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The organoleptic, physical, and chemical quality of mud crab fattening feed fermented with a microorganism mixture

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Abstract. Feed for fattening mud crabs must be able to survive in water and have high nutritional quality. High nutritional quality impacts increasing feed prices, so it is necessary to improve the quality of feed while maintaining a stable price. This study evaluated the organoleptic, physical, and chemical quality of mud crab feed fermented by adding various doses of mixed microorganisms, which cause fermentation to the raw feed materials. The microorganism mixture doses tested were: 0, 10, 20, and 30 mL 100 g⁻¹ of raw feed materials. Based on organoleptic feed data (appearance, texture, aroma, and feed color), physical data (water stability, including speed of breakdown and solids dispersion, hardness level, and sinking speed), and feed nutritional or chemical tests (protein, fat, nitrogen-free extract, crude fibre, ash, and water content, as well as the dry and organic matter digestibility percentages) it can be concluded that the microorganism mixture can be used effectively to ferment feed raw materials at a dose of 10 mL 100 g⁻¹ of raw feed materials.

1. Introduction

As an export commodity, mud crab has a fairly high selling price in domestic and foreign markets. However, it all depends on the quality of the mud crab itself (the size of the crab's fatness). The survey results in the field found that the price of mangrove crabs with 100-200 g of weight is around Rp. 140,000, and Rp. 265,000 for sizes 900-1000 g. Therefore, the fattening cultivation business is very promising.

During the rearing period, crabs are fed fresh feed, such as trash fish, snails, chicken intestines, and others. The problem faced is the continuity of the fresh feed supply and the very fluctuating price of fish (IDR 5,000-20,000 / kg (Fish auction price)). This requires applicative technological innovation. One of the most crucial is special crab feed that has high water stability (resistance in water) and a quality that can accelerate molting and increase growth and is effectively produced on a large scale. Formulated feed for crabs grow out has been found [1], which effectively accelerates molting and increases growth. The nutrients from the feed include 30.86% protein, 7.2% fat, 48.89% Nitrogen Free Extract (NFE), and 3.7% crude fiber. In feed management research, the frequency of feeding once per 2 days resulted in the highest feed efficiency of 53% [2]. Efforts to improve feed formulation have been made by utilizing *Kappaphycus alvarezii* seaweed as a source of carbohydrates and binders. Carbohydrates as a source of plant-based nutrients and binders as an adhesive to increase water stability. The feed produced can survive in water for more than 24 hours, but it could not increase feed efficiency, with a feed efficiency



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level of 41% [3]. The higher the feed efficiency, the more quality and effective the feed in increasing growth. Based on this, efforts should be made to increase feed efficiency for crab fattening cultivation using a biotechnological approach.

One of the biotechnological approaches that can be applied is fermentation. It is fermenting the feed with the help of a fermenter and providing additives to increase the digestibility and use-value of feed nutrients. Fermentation needs to be carried out to simplify the complex nutrients contained in all raw materials, especially crude fiber. As reported [4], on Azolla aquatic plant flour with the fermenter *Rhizopus* sp. Used a mixture of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. with a composition of 1 mL + 1 g + 1 g 100 g⁻¹ flour and proven to improve the quality of seaweed as a feed ingredient [5]. Microorganisms mixture added to milkfish feed at a dose used 10 mL / 100 g of feed [6].

Fermentation of raw materials with the right dose is expected to improve the quality of feed for crab fattening. Thus, feed will be produced with an efficient level of feed utilization and increasing crab growth and molting performance. This study aims to evaluate the organoleptic, physical, and chemical quality of crab feed fermented with various doses of mixed microorganisms as a fermenter.

2. Methods

2.1. Research Location and time

This study was carried out from May to July 2020. Feed manufacturing, organoleptic and physical tests were conducted in the Laboratory of Nutrition and Feed Management, Faculty of Marine and Fisheries Science, and in Laboratory of Fisheries Biotechnology, Centre of Research, and Nutrition Laboratory, Faculty of Veterinary, Universitas Hasanuddin.

