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The addition of papaya seed flour (*Carica papaya*) to the growth of 'Cantang' hybrid grouper fish (*Epinephelus fuscoguttatus* >< *Epinephelus lanceolatus*)

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ABSTRACT

This research was carried out to examine the effects of papaya seed flour at different doses on the growth of hybrid groupers and to determine the most optimal dose of papaya seed flour on fish growth. A complete randomized design with 4 treatments and 3 replications was used, which treatments consisted of: treatment A without papaya seed flour (Control), treatment B with 2.5 grams/kg of papaya seed flour, treatment C with 5 grams of papaya seed flour per kg feed, and treatment D of 7.5 grams papaya seed flour/kg feed. Treatment C was found to be the most optimal treatment, resulting in an absolute biomass value of 26.97 grams and feed conversion ratio of 1.19. Analysis of variance (ANOVA) of the absolute biomass development of the hybrid grouper obtained an F count of 41.3892 higher than F table of 5%, indicating significant effects on the fish growth. The water quality was set at an ideal level, with temperature ranging between 28-29 0C, pH between 7.0-7.5 and salinity between 30-33 ppt.

Introduction

Hybrid grouper (*Epinephellus fuscoguttatus-lanceolatus*) was the result of hybridization between the tiger grouper (*Epinephelus fuscoguttatus*) and the giant grouper (*Epinephelus lanceolatus*). The hybridization resulted in grouper species with identical shapes and organ structures to the two parents but with superior growth (Chrisdiana *et al.,* 2015). The hybrid grouper can adapt well to small spaces, low-quality environments and it has a faster growth rate, higher growth coefficient, better feed conversion, and higher survival rate (Folnuari *et al.,* 2017). KKP (2018) reported that the average selling price of the hybrid grouper in Indonesia ranged from 110,000 IDR to120,000 IDR. Statistics Indonesia in 2020 also noted that from January 2019 to January 2020, grouper exports in Indonesia experienced an increase. The export volume of live grouper was 277,006 tons in January 2019 and reached the highest export volume in March 2019 of 317,874 tons before it declined in August 2019 to the lowest export volume of 99,316 tons. KKP has set a minimum grouper production of 9,000 tons per year in order to increase the export volume. The consistent production progress raised an expectation that the export of grouper fish would increase (KKP, 2018).

The cultivation of hybrid grouper both in hatchery, nursery, and rearing is challenging due to diseases that cause death in fish and the high price of commercial feed. Some of the diseases are caused by pathogenic bacteria that adapt and produce harmful metabolism (Olmos *et al.,* 2020).

On the other side, the production cost should be optimized by using proper feed and an adequate amount of feed (Kartika *et al.,* 2018). Various efficiency efforts have been implemented to reduce production costs, including using alternative feed made from natural ingredients that are cheap, environmentally friendly, and broadly available. Putra *et al.,* (2019) mentioned that in order to improve the

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fish quality, additives made from natural ingredients should be added to fish feed like *Aloe vera* (Linayati *et al.,* 2022a), *Kaempferia galanga* (Linayati *et al.,* 2022b), *Curcuma zanthorrhiza* (Linayati *et al.,* 2021) and others natural ingredients. Papaya seeds have been identified as a natural ingredient to add to the fish feed.

Seeds of papaya are not consumed by humans. However, contributing 16% of the total weight of the fruit, papaya seeds contain nutrients and bioactives that are medicinal which can be beneficial for fish body resistance and utilization of feed for growth (Farrag *et al.,* 2013). Various bioactive and digestive enzymes are found in papaya seeds, including papain and lysozyme enzymes. The antibacterial agents found in papaya seeds include terpenoids, carpaine, and flavonoids which can kill bacteria by degrading the bacterial cell structure (Martiasih *et al.,* 2012). Furthermore, the concentration of vegetable pepsin contained in papaya seeds in the form of papain contributes to improving digestion (Kakkae *et al.,* 2021). These improvements can increase the fish's digestibility of the nutrients contained in the feed which in turn increases fish (Kakkae *et al.,* 2021). Papain enzyme also acts as a fish immunostimulant because it contains antioxidant compounds.

Tomasoa and Deidy (2019) recommended the addition of 5 grams of papaya seed flour for every kilogram of fish feed for an optimal growth rate of tilapia by 547 g. Sabrina (2022) also found that the dose was the most optimal dose in improving the growth rate of vanname shrimp by 6.23 grams.

Based on the results of some of these studies, it is necessary to examine the addition of papaya seed flour into the fish feed on the growth performance of hybrid grouper. This research also determined the best dose of papaya seed flour addition that can result in the most optimal growth.

