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SAND CRAB (*Emerita sp.*) MEAL AS A NOVEL FEED INGREDIENT FOR KOI CARP (*Cyprinus carpio*)

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ABSTRACT

Sand crab (*Emerita* sp.) is a marine biodiversity, but it has not been used as a fish feed ingredient. This study aimed to evaluate the nutritional value of sand crabs and to understand its effect when used as feed ingredient on the performance of Koi carp. The study was conducted in two steps, which is evaluation of the nutritional value of sand crab and its effect on fish performance. The proximate composition, amino acids, and fatty acids were measured using AOAC methods, and then the carotenoid content was determined by spectrophotometry. In the second step, the sand crab was added to fish feed at doses of 0, 5, 10, and 15 percent, and fed to koi fish for 42 days. The parameters observed were length gain (LG), weight gain (WG), and feed efficiency (FE). Nutritional data were analyzed by description and compared with fish feed ingredients from previous studies. Fish performance were analyzed by one-way ANOVA. When significant, Tukey's significant mean test was applied. The result showed that the nutritional value of sand crab was comparable to other feed ingredients with a protein content of 37.88%, while carotenoid content was superior. The best performance of Koi carp was obtained with a dose of 15% sand crab in the diet, with LG, WG, and FE values of 0.93 ± 0.05 cm, 0.48 ± 0.06 g, and 63.50 ± 7.05 %, respectively. Based on this result, it can be concluded that sand crab has a high nutritional value and can be used up to 15% in Koi carp diet.

KEYWORDS: ***Emerita*; nutritional value; Cyprinus; feed**

INTRODUCTION

Sand crab is a marine fishery resource found worldwide, including Indonesia (Efford, 1976; Haley, 1982; Apte, 2012; Mantelatto *et al*., 2023). Indonesian sand crabs generally belong to the Hipidae family, whose populations are commonly found on the west coast of Sumatra, the south coast of Java, Maluku, Sulawesi, Bali, West Nusa Tenggara, and Yogyakarta (Boyko & Harvey, 1999; Boyko, 2002; Haye *et al*., 2002; Mursyidin, 2007; Mashar *et al*., 2014; Wardiatno *et al*., 2015). One of the most commonly found and caught species on the southern coast of Java, particularly in districts Kebumen and Cilacap, is the *Emerita* sp. (Mashar *et al*., 2014; Bhagawati *et al*., 2022).

In Kebumen and Cilacap, the sand crab is one of the most valuable commodities for the economy of the coastal communities. Generally, sand crabs are consumed as snacks by visitors and locals communities in coastal tourist areas (Bhagawati *et al*., 2016). The nutritional value of sand crab as a food is reported to be relatively high, both in protein, omega-6, and omega-3 (Mursyidin, 2007), depending on the processing mechanism. The best processing of sand crab is steaming, which provide 25.00 g, 11.04 g, 71.86 g, and 2.11 g per 100 g sand crab for amino acids, minerals, fatty acids and cholesterol, respectively (Santoso *et al*., 2015). However, some researchers reported that sand crab is unfit for human consumption due to its heavy metal content, one of which is mercury (Pérez, 1999) and Pb, which ex-

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ceeds the standard limit of 0.2 mg/kg set by Indonesian National Agency of Drug and Food Control (BPOM). Due to the high Pb content, the consumption of sand crabs poses an adverse risk to human health (Ketaren *et al*., 2019).

It is therefore necessary to find an alternative to the use of sand crabs that does not endanger human health, so the resource can continue to contribute to the economy of coastal communities. Sand crab can be used in many types of products. Alternative uses of sand crab include as a source of chitosan (Wittriansyah *et al*., 2018), feed for quail male (Pratiwi, 2016), feed for laying quail (Astriana, 2013), and feed for ducks (Batoro, 2008). However, all these studies still have a risk of heavy metal residues in feed. The best alternative is to use sand crab as a product that is not consumed by humans, for example is to use sand crab as feed for ornamental fish. However, it is necessary to evaluate the nutritional value of sand crab before considering it in feed formulations. Therefore, the aim of this study was to evaluate nutritional value of sand crabs and to know it effect on the performance of Koi carp (*Cyprinus carpio*).

MATERIALS AND METHODS

Step one: evaluation nutritional value of sand crab

Sand crab sample preparation

The sand crab (*Emerita* sp.) was obtained from Jetis beach, Cilacap district, Central Java, Indonesia. Live sand crabs caught by fishermen were rinsed with clean water to remove dirt in the form of sand adhering to the growing parts. The clean sand crab was then fully cooked in a pan and then cooled to room temperature. The cooked sand crab was stored in a freezer at -4°C for 24 hours. A total of 1000 g of frozen sand crab was dried in a freeze dyer (CRIST-ALPHA-LD PLUS-101541, Martin Crist, Osterode am Harz, Germany) at temperature of -40 °C for 20 min and then freeze-dried in a vacuum in two steps: primary drying (-50 °C at 0.040 bar) for 24 h and final drying (-55 °C at 0.021 bar) for a further 24 h. Dried sand crab samples were ground to a powder, and then stored in airtight plastic at a temperature < 25 °C until used.

Analysis of the sand crab nutrient contents

Sand crab samples were analyzed for their proximate composition, i.e., moisture (M), crude protein (CP), ash, crude fat (CF), crude fibre (CF), calcium (Ca), and phosphorus (P) according to AOAC (2005). Analyses of amino acid and fatty acid profiles in the samples were performed using high performance liquid chromatography and gas chromatography (Shimadzu Corp., Kyoto, Japan), respectively, according to AOAC (2005). The total carotenoid (TC) concentration in sand crabs was determined using a UV-Vis spectrophotometer. A total of 0.5 g sand crab meal sample were homogenized in 5 ml acetone 98% (Merk, Darmstadt, Germany) containing anhydrous sodium sulfate using a homogenizer (Ultra-turrax IKA-T18 basic, Wilmington, NC, USA). The sample was stored in the refrigerator for 24 h at 4°C and then extracted with acetone two or three times until no color was obtained. The solutions were added 5 ml h-hexane and homogenized using a magnetic stirrer. The total carotenoid concentration was determined using spectrophotometer (Genesys-105 UV-Vis, USA) in n-hexane solutions using extinction coefficients (E1%, 1cm) 2500 for carotenoids at 350, 380, 420, 450, 475, 480, 500, and 663 nm. The total carotenoid (TC) concentration was calculated from the maximum absorbance according to the formulae:

$$TC\ (ppm) = \frac{n - hexane\ solution\ (ml) x \max absorbance\ x\ 10000}{0.25\ x\ weigt\ of\ sample\ (g)}$$

Step two: feeding trial of koi carp (*Cyprinus carpio*)

A randomized experimental design was used to test sand crabs in ornamental fish food. There were four treatments and three replications. The treatments tested were the level of sand crab meal in the feed formulation: feed without sand crab (T1), feed with sand crab 5% (T2), feed with sand crab 10% (T3), and feed with sand crab 15% (T4). This dose is based on Hertrampf & Pieded-Pascual (2000) suggesting that crab meal can be used at 5-10% in aquaculture diets. Four diets of similar protein content (crude protein 37.00%) were formulated for this study. The dietary composition of experimental diets for juvenile koi with different levels of sand crab is shown in Table 1.