2.2. Material and Methods

This study was designed in a completely randomized design (CRD) with 4 treatments and 3 replications. The treatments tested were various doses of mixed microorganisms, as a fermenter in fermenting feed ingredients, as follow: 0, 10, 20, and 30 mL 100 g⁻¹ of feed raw materials

2.3. Research Procedure

The initial stage in the experiment was preparing the feed ingredients. It started by grinding all the dry ingredients into a fine powder. Furthermore, fish silage is carried out by cleaning the fish and removing its scales, then washed and drained. Fish was grounded with a meat mill until it became smooth. Then the fish silage was put in a closed bucket that has been previously covered with a plastic bag. Furthermore, added 1.5 L of formic acid to 50 kg of fish ground meat and stirred until evenly mixed. Stirring was repeated 3 to 4 times every day until day 4. On day 5 the water at the top was removed. Furthermore, the mixed microorganism inoculation was carried out with a treatment dose and incubated for 7 days in a tightly closed and vacuum.

Feed raw materials are in the form of flour except for vitamin and mineral mix. It was weighed and mixed starting from the smallest amount to the largest and stirred until homogeneous. It was then inoculated with mixed microorganisms according to the treatment dose that has been previously diluted with water in a ratio of 1: 3. The mixture was stirred evenly and stored in airtight plastic. Incubation was carried out for 72 hours. After 72 hours, the raw material mixture was opened and sun-dried.

The feed dough was molded using a pellet template for crabs with a diameter of 1 cm and steamed for ± 40 minutes. Pellets were cut 2 cm in length and sun-dried. The dried feed was cooled at room temperature or air-dried, then put in a plastic bag and stored in a dry place before use for further test.

2.4. Research Variables

The variables observed in the experimental feed were organoleptic, physical, and chemical tests or feed nutrition, as well as dry matter digestibility (DMD) and organic matter digestibility (OMD).

2.4.1. Organoleptic Test. Organoleptic examination on experimental feed was including the appearance, texture, aroma, and color of feed. The appearance of feed can be seen from a smooth, fibrous, or perforated feed surface. The texture of the feed is influenced by the fineness of the raw material, the amount of fiber, and the type of binder used. The aroma of the feed determines the quality of the feed because it is closely related to the recipient or the lure of the crabs to the feed, which is determined by the type and amount of attractant added to the feed-making process. The color of the feed highly depends on the type of raw material used.

2.4.2. Physical test. The physical test carried out on the test feed was the observation of water stability, including the disintegrate speed test and solids dispersion, the level of hardness, and the immersion speed.

Water stability is the level of water resistance or how long it takes for the feed to soften and crumble, including disintegration speed test and solid dispersion.

Disintegration speed test measures how long it takes for the feed to disintegrate in the water. The disintegration test was visually observed. Five sticks of feed were put into a beaker filled with 4 L of seawater. Observations were performed every 15 minutes to find out whether the feed is soft or not. Observations were continued until the feed is broken/destroyed.

Solids dispersion was observed using the method of (1973) [7], 5 g of feed was put into a gauze box of 10 x 10 cm, with pores of about 1 mm, then immersed in an aquarium. After 6 hours, the feed that was still stuck in the gauze box was dried along with the gauze box in an oven at 105°C for 10 hours. Then it is cooled in a desiccator, then weigh until the weight was constant. The solids dispersion was calculated using the following formula:

$$\text{Solids dispersion (\%)} = \frac{\text{Final dry weigh of feed}}{\text{Initial dry weigh of feed}} \times 100 \quad (1)$$

Feed solidity level was measured by putting 5 g of feed into a 1 m pipe. Then the feed was load down with a weight of 500 g. The feed that has been loaded was then sieved using a 0.5 mm size Signet. The solidity level is calculated as the percentage of the unbreakable feed.

Immersion speed was carried out by measuring the time feed needed to move from the water's surface to the bottom of the rearing medium. Five sticks of feed were put into a beaker glass with a 20 cm bottom height from the water surface. The stopwatch was started at the exact moment the feed was dropped onto the water surface. The immersion speed is the distance divided by the time feed reached the bottom of the beaker glass.

