

# Utilization Of Soursop Leaf, *Annona Muricata* Extract For Improving Growth Performance And Reduce Cholesterol Levels In Fattening Mangrove Crab, *Scylla Olivacea*

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## Abstract

Soursop leaf, *Annona muricata* is a type of plant that contains active compounds, one of which is flavonoids, which can reduce cholesterol levels. This study aims to determine the dose of soursop leaf extract, *A. muricata* the optimum for adding to betet-bete fish as feed in mangrove crab cultivation. This research was conducted at the Takalar Brackish Water Aquaculture Fishery Center, South Sulawesi Province for 28 days. The test animals used were mangrove crabs, *Scylla olivacea* 60 males with average weight  $158.98 \pm 15.91$  g which were kept in the crab house measuring length, width, and height of 35 x 19 x 17 cm each filled with 1 crab per container. This study consisted of 4 treatments and 3 replications each with the addition of soursop leaf extract doses of 0 (control treatment/A), 200 ppm (B), 400 ppm (C), and 600 ppm (D) for the betet-bete fish. Data management uses ANOVA and W-Tuckey test. The results showed that the higher the dose of soursop leaf extract, *A. muricata* showed a significant effect on survival, cholesterol levels, hemolymph triglyceride levels, protein, fat and carbohydrate levels, and did not significantly differ on daily growth, absolute growth and feed efficiency. Based on this study it was concluded that the best additional dose of soursop leaf extract, *A. muricata*, was 400 ppm.

**Keywords:** *Annona Muricata*, Bete-Bete Fish, Cholesterol, Mangrove Crab, Soursop Leaves

## INTRODUCTION

mud crab, *Scylla* spp. is one of the fishery commodities with economic value and has considerable commercial market potential. This is because mangrove crabs have meat taste which is delicious, delicious, and has a good nutritional content. According to Karim et al. (2005), based on the results of proximate analysis it is known that mangrove crab meat contains 44.85-50.58% protein, 10.52-13.08% fat and 3.579-3.724 kcal/g energy. However, it is known that crab meat also contains high enough cholesterol which can be harmful to health. Based on research by Syafiq (2008) it is known that crab meat has a cholesterol content of 76 mg/100g, while the USA Department of Agriculture found that crabs have a cholesterol content of 78 mg/100g. The increased amount of feed consumption will also increase total cholesterol in the body (Sheen, 2000).

The feed that is often used in mud crab cultivation is trash fish because the amount of trash fish is still abundant, has an aroma that can trigger crabs to eat and contains nutrients that are good for growth. Based on this, an alternative is needed to increase the growth of mud crabs but can reduce the cholesterol content of mud crabs. Some compounds that can lower cholesterol levels are phenolics, flavonoids, sitosterol, vitamins. Furthermore, soursop leaves have quite high

levels of flavonoid compounds. Flavonoids can reduce cholesterol levels by increasing the excretion of bile acids, where the most excretion of cholesterol is through the bile so that cholesterol is converted into bile acids and used for digestion (Ujjani, 2015).

Flavonoids can also reduce blood viscosity, thereby reducing the occurrence of fat deposition in blood vessels (Wurdianing et al., 2014) In addition, flavonoids also function as antioxidants, antimicrobials, antivirals and growth regulators (Iswadi et al., 2019). The ability of soursop leaves to lower cholesterol levels is caused by the content of sitosterol. The mechanism of action of sitosterol is similar to that of ezetimibe, which is to reduce the absorption of cholesterol from the digestive tract, so in this way sitosterol can reduce blood cholesterol levels (Unepetty et al., 2013). Soursop leaves can lower blood cholesterol levels. The results of research conducted by Unepetty et al. (2013), stated that giving soursop leaf infusion had the effect of reducing blood cholesterol levels in white rats. Another study stated that administration of soursop leaf extract at doses of 100 and 200 mg/kg rat weight can reduce cholesterol in male white rats (Na'i et al., 2019). This study was conducted to determine the effect of soursop leaf extract of *A. muricata* and determine the best dose in reducing cholesterol content in mud crab meat *Scylla olivacea*.

## Methodology

### Time and Place of Research

This research has been carried out in months September to October 2022 at the Takalar Brackish Water Aquaculture Fisheries Center (BPBAP), South Sulawesi Province. Identification of flavonoid compounds and proximate analysis, cholesterol content of feed and test animals was carried out at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar. Water quality analysis in Productivity and Water Quality Laboratory, Faculty of Maritime Affairs and Fisheries, Hasanuddin University, Makassar.

### Research Materials

The test animals used in this study were mangrove crabs, *Scylla olivacea* male with average body weight  $158.98 \pm 15.91$  g, totaling 60 individuals, each of which was stocked at a density of 15 individuals per treatment. Maintenance of crabs using crab house as many as 60 pieces. Each crab house is filled with one crab. Crab maintenance is carried out for 28 days.

