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CONSUMPTION OF NATURAL FEED RICH IN CALCIUM AND PHOSPHORUS ON GROWTH, INTESTINAL CONDITION, AND HEPATOPANCREAS OF SAND LOBSTER (*Panulirus homarus*) IN PANGANDARAN REGENCY

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ABSTRACT

This study aims to investigate the stimulatory effects of natural feed with calcium and phosphorus supplementation on growth and survival via physiological poses of hepatopancreas and intestine in sand lobster (*Panulirus homarus*). A trial was done with three types of natural feed of shell rich in calcium and phosphorus for lobsters weighing 80 g (initial weight), and that contained different mineral values: shrimp (*Acetes*), with calcium/phosphorus content (mg/mg) of 757/292, anchovy (*Stolephorus* sp) with 500/500, and golden apple snail (*Pomacea canaliculata*) with 129/60. This study was conducted from October 2023 to December 2023 at the KJA of the Faculty of Fisheries and Marine Science, Universitas Padjadjaran, located on the East Coast of Pangandaran Regency. The parameters measured were survival, growth, histological health of the hepatopancreas and the intestine of the sand lobsters, and water quality. The results showed the highest survival of lobsters in the treatment fed by shrimp at 96%, and the highest specific growth rate of lobsters was found in the anchovy diet at 1.90 0.5117%. Intestinal villus area of lobster was 1857.667 μm^2; besides this, the histology of Hepato pancreas and intestine has been described descriptively. Water quality parameters in the research site varied as depth-visibility was from 3.1 m, temperatures of 27 to 29°C, dissolved oxygen was 6.4 to 8.8 mg/L, pH range was 7 to 8, and the salinity range was 34 to 35 ppt.

Keywords: Histology, Survival Rate, Hepatopancreas, *Panulirus homarus*

1. INTRODUCTION

The Indonesia is the second-largest lobster producer in the world at 556 tons, behind Vietnam (1,100 tons), followed by Singapore at 58 tons and Sri Lanka at 19 tons. Nonetheless, lag in Indonesian lobster production is primarily based on Capture Fisheries rather than Aquaculture[1]. Aquaculture is required to supply the demand for lobsters.

Although factors like poor survival due to failed molting leading to death are still among the concerns in lobster farming, most of us have heard about the "crazed mating sea monsters" which refers to how lobsters mate, and how during molting they will kill and eat each other[2-3]. During molting, lobsters need enough nutrients and energy to act as food reserves during molting and for building a new shell[4]. Following a molt, lobsters have a new shell that is not yet fully formed. The shell hardening process is time-consuming and is supported by the existing shell content of

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calcium carbonate, protein, and chitin[5]. Dietary calcium deficit retards ecdysis as reported by Stein[6]. Therefore, the soft-shell period can be extended, during which the lobsters are weaker and slower in feeding than in the hard-shell condition.

In crustaceans, phosphorus is associated with exoskeleton mineralization and the concurrent formation of endocuticle after molting[7]. Phosphorus is also closely related to other minerals such as calcium[8]. Research by Kunkel et al.[9] reported that the cuticle structure is composed of phosphorus and calcium constituents, which bolster the mechanical stiffness of the cuticle due to its heterogeneous mineral content. Calcium and phosphorus originating from feed or the environment are taken up in the pre-molting period and incorporated into the gastrolith for utilization during the shell hardening (ecdysis) process[10].

Dietary and environmental calcium are readily absorbed from lobsters[11]. Lobsters cannot fulfill the calcium requirements either internally or externally. The calcium level in water was 0.34%, meaning that calcium should be incorporated into the diet[12]. Research by Anggraini et al.[13] reported that feeding calcium-enriched feed might increase growth up to 59.17 g; however, Hadie et al.[12] reported growth of 5.83 g with the addition of calcium in culture media for aquaculture.