Materials and Methods

Location and time of research

The research was conducted from November 30th – December 29th, 2021 at Laboratorium Penelitian Perikanan, Pekalongan University addressed at Pantai Dewi Street, Krapyak Lor, North Pekalongan District, Pekalongan City, Central Java

Equipment and materials

Several tools were used in this research, including jars, digital scales, hoses and aeration stones, warings, measuring cups, refractometers, pH meters, thermometers, scopnets, stationery, cameras, and hoses. Hybrid grouper seeds of 6 cm in size, fish pellets, and papaya seed flour were also used in this research.

Treatments

In this research, a complete randomized design with four treatments and three replications was employed. Different papaya seed flour doses were applied as follows:

A: Feed without being added with papaya seed flour
B: Feed + papaya seed flour of 2.5 g/kg of feed
C: Feed + papaya seed flour of 5 g/kg of feed
D: Feed + papaya seed flour of 7.5 g/kg of feed

Those doses were determined as suggested by Tomasoa and Deidy (2019) in their attempt to improve the production of tilapia, in which the addition of 5 g of papaya seeds flour for each kilogram of feed was the most optimal treatment.

Preparation of the rearing media

10L plastic jar with a volume of ± 5 L of 12 units of water were used. Each maintenance medium was equipped with an oxygen channel through an aeration hose. Before being filled with water, the container had been washed and then rinsed to clean. The jars were then dried up and rinsed to ensure their hygiene. The clean jars were then filled with water to reach a volume of ± 5 L. The half-filled jars were filled with 5 hybrid grouper seeds.

Feed preparation

Feed pellets were added with papaya seed flour. Papaya seeds were first separated from the flesh and fiber before being washed. After that, papaya seeds were dried under the sun for 3 days and were blended and sieved into flour. Papaya seed flour was then weighted to the predetermined doses and mixed with ± 2 to 3 g adhesive to be added to the feed. The water of 150 ml/kg feed was added afterward. The mixture was poured into the feed (pellets), then stirred until papaya seed flour stick with the feed (pellets) and was let dry for 60 minutes. Any change in color and smell during this process indicates failure in the mixing process and that the process should be started over again. The feed making procedure refers to the research of Sitorus *et al.,* (2015).

Fish rearing

The test animals used were certified hybrid grouper fish which was obtained from the Center for Brackish Water Cultivation Fisheries (BPPBAP) Situbondo. Observation of seed rearing was done within 30 days. Feed for *at satiation* (Firdausi and Mubarak, 2021) was administered three times a day at 08.00, 13.00, and 17.00 of West Indonesian Time. According to Amadou *et al.,* (2019), feeding the hybrid grouper three times a day will help in better feed utilization. The volume was determined regarding to the capacity of the hybrid grouper fry. Nuraini *et al.,* (2020) emphasized that to gain optimal fish growth, the eating capacity of the fish and the

feeding time should be taken into consideration in feeding the fish.

Data analysis

The total absolute biomass growth was calculated using a formula proposed by Effendi (1997):

$$Wm = Wt - Wo$$

Information: Wm= absolute biomass growth of Hybrid grouper (g); Wt = Weight of Hybrid grouper at the end of study (g); Wo= Weight of Hybrid grouper at the beginning of study (g).

Effendi (1997) also proposed a formula to measure the Feed Conversion Ratio as follows:

$$FCR = \frac{Feed\ consumed\ (gram\ dry\ weight)}{Live\ weight\ gain\ (gram)}$$

The formula in calculating the survival rate of fish proposed by Effendie (1997) was also used as follows:

$$SR = (Nt/No) \times 100\%$$

Information: SR = Survival (%); Nt = Number of live fish at the end of rearing; No = Number of fish at the beginning of maintenance.

Parameters of the water quality measured in this research were the pH level measured using a pH meter, the temperature measured using a thermometer, and the water salinity measured using a refractometer.

The data gained in this research were first tested for data normality and homogeneity. The liliefors test (Nasoetion and Barizi, 1983) was employed to ensure that the growth rate in each treatment was normally distributed. Meanwhile, the homogeneity of the data was tested using the Barlett test (Sudjana, 1996). After that, an analysis of variance (ANOVA) was performed to identify the significance of the treatments followed by the Student Newman Keuls (SNK) test. Afterwhile, the parameters of the water quality were descriptively observed.

Results

Biomass growth

The data on the absolute biomass growth are presented in Table 1. Table 1 presents the growth of hybrid grouper in each treatment, with the highest absolute biomass yield found in treatment C with a dose of papaya seed flour 5 g/kg feed with an absolute biomass value of 26.97±0,35^{d} g. Treatment D with an additional dose of papaya seed flour 7.5 g/kg feed ranked two with an absolute biomass value of 24.70±0,95^{c} g, followed by treatment B with an additional dose of papaya seed flour 5 g/kg feed with an absolute biomass value of 23.30±0,36^{b} g, while treatment A without the addition of papaya seed flour obtained the least absolute biomass value of 21.90±0,44^{a} g.