All feed ingredients in the form of flour are ground in a blender to a uniform size and passed through a standard US sieve mash #80. The raw materials are then weighed according to the formula, the raw materials that are in small quantities (wheat flour, fish oil, premix and sand crab) are mixed until they are homogeneous, then they are mixed with the raw materials that are in large quantities in the feed formula (soybean meal, wheat pollard, fish meal) until they are homogeneous. The powder feed mixture is mixed with 12-15% water before being formed into pellets using a meat mincer with a 1 mm die diameter. Subsequently, the pellets are dried by hot air spraying at 37°C for 24 hours. The dry pellets were broken down into crumbles using a coffee grinder. Only crumble that passed the US standard mash #60 and retained the #80 mash standard was used in this study.

The juvenile koi carp used in the experiment were obtained from the same broodstock and the same batch. A total of 288 fish juvenile fish with a mean initial body weight of 0.55 ± 0.15g and an initial length of 3.09 ± 0.23cm were used in the experiment. The fish were placed in twelve aquaria (40 × 30 × 30 cm^3) filled with 18 L of water. Twenty juveniles were stocked in each aquarium. Fish were acclimated for one week and fed a control artificial diet (without sand crab), twice a day up to 3% of the biomass. The fish were then fed the experimental diet according to the treatment. The fish were fed twice a day, morning and evening, for 42 days. The remainder feed was siphoned after 1 hour of feeding to maintain good water quality in aquarium.

Fish were sampled once a week to determine body weight gain (WG), length gain (LG), specific growth rate (SGR), and feed efficiency (FE). In addition, water quality was measured at the beginning and the end of the study to check for similar environmental factors among treatments.

Data analysis

Nutritional data of sand crab were presented as mean ± standard deviation, and then compared with the nutritional composition of crab meal, shrimp head meal, krill meal, and soybean meal from previous studies. Koi carp performance data were analyzed by one-way ANOVA to know the effect of different sand crab meal levels in diet. A Tukey's post-hoc test analysis was used where the analysis indicated significant difference among treatments. Statistical analysis was performed using Minitab software version 16.

Table 1. Composition of experimental feed (g/100 g)

Ingredient	Ingredient Composition of Experimental Diet			
	T1 (0)	T2 (5)	T3 (10)	T4 (15)
Soybean Meal	38.04	35.25	31.01	21.90
Fish Meal	18.70	18.20	19.20	24.33
Sand Crab	0	5	10	15
Wheat Pollard	30.00	30.00	25.05	12.77
Wheat Flour	7.26	5.55	8.74	20.00
Fish Oil	3	3	3	3
Premix	3	3	3	3
Total	**100**	**100**	**100**	**100**
Nutrient Composition of Experimental Diet (%)				
Dry Matter	89.70	90.00	90.30	90.60
Protein	37.01	37.01	37.01	36.99
Fiber	5.73	5.81	5.36	3.96
Fat	4.00	5.03	5.98	6.84
Ash	9.34	10.18	11.07	12.31
Calcium	1.77	2.16	2.67	3.49
Phosphorus	1.05	1.02	0.98	0.95

RESULTS AND DISCUSSION

Nutritional value of sand crab

The results of the laboratory analysis are shown in Table 2. The protein content of sand crab in this study was 37.89%. This is similar to the results of previous studies, the protein content of crab meal, but lower than the protein in shrimp head meal, krill meal and soybean meal. The mean sand crab has a very high potential as a source of protein in aquafeed. In general, the protein source used in freshwater fish feeds is fishmeal, which accounts for 5-25% (Tacon & Mentian, 2008) and can currently be replaced by soybean meal (Hodar *et al*.,2020). Due to its protein content, sand crab has the potential as an alternative to reduce the use of one of the two ingredients, as protein sources in feed cannot be eliminated, but can be replaced by other ingredients. As we know that aquatic animals require protein for tissue protein building, enzyme synthesis, hormones, growth, tissue repair, maintenance, and optimal health status (Jia *et al*., 2022; NRC, 2011; Paul *et al*., 2019; Teles & Couto, 2019).

Table 2. Proximate composition of the sand crabs compared to others ingredient (in dry matter basis)

Nutrient	Sand Crab[1]	Sand Crab (previous study)	Crab Meal [5,6,7]	Shrimp Head Meal [5]	Krill Meal [5,8]	Soybean Meal [5]
Protein (%)	37.89 ± 4.36	35.13-38.52[2] 30-40[3]	31.0-37.6	43.3	52-67.3	42.5-48.6
Ash (%)	23.86 ± 0.25	31.59-35.65[2] 26.4[3] 40.03[4]	36.1-41.2	33	9.7-15.9	3.20-6.20
Fat (%)	24.56 ± 0.98	2.95-8.76[2] 17.22-21.56[3] 10.2[4]	2.0-5.0	5.6	4.2-15.9	0.82-5.60
Fiber (%)	6.00 ± 0.13	4.9[4]	10.5-10.7	15.8	-	1.00 -7.40
Calcium (%)	8.77 ± 0.10	9.3[3] 8.74[4]	16.03	6.3	1.33	0.25-0.28
Phosporus (%)	0.82 ± 0.15	1.6 [3] 0.63[4]	1.72	1.6	1.4	0.59-0.68
Carotenoid (µg/g)	1696 ± 2.50	-	800	7-72	130-330	-

Note:
[1] present study
[2] data adopted from Santoso *et al*. (2015)
[3] data adopted from Kardaya *et al*. (2011)
[4] data adopted from Haq *et al*. (2018)
[5] data adopted from Hertrampf & Pieded-Pascual (2000)
[6] data adopted from Kuo *et al*. (1976)
[7] data adopted from Vijayalingam & Rajesh (2020)
[8] data adopted from Chen *et al*. (2009)

A detailed comparison of the protein content of the sand crab (37.89%) with the previous studies, as well as comparisons with other ingredients, shows that the sand crab can be considered to be equivalent to other ingredients. According to Mursyidin *et al.* (2002), the protein content of sand crab ranges from 30-40%, which is also consistent with the report of Kardaya *et al*. (2011) that the protein content of sand crab is 32.5%. Variations in the protein content of sand crab can occur in fresh, boiled, steamed and grilled conditions of 38.52, 38.40, 37.20 and 35.13% respectively (Santoso *et al*., 2015). The protein content of sand crab is also similar to that of crab processing waste, which is 31.0-37.6%, but lower than that of shrimp meal, which is 43.3%, krill meal 52-67.3% and soybean meal 42.5-48.6% (Chen *et al*., 2009;

Hertrampf & Piedad-Pascual, 2000; Kuo *et al*., 1976; Vijayalingam & Rajesh, 2020).

