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Effect of Using Fern Leaf Extract (*Diplazium esculentum*) in Raw Fish Feed on Growth and Feed Conversion Ratio of Mud Crab (*Scylla serrata*)

Olivia Ningsih [a], Muhammad Junaidi [a*] and Damai Diniawirisan [a]

[a] Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. Author ON designed the research, conducted the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MJ and Author DD managed the research analysis and managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Mangrove crab or known as *Scylla* sp. is one of the biota whose habitat is in coastal waters, especially in mangrove forest areas. The need for mangrove crabs still largely relies on natural catches that are fluctuating. For this reason, it is necessary to cultivate mangrove crabs in a controlled manner that can support an effective, efficient and economically profitable mangrove crab farming business. Cultivation can increase mangrove crab production to meet domestic and foreign

**Corresponding author: Email: m.junaidi@unram.ac.id;*

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market demand. An alternative that can be chosen for mangrove crab cultivation is an apartment system that is arranged vertically in the form of a storage rack. The water medium used in this apartment system cultivation uses a recirculation system. One of the efforts to accelerate the growth of crabs is by using steroid hormones. Ecdysteroid is the main steroid hormone that has the main function as a skin replacement hormone. In addition, this hormone also regulates physiological functions, such as growth, metamorphosis, and reproduction. Therefore, this study was conducted to determine the effect of using fern leaf extract (*Diplazium esculentum*) in raw fish feed with different doses on growth and feed conversion ratio in mud crabs. This research method uses a field experimental method with a completely randomized design (CRD) consisting of 5 treatments and 3 replicates with different percentage doses, namely: PA: Percentage of 0 ml/Kg, PB: Percentage of 100 ml/Kg, PC: Percentage of 125 ml/Kg, PD: Percentage of 150 ml/Kg, PE: 175 ml/Kg percentage. The results showed that the use of fern leaf extract in mangrove crab (*Scylla serrata*) feed had a significant effect on the growth of absolute weight, Absolute Length, SGR (Specific Growth Rate) and FCR (Feed Conversion Ratio) where the most influential treatment was treatment E which was the highest dose of 175 ml/Kg. This indicates that treatment E is the best dose treatment for growth and feed conversion ratio of mud crab (*Scylla serrata*).

Keywords: Mangrove crab (Scylla serrata); leaf extract (Diplazium esculentum); apartment system.

1. INTRODUCTION

Mangrove crab or known as Scylla sp. is one of the biota whose habitat is in coastal waters, especially in mangrove forest areas. Currently, the demand for mangrove crabs mostly relies on capture in nature, which fluctuates. The current world production of Scylla sp. is mostly derived from mangrove crab catches in the world in 2011 reaching 44,670 tons but began to decline to 38,055 tons in 2013. The reduction in catches also affected the number of Indonesian mangrove crab exports which decreased from 34,172 tons in 2013 to 28,080 tons in 2014 (Kiswanto, 2015).

It is necessary to carry out mangrove crab cultivation in a controlled manner that can support an effective, efficient and economically profitable mangrove crab farming business (Ario et al., 2019). In addition, cultivation can increase mangrove crab production to meet domestic and foreign market demands. An alternative that can be chosen for mangrove crab cultivation is an apartment system that is vertically arranged in the form of a storage rack. The water medium used in this apartment system cultivation uses a recirculation system (Kurniawan et al., 2022).

One of the efforts to accelerate growth in crabs is by using steroid hormones. Ecdysteroid is the main steroid hormone that has the main function as a molting hormone, besides that it also regulates physiological functions, such as growth, metamorphosis, and reproduction (Ario et al., 2019). Fern leaves are one type of fern plant that contains the hormone ekdisteroid, which is useful for accelerating molting of mangrove crabs (Romadhon et al., 2022). Therefore, this study was conducted to determine the effect of using fern leaf extract (*Diplazium esculentum*) in raw fish feed with different doses on growth and feed conversion ratio in mud crabs.

2. METHODOLOGY

This research was conducted in June-July 2024 at Empol Preparation Village, Sekotong Tengah, Sekotong District, West Lombok Regency, Indonesia. This research method uses a field experimental method with a complete randomized design (CRD) consisting of 5 treatments and 3 replications with different dosing percentages, namely:

1. PA : Persentase 0 ml/Kg
2. PB : Persentase 100 ml/Kg
3. PC : Persentase 125 ml/Kg
4. PD : Persentase 150 ml/Kg
5. PE : Persentase 175 ml/Kg

The five treatments were repeated 3 times, resulting in 15 experimental units. Each experimental unit was then invited into a crab apartment unit. The construction of the research layout design can be seen in Fig. 1.