Shrimp (*Acetes*) contains high levels of calcium and phosphorus. There are 757 mg of calcium and 292 mg of phosphorus per 100 g of shrimp[14]. The high mineral content was also found in golden apple snail (*Pila ampullacea*), 129.18 mg of calcium and 60.52 mg of phosphorus per 100 g[15]. Previous studies reported that golden apple snails have been used as an alternative feed for several marine species, including crabs[16] and sand lobster[13]. There has been growing interest in barnacles, as coral animals, for producing alternative feeds for other animals because they are readily available and inexpensive[17]. Several barnacle species have been utilized for flour and as an alternative feed for some fish species, including freshwater pomfret[18].

Therefore, we should consider the supply of natural calcium and phosphorus sources for the sand lobster during the growing phase. The significance of this study was to facilitate the production of sand lobsters and to expand the other natural feeds that can be used as natural trash fish advantages to help the lobster grow properly. This study aims to find optimum natural feeding sources to enhance sand lobster's survival, growth, and gut condition.

2. RESEARCH METHOD

Time and Place

The study period was between August and December 2023. The lobster rearing was conducted on a Submerged Cage (L-size) with a longline system at the Floating Net Cage (KJA) FPSP Universitas Padjadjaran, floating off the coast of Pangandaran, West Java.

Method

The research was conducted using a Completely Randomized Design (CRD). The experimental method is based on experiments to work out effects or phenomena resulting from fixed factors. The study used the Completely Randomized Design (CRD), and the variables were four treatment levels repeated 5 times with the feed provision rate 20% of the biomass[19]. The treatments applied were:

Treatment A	:	Diet based on golden apple snail feeding
Treatment B	:	Through barnacle ingestion
Treatment C	:	Shrimp (*Acetes*) feed
Treatment D	:	Anchovies feeding

Procedures

Container Preparation

The maintenance container was a partially submerged cage (enclosure) of length (L), a cube 272 cm long, 250 cm wide, and 135 cm high. The enclosure also comprised High-Density Polyethylene (HDPE) square poles. The lobster research container was located at a depth of 5m from the water surface, known as an optimal depth for inducing the growth of lobsters. At 5 m depth, the temperature is not too high, the wind is not too strong, the waves are not too high, and zooplankton is abundant[Error! Reference source not found.].

Lobster Dispersion

The lobsters were scattered in the afternoon by the opening of the transport cardboard. Lobsters were then allowed to acclimate for 2 days before the cage was lowered to a depth of 5 m.

Lobster Maintenance

The lobsters used in this study were sand lobsters with 100 individuals (mean weight of 70-90 g) kept in an L-sized cage at a fixed depth of 5 m.

Feeding

All lobsters were fed different feeds depending on the categories: golden apple snails, barnacles, shrimp, and trash fish. Feeding was performed daily in the afternoon with 20% biomass in each tank. The food was cut into small pieces with a knife before being fed. The feed was distributed slowly through a funnel on the water surface and slowly provided to the cage at the maintenance level.

Data Collection

Data were taken in 60 days, with the length and weight measured every 10 days until the end of the maintenance phase. Lobsters' length and weight were assessed on five individuals/treatment. Body length was measured with a 1 mm precision millimeter block, and weight was measured using a digital scale (0.1 g).

Intestinal Sample Collection

At the end of the maintenance period, one lobster per treatment was sampled for the intestine. Then the lobsters of all the treatments were dissected to remove their intestines. The intestine was immediately transferred to Bouin's solution for histological processing. The luster of histological sand lobster intestines samples made in the Biology Laboratory of FMIPA, Universitas Padjadjaran.

The villi surface area, villi height, and intestinal diameter were measured in histological sections. The histological procedure could be divided into dehydration, impregnation, embedding, sectioning, mounting slides, and staining. Preparations were viewed under a microscope.

Water Quality Measurement

Water quality variables tested for during the course of the study were clarity (m), current velocity (cm/min), temperature (ºC), pH, salinity (ppt), and dissolved oxygen (mg/L).