The ash content of sand crab varied when compared to previous studies. The ash content of sand crab in the current study was 23.86%, which was similar to the report of Kardaya *et al*. (2011), which was 26.4%, but lower than the report of Haq *et al*. (2018), which was 40.03%. The ash content of sand crab may decrease with different processing methods. According to Santoso *et al*. (2015), the ash content of fresh, boiled, steamed and grilled sand crab was 35.13, 35.63, 34.63, 32.50, and 31.59%, respectively. Despite the variation in ash content, the Ca and P mineral contents of 8.77 and 0.82% in this study are similar to those reported by Kardaya *et al*. (2011) and Haq *et al*. (2018), and close to the amounts of Ca, Mg, Fe, and Cu of 10-11% (Santoso *et al*., 2015). Ca and P are known to be essential for the development and maintenance of the skeletal system and for many other physiological functions in aquatic organisms (Davis & Gatlin, 1996; Davis & Lawerence, 1997; Prabhu *et al*., 2016; Lall & Kaushik, 2021). Given that sand crab habitats are known to be sandy intertidal areas (Wardiatno *et al*., 2014) and sand is an inorganic compound, it is possible that less clean sand was contaminated during sample preparation, thus increasing the amount of ash but not Ca and P, which occurred in previous studies. Another possibility is due to species differences or the phenomenon of molting in sand crabs.

The fat content of sand crabs varies widely, but this component is very important as a source of energy and for reproduction (Hu *et al*., 2009; Tocher, 2003). The fat content of sand crab in this study was 24.56%, similar to the report of Mursyidin *et al*. (2002) with a range of 17.22-21.56% in *Emerita analoga* and *Emerita talpoida* species. This value is higher than that reported by Kardaya *et al*., (2011), which was 10.2%, and in *Emerita emeritus*, which was reported as 8.76, 2.95, 4.32 and 5.65% for fresh, boiled, steamed and roasted conditions, respectively (Santoso *et al*., 2015).

The difference in research findings on fat content of sand crabs compared to previous studies may be due to several factors. The first factor is the difference in the species studied, as Balzona *et al*. (2017) stated that the fat content and profile of shrimp and crabs is influenced by the species type. The second factor is the different processing methods. The processing of fishery products affects the quality and quantity of nutrients (Abraha *et al*., 2018; Akonor *et al*., 2016; Hairol *et al*., 2022; Wang *et al*., 2011; Wu & Mao, 2008), especially the drying step (Chukwu & Shaba, 2009). The third factor is when the crab is caught. This is related to the size, age and stage of the crab itself. Just 1 month before hectic breeding activity (February and August), the percentage of fat in the sand crab body was observed to increase and then decrease sharply during and after spawning (Nagabhushanam & Kulkarni, 1977).

Other parameters presented in Table 2 are fibre content and total carotenoids. The fibre content of 6.0% in sand crab was similar to that reported by Kardarya *et al*. (2011), which was 4.9% and lower than other crustacean feed ingredients. While carotenoids are an essential nutrient for any biological function in crustaceans, which can be seen when sand crab is heated or boiled, it turns a reddish colour like other crustaceans (Helliwell, 2010; Manikandan *et al*., 2020; Mohamad-Zuki *et al*., 2022; Pan *et al*., 2020). The results of the present studies also showed that the concentration of carotenoids in sand crabs was as high as 1696 µg/g, much higher than the last reported with a range of 40-50 µg/g (Gilchirst & Lee, 1972). This different migh caused by many factors such as an age, developmental stages, metabolism, sex, molting, environmental conditions, carotenoid concentration in feed, and diseases that affect carotenoid deposition in the same aquatic animal species (Contancio, 2011; Meyer & Latscha, 1997; Sukarman *et al*., 2023). If compared with other fish feed ingredients from crustacean by-product, the sand crab has better carotenoid concentration than crab meal of

Figure 1. Colors of fresh Sand Crabs (left) and Sand crabs that have been processed using the freeze- dried method (right).

800 µg/g (Kuo *et al*., 1976), shrimp head meal of 7-72 µg/g, and krill meal 130- 330 µg/g (Hertrampf & Piedad-Pascual, 2000). Based on the data of carotenoid content, sand crab is more valuable as an feed ingredient for ornamental fish because fish cannot produce these compounds and must be supplied from their feed (Choubert, 2010; Simpson *et al*., 1981). Therefore, ornamental fish need to be fed with high carotenoids content such as sand crabs.

A more in-depth study of the nutritional content of the sand crab is related to the amino acids and the fatty acids profile. The results of the present studies showed that the total essential amino acid (TEAA) content of sand crab (12.84%) was higher than that of crab meal (4.72%), but lower than that of shrimp meal, krill meal and soybean meal, as shown in Table 3. This indicates that the protein present in sand crab is not only composed of essential amino acids, but also non-essential amino acids, and may also be nitrogen-bonded by chitin compounds. As reported by Wittriansyah *et al*. (2018), sand crab contains 18.87% chitin, and chitin is known as a nitrogenous polysaccharide found in many crustaceans (Boriæ *et al*., 2020; Kumari *et al*., 2015; Mohan *et al*., 2021). Chitin has also been found in crab meal at 17-24% (Broke *et al*., 2023), shrimp meal at 12.6-17.0% (Hertrampf & Piedad-Pascual, 2000), and krill meal at 2-10% chitin (Landymore *et al*., 2019). The nitrogen in chitin cannot be utilised by fish, but the presence of chitin in the diet does not affect the growth of common carp *Cyprinus carpio* (Ringø *et al*., 2012). Therefore, the authors focus only on the availability of essential amino acids, specifically lysine and methionine.

Lysine and methionine are the main limiting amino acids for the fish growth (Giri *et al*., 2006; NRC, 2011; Mukhtar *et al*., 2017; Li *et al*., 2021). Previous research on lysine requirements in fish diets has varied widely depending on the species. NRC (2011) has summarised the estimated lysine requirements for fish, which range from 1.2-2.5% of the diet, or 4.0-5.8% of the dietary protein. Meanwhile, the methionine requirement for fish ranges from 0.7-1.2% in feed or 1.73-3.3% of feed protein. More specifically, the lysine requirement of *Cyprinus carpio* is 5.9% of dietary protein (Zhou *et al*., 2008), while its methionine requirement is 0.86% of diet or 2.13% of dietary protein (Schwarz *et al*., 1998). The present study showed that sand crabs contained only 0.99 and 0.35% lysine and methionine respectively and are unlikely to be the main source of lysine and methionine in feeds for *Cyprinus carpio*. However, up to 30% may potentially be used in feeds such as crustaceans or other crustacean by-products (Hertrampf & Piedad-Pascual, 2000; Yi *et al*., 2015; Hairol *et al*., 2022).