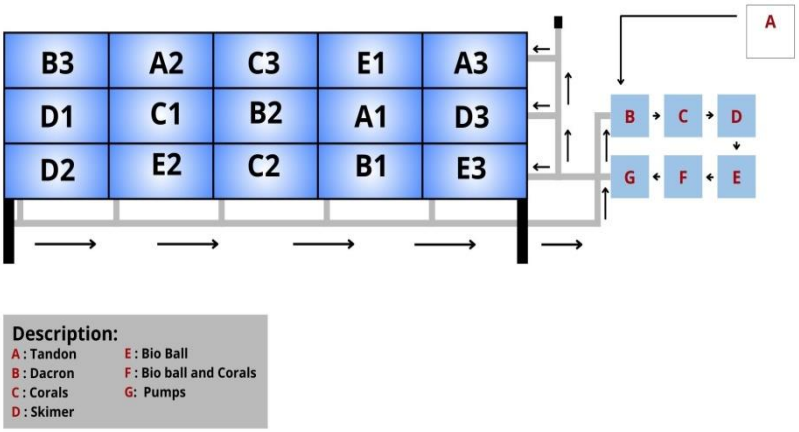

Fig. 1. Experimental designs

2.1 Research Procedure

The containers used in the study were apartments assembled using jirgens arranged vertically in the shape of a storage rack. Before stocking, it should be cleaned using soap and rinsed with fresh water. The apartment will be filled with seawater that has been diluted with fresh water so that it becomes brackish water with a salinity of 22-25 ppt. The maintenance container is filled with water to a height of 3-5 cm. Subsequently, the rearing container is immersed for 1 day.

The test animals are mangrove crabs (*Scylla serrata*) which are selected before stocking. Furthermore, the crabs that have been stocked will be acclimatized for 2-3 days. The crabs used had an average initial weight of 30-40 g/head, which was obtained from the catch of the community in the village of Empol Preparation, Central Sekotong, Sekotong Sub-district, West Lombok Regency, Indonesia. The fern leaves used are old fern leaves and are not eaten (not consumed). Then washed and air dried, then dried in the hot sun 3-4 days and then ground until smooth. Extraction was carried out at the Basic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University.

The feed used is kurisi (*Nemipterus* sp) mixed with fern leaf extract. Before mixing, the fish was first cut into smaller sizes. Then given the addition of fern leaf extract with as much as 0 ml / kg, 100 ml / kg, 125 ml / kg , 150ml / kg, 175ml / kg according to the treatment dose. Feed that has been mixed with fern leaf extract is stirred until homogeneous. Feeding is given once a day at 17:00 WITA. Measurement of absolute weight and length of mangrove crabs is done every 1 week. To measure the weight using analytical scales. Meanwhile, to measure the absolute length of the crab, the crab used a vernier caliper. Then water quality observations were made, namely measuring physical parameters such as temperature, chemical parameters such as pH, salinity and dissolved oxygen (DO) which were carried out once a week except for DO parameters carried out at the beginning and end of maintenance.

2.2 Research Parameters

Parameters measured in this study include absolute weight growth, absolute length measurement, survival rate (SR), daily growth rate (SGR), feed utilization efficiency (EPP), feed conversion ratio (FCR), blood profile and data analysis.

Absolute body weight growth is measured by the formula Wm = Wt - Wo. Wm = Absolute weight growth (gr); Wt = Final average weight (gr); Wo = Initial average weight (gr) (Samidjan et al., 2015).

Percentage of survival with the formula SR= Nt/No x 100%; SR=Survival rate (%); Nt=Number of mud crabs at the end of rearing (fish); No=Number of mud crabs at the beginning of rearing (fish) (Sagala et al., 2013).

Specific growth rate formula SGR = In Wt - In Wo/ T x100%; SGR = Daily growth rate (%); Wo

= Weight of test animals at the beginning of the study (g); Wt = Weight of test animals at the end of the study (g); T = Study time (days) (Asyhariyati et al., 2013).