The test feed used was trash fish obtained from the fish auction site (TPI) with dosage 10% of body weight/day added with various doses of soursop leaf extract, *A. muricata*. Flavonoid levels in the bete-bete fish which have been added to various doses of soursop leaf extract, *A. muricata*, cholesterol levels and nutritional composition of the bete-bete fish are presented in tables 1 and 2.

**Table 1** Flavonoid content of the test feed (bete-bete fish) which has been added to soursop leaf extract at different doses in mangrove crab cultivation.

Dosage of Soursop Leaf Extract (ppm)	0	200	400	600	Pure Extract
Flavonoids (mg/kg)	0	61,31	86,18	118.76	962.86

Remarks: results based on original sample

<sup>a</sup>= Extract Ingredients Without Nitrogen

**Table 2** Cholesterol levels and nutritional composition of feed (fresh bete-bete)

Fresh food for bete-bete fish	Cholesterol Levels (mg/100gr)	Nutrients (%)					
		Water	Crude protein	Crude Fat	Coarse Fiber	BETN*	Ash
	78.08	76.39	76,2	2.65	0.13	1.68	19.34

Description: \*Nitrogen-Free Extract Ingredients

### Research procedure

The preparation of the research container started with cleaning all the crab houses that had been provided at BPBAP Takalar from dirt particles with clean water. Next is to check the water circulation pipe to ensure that there is no clogged dirt. The crab house used measures length, width and height, respectively, namely 35 x 19 x 17 cm. before stocking, the crabs are first weighed and sorted then put into the crab house and marked. Before the crabs are treated, the crabs are acclimatized and fasted for 3-4 days according to environmental conditions. The soursop leaves used are not too young. The leaves (*Annona muricata* L) were weighed as much as 6 kg then washed and then aired without direct sunlight for 5 days. The dried leaves are separated from the midrib of the leaf, then cut into small pieces after that the leaves are mashed using a blender and then weighed as much as 1 kg. After weighing as much as 1 kg. Then the soursop

leaf powder is macerated to dissolve its chemical compounds using 5 liters of 96% ethanol solution for 24 hours and then filtered until it is separated from the dregs. 2,050 ml of the solution which was filtered using filter paper measuring 0.45 µm was then evaporated using an evaporator to obtain 104.275 grams of 104.275 gram pure extract of 100% soursop leaves.

The feed used in this study was trash fish, fresh bete-bete fish. Nugroho (2006) reported that the proximate composition of bete-bete fish was 13.52% protein, 77.07% water, 3.95% fat, 4.56% ash and 0.90% carbohydrates. Before being given treatment, trash fish that have been obtained from fish auction places or local traders are removed from the entrails, head and scales and then washed clean. After that, it is cut into 1-2 cm sizes to make it easier for the crabs to pick up and eat it. After being cut into small pieces, trash fish is soaked for 20-30 minutes until the soursop leaf extract solution is absorbed, then sprayed with 3% chitosan solution and then air-dried. The maintenance process starts with pre-selection of test crabs based on weight and physical condition, namely not disabled and according to the required size. Furthermore, the test crabs were stocked into the crab house as much as one for each crab house and acclimatized for 3-4 days. During the acclimatization process, the test crabs were fed trash fish. The day before giving the test feed, the test crabs were fasted for 12 hours. Next, done feeding 2 times a day in the morning as much as 30% and in the afternoon as much as 70% of the total feed 10% of body weight (Karim, 2013). Water quality measurements carried out during maintenance included temperature, salinity, pH, DO and ammonia. Observations on water quality were carried out every day including temperature, salinity and pH while DO and ammonia were measured at the beginning, middle and end of the study and for cholesterol analysis, sampling was carried out at the beginning and end of the study.

This study used 4 treatments and each treatment had 3 replications. Thus, this study consisted of 12 experimental units. The treatments tested were various doses of soursop leaf extract, *A. muricata* which were added to the feed (bete-bete fish), namely:

- A. 0 ppm (Control)
- B. 200 ppm
- C. 400 ppm
- D. 600 ppm

### Observed Parameters

#### Daily and Absolute Growth

Daily growth will be calculated by Effendi's formula (1979):

$$G = \frac{W_t - W_0}{t} \times 100\%$$

Information:

G = Daily growth rate (%)

W<sub>t</sub> = average weight of test animals at the end of the study (g)

W<sub>0</sub> = average weight of test animals at the start of the study (g)

t = maintenance time (days)

Absolute growth is calculated according to the formula:

$$h = W_t - W_0$$

Information :

h= individual absolute weight growth (g)

W<sub>t</sub> = individual final weight (g)

W<sub>0</sub> = individual initial weight (g)

#### Life sustainability

Survival or survival rate is calculated using the formula:

$$SR (\%) = \frac{N_t}{N_0} \times 100$$

## NO

Information:

SR = survival of test animals (%)

Nt = Number of live animals at the end research (tail)

N<sub>0</sub> = The number of live animals at the beginning research (tail)

### Cholesterol Levels

Cholesterol levels of the test animals will be observed at the beginning and end of the study. The part of the test animal that will be sampled is mangrove crab meat. Determination of cholesterol content carried out by the Liebermann Burchard method (Schunack et al., 1990).