Feed conversion ratio (FCR)

Quantitative data on the Feed Conversion Ratio of the hybrid grouper are shown in Table 1. The Feed Conversion Ratio data are presented in Table 1. As seen in the Table, the highest feed conversion ratio (FCR) of hybrid grouper fry was obtained in treatment A with no addition of papaya seed flour with an average value of 1.40±0,026^{c}, followed by treatment B with a dose of 2.5 g papaya seed flour/kg feed resulting in an average value of 1.34±0,040^{bc}, treatment D with a dose of papaya seed flour 7.5 g/kg feed resulting in an average value of 1.29±0,050^{b}, and the lowest average value of 1.19±0,038^{a} in treatment C with a dose of papaya seed flour if 5 g/kg feed.

Survival rate

The data on the survival rate of the hybrid grouper are presented in Table 1. The observation was done in each treatment resulted in 100% survival rates of hybrid grouper fry in treatments A, B, C, and D, which indicating that the rearing media quality was excellent.

Water quality

Observations of the quality of the hybrid grouper rearing media were done to gain data on water temperature, pH, and salinity as presented in Table 2. The water temperature ranged around 28-29°C and the water salinity was between 30-32 ppt. Whereas, the pH value was between 7.0-7.5. Therefore, the water media was considered feasible for grouper fish to grow. The water control was also carried out by changing 100% of the water in the morning and siphoning in the afternoon daily.

Table 1. The absolute growth rate, food conversion ratio and survival rate of the hybrid grouper fish in various treatments with the addition of papaya flour.

Treatment	Absolute Growth Rate (g)	Food Conversion Ratio	Survival Rate(%)
A	21,90±0,44^{a}	1,40±0,026^{c}	100±0,00^{a}
B	23,30±0,36^{b}	1,34±0,040^{bc}	100±0,00^{a}
C	26,97±0,35^{d}	1,19±0,038^{a}	100±0,00^{a}
D	24,70±0,95^{c}	1,29±0,050^{b}	100±0,00^{a}

Table 2. Water quality

Parameter	Result	Optimum	Reference
Temperature (°C)	28–29	28–32	SNI (2014)
Salinity (ppt)	30–33	24–33	SNI (2014)
pH	7,0–7,5	7,0–8,5	Hendriansyah *et al.*, (2018)

Discussion

Growth

Treatment C resulted in an absolute biomass value of 26.97±0,35[d] g, and a daily growth rate of 3.16%, which is the most optimal one among other treatments. On the other hand, treatment A without papaya seed flour had the least absolute growth rate and the lowest specific growth rate of 21.90±0,44[a] g and of 2.80% respectively. The feed given in treatment C can be decomposed better into a more conventional form, namely amino acids and the contents in the papaya seeds are able to fight against bacteria, allowing fish to grow well.

The calculation of variance on the growth of hybrid grouper seed weight obtained a calculated F value (41.3892) > from the F table values of 5% and 1% (4.07 and 7.59) showing significant effects on hybrid grouper growth.

Various bioactive and enzymes consisting of terpenoids, tocopherols, flavonoids, and alkaloids such as carpaine, papain enzymes, chymopapain enzymes, and lysozyme were found in papaya seeds. Papaya seeds contain flavonoids, terpenoids, and carpaine which have antibacterial properties that can degrade the composition of bacterial cell membranes and kill the bacteria. The antibacterial properties inhibit bacterial growth in fish (Martiasih *et al.*, 2012). According to Rahmawati et al. (2020) papain enzyme from papaya seeds stimulates optimization of feed utilization by hydrolyzing protein into amino acids that are useful for increasing fish growth.

Papain enzyme can also break down the complex protein into simple proteins such as amino acids and peptides. Yogiraj *et al.* (2014) explained that chymopapain enzymes and papain enzymes have similarities as both are able to hydrolyze proteins. Lysozyme enzyme is an enzyme that is small in size but has antibacterial properties that degrade the extracellular matrix of bacteria, causing bacterial cells to lose the protection of their life (Oliver and Wells, 2015).

Amalia *et al.* (2013) also found papain enzyme in papaya seeds capable of catalyzing the hydrolysis reaction of a substrate that can stimulate the hydrolysis process of protein peptide bonds to produce amino acids. Through acid circulation and urea circulation, the output of the enzymatic process in the form of amino acids is processed to form energy.