Table 3. Composition of Essential Amino Acids of Sand Crabs compared to Other Protein Sources (% of sample)

Amino Acid	Sand Crab[1]	Crab Meal[2]	Shrimp Meal[2]	Krill Meal[2]	Soybean Me
Crude Protein	37.89 ± 4.36	32.20	43.20	58.80	44.00
Lysine	0.99 ± 0.12	0.73	2.06	4.11	2.83
Methionine	0.35 ± 0.01	0.28	0.56	1.74	0.61
Threonine	0.95 ± 0.05	0.33	1.55	2.49	1.73
Tryptophan	0.14 ± 0.00	0.22	1.55	0.52	0.61
Arginine	1.38 ± 0.02	0.61	2.41	3.46	3.24
Histidine	1.14 ± 0.07	0.29	0.81	1.21	1.17
Leucine	2.32 ± 0.12	0.71	2.53	4.35	3.42
Iso leucine	1.49 ± 0.20	0.52	2.70	2.78	1.99
Phenylalanine	2.05 ± 0.01	0.44	1.76	2.49	2.18
Valine	2.03 ± 0.03	0.59	2.32	2.78	2.40
Total EAA	12.84	4.72	18.25	25.93	20.18

Note:

[1] result of Present study

[2] adopted from Hertrampf & Piedad-Pascual (2000): original data as percentage of protein content, converted to percentage of samples

[3] adopted from NRC (2011)

The last nutritional parameter analyzed in this study was the content of essential fatty acids, as shown in Figure 2. Based on the results of the study, the omega-3 components in this form of EPA and DHA of dry sand crab were 1.86% and 0.78%, corresponding to 7.79% and 3.26% of the total fat, respectively. The results are in line with previous research reports, which found that the EPA and DHA content of sand crab was 6.41-8.43% and 1.34-6.57% of total fat, respectively (Mursyidin, 2007). In general, omega-3 fatty acids in the form of EPA and DHA are readily available to marine life, as they are consumed by shrimps and sea fish, as well as by micro-organisms that are rich in these compounds (Adarme-Vega *et al*., 2014). Similarly, it is thought that sand crab obtains its omega-3s from the same source. At the same time, the other omega-3 components in the form of APA are low in concentration. Alpha-linolenic acid (APA) is usually easily obtained from nuts such as flaxseed (Basch *et al*., 2007). While omega-6 fatty acids from research results amounted to 0.52% of the total ingredients or equivalent to 2.17% of total fat, much smaller than in species *Emerita talpoida* and *Emerita analoga* contained 11.80% and 12.94% omega-6 (Mursyidin, 2007). However, the omega-9 content of sand crabs is relatively high at 1.99% in sand crabs or 8.34% of total fat. The three types of fatty acids in omega-3, omega-6, and omega-9, as well as potential human health supplements, significantly reduce the risk of a heart attack. In addition, when used as feed, these three compounds play an essential role in fish reproduction and seed growth (Hertrampf & Piedad-Pascual, 2000; NRC, 2011).

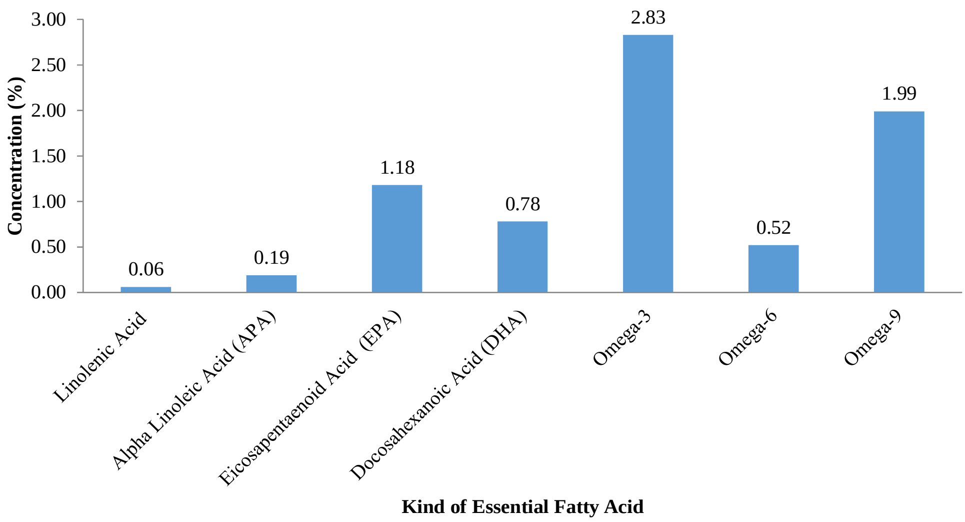

Figure 2. Essential Fatty Acids in Sand crabs (% of sample).

Feeding trial different level sand crab in feed to juvenile of koi carp (*Cyprinus carpio*)

The study on ornamental Koi carp revealed that incorporating sand crabs up to 15% in their diet resulted in a significant increase in both body weight and length, compared to other treatments ($p<0.05$). However, different levels of sand crab meal in diets did not significantly affect the specific growth of these fish during 42 days of feeding experiment. The highest to lowest increase in body weight of Koi carp ranged from 0.44 ± 0.09 g to 0.26 ± 0.08 g (T4 to T1 treatments, respectively). The highest to lowest increase in body length was observed in T4 to T1 treatments, respectively, with consecutive values of 0.93 ± 0.11 cm to 0.66 ± 0.19 cm. The specific growth rate (SGR) of fish did not differ significantly between treatments ($P>0.05$) and ranged from 0.95-1.45% per day. Figure 3 presents the overall performance data of Koi carp fed with sand crab.

Based on Figure 3, it can be reported that 15% sand crab in the diet (treatment T4) produced the best performance in Koi carp. Until the results of this study were published, there had been no previous studies using sand crab as a fish feed ingredient. However, the use of 40% sand crab in quail feed was reported to produce the best growth (Pratiwi, 2016). Meanwhile, 30% of sand crab meals produced eggs with the highest linoleic acid levels (Astriana, 2013). Experiments have also been carried out on mice to test the efficacy of sand crab. The most effective dose for promoting growth in mice was found to be a 25% concentration in the diet (Kardaya *et al*., 2011). Similar to this report, another marine crab *Chaceon affinis* is reported to be able to replace other protein sources in catfish feed (Keremah, 2013), and added up to 15% in shrimp feed can improve growth, feed conversion and protein efficiency of *L. vannnamei* juveniles (Goytortúa-Bores *et al*., 2006). In others report, inclusion of marine crab meal at 10-20% of the diet has been reported to improve growth performance, promote yellow skin colour and delay lipid oxidation in muscle of red porgy *Pagrus pagrus* (García-Romero *et al*., 2014a, García-Romero *et al*., 2014b).