### Hemolymph Triglycerides

Observation of triglyceride levels was carried out at the end of the study with previously fasted crabs to see at what hour the hemolymph triglyceride levels decreased after being fed. Then crab blood was taken at 0, 6, 12 and 18 hours after feeding. The triglyceride analysis method for crab blood was carried out, namely blood was taken and put into a centrifugation tube at 3000 rpm for 30 minutes (this is the optimal time and speed in separating blood plasma from serum). (Siahaan et al., 2015). After the serum was obtained, 10 µL was added with 1000 µL reagent, stirred until homogeneous, and carried out 3 times. The addition of 10 µL of distilled water and 1000 µL of reagent. Standard solution containing 10 µL of 200 mg/dL triglyceride standard and 1000 µL of reagent. Samples were kept for 10 minutes. After that, serum activity was carried out with LOT D393 Biocon Germany with a wavelength of 546 nm.

### Proximate Composition

The proximate test is a test for determining the nutritional composition including fat protein, nitrogen-free extract (BETN), crude fiber, ash and water in the trash fish trial feed that has been treated by soaking in soursop leaf extract solution as well as in the test crab meat in units of weight dry (Takuchi, 1998).

### Water quality

As supporting data, during the research, several physical and chemical parameters of water will be measured, including: salinity, temperature, pH, dissolved oxygen, and ammonia. Salinity was measured using a refractometer, temperature with a thermometer, pH with a pH meter, dissolved oxygen with a DO meter and ammonia using a spectrophotometer. Measurement of the physical and chemical parameters of water in the morning and evening including temperature, pH, salinity and DO and ammonia were measured at the beginning, middle and end of the study.

### Data analysis

Obtained data in the form of cholesterol levels in mangrove crab meat, feed conversion ratio and media water quality will be analyzed descriptively according to the needs and feasibility of mud crabs.

## Results and Discussion

### Growth of Mangrove Crab

The average daily growth in mud crabs kept for 28 days in each treatment can be seen in Table 3.

**Table 3** The average growth of mud crabs after being reared for 28 days

NO	Dosage of Soursop Leaf Extract (ppm)	Average Daily Growth (%)	Average Absolute Growth (%)
1	0	61.46±6.47a	15.80±0.28a
2	200	62.38±5.19a	15.87±0.23a
3	400	58.31±3.34a	16.07±0.50a
4	600	59.05±1.09a	16.53±0.31a

Absolute and specific weight gain of mud crabs were obtained within 28 days of study. Absolute and specific weight gain data were tested using ANOVA with a level of confidence ( $p > 0.05$ ) and showed that the feeding of bette-bete trash fish had no significant effect on each treatment with values between 57.60-62.38% for growth. specific and 15.80–16.53 g for absolute growth. Even though various doses of soursop leaf extract had been added, the crabs reared during the study continued to eat the trash fish voraciously so that there was no difference in the amount of nutrient intake. Then the frequency of feeding for each treatment is also the same 2 times a day in the morning as much as 30% and in

the afternoon as much as 70% of the total feed 10% of body weight so that there is no significant difference between each treatment. Meanwhile, according to Tacon (1987) the thing that causes a decrease or slow growth in crabs is because the amount of feed given is too little so that cultivators will utilize the protein in their bodies to maintain energy needs. Although there was no significant difference in each treatment, the average daily and absolute growth of mud crabs was 60.12% for specific growth and 16.02 g for absolute growth. This is due to a supportive environment and appropriate feed quality. When viewed from the nutritional content of the feed used, the bete-bete fish contains carbohydrates of 1.81%, 76.2% crude protein, 2.65% crude fat, 19.34% ash and 76.39% moisture content (table 3). This is related to the nutritional needs of mangrove crabs which include carbohydrates, proteins, fats, vitamins and minerals (Praing et al., 2014), where protein is an important nutritional component that can form and repair damaged body tissues, as a component of enzymes and a source of energy for metabolism (Fujaya et al., 2019). Then good growth is also supported by the easy digestibility and bioavailability of the bete-bete fish (Praing et al., 2014).

### Life Sustainability

The average survival of mud crabs reared for 28 days in each treatment can be seen in Table 4.

