score: 0.

Liver Histology

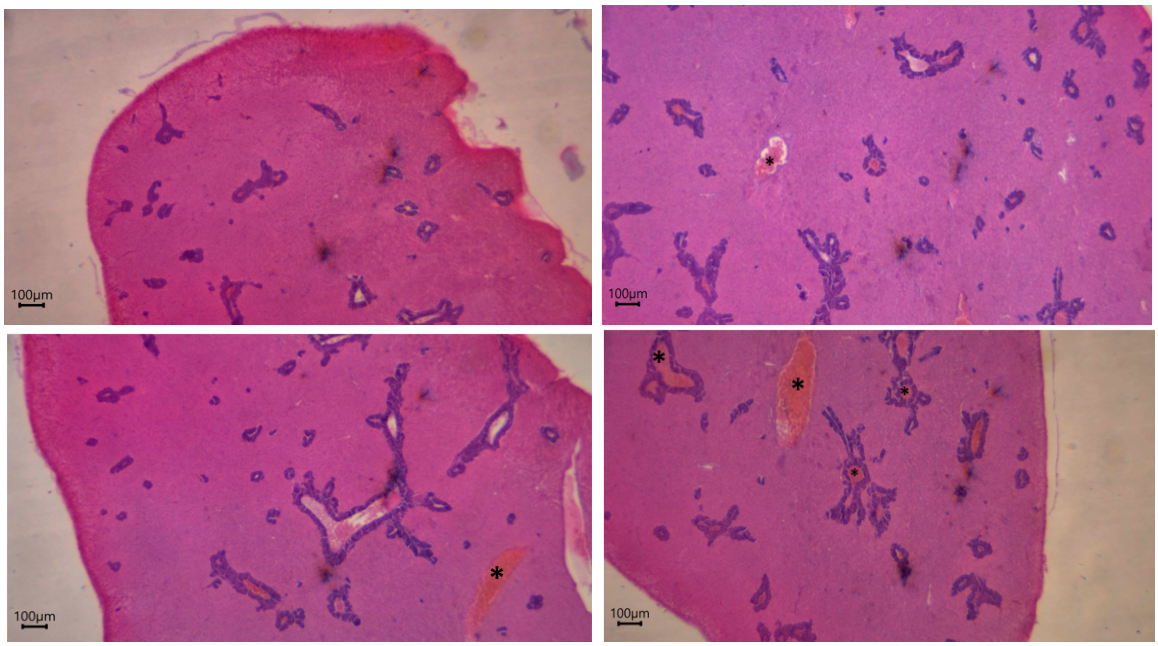
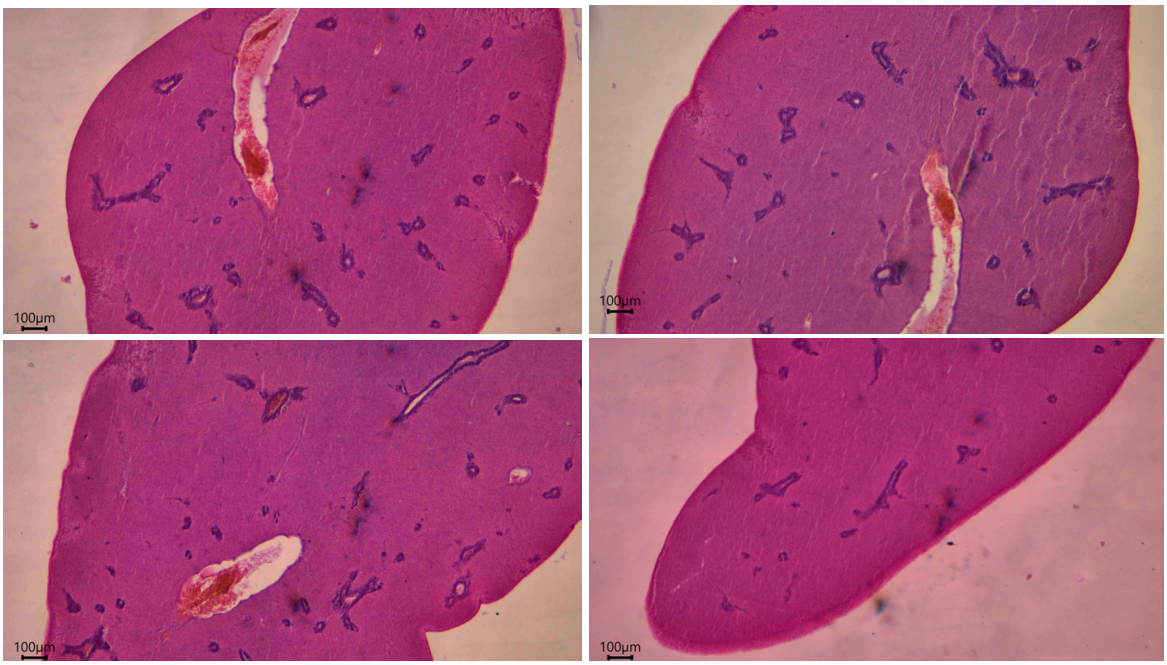


Fig 5. Liver histology (Fish fed without the addition of Sargassum sp. extract or Treatment A), (40x magnification).
Damage: *Congestion in the blood vessels.