The positive effect of sand crab on the growth of ornamental Koi carp cannot be separated from its high nutrient content, especially fat and calcium. In this study, the protein content was made equal between the treatments, while the other nutrients followed the composition of the raw materials. Adding sand crab to the koi feed at levels 5, 10 and 15% increases the calcium content from 1.77 (control) to 2.16, 2.67 and 3.49% respectively. Also increase the fat content in the feed from 4.00 (control) to 5.03, 5.98 and 6.84%, respectively (Table 1). This means that the fat and calcium content in the treatment was 15% higher than in the other treatments, indicating that the difference in growth between treatments was not due to protein content, but rather to other nutrients such as fat and calcium. This follows the theory that calcium and phosphorus affect the growth of juvenile fish (Liu *et al*., 2021) and essential fats meet the energy needs of fish (NRC, 2011), thus indirectly increasing fish growth. It also improves feed efficiency (Figure 4).

The feed efficiency for each treatment was 40.62, 46.78, 51.24 and 63.5% for T1, T2, T3 and T4, respectively. This is in line with previous reports for the koi carp *Cyprinus carpio*, which was ranged from 48.59-73.24% (Winarti *et al*., 2017), and when beta carotene was added in diet, feed efficiency range from 51.35-61.42% (Purba *et al*., 2020). In general, the diets containing 10 and 15% sand crab could be well utilized by koi carp, as the efficiency value was more than 50%

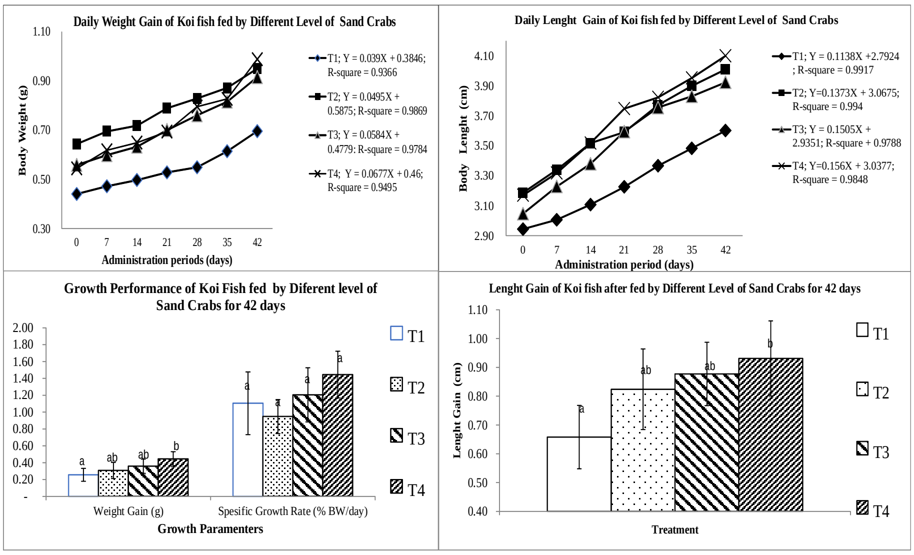

Figure 3. The growth performance of Koi carp was evaluated over a 42-day period by feeding them with varying doses of sand crab in diets. T1: feed without sand crab, T2: feed with sand crab 5%, T3: feed with sand crab 10%, T4: feed with sand crab 15%.

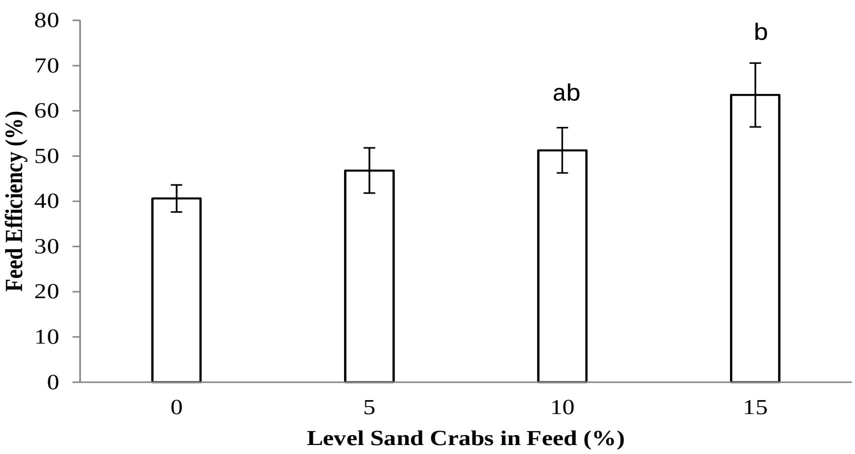

Figure 4. Koi Fish Feed Efficiency using different sand crab levels.

(Purba *et al*., 2020), but the best value was obtained at a dose of 15%, which was significantly different from the others ($p<0.05$). Based on these results, the use of sand crab up to 15% in the diet resulted in the best performance of juvenile Koi carp, and there is still an opportunity to increase its use.

CONCLUSION

The protein content of sand crab is quite high at 37.88%, but the essential amino acid (EAA) content is lower compared to shrimp meal, shrimp head meal, krill meal and soybean meal. While the fat content is higher than the four ingredients, the omega-3 and omega-9 content is 2.83% and 1.99% respectively. Sand crab has a high carotenoid content of up to 1696 µg/g, making it a promising source of aquafeed pigments. The inclusion of sand crabs at a level of 15% in the diet of koi fish resulted in the highest values of LG, WG and FE, which were 0.93 ± 0.05 cm, 0.48 ± 0.06 g and 63.50%. These findings lead that sand crab has good nutritional content, and which makes it suitable for Koi carp feed at concentrations of up to 15%.

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REFERENCES

Abraha, B., Admassu, H., Mahmud, A., Tsighe, N., Shui, X. W., & Fang, Y. (2018). Effect of processing methods on nutritional and physico-chemical composition of fish: a review. *MOJ Food Processing & Technology*, 6(4), 376–382. https://doi.org/10.15406/mojfpt.2018.06.00191

Adarme-Vega, T. C., Thomas-Hall, S. R., & Schenk, P. M. (2014). Towards sustainable sources for omega-3 fatty acids production. *Current Opinion in Biotechnology*, 26, 14–18. https://doi.org/10.1016/j.copbio.2013.08.003

Akonor, P. T., Ofori, H., Dziedzoave, N. T., & Kortei, N. K. (2016). Drying characteristics and physical and nutritional properties of shrimp meat as affected by different traditional drying techniques. *International Journal of Food Science,* 1–5. https://doi.org/https://doi.org/10.1155/2016/7879097

AOAC. (2005). Official Methods of Analysis (16th ed.). Association of Official Analytical Chemists.