Feed efficiency is calculated based on the formula EP = Wt-Wo/F x 100%; EP = Feed efficiency; Wt = Weight of test animals at the end of the study (g) Wo = Weight of test animals at the beginning of the study (g) F = Feed given (g) (Asyhariyati et al., 2013).

Food Conversion Ratio (FCR) refers to the formula FCR = F/Wt - Wo; FCR = Feed Conversation Ratio; F = Amount of feed consumed (g); Wt = Weight of test animals at the end of the study (g); Wo = Weight of test animals at the beginning of the study (g) (Adila et al., 2020).

The hemocyte assay can be calculated using the formula Hemocyte Cell Type Percentage = Number of Each Hemocyte Cell / Total Hemocytes x 100% (Sari and Ekawaty, 2016).

2.3 Data Analysis

The data obtained from this study are absolute weight growth, absolute length measurement, daily growth rate (SGR), feed utilization efficiency (EPP), feed conversion ratio (FCR) and blood profile. Will be analyzed using Analysis of Variance (ANOVA) with 95% confidence level. Furthermore, it is continued with the Ducan test and homogeneity test if significantly different results are obtained. While water quality data is presented descriptively.

3. RESULTS AND DISCUSSION

3.1 Absolute Weight Growth

The research was conducted for 43 days in Empol Preparation Village, Central Sekotong, Sekotong District, West Lombok Regency. Feeding the crab with fern leaf extract at different doses showed that the average absolute weight of the crab obtained ranged from 5.37%-11.62% as can be seen in Fig. 2.

Anova test results showed that there were significant differences between treatments. The results of the Duncan test analysis showed that PA (5.37±0.77a) was not significantly different from PB (7.38±1.97ab) and significantly different from PC (8.63±1.73bc), PD (9.87±2.33bc) and PE (11.62±0.78c). PE was significantly different from PA (5.37±0.77a) and PB (7.38±1.97ab). Based on the results obtained, the best treatment is the PE treatment (11.62±0.78c).

Based on the results of observations on the growth parameters of absolute weight, the highest was produced from treatment E (11.62 ± 0.78c) with a dose of 175mg / kg. while the lowest absolute weight growth was in treatment A control (5.37 ± 0.77a). this shows that PE energy obtained in consumed feed can be utilized properly. Fern leaves contain ecdysteroids which are not only useful for accelerating molting but also stimulating growth. Fern leaves, one type of fern plant, contain the hormone ekdisteroid which is useful for accelerating molting (Romadhon et al., 2022). Ecdysteroid is a steroid hormone that has a main function as a molting hormone, but also regulates physiological functions, such as growth, metamorphosis, and reproduction. Judging from the results of the study, the dose of fern leaf extract was 175ml/kg (Ario et al., 2019).

The results of the anova test of absolute length of mangrove crabs showed that there were significant differences between treatments. The results of Duncan test analysis showed that PA (0.0±0a) was not significantly different from PB (0.0±0a), PC (1.0±1.73a), PD (2.5±2.14ab) and significantly different from PE (4.2±0.72b). Based on the results obtained, the best treatment is the PE treatment (4.2±0.72b).

3.2 Absolute Length

Based on the research conducted, the highest absolute length gain was PE (4.2 ± 0.72b) and the lowest was PA and PB (0.0 ± 0a). It is suspected that the absolute length growth of mangrove crabs increased in PE because some mangrove crabs in PE experienced molting and the dose of fern leaf extract was greater than other treatments. Absolute length increase is seen if the molting process has occurred (Ode et al., 2019). Absolute length increase does not occur in the absence of molting, this molting phenomenon is periodic, because for growth crustaceans must expand their body volume by molting. The growth process must be assisted by the absorption of large amounts of water (Nova et al., 2023).

The results of the analysis showed that PA-PE did not experience a decrease or death during maintenance. The survival rate value obtained in each treatment with a percentage of 100%.