Observation Parameters

Survival Rate (SR)

Survival rate (%) was calculated by the method of Huisman[21] as:

$$SR = \frac{Nt}{No} x100\%$$

Description:

SR = Survival rate (%)
Nt = number of lobsters at the end of the final study (individuals)
No = Initial number of lobsters (individuals)

Specific Growth Rate (SGR)

The growth rate was defined by the equation given by Huisman[21]:

$$LPS = \frac{Wt\text{-}Wo}{t} x100\%$$

Description:

SGR Specific Growth Rate (%)
Wt = Mean weight of lobsters at the end of maintenance (ind)
W0 = Mean body weight of lobsters at start of maintenance (ind)
t = Duration of maintenance (days)

Lobster Intestine Histology

Histology score was determined by the formula reported by Iji et al.[22]:

$$LV=\frac{b+c}{c}\times a$$

Description:
LV = Surface of the villi (µm²)
a = Height of the villi (µm)
b = width of the apical villi (µm)
c = diameter of the basal villi (µm)

The histological preparation of the intestines of the sand lobster was carried out at the Biology Laboratory, FMIPA, Universitas Padjadjaran.

Data Analysis

Descriptive analysis was performed based on a review of the studies collected and related supporting data. A descriptive analysis of water quality parameters and digestive tract conditions was also conducted using images or tables. The production performance parameters, SR and SGR, were statistically analyzed by one-way analysis of variance (ANOVA).

The statistical parameters obtained were studied using Microsoft Excel Office 2010. Then the data that had been received were analyzed by this test model in the form of analysis of variance by F-test to know whether there was a significant difference between treatment groups, and continued with the analysis by Duncan's new multiple range test (DMRT) at a confidence level of 95%[23].

3. RESULT AND DISCUSSION

Survival Rate

The survival rate is given as a percentage of the number of organisms alive at the beginning of the experiment to the number alive at the end of the experiment[24]. The more organisms surviving during maintenance, the larger the percentage of the survival rate achieved. Figure 1 shows the survival of sand lobsters.

After the maintenance phase, the survival rate information varied from 72% to 96%. The best survival was obtained from treatments A (golden apple snails) and C (shrimp) (each at 96%), followed by treatment B (barnacles, 92%), and the lowest was from treatment D (anchovies, 72%).

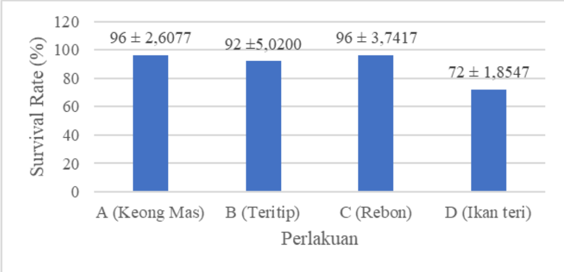


Figure 1. Survival rate 60 Days

As seen in the above figure, treatment D with anchovy showed survival rates lower than A, B, and C, which obtained higher survival rates. The high survival rate may be due to the volume and frequency of feeding delivered, which significantly suppressed cannibalism within the lobsters. Research by Ikhsan et al.[19] revealed that average survival (80–100%) was good for juveniles in which fish were fed once at a level of 20% body weight, whereas the other studies of Rihardi et al.[25] observed 80% survival rates even with a similar frequency of feeding.

Natural feeding in treatments A, B, and C (including the shell) contributed to calcium and phosphorus in these shells, which led to reduced cannibalism and increased survival of lobsters. This agrees with Suptijah[26], who reported that the body easily absorbs calcium from crustacean shells, leading to better functionality.

It is believed that cannibalism during the maintenance period was the reason for the high mortality found in treatment D, as some empty lobster shells due to cannibalism were observed. Lobster predation is an example of aggression within a common environment to each competitor, representing a dominance[27]. Cannibalism of lobsters increases when they molt as the lobster's body becomes soft, thus attracting others due to the unique scent of molting[28].

Cannibalism could potentially ensue when shell hardening is delayed and a "drift of smell," a characteristic scent emitted by the molting lobsters, attracts conspecific lobsters to attack molting ones[3]. So, calcium is very important in reducing the time taken for the hardening of the carapace of lobsters and in reducing case fatality rates of molting stages to avoid predation[28]. Hakim[29] found that lobsters that molted more frequently had a lower survival rate due to cannibalism, because they do not have shelter and are more vulnerable to attack by other lobsters.