If the energy is sufficient for body activities and maintenance, the excess energy will be diverted for the growth process in fish. Likewise, Harahap *et al.* (2019) also explained that to boost the growth process in fish, the energy intake should exceed the minimum energy intake. Furthermore, the accumulation of additional doses of papaya seed flour into the feed is highly vital in this study. Sari and Andriani (2018) found that the dose of feed supports the protein simplification activity, which will stimulate the acceleration of protein absorption such as amino acids in fish metabolism.

Hardiyanti *et al.* (2020) found the addition of *lemuru* fish flour increased the growth of hybrid grouper and resulted in the highest absolute biomass growth value of 16.01 g. In addition, Harsoyo (2021) stated that earthworm flour on pellets improved the growth and survival rates of hybrid grouper fry, resulting in the highest absolute biomass growth value of 12.5 g. The addition of papaya seed flour to feed on the growth of hybrid grouper fry compared to the ones used by Hardiyanti *et al.* (2020) and Harsoyo (2021) was favored as it produced the highest absolute biomass value of 26.97 g.

The decrease in growth rate in treatment D (7.5 g/kg feed) occurred allegedly due to excessive papaya seed flour dose. Saputri (2020) pointed out that the bioactive contained in papaya seeds such as alkaloids can be toxic in excessive amounts. Cell death will occur when exposure to toxic substances in cells occurs for some time. In addition, feed that contains excessive saponins will be toxic to the body of the fish. Similarly, Kinasih *et al.* (2013) found that saponins can hemolyze the red blood cells, obstructing oxygen distribution which can lead to cell death as saponins are toxic to cold-blooded animals.

Feeds that contain excess tannins can cause liver damage due to increased oxidative levels in organs. Wiranatha *et al.* (2019) also found long-term exposure to many tannins damages the cells, in which tannins become harmful nutrients.

Feed conversion ratio (FCR)

The calculation of variance on the FCR of hybrid grouper seed weight obtained a calculated F value (14.5035) > from the F table values of 5% and 1% (4.07 and 7.59) showing significant effects on FCR hybrid grouper

The highest feed conversion ratio (FCR) was found in treatment A with an average value of 1.40±0,026[c], and the lowest one was found in treatment C with an average score of 1.19±0,038[a]. This is in accordance with the research of Shofura *et al.* (2017), the feed conversion ratio (FCR) is regarded as sufficient within the range of 0.8-1.6. The addition of papaya seed flour into the feed increases the feed quality, as the papain enzyme optimizes nutrient absorption and increases the nutritional content in the feed. The mixing of papaya seed flour and feed produces changes in the aroma and taste of the feed that the fish tend to eat more. Arief *et al.* (2016) also

stated that the papain enzyme can increase the amino acid content in the feed which is beneficial to the growth process and physiological condition of the fish. Taqwdasbriliani *et al.* (2013) also believed that the papain enzyme can break down the protein in feed to become easy-to-digest protein, such as amino acids and peptides. Feed containing the papain enzyme can make feed consumption more efficient.

Survival rate

The survival value of the hybrid grouper in this research reached 100%. None of the treatments applied in this research caused death or stressed the fish out. Besides that, the water was changed to 100% in the morning, and siphoning was administered in the afternoon. Therefore, the water was clean and free from pathogens that could cause mass death.

The prevention of cannibalism among hybrid grouper fry has been successful in this research. According to Tan (2021), cannibalism can be avoided because of regular feeding and uniform size of the fish.

Water quality

The maintenance of the rearing media included keeping the temperature at the optimal limit between 28-29˚C which was considered ideal for hybrid grouper hatchery. Indonesian Standards (2014) showed the optimal temperature for the survival and growth of hybrid grouper fry ranges between 28-32 C. The pH of the water in the rearing media ranged between 0.0-7.5 (good). As stated by Hendriansyah *et al.* (2018), the ideal pH value for grouper cultivation activities is between 7.0-8.5. If the pH value is below or above the normal threshold, the fish will die. Furthermore, the water salinity was maintained within a range of 30-33 ppt as determined in indonesian Standards (2014) that the ideal salinity value for hybrid grouper fry is 24-33 ppt.

Conclusion

Conclusions were drawn based on the results of this study as follows.

1. The addition of papaya seed flour into the fish feed significantly improved the growth of the hybrid grouper fish.
2. Treatment C with papaya seed flour of 5 g/kg feed resulted in the highest absolute biomass of 26.97 g, and treatment C was found as the most ideal treatment in this research.

In regard to the results of this research, it is recommended to add papaya seed flour into the commercial feed of hybrid grouper fish at a dose of 5 g/kg feed.

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