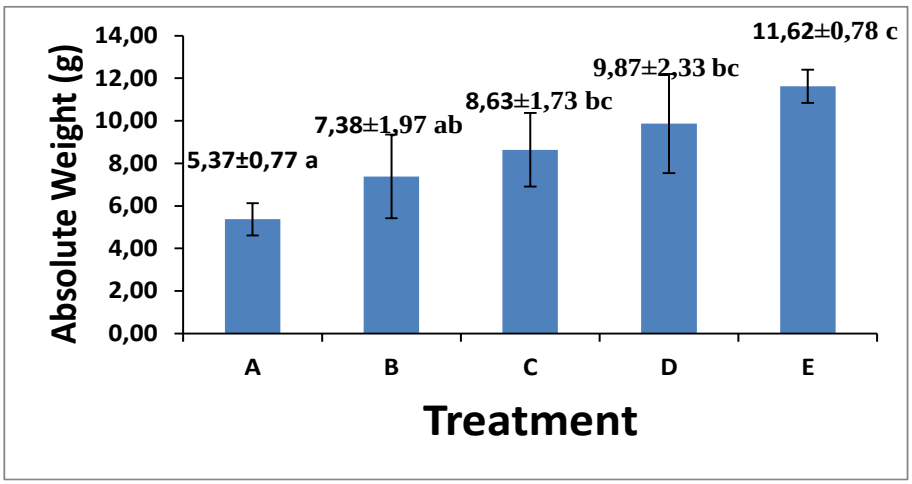

Fig. 2. Absolute Weight Chart

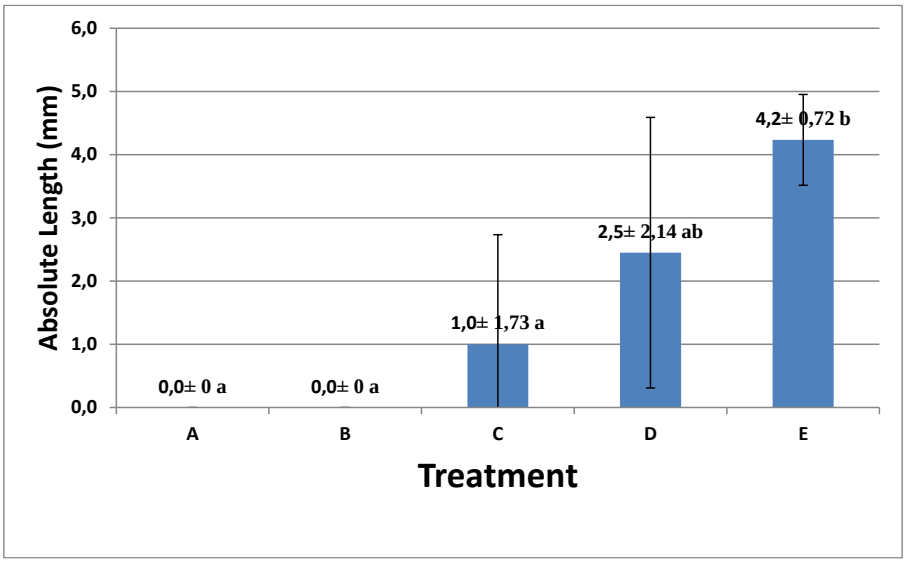

Fig. 3. Absolute Length

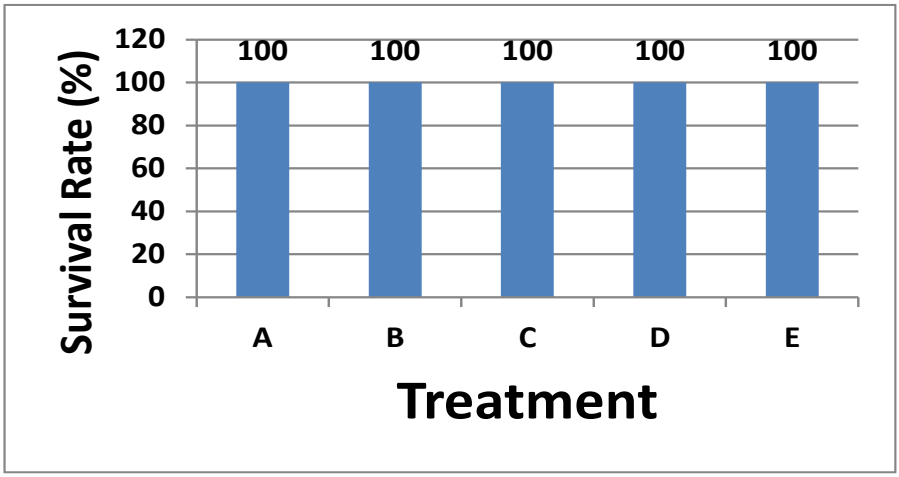

Fig. 4. SR (*Survival Rate*)