Specific Growth Rate

The specific growth rate is a parameter that can be derived from measuring the percentage of growth of lobsters per day. The mean specific growth rate of sand lobsters for the 60 days is shown in the Figure 2.

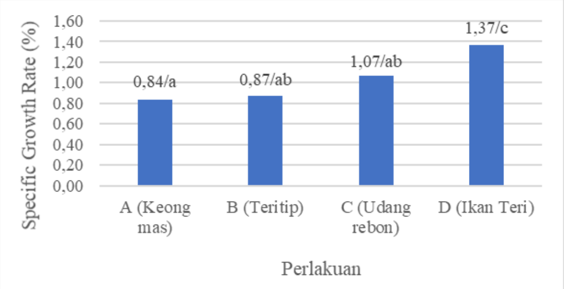


Figure 2. Specific growth rate

From the 60-day observation period, the specific growth rate for sand lobsters was between 0.84% and 1.37%. The highest specific growth rate was reached with treatment D with anchovy feed at 1.37%, while the lowest specific growth rate was obtained in treatment A with golden apple snail feed at 0.84%.

All treatments showed an improvement in the specific growth rate due to the performance of the feed on the body. Anchovies, in addition, can also meet calcium and phosphorus needs in the course of stimulating lobster development, as minerals like calcium and phosphorus are significant contributions from anchovies. In such a process, calcium and phosphorus may also facilitate molting, as evidenced by the enhancement of growth in sand lobsters.

The high value of specific growth rate in anchovy-fed lobster is attributed to the rich contributions, including amino acids, calcium, phosphorus, minerals, and iron[30]. Kurniasih[31] states that the rate of molting of crustaceans will be more rapid if the intake of nutrients is adequate, whether converted to energy reserves or growth and maintenance. Molting plays an important part in the life history of lobsters. Successful molting governs lobster size[29], and calcium is significant to the molting because it is a gastrolith that lobsters reabsorb to harden their shells after molting[9].

Larger lobsters will have a more extended intermolt period than smaller ones[27]. In intermolt lobsters, calcium is stable because the exterior skeleton has been completed. Lobsters will also mate, feed, and engage in other activities during this time. Going into the premolt, calcium will be mobilized from the shell into the blood via the integument epithelium, with much of the calcium moving from the blood into the cells and tissues, and the gastrolith as a temporary calcium sink to be used for the next exoskeleton. Subsequently, the gastrolith will, during postmolt release, release its calcium into the haemolymph, the cations will be transported through the integument epithelium out into the new hardening cuticle, and while also there, it will be of importance to the stiffness and to the strength of the newly formed exoskeleton.

The high specific growth rate in treatment D was also thought to be due to the timing and feeding frequency of feed for the growth of sand lobster fit. Once, in the afternoon, the feeding frequency was considered to be following lobster behavior. Masser & Rouse[32] reported that the peak period of daily activity in lobsters is from the evening to the morning between 18:00 and 9:00. Moreover, Rihardi et al.[25] reported that feeding during distractive periods of the lobsters' natural feeding cycle could result in low feed intake, and that with increasing feeding activity, the amount of food fed at the hungry period may be low, causing also low feed intake.

The least specific growth rates were observed in treatments B and A with barnacles and golden apple snails, respectively. This was suspected because these feeds are lower in dietary calcium and phosphorus than anchovies and shrimp. This is in line with Hakim[29], who has also expressed that calcium may be optimal for lobsters if the calcium provided meets the concentration requirement.

Intestinal Histology

Intestinal histology is a good benchmark for evaluating an animal's nutritional state. Intestinal tract monitoring is an essential factor for examining digestive performance, as the intestine plays a key role in the absorption and digestion of nutrients in food33. Lobsters have a unique digestive system compared to fish. The hepatopancreas is the site of digestion and feed utilization. Lobster digestive system includes the mouth, gizzard, digestive gland, and intestine. The foregut is the hepatopancreas, and the hindgut in lobsters is the intestinal part[34].

Table 1 presents the values for the number and the surface area of the villi obtained from the observations for each treatment.