Fih 6. Liver histology (Fish fed with added Sargassum sp. extract or Treatment D), (40x magnification).
Description: No damage.

DISCUSSION

Survival Rate

Based on the results obtained, the survival rate (SR) value of Siamese catfish seeds that were reared for 40 days showed no difference or had the same percentage, namely 100% (Figure 2). Thus, these results state that Siamese catfish seeds show good adaptability during

rearing so that there is no real effect of the treatment given on fish survival parameters. Mulyani (2014) in Herlina & Widayarti (2020) states that fish survival is very dependent on the fish's adaptability to food, the environment and the fish's health status. Arzad et al. (2019) stated several factors that play a role in fish survival rates, including stocking density, feed supply, disease and quality of cultivation media.

Histopathology

Based on the results of observations on the histopathology of the intestines and liver of Siamese catfish fry, it showed that damage occurred in fish fed without the addition of Sargtassum sp. extract, while fish fed with the addition of Sargassum sp. extract. did not show any damage to either the intestinal organs (Figure 4) or the liver (Figure 6). Treatment A, which is a control treatment or feed without the addition of Sargassum sp extract. Damage to the intestinal organs includes: Leukocyte infiltration (IfL), necrosis (Ne), goblet cell proliferation (Psg), hyperplasia (Hpl), hemorrhage, with a damage score of 1 (Figure 3). The liver in treatment A showed congestion in the blood vessels (Figure 5).

GALT in Fish fed Algae Extract Supplements

The major mucosa-associated lymphoid tissues (MALT) in teleosts include the skin-associated lymphoid tissue (SALT), which encompasses both diffuse lymphoid tissue and microbiota (Salinas 2015, Mitchell & Criscitiello 2020), the gill-associated lymphoid tissue (GIALT), the intrabronchial lymphoid tissue (ILT) (Koppang *et al*., 2010), the recently identified nasopharynx-associated lymphoid tissue (NALT) situated in the olfactory organ (Salinas 2015, Sepahi & Salinas 2016), and the buccal-pharyngeal-associated lymphoid tissues (OFALT) (Yu, Y-Y., *et al.,* 2019). Among these, the gut-associated lymphoid tissue (GALT) is extensively researched in teleosts due to its critical role in fish health (Lee *et al*., 2021, Gomez *et al*., 2013, Tacci *et al*., 2014, Parra *et al*., 2016, Salinas *et al*., 2021). While the GALT generally exhibits similar morphology across different fish species, variations in structure can occur depending on whether the fish are herbivorous, carnivorous, or omnivorous (German & Horn, 2006). Teleost fish are among the earliest organisms possessing elements of an adaptive immune system, including major histocompatibility complex (classes I and II) and B and T cells (Parra et al., 2016, Sunyer 2013). Studies on adaptive mucosal immune responses in teleost fish, particularly in rainbow trout (*Oncorhynchus mykiss*) and plaice (*Pleuronectes platessa*), have concentrated on immunization via oral and parenteral routes (Guerrera *et al.,* 2022, Wilson & Castro 2010, Salinas 2015). The GALT in fish comprises various leukocytes such as intraepithelial lymphocytes (IELs) and lamina propria leukocytes (LPLs), including lymphocytes and phagocytic cells like granulocytes, macrophages, and dendritic-like cells (Lee *et al*., 2021).

CONCLUSSION

The conclusion of this research is the effect of adding bioactive compounds from Sargassum sp extract. on the feed on the health picture of Siamese catfish based on the results of observations on the histopathology of the intestines and liver showing that fish fry were fed with Sargassum sp extract. experienced damage than fish seeds fed without the addition of Sargassum sp extract. (control). Damage that occurs to the intestinal organs includes leukocyte infiltration (IfL), necrosis (Ne), goblet cell proliferation (Psg), hyperplasia (Hpl), and hemorrhage, with a damage score of 1, while damage to the liver of fish fry Siamese catfish experiences congestion in the blood vessels.

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