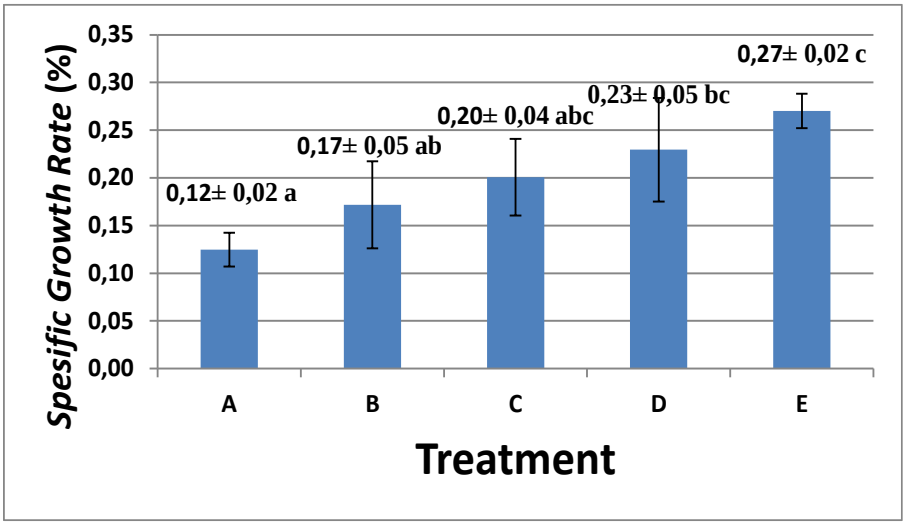

Fig. 5. SGR (*Spesific Growth Rate*)

3.3 SR (Survival Rate)

Based on the results of the study, it shows that feeding the type of fish with and without giving different doses of fern leaf extract has no significant effect (P>0.05) on the survival rate of mangrove crabs. The high survival rate is thought to be due to the controlled environment, adequate feeding and the absence of competition between other crabs. A well-controlled maintenance environment, sufficient amount of feed, no competition for space and feed can support high survival of mangrove crabs during the maintenance period (Agus et al., 2015).

The specific growth rate in mud crabs ranged from 0.12%-0.27% as can be seen in Fig. 5. Anova test results show that there are significant differences between treatments. The results of the Duncan test analysis showed that PA (0.12 ± 0.02a) was not significantly different from PB (0.17 ± 0.05ab), PC (0.20 ± 0.04abc) and significantly different from PD (0.23 ± 0.05bc) and PE (0.27 ± 0.02c). However, PE was significantly different from PA (0.12±0.02a) and PB (0.17±0.05ab). Based on the results obtained, the best treatment is PE (0.27±0.02c).

3.4 SGR (Spesific Growth Rate)

Based on the results of the research conducted that the highest growth rate of PE (0.27 ± 0.02c) and the lowest PA 0.12 ± 0a) giving a dose of fern leaf extract of 175ml / kg to PE into the raw fish feed can accelerate growth compared to other treatments given a lower dose of extract and without the use of fern leaf extract. The specific growth rate is closely related to the body weight gain derived from the feed consumed. The greater the specific growth rate, the better the feed is utilized for growth (Aditya et al., 2012). Protein plays an important role in feed for the growth of cultivated animals. Raw fish feed has high completeness and nutritional value, so that it can meet the nutritional needs of the mangrove crab body (Samidjan et al., 2015). Fresh raw fish has a high nutritional content, which has a crude protein content of 64.33%, carbohydrates 1.14%, fat 7.40%, and Ca 4.15% (Hanif and Herlina, 2021).

In addition, fern leaves contain echdisteroids that can accelerate growth in mud crabs. ekdisteroid is the main steroid hormone in arthropods which has the main function as a molting hormone, besides that it also regulates physiological functions, such as growth, metamorposis, and reproduction and good feed utilization can maintain the body condition of mud crabs so that the feed given is used for growth (Aslamyah and Fujaya, 2010). the growth rate of cultural organisms depends on the species, feed and environment (Abadi et al., 2020)

The average feed utilization efficiency (EPP) in mud crabs ranged from 24.06%-38.66% as can be seen in Fig. 6. The results of the ANOVA test showed that there were significant differences between treatments. The results of the Duncan test analysis showed that PA (24.06±3.09a) was not significantly different from PB (27.93±5.84ab), PC (32.03±5.92abc) and significantly different from PD (34.50±6.58bc)

and PE (38.66±3.61c). PE was significantly different from PA (24.06±3.09a) and PB (27.93±5.84ab). Based on the results obtained, the best treatment was PE (38.66±3.61c).