Table 1. Observations of intestine condition in lobsters

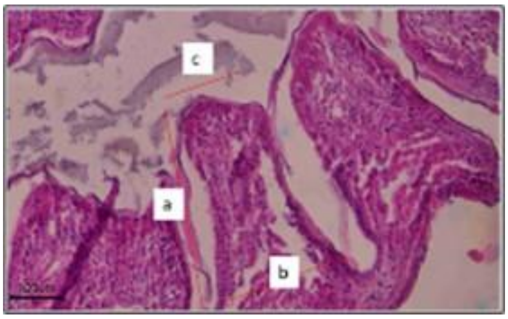
Treatment	Number of Villi	Area per Villi (μm^2)
A	10.200 ± 0.7483 a	505.908 ± 531.6347 a
B	8.400 ± 1.0198 b	441.290 ± 528.6376 b
C	14.600 ± 1.2000 c	1631.468 ± 426.6589 c
D	15.000 ± 1.0954 c	1857.667 ± 303.9936 c

Notes: The same letter in each column is not significantly different by Duncan's multiple range test at 5% level

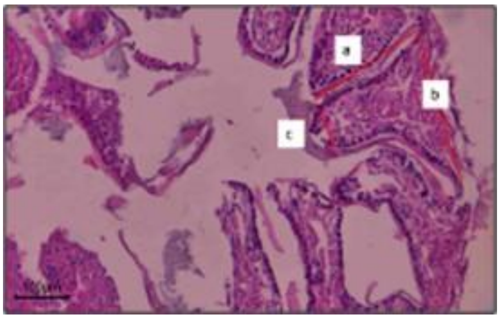
According to the histology, after 60 days of lease, the villi surface of star pomfret was 505.908-1857.667 µm². The maximum villus ratio was calculated as 1857.667 μm^2 in anchovy feeding (treatment D). The villi were significantly wider when the fish were fed anchovies, histologically. This contrasts with the histology of the intestines of treatments A and B, in which the villus surface area is reduced. An increase in villus height and width means more surface area for nutrient uptake; an increase in the submucosal layer may enhance the digestive capacity and absorption35. The histology results of the intestines of the sand lobster after being maintained for 60 days are as follows.

Aryanti[36] states that balanced nutrition in the feed can maintain a healthy digestive tract. Some of these nutrients are calcium and phosphorus. Calcium and phosphorus deficiencies can reduce the growth and repair of villi, consequently, impairing intestinal absorption[37].

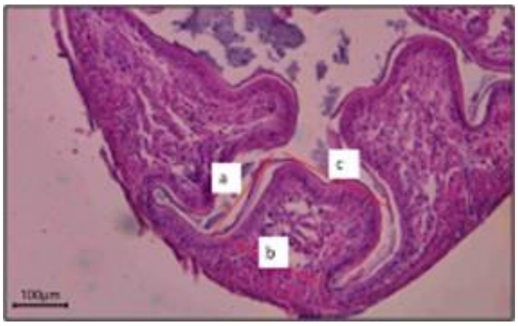
The width of the villi is related to processes such as nutrient absorption; the broader the villi, the more nutrients enter the body, which affects the development of the internal organs. Conversely, the villus length also carries nutrients away through specific cells conveyed through capillary networks and lymphatic vessels, then carried via blood to the tissues of the body.



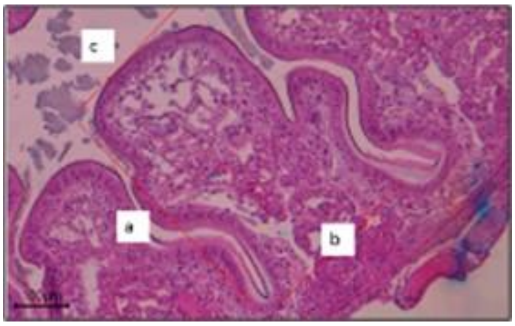
Histology of Sand Lobster Intestines A (Golden Apple Snail). a: the villus's height, b: the base's width, c: the top.



Histology of the sand lobster intestines B) barnacle. a: villus height, b: basal width, c: apical width.



Histology of sand lobster intestine C (shrimp-*Acetes*). a: height of villi, b: width at the base, c: width at the apex.