**Table 4** Average survival of mud crabs for 28 days of rearing in each treatment

NO	Dosage of Soursop Leaf Extract (ppm)	Average Survival (%)
1	0	73.3±11.54b
2	200	86.7±11.54ab
3	400	93.3±11.54ab
4	600	100.0±0.00a

*Note: Different letters in the average survival column indicate differences significant between each treatment ( $p < 0.05$ ).*

Giving bete-bete fish with the addition of various doses of soursop leaf extract of *A. muricata* had a significant effect on the survival of mud crabs *S. olivacea* ( $p < 0.05$ ), which ranged from 73.3-100% (table 4). The best survival was in treatment D with the addition of 600 ppm soursop leaf extract. This is because in treatment D the feed given contains the highest soursop leaf extract when compared to the other treatments. Even though the feed and maintenance conditions given were the same, soursop leaf extract added at different concentrations played a good role in supporting the survival of mangrove crabs. It is known that soursop leaf extract contains secondary metabolites such as phenols, flavonoids, tannins, phytosterols, calcium oxalate, alkaloids, steroids, cardiac glycosides, anthraquinones, saponins, coumarins and steroid oils (Sunarjono, 2005). These compounds have a function as a disinfectant-antiseptic (Hasmila, 2019).

Soursop leaves of *A. muricata* contain acetogenin compounds which have many benefits, including anticancer, antitumor, anti-inflammatory, anti-depressant, antiviral and antibacterial (Zuhud, 2011). The higher the dose of soursop leaf extract added to the mangrove crabs, the better the survival. Crabs that were reared for 28 days in treatment D or the highest dose did not experience death, in contrast to treatment A or control, which had the lowest survival rate, which was around 73.3%. (Londok & Mandey, 2014), revealed that in 1997 a clinical study found that a novel alkaloid in soursop fruit was an antidepressant in animals. It was also stated that the acetogenin compounds in soursop generally have antitumor, antiparasitic, insecticidal, and antimicrobial activities. It is suspected that it is these secondary metabolites that have the ability to protect, then as antioxidants that can neutralize the instability that occurs due to the presence of reactive molecules called free radicals. These free radicals can attack body cells every day, but the presence of flavonoid compounds can sweep away free radicals (Londok & Mandey, 2014). Then another thing that supports the high survival of the crabs kept during the study is the container used. Crab house length, width and height respectively, namely 35 x 19 x 17 and 1 crab was stocked per container in each treatment. This can reduce the occurrence of cannibalism which is a striking characteristic of crabs which can cause low survival (Tulangow et al., 2019). This is supported by the results of Haikal's research (2022) which achieved 100% survival rate for local crabs using the apartment model. Crab isolation is considered to have an effect on their survival, because cultivation using ponds produces a survival rate of 77-80% (Khotimah, 2018).

### Cholesterol Levels in Crab Meat

The average cholesterol levels in mud crabs reared for 28 days in each treatment can be seen in Table 8.

**Table 5** Average cholesterol levels in mud crabs for 28 days of maintenance in each treatment

NO	Dosage of Soursop Leaf Extract (ppm)	Average Cholesterol Level (MG/100g)
1	0	96.1±5.14a
2	200	59.44±5.88b
3	400	53.56±9.89b

4	600	68.46±9.64b
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Note: Different letters in the average cholesterol column indicate a significant difference between each treatment ( $p < 0.05$ ).

The addition of soursop leaf extract, *A. muricata* to bette-bette fish as feed for mangrove crabs, *S. olivacea* had a significant effect on cholesterol levels in crab meat ( $p < 0.05$ ), which ranged from 53.56 to 96.10 mg/100g (Table 5). The highest meat cholesterol content was produced by control treatment crabs which did not receive any addition of soursop leaf extract, *A. muricata*. The high levels of cholesterol in treatment A crabs were due to the relatively high cholesterol content of bette-bette fish, which was 78.08, in line with what was stated by Silalahi (2006), that cholesterol is produced in the body in the liver about 80% and the rest will be found in foods high in cholesterol consumed. Pramudya et al. (2013) who measured the cholesterol levels of mangrove crabs in two different areas found that male crabs had a relatively higher cholesterol content than females reared under controlled conditions by giving trash fish containing 70 mg/g cholesterol twice a day.

The high cholesterol level is thought to be because male crabs have a higher metabolic rate than female crabs, so their eating activity is greater. Then the energy stored in the male crab's body is only used for the growth and enlargement of the claws. Then the media used for maintenance, namely the crab house also supports it so that the movement of the crabs becomes limited causing the mud crabs to not expend a lot of energy to move, find food and defend themselves from predators. Energy that should be wasted to carry out activities will be stored as food reserves in the form of fat which contains cholesterol (Pramudya et al., 2013).