2.4.3. Chemical test. A chemical test determines the quantity and the quality of nutrients in feed, including: **Proximate composition determination** of protein content, lipid, NFE, fiber, ash, and water of the experimental feed. **Dry matter digestibility (DMD) and organic matter digestibility (OMD).** The formula for determining the percentage of dry matter and organic matter digestibility in vitro [8] is as follow:

$$\text{Dry matter digestibility (DMD)} = \frac{\text{Initial DM sample} - (\text{DM residue} - \text{DM Blanco})}{\text{Dry Matter initial weigh}} \times 100 \quad (2)$$

$$\text{Organic matter digestibility (OMD)} = \frac{\text{Initial OM sample} - (\text{OM residue} - \text{OM Blanco})}{\text{Initial OM sample}} \times 100 \quad (3)$$

Where:

DM = Dry matter

OM = Organic matter

2.5. Data analysis

All observational data obtained in this study were analyzed descriptively by comparing the results obtained between treatments and also comparing with supporting literature.

3. Results and Discussion

3.1. Organoleptic and physical test

The results of organoleptic observations (appearance, texture, aroma, and color of the feed) and physical tests (water stability, including disintegration speed and solids dispersion, solidity level, and feed immersion speed) for each treatment are presented in Table 1.

Based on the organoleptic test (Table 1), the four experimental feeds have the potential as crab feed and can attract crabs to eat them. Furthermore, based on the water stability test on various experimental feeds, it was observed that all experimental feeds had the ability to survive in water for more than 24 hours of observation. The lowest percentage of solids dispersion was shown by the unfermented feed with mixed microorganisms, followed by the mixed microorganisms dose of 10 and 20 mL 100 g⁻¹ of feed ingredients. The highest was at the dose of 30 mL 100 g⁻¹ of raw materials. All experimental feeds had a high solidity level, ranging from 83–86%. The results of the immersion velocity test showed that it ranged from 7.6–8.7 cm/sec.

Table 1. Organoleptic and physical test data of experimental feeds

Parameters	Dose of microorganism mixture (mL 100 g ⁻¹ feed)			
	A (0)	B (10)	C (20)	D (30)
Organoleptic				
Appearance	Smooth surface and very few fiber	Smooth surface and very few fiber	Smooth surface and very few fiber	Smooth surface and very few fiber
Texture	Compact, hard outside and crunchy inside	Compact, hard outside and crunchy inside	Compact, hard outside and crunchy inside	Compact, hard outside and crunchy inside
Aroma	Very tangy with distinctive aroma like shrimp-paste	Very tangy with distinctive aroma like shrimp-paste	Very tangy with distinctive aroma like shrimp-paste	Very tangy with distinctive aroma like shrimp-paste
Colors	Blackish brown	Blackish brown	Blackish brown	Blackish brown
Physical test				
<i>Water stability</i>				
Disintegration speed (hours)	>24	>24	>24	>24
Solids dispersion (%)	10.30	11.60	11.98	12.77
Solidity level (%)	83	86	85	86
Immersion speed (cm/sec)	7.6	7.6	8.3	8.7

Water stability is a major consideration in crab feed formulation due to the eating habits of crabs that hold and shred the food before putting it in the mouth. An artificial feed with low water stability causes the feed to be easily crushed and dispersed, make it difficult to be held by crabs. In general, the experimental feed already has a perfect level of stability in water, which is above 5 hours. According to [7], in general, the stability of feed in water ranges from 3–5 hours. However, at the solids dispersion rate, it was higher than recommended [9]. The solid dispersion rate should not be more than 10% because it greatly affects the quality and quantity of nutrients in the feed. The stability of the feed in water describes the cohesiveness of the feed. The longer it takes to destroy the feed, it means the higher the cohesiveness of the feed. According to [10], several factors affect the stability of feed in the water, such as the fineness of feed raw materials and the process of mixing materials in the feed-making process. The finer the feed ingredients, the better the feed produced. The feed ingredients will be evenly mixed to produce a product that is more compact and stable in the water.

The level of solids dispersion was increased in feed with raw materials fermented with mixed microorganisms, compared to controls, and increased with increasing dose of mixed microorganisms. The fermentation process causes the feed nutrients to be degraded, which causes the components of the feed ingredients to become smoother and easier to dissolve and has reduced binding ability. Stated that besides the manufacturing process, the right binder material also greatly determines the water stability and other physical properties [9]. Pollart is a waste of wheat flour. Apart from being a source of nutrients, it also functions as good quality and digestible binder [11]. The type of raw material and water quality also affects water stability. Several kinds of feed raw materials positively affect feed stability, such as soybeans, corn, and pollart [12]. It is further explained that the process of making feed using steam will increase water stability. This is because the hot steam will convert carbohydrates and other binding materials into gelatin mass. This material will become sticky, creating a more compact feed dough.