Apte, S. K. (2012). Impact assessment on the aquatic ecosystem in the vicinity of an operating nuclear power plant at the Kalpakkam Coastal Site. In *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 349–355. https://doi.org/10.1007/s40011-012-0103-4

Astriana, Y. (2013). Increase in egg yolk colour intensity and omega-3 levels in quails fed with sand crab (*Emerita sp*). *Undergraduate Thesis*. University of Semarang. https://lib.unnes.ac.id/20193/

Balzano, M., Pacetti, D., Lucci, P., Fiorini, D., & Frega, N. G. (2017). Bioactive fatty acids in mantis shrimp, crab and caramote prawn: Their content and distribution among the main lipid classes. *Journal of Food Composition and Analysis*, 59, 88–94. https://doi.org/10.1016/j.jfca.2017.01.013

Basch, E., Bent, S., Collins, J., Dacey, C., Ulbricht, C., Vora, M., & Weissner, W. (2007). Flax and flaxeed oil (*Linum usitatissimum*)/ : a review by the natural standard research collaboration. *Journal of the Society for Integrative Oncology*, 5(3), 92–105.

Bhagawati, D., Anggoro, S., Zainuri, M., & Sya'rani, L. (2016). Taxonomic contribution to species utilisation: study of morphological characteristics and key dichotomies of the Yutuk crab (Crustacea: Hippoidea) from the Cilacap coast. In *Proceedings of the 5th National Annual Seminar, Results of Fisheries and Marine Science*, 550-564, Diponegoro University.

Bhagawati, D., Nuryanto, A., Winarni, E. T., & Pulungsari, A. E. (2022). Morphological and molecular characterization of mole crab (Genus: *Emerita*) in the Cilacap coastlines of Indonesia, with particular focus on genetic diversity of *Emerita sp. Biodiversitas*, 23(5), 2395–2404. https://doi.org/10.13057/biodiv/d230517

Batoro, J. (2008). Omega 3 Eggs. http//www.malangkab.go.id./artikel/artikel.cfm.

Boriæ, M., Vicente, F. A., Jurkoviæ, D. L., Novak, U., & Likozar, B. (2020). Chitin isolation from crustacean waste using a hybrid demineralization/DBD plasma process. *Carbohydrate Polimers,* 246 (2020), 116648.

Boyko, C. B. (2002). A worldwide revision of the recent and fossil sand crabs of the *Albuneidae stimpson* and *Blepharipodidae*, new family (Crustacea: Decapoda: Anomura: Hippoidea). *Bulletin of the American Museum of Natural History*, 272, 3–392.

Boyko, C. B., & Harvey, A. W. (1999). Crustacea Decapoda: *Albuneidae* and *Hippidae* of the tropical Indo-West Pacific region. *Mémoires Du*

Muséum National d'Histoire Naturelle, 20(180), 379–406.

Chen, Y. C., Tou, J. C., & Jaczynski, J. (2009). Amino acid and mineral composition of protein and other components and their recovery yields from whole antarctic krill (*Euphausia superba*) using isoelectric solubilization/precipitation. *Journal of Food Science*, 74(2),31–39. https://doi.org/10.1111/j.1750-3841.2008.01026.x

Choubert, G. (2010). Response of rainbow trout (*Oncorhynchus mykiss*) to varying dietary astaxanthin/canthaxanthin ratio: colour and carotenoid retention of the muscle. *Aquaculture Nutrition*, 16(5), 528–535. https://doi.org/10.1111/j.1365-2095.2009.00692.x

Chukwu, O., & Shaba, I. M. (2009). Effects of drying methods on proximate compositions of catfish (*Clarias gariepinus*). *World Journal of Agricultural Sciences*, 5(1), 114–116.

Contancio, L. F. (2011). Effects of season, size and parasitism by the acanthocephalan, profilicollis altmani, on the carotenoid concentration and composition of the pacific mole crab, *Emerita analoga*. *M.Sc.Thesis*. Faculty of California Polytechnic State University.

Davis, D. A., & Gatlin, D. M. (1996). Dietary mineral requirements of fish and marine crustaceans. *Reviews in Fisheries Science*, 4(1), 75–99.

Davis, D. A., & Lawerence, A. I. (1997). Minerals. In L. R. D'Abramo, D. E. Conklin, & D. M. Akiyama (Eds.), *Crustacean Nutrition* (pp. 150–159).

Efford, I. E. (1976). Distribution of the sand crabs in the genus *Emerita* (Decapoda, Hippidae). *Crustaceana*, 30(1), 169–183.

García-Romero, J., Ginés, R., Izquierdo, M., & Robaina, L. (2014a). Marine and freshwater crab meals in diets for Red porgy (*Pagrus pagrus*): effect on fillet fatty acid profile and flesh quality parameters. *Aquaculture*, 420–421(2014), 231–239. https://doi.org/10.1016/j.aquaculture.2013.10.035

García-Romero, J., Ginés, R., Izquierdo, M. S., Haroun, R., Badilla, R., & Robaina, L. (2014b). Effect of dietary substitution of fish meal for marine crab and echinoderm meals on growth performance, ammonia excretion, skin colour, and flesh quality and oxidation of Red porgy (*Pagrus pagrus*). *Aquaculture*, 422–423(2014), 239–248. https://doi.org/10.1016/j.aquaculture.2013.11.024

Gilchirst, B. M., & Lee, W. L. (1972). Carotenoid pigments and their possible role in reproduction in the sand crab, *Emerita analoga* (Stimpson, 1857). *Comp. Biochem. Pysiol*, 42B, 263–294.

Giri, N., Suwirya, K., & Marzuqi, M. (2006). Dietary methionine requirement for growth of juvenile Humpback grouper (*Cromileptes altivelis*). *Indonesian Aquaculture Journal*, 1(2), 79–86.

Goytortúa-Bores, E., Civera-Cerecedo, R., Rocha-Meza, S., & Green-Yee, A. (2006). Partial replacement of red crab (*Pleuroncodes planipes*) meal for fish meal in practical diets for the white shrimp *Litopenaeus vannamei*. Effects on growth and in vivo digestibility. *Aquaculture*, 256 (1–4), 414–422. https://doi.org/10.1016/j.aquaculture.2006.02.035

Hairol, M. D., Nian, C. T., Imlani, A. H., Tikmasan, J. A., & Sarri, J. H. (2022). Effects of crab shell meal inclusions to fishmeal replacement on the survival, growth, and feed utilization of mangrove crab *Scylla serrata* (Forsskal 1775). *Journal of Agricultural Sciences*, 32(4), 714–726. https://doi.org/10.29133/yyutbd.1131220

Haley, S. R. (1982). Zonation by size of the pacific mole crab, *Hippa pacifica* dana (Crustacea:Anomura:Hippidae), in hawaii. *Journal of Experimental Marine Biology and Ecology*, 58(2–3), 221–231. https://doi.org/10.1016/0022-0981(82)90131-9

Haq, M., Irmansyah, Maddu, A., Riyanto, B., Wardiatno, Y., & Zakiah, A. F. N. (2018). Exploration of composition, elements, and microstructure of body and shell on tropical mole crab (Emerita emeritus). *Conference Series: Earth and Environmental Science*, 187(1), 0–9. https://doi.org/10.1088/1755-1315/187/1/012021

Haye, P. A., Tam, Y. K., & Kornfield, I. (2002). Molecular phylogenetics of Mole crabs (Hippidae: *Emerita*). *Journal of Crustacean Biology*, 22(4), 903–915.