Histology of sand lobster intestines D (anchovy). a: villi height, b: base width, c: apex width.

Water Quality Parameters

Survey results show that the water quality in the culture pond of sand lobster is below quality standards for sand lobster maintenance. Water Quality Variability in Floating Net Cages can be seen in Table 2.

Table 2. Variability of water quality in floating net cages

Parameter	Unit	Measured value	Quality standard
Clarity	M	3.1	>3**
Flow velocity	cm/det	19.5	20-40**
Temperature	°C	27 – 29	27 – 32*
DO	mg/L	6.4 – 8.8	>5*
pH	-	7 – 8	8-8.5*
Salinity	Ppt	34 – 35	34-36*

Notes: * SNI 8116:2015[38]; **Affan[39]; Radiarta et al.[40]

The clarity value of the KJA system on the east coast of Pangandaran is 3.1 m. Therefore, based on the suitability value of the lobsters' KJA cultivation area, it has clear water greater than 3 m. The clarity index indicates suspended particles that are dissolved in the water. The deeper the water, the more particles it contains, and the less light that can pass through it (and the muddier the water will be). These particles may be of the organic material, which microorganisms can decompose and consume dissolved oxygen (DO) in water, reducing DO levels in the water[41].

The observed current velocity on the East Coast of Pangandaran was 19.5 m/s. While selecting a location for cultivation purposes, we should also consider the current conditions. Strong currents are considered threats to the growth and structure of fishponds (KJA), while very weak currents are deemed inappropriate to culturing activity[42]. Optimum flow rate for lobster rearing with the KJA system is 20-40 cm/s[43]. The role of water circulation in KJA growth is crucial. Feed residues in the maintenance containers are inadequately dissolved by the weak currents and accumulate, which can have toxic effects on the cultured organisms[41].

The temperature regulates the state of the ecosystem. Temperature variations can have an impact on the chemical, physical, and biological characteristics of water. The temperature, which was measured in the rearing, 27-29°C, showed that the water condition at the East Coast of Pangandaran is highly suitable for the culture of lobster with the KJA system. The range of temperatures for the best autoclave treatment is 24–31°C without dramatic variation[44]. Any temperature fluctuation can also affect growth and molting[45].

This study measured dissolved oxygen (DO) concentrations of 6.4–8.8 mg/L. According to the seawater quality standard in KJA for the cultivation system, the suitable DO should be >5 mg/L, so the DO concentrations in this study are very safe for lobster growth. In some water, the level of dissolved oxygen may decrease; this can influence growth, metabolism, molting, and a suppression of the immune system, which lowers the defense against diseases in the lobster. Molting is the shedding of the skin in shellfish such as crabs, shrimp, crawfish, and lobsters. This chore is directly related to water quality. A high frequency of molts indicates good growth[41].

The concentration of hydrogen ions measures the acidity (pH) level and water's basic and acidic properties. The pH values found in this study ranged from 7 to 8. These values are also within an acceptable level near the seawater quality values. Based on the Government Regulation No. 22 of 2021, the pH quality of the waters at the time the research was conducted was in the City's good category (pH of between 7–8.5), which is suitable for marine life organisms. So, it can be concluded that the water of the East Coast of Pangandaran (study location) is appropriate for lobster growth in the KJA system.

Salinity is one factor that can govern lobsters' behavior and survival. The salinity during all the surveys was 34-35 ‰. Considering water quality standards, such values are suitable for lobster growth.

4. CONCLUSION

The results of this study indicated that the highest survival of lobsters was in shrimp feed treatment at 96% and the highest specific growth rate of lobsters was in anchovy feed at 1.90± 0.5117%. Natural feed supplemented with different calcium and phosphorus sources impacted the intestinal histology of lobsters. Lobsters fed on anchovies attained a villus surface area of 1857.667 μm^2, and the structure of the hepatopancreas and intestines was analyzed descriptively. The water quality range at the research site was clear up to 3.1 m in depth, temperature at 27–29°C, dissolved oxygen of 6.4–8.8 mg/L, pH from 7–8, and salinity from 34–35 ppt.

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