Feed stability in water is also closely related to the level of solidity of the feed. Suggests by [10] that good quality feed must have a high level of solidity. This is largely determined by the fineness of the raw material and the type of binder used. The hardness level of the experimental feed in this study was very high, ranging from 83–86%. This means that only about 14-17% were destroyed, and the rest was still intact.

Crab feed should have a density greater than seawater, so it sinks as soon as it is administered. This is important, considering that crabs have the nature of taking food at the bottom. The experimental feed in this study shows the fastest immersion rate. According to [13], the immersion rate is closely related to other physical test parameters. It is very dependent on the fineness of the raw material, the number, and the type of binder used.

3.2. Chemical test

Proximate analysis data of each experimental feed is presented in Table 2, while the percentage of dry matter digestibility and organic matter digestibility is presented in Table 3.

Table 2. Proximate analysis data of experimental feed

Composition	Dose of microorganism mixture (mL 100 g ⁻¹ feed)			
	A (0)	B (10)	C (20)	D (30)
Water (%)	9.22	8.9	9.65	9.47
Ash (% bk)	13.84	13.49	13.43	13.09
Protein (% bk)	39.84	41.93	41.98	41.96
Lipid (% bk)	8.32	7.43	7.42	7.48
Fiber (% bk)	8.87	7.82	7.8	7.91
NFE (% bk)	29.13	29.33	29.37	29.56
Digestible Energy (kcal kg ⁻¹) ^a	2761.57	2767.63	2769.57	2,778.48

C/P (DE g ⁻¹ Protein)	7.11	6.76	6.76	6.78
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Where : *) Calculated based on energy equation [14]: 1 g Carbohydrate = 2.5 kcal DE; 1 g protein = 3.5 kcal DE; 1 g lipid = 8.1 kcal DE

The nutritional composition of all experimental feeds (Table 2) produced was sufficient and balanced for the requirements of crabs. Crabs require feed to maintain a living and growing existence. Hence the available feed should contain all the nutrient components needed. According to [15], the composition of essential feed nutrients will determine the growth and feed efficiency of organisms.

Like other organisms, the nutrient requirements of crabs are protein, carbohydrates, lipids, vitamins, and minerals. The range of nutrient composition in crab feed is 34–54% protein; lipid 4.8–10.8; fiber 2.1–4.3%; NFE 18.7–42.5%; ash 0.6– 22.0% [16]. The moisture content level is classified as good dry feed if the moisture content is less than 10%. Recommended [17] that a feed containing 35% protein increased the daily weight growth rate, biomass production, and body nutrient retention (protein, lipid, energy, calcium, and phosphorus) of female mud crabs.

Besides the nutrient levels, protein and energy balance in the feed also greatly affect the growth. Suggested [18] that a feed with an optimum energy per protein ratio illustrates the balance point between the amount of energy required for basal metabolism and growth. The balance between protein and energy for growth is one of the keys to obtain an appropriate feed. Energy deficient feed will cause most of the feed protein to be used as energy for metabolic purposes. Conversely, if the feed energy content is excessive, it will cause a decrease in feed consumption rate, the acquirement of other nutrients, including protein required for growth, is also reduced.

The energy protein ratio of the experimental feed ranged from 6.76–7.11. According to [19], carp's energy/protein ratio is about 8 kcal DE / g protein. According to [20], milkfish requires an energy/protein ratio of about 6.5 Kcal DE / g protein. The energy and protein requirements of crabs range from 6.2–8.58 Kcal DE / g protein.

The percentage of dry matter digestibility (DMD) produced in this experiment ranged from 40.34–55.5% while the percentage of organic matter digestibility (OMD) ranged from 42.43–57.72% (Table 6). The lowest percentage of DMD and OMD were observed in the control treatment, where the feed was unfermented. The percentage of DMD and OMD increased with the fermentation process carried out by mixed microorganisms on feed raw materials.