In certain levels, cholesterol is needed by the body as a precursor for many physiological components, such as steroid hormones and molting hormones, adrenal corticoids, bile acids, and vitamin D (Sheen, 2000). Cholesterol is also needed to fulfill several endocrine functions, namely for the process of gonadogenesis, ovary maturation and larval development (Wouters et al., 2001). However, it is different from its function when cholesterol levels are normal, the higher the cholesterol level in the blood, the greater the risk of atherosclerosis. Atherosclerosis is a buildup of fat, cholesterol and other substances in and on the walls of the arteries which can block blood flow throughout the crab's body (Sargowo, 2015). Atherosclerosis is caused by arterial muscular enlargement and is characterized by endothelial dysfunction, vascular inflammation, accumulation of lipids, cholesterol, calcium, cellular debris in the intima of blood vessels. The accumulation will cause blood flow abnormalities and decreased oxygen supply to target organs (Murwani et al., 2013).

High cholesterol levels also had an impact on the survival of treatment A crabs, it was seen that during the study the crabs that died the most were those that were not given a dose of soursop leaf, *A. Muricata* extract in line with what was stated Guyton and Hall (2011), that cholesterol is also thought to be responsible for increased oxidative stress. Oxidative stress is a condition where the number of free radicals in the body exceeds the body's capacity to neutralize them. As a result, the intensity of the oxidation process of normal body cells becomes higher and causes more damage. Ismawati et al. (2017), also reported that cholesterol is transported in lipoproteins in plasma, the largest proportion of which is in LDL. Giving bette-bette fish which contains high cholesterol will increase the formation of plasma LDL where LDL has atherogenic properties so that it can stick to blood vessel walls and can cause accumulation of lipid components, so that this condition facilitates the process of oxidation by free radicals and causes oxidative damage to lipids.

Wurdianing et al. (2014) reported that soursop leaf extract played an important role in reducing cholesterol levels in mangrove crab meat. Soursop leaves contain active compounds, including flavonoids, alkaloids, fatty acids, phytosterols, myrsin and alcohol. Flavonoids play a role in lowering cholesterol levels by increasing the excretion of bile acids and reducing blood viscosity, thereby reducing the occurrence of fat deposition in blood vessels (Ranti et al., 2013).

The tannin content in soursop leaf extract is thought to also play a role in reducing cholesterol levels in mangrove crab meat by preventing reabsorption and increasing excretion. Cholesterol Creation. Tannins are antioxidants that act as anti-free radicals and activate antioxidant enzymes. Tannins also prevent the oxidation of LDL cholesterol, stimulate the secretion of bile salts and remove cholesterol through the feces (Lajuck, 2012). In addition, the content of saponin compounds in soursop leaf extract is thought to reduce cholesterol levels in mangrove crab meat by binding to cholesterol in the digestive tract so as to prevent cholesterol reabsorption. In addition, saponins reduce the absorption of bile by forming micelle complexes that are too large to be absorbed. Saponins and cholesterol also have the same receptors so competition for cholesterol receptors on cells can occur (Na'i et al., 2019).

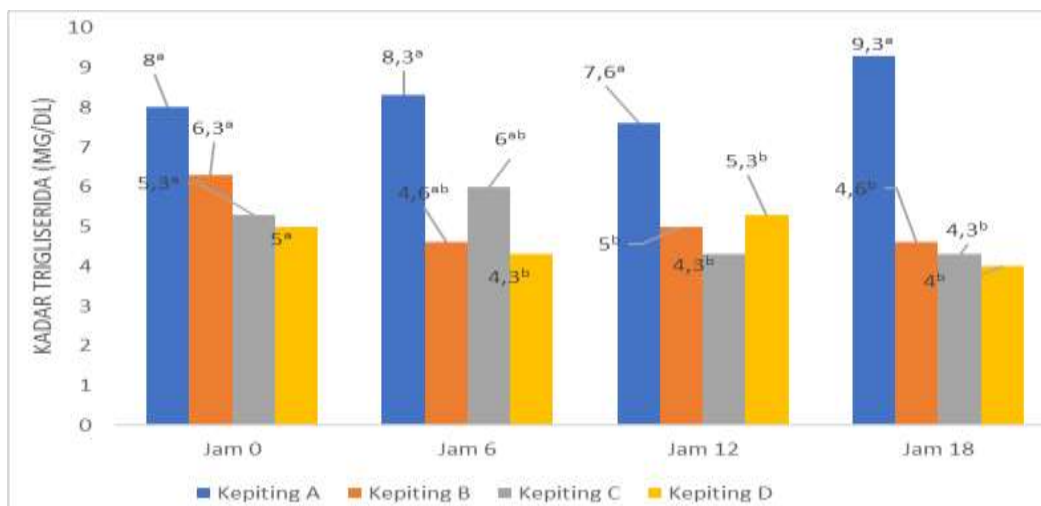
In this study, there was an increase in crab meat cholesterol levels in treatment D with the addition of a dose of soursop leaf extract, which was 600 ppm higher than at doses of 200 and 400 ppm. The increase in cholesterol levels that occurs is thought to be caused by sitosterol contained in soursop leaf extract. Sitosterol acts as a plant fat that can increase HDL or good cholesterol (Unepatty et al., 2013). Wurdianing et al. (2014), also reported that soursop leaf extract was

able to increase HDL cholesterol levels. HDL plays a role in transporting cholesterol from peripheral tissues and decomposed back in the liver.