Helliwell, J. R. (2010). The structural chemistry and structural biology of colouration in marine crustacea. *Crystallography Reviews*, 16(3), 231–242. https://doi.org/10.1080/08893111003747084

Hertrampf, J. W., & Piedad-Pascual, L. (2000). Handbook on Ingredient for Aquaculture Feeds. Kluwer Academic Publishers, London.

Hodar, A. R., Vasava, R., Joshi, N. H., & Mahavadiya, D. R. (2020). Fish meal and fish oil replacement for alternative sources: a review. *Journal of Experimental Zoology India*, 23(January), 13–21. https://www.thepharmajournal.com/archives/2021/vol10issue9/PartI/10-8-322-305.pdf

Hu, E., Wang, R. J., Pan, C. Y., & Yang, W. X. (2009). Fatty acids: composition and functions for reproduction. *Aquaculture Research Progress*, May, 127–146.

Jia, S., Li, X., He, W., & Wu, G. (2022). Protein-sourced feedstuffs for aquatic animals in nutrition research and aquaculture. In *Advances in Experimental Medicine and Biology*, 1354, 237-261. https://doi.org/10.1007/978-3-030-85686-1_12

Kardaya, D., Ralahalu, T. N., Zubir, Purba, M., & Parakkasi, A. (2011). Test on *Emerita analoga* as cholesterol reducing agent on *Mus musculus* BALB/*C. JITP*, 1(2), 74–87.

Keremah, R. I. (2013). The effects of replacement of fish-meal with crab-meal on growth and feed utilization of African giant catfish *Heterobranchus longifilis* fingerlings. *International Journal of Fisheries and Aquaculture*, 5(4), 60–65. https://doi.org/10.5897/IJFA2013.0332

Ketaren, C. B. B., Hakim, A. A., Fahrudin, A., & Wardiyatno, Y. (2019). The concentration of the heavy metal lead (Pb) in sand crabs and its impact on human health. *Journal of Biology Topic*, 19(1), 90–100. https://doi.org/10.29303/jbt.v19i1.1066

Kumari, S., Rath, P., Sri Hari Kumar, A., & Tiwari, T. N. (2015). Extraction and characterization of chitin and chitosan from fishery waste by chemical method. *Environmental Technology and Innovation*, 3, 77–85. https://doi.org/10.1016/j.eti.2015.01.002

Kuo, H. C., Lee, T. C., Kamata, T., & Simpson, K. L. (1976). Red crab processing waste as a carotenoid source for rainbow trout. *Alimenta*, 15, 47–51.

Lall, S. P., & Kaushik, S. J. (2021). Nutrition and metabolism of minerals in fish. *Animals*, 11(9), 1–41. https://doi.org/10.3390/ani11092711

Landymore, C., Durance, T. D., Singh, A., Singh, A. P., & Kitts, D. D. (2019). Comparing different dehydration methods on protein quality of krill (*Euphausia Pacifica*). *Food Research International*, 119, 276–282. https://doi.org/10.1016/j.foodres.2018.12.001

Li, X., Zheng, S., & Wu, G. (2021). Nutrition and functions of amino acids in fish. In *Advances in Experimental Medicine and Biology*, 1285, 133-168. https://doi.org/10.1007/978-3-030-54462-1_8

Liu, Y., Liu, Y. N., Tian, X. C., Liu, H. P., Wen, B., Wang, N., Gao, J. Z., & Chen, Z. Z. (2021). Growth and tissue calcium and phosphorus deposition of juvenile discus fish (*Symphysodon haraldi*) fed with graded levels of calcium and phosphorus. *Aquaculture*, 541, 1–7. https://doi.org/10.1016/j.aquaculture.2021.736755

Manikandan, K., Felix, N., & Prabu, E. (2020). A review on the application and effect of carotenoids with respect to canthaxanthin in the culture of fishes and crustaceans. *International Journal of Fisheries and Aquatic Studies*, 8(5), 128–133. https://doi.org/10.22271/fish.2020.v8.i5b.2314

Mantelatto, F. L., Paixão, J. M., Robles, R., Teles, J. N., & Balbino, F. C. (2023). Evidence using morphology, molecules, and biogeography clarifies the taxonomic status of mole crabs of the genus *Emerita scopoli*, 1777 (Anomura, Hippidae) and reveals a new species from the western Atlantic. *ZooKeys*, 2023(1161), 169–202. https://doi.org/10.3897/zookeys.1161.99432

Mashar, A., Wardiatno, Y., Boer, M., Butet, N. A., & Farajallah, A. (2014). Diversity and abundance of sand crabs on the south coast of Central Java. *Indonesian Journal of Marine Sciences*, 19(4), 226–232. https://doi.org/10.14710/ik.ijms.19.4.226-232

Meyer, S. P., & Latscha, T. (1997). Carotenoids. In L. R. D'Abramo, D. E. Conklin, & D. M. Akiyama (Eds.), *Crustacean Nutrition* (pp. 164–193). Advance in World Aquaculture, Vol 6. Baton Rouge: World Aquaculture Society.

Mohamad-Zuki, N. A., Redhwan, A. I., Rashid, Z. M., Zalilawati, M. R., Siew, I. N., Assis, K., Rex, F. M. T., & Komilus, C. F. (2022). Crustacean shell waste as a potential feed material. *Bioscience Research*, 19(SI-1), 360–372. http://www.isisn.org/BR-19-SI-1-2022/360-372-35-BR-SI-FBIM-2022-Mohamad-Zuki.pdf

Mohan, K., Muralisankar, T., Jayakumar, R., & Rajeevgandhi, C. (2021). A study on structural comparisons of á-chitin extracted from marine crustacean shell waste. *Carbohydrate Polymer Technologies and Applications*, 2, 100037. https://doi.org/10.1016/j.carpta.2021.100037

Mukhtar, B., Malik, M., Shah, S., Azzam, A., Slahuddin, & Liaqat, I. (2017). Lysine supplementation in fish feed. *Journal of Applied Biology and Forensics*, 1(2), 26–31.

Mursyidin, D.H. (2007). Omega-6 fatty acid content of sand crabs (*Emerita* spp.) on the south coast of Yogyakarta. *Bioscientiae*, 4(2), 79–84.

Mursyidin, D.H., Muhammad, S., Perkasa, D., Sekendriana, & Prabowo. (2002). Evaluation of the omega-3 fatty acid content of sand crab (*Emerita* sp.) from the south coast of Yogyakarta. *Taken from the Internal report of the Student Creativity Programme (PKM) of Gadjah Mada University*, funded by the Directorate General of Higher Education.