Table 3. Dry matter digestibility (DMD) and Organic matter digestibility (OMD) percentage of the experimental feed.

Dose of microorganism mixture (mL 100 g ⁻¹ feed)	Percentage (%)		
	Dry Matter Digestibility (DMD)	Organic Matter Digestibility (OMD)	
A (0)	1	40.34	45.56
	2	42.87	42.43
	3	45.12	43.76
Average	42.78	43.92	
B (10)	1	53.65	54.79
	2	52.51	56.65
	3	52.28	56.88
Average	52.81	56.11	
C (20)	1	55.5	54.34
	2	53.14	57.72
	3	52.76	56.98
Average	53.80	56.35	
D (30)	1	52.14	54.56
	2	52.98	54.85
	3	53.79	55.42
Average	52.97	54.94	

The high digestibility of fermented feed was assumed to be due to the contribution of different extracellular enzymes released by each microorganism. According to [21], *Bacillus* sp. is a microbe that produces alkaline extracellular protease enzymes. Production can reach 10 U mL⁻¹. Alkaline protease are enzymes that hydrolyze protein substrates under alkaline conditions. *Rhizopus* sp. is a good producer of amylolytic enzymes, especially glucoamylase (GA). This enzyme can decrease both amylose and amylopectin by hydrolyzing α -1,4 and α -1,6, linking both starch glucosides and producing glucose [22]. Reported [23] that during the ensilage activity, degradation of cellulose and hemicellulose components occurred by microorganisms involved in the fermentation process. Meanwhile, bacteria will convert simple sugars into organic acids (acetic, lactic, propionic, and butyrate) during the ensilage. As a result, the resulting final product is highly digestible when compared to non-fermented materials. Besides, the organic acid products produced can degrade fiber components, especially cellulose and hemicellulose.

Thus, digestibility is an early indication of various nutrients in certain feed ingredients for the fish to consume them. High digestibility reflects the amount of contribution of certain nutrients. Meanwhile, feed that has low digestibility indicates that the feed cannot supply nutrients for basic living and fish production purposes. The higher the DMD, the higher the OMD, and the higher the opportunities for nutrients that can be utilized for production, and vice versa, the lower the DMD, the lower the OMD, then the lower the chances for nutrients that can be used [24].

The DMD and OMD of this study were relatively similar to the research results [25], which used different substrates, namely com cobs treated with urea, *Aspergillus niger*, and *Aspergillus niger* + 0.5 urea resulted in a higher DMD of 59.7; 47.2 and 50.9 respectively. Observed that the cacao pod husks without urea treatment resulted in a dry matter digestibility percentage of 46.37%, while cacao pod husks treated with 6% urea ammoniation had a higher DMD of 52.80% [26]. It was even much higher when compared to the results of the study [27] who fermented the cocoa skin with *Aspergillus niger*, and the DMD ranged from 18.6-25.1% and OMD ranged from 3.4%-11%.

The digestibility rate of organic matter is relatively higher than the dry matter digestibility. This was because dry matter still contains ash, while organic matter does not contain ash. Thus materials without ash content are relatively easy to digest. This is evidenced [28], who stated that 40 ppm Mn and 10 ppm Cu can increase the ash content. As a result, it can slow down or inhibit the digestion of dry ingredients. Organic material composition, which consists of carbohydrates, proteins, lipids, and vitamins. Carbohydrates are part of the leading organic matter and have the highest composition (50-70%) of the dry matter [29].

The organic matter is part of dry matter, so if the dry matter increases, it will increase organic matter and vice versa [28]. Therefore, this will also apply to the digestibility value. If the digestibility of dry matter increases, the digestibility of organic matter will also increase.

4. Conclusion

Based on the data in the first experiment, feed fermentation with various doses of mixed microorganisms. As a fermenter, it includes organoleptic feed data (appearance, texture, aroma, and color of feed), physical data (water stability has disintegration rate and solids dispersion, solidity level, and immersion rate), and nutritional or chemical tests (the proximate composition of protein content, lipid, NFE, crude fiber, ash, and water from the experimental feed, as well as the percentage of dry matter and organic matter digestibility) it can be concluded that the mixed microorganisms effectively used as a fermenter to ferment feed raw materials at a dose of 10 mL 100 g⁻¹ of feed raw materials.

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