Research conducted by Wurdianing et al. (2014), which used soursop leaf extract was shown to reduce cholesterol, triglyceride and LDL levels but could increase HDL levels in male white rats. This is presumably because the flavonoid compounds contained in soursop leaf extract can increase lecithin cholesterol acyl transferase (LCAT). LCAT is an enzyme that converts free cholesterol into cholesterol esters and is essential for the maturation of HDL metabolism and the cholesterol esters collected by HDL cholesterol are returned to the liver. There has been no previous research using soursop leaf extract to reduce cholesterol levels in mangrove crabs. However Ali (2021), using seaweed, *Kappaphycus alvarezii* which contains flavonoid compounds can reduce cholesterol levels in mangrove crabs, *Scylla* spp.

### Hemolymph Triglycerides

The average hemolymph triglyceride content of mud crabs consuming trash fish with the addition of various doses of soursop leaf extract, *A. muricata* is shown in Figure 5



**Figure 5** The average crab hemolymph triglyceride levels were fattening mud crabs that consumed trash fish with the addition of various doses of soursop leaf extract, *A. muricata*.

The addition of soursop leaf extract, *A. muricata* to the bete-bete fish had a significant effect on reducing blood triglyceride levels of mud crabs ( $p < 0.05$ ). Hemolymph triglyceride levels of mud crabs fluctuated in each good treatment which did not receive the addition of soursop leaf extract *A. muricata* but in treatments B, C and D which consumed bete-bete fish with the addition of soursop leaf extract, *A. muricata* tended to have higher triglyceride levels. lower when compared to the control treatment (Figure 5).

At hour 0 there was no significant difference between each treatment ( $p > 0.05$ ). Lestari (2021), who also observed triglyceride levels of mud crabs reported that at 0 hours of feeding gel, triglyceride levels of mud crabs did not decrease. This is because previously the crabs were given the same treatment, namely being fasted and then given food. The feed is consumed at 0 o'clock so that the new crabs do eating activities so that digestion in the intestine has not occurred. At the 18th hour it was seen that the triglyceride levels of the control treatment crabs had increased. The cause of increased triglyceride levels is caused by the food consumed which undergoes a process of digestion in the intestine into free fatty acids, triglycerides, phospholipids and cholesterol which are absorbed into chylomicrons (Sari, 2012).

Chylomicrons carry triglycerides and cholesterol into the bloodstream. Then triglycerides will experience decomposition by enzymes lipoprotein lipase to form free fatty acids and chylomicron remnants. The remaining chylomicrons will be metabolized in the liver to produce free cholesterol. Removal of cholesterol from the body through bile acid synthesis which takes place in the liver, so that the more cholesterol is excreted, the more bile acid production will increase (Murray et al., 2009).

Triglycerides are part of total cholesterol. When cholesterol is eliminated from the body, it will first be converted into bile acids. The  $7\alpha$ -hydroxylation reaction is an obligatory first step in bile acid biosynthesis, while limiting the rate of this reaction. The reaction is catalyzed by  $7\alpha$ -hydroxylase, a microsomal enzyme. This reaction is a change in cholesterol plus NADPH, oxygen and cytochrome P-450 into bile acids. This  $7\alpha$ -hydroxylation reaction requires oxygen, NADPH, and cytochrome P-450 oxidase. In this cholesterol hydroxylation reaction, oxygen is easily reduced to superoxide anion free radicals ( $O_2^{\cdot-}$ ). The chemical effect of  $O_2^{\cdot-}$  in the network is strengthened by its nature which causes free radical chain reactions. Murray et al. (2003) suggested that  $O_2^{\cdot-}$  bound to cytochrome P-450 is an

intermediate in the activation of oxygen in various hydroxylation reactions. So with an increase in the activity of cytochrome P-450 in mediating hydroxylation reactions, more and more free radicals are formed. More and more free radicals will disrupt Apo-C2 which is a cofactor for the lipoprotein lipase enzyme. Then chylomicrons consisting of triglycerides will accumulate in the serum (Murray et al., 2003).

Mud crab triglyceride levels in the treatment that received an additional dose of soursop leaf extract tended to be lower when compared to the other treatments even though there was no difference at 0 hour. However, at the 6th, 12th and 18th hours it was clear that the triglyceride levels of the crabs treated B, C and D were lower. The low levels of triglycerides in mud crab hemolymph are thought to be caused by soursop leaf extract which contains high antioxidants. The decrease in blood triglyceride levels in crabs can occur due to an increase in lipoprotein lipase, this increase is due to the antioxidants found in soursop leaf extract which work by inhibiting the occurrence of free radicals.