Nagabhushanam, R., & Kulkarni, K. M. (1977). Seasonal changes in biochemical cof the sand crab *Emerita holthuisi* sankolli (Decapoda anomura).

Monitore Zoologico Italiano - Italian Journal of Zoology, 11, 57–64. https://doi.org/10.1080/00269786.1977.10736290

NRC. (2011). Nutrient Requirements of Fish and Shrimp. The National Academies Press. https://doi.org/10.1007/s10499-011-9480-6

Pan, C., Liang, X., Chen, S., Tao, F., Yang, X., & Cen, J. (2020). Red color-related proteins from the shell of red swamp crayfish (*Procambarus clarkii*): isolation, identification and bioinformatic analysis. *Food Chemistry*, 327(2020), 127079. https://doi.org/10.1016/j.foodchem.2020.127079

Paul, M., Sardar, P., Sahu, N. P., Varghese, T., Shamna, N., Harikrishna, V., Deo, A. D., Jana, P., Singha, K. P., Gupta, G., Kumar, M., & Krishna, G. (2019). Optimal dietary protein requirement of juvenile GIFT tilapia (*Oreochromis niloticus*) reared in inland groud saline water. *Journal of Enviromental Biology*, 43, 205–215.

Pérez, D. (1999). Mercury levels in mole crabs *Hippa cubensis*, *Emerita brasiliensis, E. portoricensis*, and *Lepidopa richmondi* (Crustacea: Decapoda: Hippidae) from a sandy beach at Venezuela. *Bulletin of Environmental Contamination and Toxicology*, 63(3), 320–326. https://doi.org/10.1007/s001289900983

Prabhu, A. J., Schrama, J. W., & Kaushik, S. J. (2016). Mineral requirements of fish: A systematic review. *Reviews in Aquaculture*, 8(2), 172–219. https://doi.org/10.1111/raq.12090

Pratiwi, I. (2016). The effect of commercial feed substitution with mole crab (*Emerita* sp.) meal toward the body weight of male quail (*Coturnix coturnix japonica* l). *Jurnal of Biology*, 5(3), 1–8.

Purba, M., Putriningtias, A., & Komariyah, S. (2020). Addition of â-carotene natural flour sources in feed on improvement of color brightness and growth of koi (*Cyprinus carpio*). *Journal of Akuakultura*, 4(2), 10. https://doi.org/10.35308/ja.v4i2.3454

Ringø, E., Zhou, Z., Olsen, R. E., & Song, S. K. (2012). Use of chitin and krill in aquaculture - the effect on gut microbiota and the immune system: A review. *Aquaculture Nutrition*, 18(2), 117–131. https://doi.org/10.1111/j.1365-2095.2011.00919.x

Santoso, J., Hanifa, Y. N., Indariani, S., Wardiatno, Y., & Mashar, A. (2015). Nutritional values of the Indonesian mole crab, *Emerita emeritus*: are they affected by processing methods? *AACL Bioflux*, 8(4), 579–587.

Schwarz, F. J., Kirchgessner, M., & Deuringer, U. (1998). Studies on the methionine requirement of carp (*Cyprinus carpio* L.). *Aquaculture*, 161(1–4), 121–129. https://doi.org/10.1016/S0044-8486(97)00262-7

Simpson, K. L., Katayama, T., & Chichester, C. O. (1981). Carotenoids in fish feeds. In *Carotenoids as Colorants and Vitamin A Precursors*. (Ed. by JC Bauernfeind). Academic Press Inc, USA.

Sukarman, Ginanjar, R., Zamroni, M., Ardi, I., Musa, A., Rini Fahmi, M., Priyadi, A., & Yamin, M. (2023). Effect of different enriched crickets (*Gryllus sigilatus*) on growth and pigmentation of Asian arowana (*Scleropages formosus*) Var. super red. *HAYATI Journal of Biosciences*, 30(2), 392–403. https://doi.org/10.4308/hjb.30.2.392-403

Teles, A. O., & Couto, A. (2019). Dietary protein requirements of fish – a meta - analysis. *Reviws in Aquaculture*, 1–33. https://doi.org/10.1111/raq.12391

Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285(1–4), 146–158. https://doi.org/10.1016/j.aquaculture.2008.08.015

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, 11(2), 107–184. https://doi.org/10.1080/713610925

Vijayalingam, T. A., & Rajesh, N. V. (2020). Analysis of nutritional value of crab meal as feed supplement for livestock and poultry. *Chemical Science Review and Letters*, 9(35), 773–776. https://doi.org/10.37273/chesci.CS205107195

Wang, Y., Zhang, M., & Mujumdar, A. S. (2011). Trends in processing technologies for dried aquatic products. *Drying Technology*, 29(4), 382–394. https://doi.org/10.1080/07373937.2011.551624

Wardiatno, Y., Ardika, P. U., Farajallah, A., Butet, N. A., Mashar, A., Kamal, M. M., Renjaan, E. A., & Sorang, M. . (2015). Biodeversity of Indonesian sand crab (Crustacea, Anomura, Hippidae) and assessment of their phylogenetic relationships. *AACL Bioflux*, 8(2), 224–235. https://doi.org/10.1163/9789004387584_011

Wardiatno, Y., Nurjana, I. W., & Mashar, A. (2014). Habitat characteristics of the sand crabs (Family Hippidae) in sandy coast of Cilacap District. *Jurnal Biologi Tropis*, 14(1), 1–8. https://doi.org/10.29303/jbt.v14i1.1947

Winarti, W., Subandiyono, S., & Sudaryono, A. (2017). Use of fermented *Lemna Sp*. meal in artificial diets for the growth of carp fish (*Cyprinus carpio*). *Journal of Science Aquaculture Technology*, 1(2), 88–94.

Wittriansyah, K., Handayani, M., & Dirgantara, D. (2018). Characterization of chitin and chitosan *Emerita Sp.* from Widarapayung coast, Cilacap, Central Java. *Jurnal Ilmiah Samudra Akuatika*, 2(1), 45–51.

Wu, T., & Mao, L. (2008). Influences of hot air drying and microwave drying on nutritional and odorous properties of grass carp (*Ctenopharyngodon idellus*) fillets. *Food Chemistry*, 110(3), 647–653. https://doi.org/10.1016/j.foodchem.2008.02.058

Yi, X., Li, J., Xu, W., Zhou, H., Smith, A. A., Zhang, W., & Mai, K. (2015). Shrimp shell meal in diets for large yellow croaker *Larimichthys croceus*: effects on growth, body composition, skin coloration and anti-oxidative capacity. *Aquaculture*, 441, 45–50. https://doi.org/10.1016/j.aquaculture.2015.01.030

Zhou, X. Q., Zhao, C. R., Jiang, J., Feng, L., & Liu, Y. (2008). Dietary lysine requirement of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition*, 14(5), 381–386. https://doi.org/10.1111/j.1365-2095.2007.00535.x