3.5 EPP (Feed Utilization Efficiency)

Based on the observations made, the highest feed utilization efficiency (EPP) value of mud crab (*Scylla serrata*) in PE was 38.66% and the lowest in PA was 24.06%. This shows that PE utilizes feed well for higher growth of mangrove crabs compared to other treatments given lower extracts and without the use of fern leaf extract. The high value of feed utilization efficiency in PE indicates that the highest dose of 175ml/kg has an effect on growth supported by the use of fresh fish feed which has good nutrition and is not easily destroyed in water compared to artificial feed so that the efficiency of feed utilization is quite high.

The quality of feed can be seen from the EPP value which states that the smaller the EPP value, the lower the quality of feed consumed. The efficiency of food use by cultivated cultivars shows the percentage of food that can be utilized by the body. The amount and quality of food greatly affects growth and lack of essential fatty acids can be shown by symptoms of weight loss, small feed efficiency, increased mortality (Wahyuningsih et al., 2015). High feed consumption will provide high growth for the body (Ambarwati et al., 2014).

Feed Conversion Ratio (FCR) in mud crabs ranged from 20.84%-88.80% as can be seen in Fig. 7. Anova test results showed that there were significant differences between treatments. The results of Duncan test analysis showed that PA (88.80±18.49c) was significantly different from PB (54.44±16.37b), PC (38.08±6.31ab), PD (32.32±5.37ab) and PE (20.84±0.98a). PE was significantly different from PA (88.80±18.49c) and PB (54.44±16.37b). Based on the results obtained, the best treatment is the PE treatment (20.84±0.98a).

3.6 FCR (Feed Conversion Ratio)

Based on the observations made, the highest feed conversion ratio (FCR) value of mud crab (*Scylla serrata*) in PA was 88.80% and the lowest in PE was 20.84%. This shows that the difference in specific growth rate (SGR) in each treatment is supported by the difference in feed conversion ratio (FCR) results. Growth is closely related to feed conversion ratio (FCR) (Aditya et al., 2012). The difference in the amount of feed consumed by mangrove crabs to the weight produced in each treatment, influences the difference in the average value of feed conversion ratio (FCR). The higher the feed conversion ratio, the less efficient the feed utilization for growth (Abadi et al., 2020).

The level of feed consumption can also affect growth, where the quality and quantity of feed consumed will determine the metabolic process in the cultivator's body, the amount of energy stored in the body. In addition, the amount of energy used for body activities also determines the ability of the cultivator's body to utilize the nutrients obtained from the feed consumed (Ode et al., 2019).

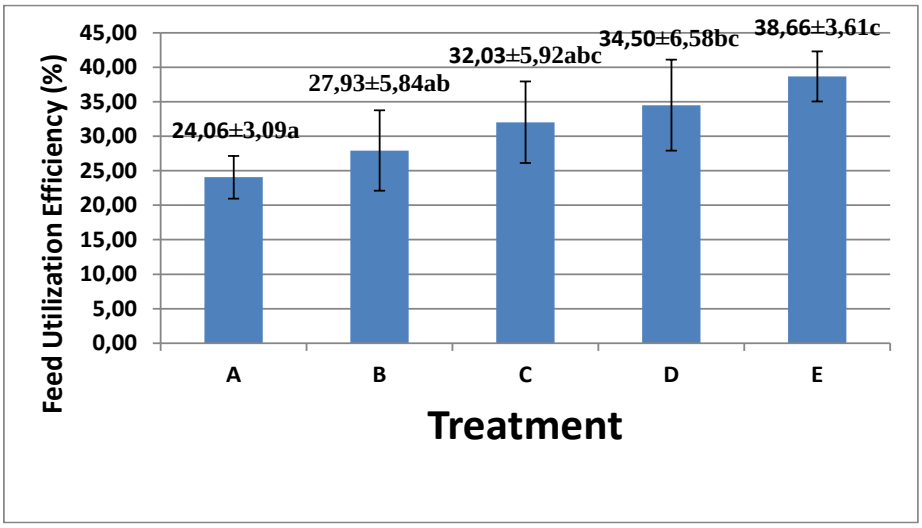

Fig. 6. EPP (Feed Utilization Efficiency)