Many flavonoid compounds contained in soursop leaf extract act as free radical scavengers that have hydroxyl groups (OH-) on the aromatic ring and stop the lipid peroxide chain reaction by protecting cells and chemicals in the crab's body. The mechanism of action of antioxidants such as flavonoids plays a role in lowering cholesterol levels by inhibiting the absorption of cholesterol in the digestive tract and increasing the formation of bile acids from cholesterol to be excreted in the feces (Yokozawa et al., 2002).

### Proximate Composition

The results of testing the nutritional content of mangrove crab meat that consumed trash fish with the addition of various doses of soursop leaf extract, *A. muricata* can be seen in table 9.

**Table 6** Nutritional content in mangrove crab meat for 28 days of rearing in each treatment

Dosage of Soursop Leaf Extract (ppm)	Nutritional content (%bk)					
	Water	Crude protein	Crude Fat	Coarse Fiber	BETN	Ash
Early Crab	75.86	80,36	3.97	0.21	6.99	8,47
A crab	71.66	78,73	3.49	0.11	10.31	7,36
B crab	76.58	74,27	2.45	0.17	12.59	10.52
C crab	80.43	77,43	2.01	0.26	9.58	10.72
D crab	75.58	77,53	2.75	0.12	11,16	8.40

Notes: 1) All fractions are stated in dry matter

2) BETN = Extract Material Without Nitrogen.

The addition of soursop leaf extract, *A. muricata* to the bete-bete fish had an effect on the protein content of mud crab meat. Crab meat protein ranged from 74.27-78.73% (Table 9). In treatment A, the protein content of mud crab meat was higher but there was no significant difference with the other treatments. This is because the bete-bete fish consumed contains a fairly high protein, namely 76.2%. Aslamyah and Fujaya (2010), stated that the protein content needed by crabs in feed ranged from 34-54%. Crab growth requires a higher percentage of protein than fat and carbohydrates. Where protein is the most important component of feed that functions in the formation of body tissues, repairing damaged body tissues, as a component of enzymes and a source of energy for metabolism.

Explained by Ekawati (2008), that the low activity of crabs causes the existing energy to be used in the process of anabolism (a process that requires energy to convert small compounds into larger molecules such as amino acids into proteins). Therefore, the availability of sufficient energy from carbohydrates can increase protein levels in crab meat and also achieve high survival and growth. Protein levels in treatments B, C and D were suspected to be due to the administration of chitosan with the addition of 1% acetic acid according to what was stated Yuliasari et al. (2011) that although acetic acid is a weak acid with a pKa of 10-5, it is capable of dissolving a protein.

The addition of soursop leaf extract, *A. muricata* effect on the body of crab without nitrogen extracts (BETN). BETN of crab meat ranged from 9.58-12.59% (dry matter). The highest BETN was in treatment B and the lowest was in treatment C. Crab meat carbohydrates were derived from total crude fiber and extract material without nitrogen. This is related to the low levels of cholesterol in crab meat, because carbohydrates act as precursors in internal metabolic processes whose products are needed for growth, for example non-essential amino acids and nucleic acids. It can be seen that the lowest carbohydrates were in treatment C and the lowest cholesterol levels were also in treatment C, this is in line with what was stated by Harisud et al. (2019), under the high carbohydrate feed can affect the fat content. This is because carbohydrates apart from being a source of energy, can also be converted into body fat.

The addition of soursop leaf extract *A. muricata* effect on the levels of crude fiber, ash and crude fat of mangrove crab meat. Crude fiber, ash and fat content of crab meat ranged from 0.11-0.26%, 7.36-10.72%, 2.01-3.49%, respectively. A small amount of crude fiber is very good for crab growth because crude fiber can cause satiety and reduce feed

consumption. In addition to having the lowest cholesterol content, Crab treatment C crude fiber also had the lowest crude fat content when compared to other treatments. Although the fat content of the bete-bete fish was given the same in each treatment, the decrease in fat content was thought to be due to the addition of soursop leaf extract which contains antioxidant compounds (Wurdianing et al., 2014). also has a fraction that is difficult to digest and is a low energy source (Londok & Mandey, 2014), so that it can affect the growth of mangrove crabs.

Saponin compounds which are also contained in soursop leaf extract can inhibit pancreatic lipase and inhibit the absorption of free fatty acids. Pancreatic lipase functions to hydrolyze fatty acids of all long chains from positions 1 and 3 of the glycerol group on triacylglycerol to produce free fatty acids and 2-monoacylglycerol. In intestinal epithelial cells, fatty acids and 2-monoacylglycerols form triacylglycerols. So the process of inhibiting pancreatic lipase activity has an impact on decreasing triacylglycerol levels in the intestine. Triacylglycerol is a type of fat that can be found in the blood and is the result of the breakdown of the body from food (Setyawan et al., 2016).