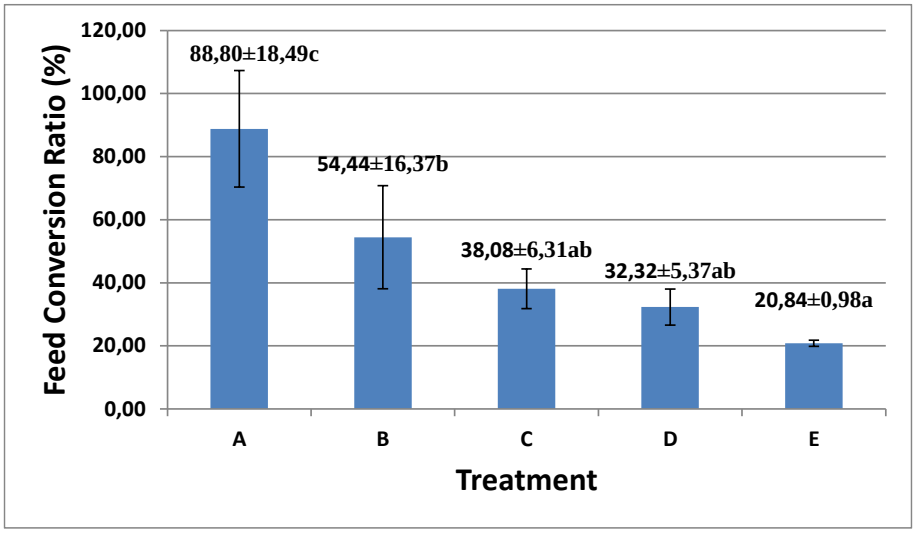

Fig. 7. Feed Convertion Ratio

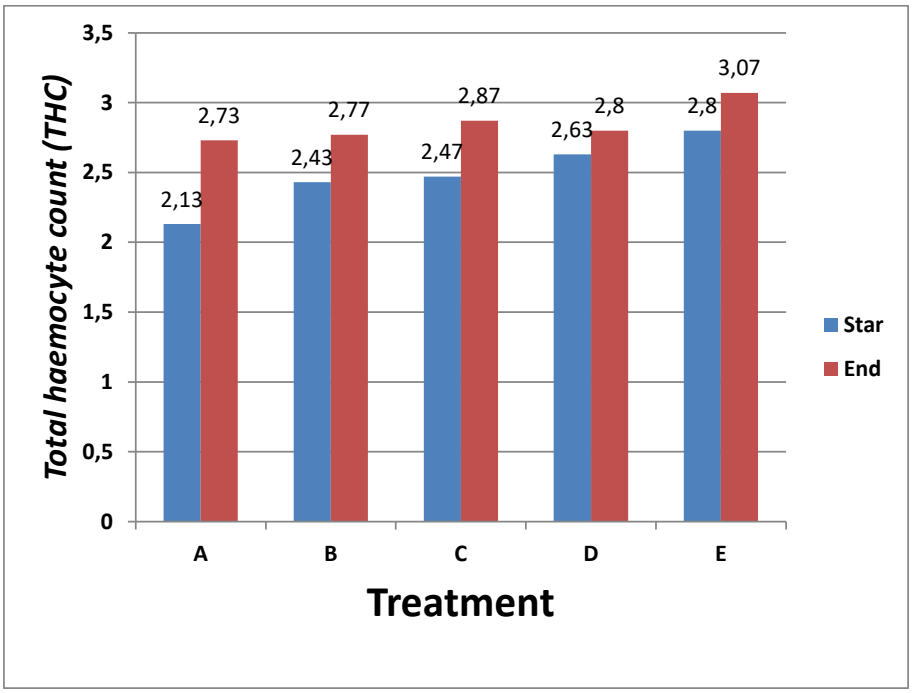

Fig. 8. Total haemocyte count (THC) of mud crab (*Scylla serrata*)

Table 1. Differential haemocyte count (DHC) of mud crabs (*Scylla serrata*)

Perlakuan	DHC					
	Stars			End		
	Hialin %	Granulosit%	Semi Granulosit	Hialin	Granulosit	Semi Granulosit
A	35	35	30	38	33	29
B	39	35	27	38	35	27
C	37	31	31	38	33	30
D	47	31	22	46	31	23
E	38	35	27	39	35	27

Table 2. Water quality parameters of mud crab (*Scylla serrata*)

Parameters	Obtained Range	Ideal Range	Reference
Salinitas (ppt)	22-25	15-25	Winestri *et al.,* (2014)
Suhu (^{O}C)	27-29	29-30	Adila *et al.,* (2020)
pH	7,0-7,7	7-8	Asyhariyati *et al.,* (2013)
DO (ppm)	5,2-6,3	3-15	Suryani *et al.,* (2017)

The blood profile of mangrove crabs (*Scylla serrata*) during the study was in the range of normal limits so that it could support the growth and survival rate of mangrove crabs (*Scylla serrata*).

3.7 Mangrove Crab Blood Profile

Total haemocyte count (THC) is a picture of blood in crustaceans to show the anti-body response in the body, especially in fighting diseases that attack the body or environmental conditions. Total haemocyte count analysis results obtained at the beginning of maintenance ranged from 2.13×106- 2.80×106 and at the end of maintenance ranged from 2.70×106-3.07×106. Shows that the results obtained are still within the normal range of THC of mangrove crabs. The normal hemocyte count in Crustaceans under normal circumstances is 1.85 x 106 cells/ml. The abundance of the number and type of hemocyte cells in crustaceans can vary due to various things including sex, water temperature, molting cycles, starvation conditions. In addition, the increased THC is the mangrove crab's effort to increase the body's immunity to the surrounding environment or foreign objects that enter the body (Pratiknyo, 2018).

The observation results obtained the value of differential haemocyte count obtained at the beginning of maintenance, namely hyaline ranging from 35-47%, granulocytes ranging from 31-39%, and semi granulocytes 20-30% and at the end of maintenance, namely hyaline ranging from 35-49%, granulocytes ranging from 29-43%, and semi granulocytes 20-31%. These 3 types of hemocyte cells, namely hyaline, semigranulocytes and granulocytes have different shapes and sizes. Hyaline cells have a smaller size, spherical without granules or few granules that have a phagocytic role while oval-shaped semigranulocytes play a role in encapsulation, phagocytosis and cytotoxic response, Granulocytes are round, many granules play a role in cytotoxic activity (Sari and Ekawaty, 2016).

3.8 Water Quality Parameters

Water quality is one of the important factors supporting the success of mangrove crab cultivation and also an important factor affecting the survival rate of mangrove crabs apart from the feed factor. The salinity range obtained during the study ranged from 22-25 ppt. where this range is still within normal limits for mangrove crab cultivation. The appropriate salinity for mangrove crab rearing is 15-25 ppt (Winestri et al., 2014). Mangrove crabs can live in a salinity range of less than 15 ppt to greater than 30 ppt (Katiandagho, 2014).

The results of temperature measurements during the study ranged from 27-29ºC, which is within the optimal range for mangrove crab cultivation. The temperature tolerance limit for mangrove crab life is 29-30 ºC (Adila et al., 2020). Measurement of acidity (pH) during the study ranged from 7.0 to 7.7, the range is still within the optimal range. Good acidity (pH) levels for crab farming range from 7-8 (Asyhariyati et al., 2013).

Dissolved oxygen (DO) measurements during maintenance were found to range from 5.2-6.3 ppm. The range of dissolved oxygen suitable for mangrove crab life ranges from 3-15 ppm (Suryani et al., 2017).

4. CONCLUSION

The conclusion of the research that has been done is that the use of fern leaf extract in mangrove crab (*Scylla serrata*) feed has a significant effect on the growth of absolute weight, Absolute Length, SGR (Specific Growth Rate) and FCR (Feed Conversion Ratio) where the most influential treatment is treatment E, which is the highest dose of 175 ml/kg. This shows that treatment E is the best dose treatment for the growth and feed conversion ratio of mangrove crabs (*Scylla serrata*).

ETHICAL APPROVAL

The principles of laboratory animal care" (SNI 9057-3:2023) have been followed, as well as

applicable national laws. All experiments were reviewed and approved by the appropriate ethics committee.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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