### Water Quality

Water quality is one of the environmental factors that also influence the successful process of mud crab cultivation. In this research several physical and chemical parameters of water were measured including: temperature, pH, salinity, DO, and ammonia. The results of water chemistry physics measurements observed during the 28 days of rearing can be seen in Table 10.

**Table 8** Average water quality of crab rearing media fed with the addition of various doses of soursop leaf extract to reduce crab cholesterol levels in mud crab fattening

Parameter	Average value	Optimum Value	Reference
Temperature (0C)	26.5	25-35	(Karim, 2013)
Degree of Acidity (pH)	7	7.0-9.0	(Karim, 2013)
Salinity (ppm)	33.5	15-35	(Soim, 1999)
Dissolved Oxygen (ppm)	6	≥ 3	(William, 2003)
Ammonia (ppm)	0.004	0.003-0.005	(Misbah et al., 2017)

The water quality of the mud crab rearing medium measured was temperature, salinity, degree of acidity (pH), dissolved oxygen and ammonia (Table 10). The average temperature of the maintenance media is 26.50C. The media temperature is included in normal conditions because it is still in the optimum range, which is around 26-32. Abnormal temperatures can affect the growth of crabs because a lot of energy is wasted just to adapt to the environment. Fluctuating temperatures can also cause mud crabs to become stressed and can even result in death (Karim, 2013). Water temperatures lower than 200C will drastically reduce the activity and appetite of mud crabs. At that time growth will stop even though the crabs can still live (Katiandagho, 2014). Conversely, crab appetite will increase if the ambient temperature is normal (Tulangow et al., 2019). The average salinity of the rearing medium is 33.5 ppm. The salinity of the maintenance medium is still in the optimal range. Misbah et al. (2017), argued that Salinity is one of the most important abiotic factors affecting aquatic organisms. According to Fujaya (2008), mud crabs can live in the salinity range of 5-36 ppt, although during growth the crabs prefer salinity which tends to be low between 5-25 ppt. Kordi and Gufon (2007), also stated that mud crabs are classified as euryhaline animals (that is, they are able to tolerate salinity in a wide range), namely 0-35 ppt.

According to Katiandagho (2014), the salinity of waters is thought to affect the structure and function of aquatic organisms through osmotic pressure changes in the relative proportions of solvents, absorption coefficient and solubility saturation, density and viscosity, changes in light absorption, sound conductivity and electrical conductivity so that this will change species composition in ecological situations certain. The level of salinity during the study was relatively high, but the crabs were able to adapt and grow well because of the ability of the crabs to regulate osmosis properly (Soim, 1995). Salinity is a masking factor for the physical and chemical variables of water into a single unitary influence that has an osmotic impact on osmoregulation and bioenergetics (Karim, 2013).

The degree of acidity or often called the pH in the rearing medium during the study was 7. The normal pH is because the media and location of the rearing are under controlled conditions and are indoors so that the factors that can affect changes in water conditions are fewer when compared to rearing in ponds. Low pH cannot be used for the maintenance of mud crabs because it can cause stress on mud crabs and have an impact on growth (Edwards et al., 2004). The optimum pH for crab maintenance is in the range of 7-9 (How et al., 1992).

Dissolved oxygen levels (Dissolved Oxygen) the average media during maintenance is 6. Dissolved oxygen is needed by all living organisms for respiration, metabolic processes or exchange of substances which then produce energy for growth and development. Mwaluma (2002), stated that basically mud crabs can live in an aquatic environment with an oxygen range of 2.65-4.00 mg/l and for the best growth is >5 mg/l. How et al. (1992), also reported that good dissolved oxygen for mud crabs is more than 4 ppm.

The average ammonia level of the rearing medium is 0.004 ppm. This range is considered still feasible for crab cultivation. Ammonia is the main product compound of nitrogenous waste in waters originating from aquatic organisms (Cavalli et al., 2000). In decapod crustacea, the presence of ammonia in water is an indication of amino acid catabolism. Ammonia is toxic so that in high concentrations it can poison organisms. If the concentration of ammonia increases, it will affect the permeability of organisms and reduce the concentration of neutral ions, affecting growth and oxygen consumption (Boyd, 1990). Therefore, when rearing mud crabs, the ammonia level in the media is no more than 0.1 ppm (Kuntiyto & Supratno, 1994).

## Conclusion

Based on the results of this study it was concluded that; (1) The addition of various doses of *A. muricata* soursop leaf extract gave a good effect although it did not differ in each treatment; (2) The best dose of adding soursop leaf extract, *A. muricata* to betete fish to lower cholesterol levels is 400 ppm.

## Suggestion

For efforts to reduce cholesterol in mud crab meat, a dose of 400 ppm should be used which is added to feed for mud crab cultivation.

## Thank-You Note

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