JURNAL PERIKANAN
UNIVERSITAS GADJAH MADA
Terakreditasi Ristekdikti No: 158/E/KPT/2021
ISSN: 2502-5066 (Online)
ISSN: 0853-6384 (Print)
Vol. 26 (1), 61-69
DOI 10.22146/jfs.91538

The Fermented Cassava (*Manihot esculenta* C.) Flour and Bioimun® as Additional Feed for the Juvenile Catfish (*Clarias gariepinus*) to Improve Growth, Immunity, and Survival

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Submitted: 07 December 2023; Revised: 27 February 2024; Accepted: 24 June 2024; Published: 30 June 2024

ABSTRACT The availability of fish feed has remained an obstacle in the fish cultivation until now. The dependence on fish flour, soy flour, the imported cornstarch has not been solved, though the natural resource in Indonesia supports the dependence of this raw material availability. This research was conducted by testing the use of the fermented cassava flour to substitute cornstarch as the catfish feed, by adding natural drug product, BIOIMUN , which positively affected growth, immunity, and production. Research methodology used was the Completely Randomized Design with six treatments and three repetitions. Fishes with average weight of 10 g and length of 10 cm were raised in the aquarium with dimension of 60x40x40 cm, with the density of 10 fishes per aquarium. The research was conducted for 60 days with observation every 14 days. Compositions of cornstarch and cassava flour were P1 (0/200), P2 (100/100), P3 (75/125), P4 (50/150), P5 (25/175), and P6 (commercial feed), added with BIOIMUN® at 20 ml/kg from the extracts of Solanum ferox and Zingiber zerumbet. Feed was given three times at a percentage of 5%. This research showed the result that the best growth was in treatment (P3), with 75 g of cornstarch and 125 g of cassava flour (75/125). The highest absolute weight gain was in P3 at 132.67 ± 8.33 g. The immunity system testing was in accordance with the result of phytochemical test of positive alkaloid, negative flavonoid, and negative steroid. Survival Rate (SR) based on the variance analysis (ANOVA) had an insignificant effect (P>0.05) 93.00-100.00%. P3 feed conversion rate was 5.35±0.23. Hematocrit level and total erythrocyte with a decent score and total leukocyte increased from P6. Feed proximate test was in accordance with the standard. Total bacteria were in the normal range, the contamination test on heavy metal proved no harm against the catfishes, and the water quality was normal. In terms of economic analysis, P1-P5 were more affordable than (P6), so that it can be concluded that the fermented cassava flour can be used as a replacement of corn by adding BIOIMUN® as the catfish feed.

Keywords: Cassava flour; catfish feed; growth; immunity; increase in production

INTRODUCTION

The role of artificial feed in the fish cultivation is highly dominant, particularly in an intensively managed cultivation. Artificial feed is one of the issues in the field of fishery since its cost is relatively high, at 60-70% of the production component, and its availability is limited. Besides for meeting the need in a farm, the resulted feed can be marketed to other fish farmers (Sahwan, 2003).

Based on a report from the Ministry of Marine Affairs and Fisheries (MMAF, 2022), the development of fish feed formulation with local raw materials has started to be encouraged for the time being. Local raw material for making feed is a raw material, not in conflict with the allocation of other industries, available throughout the year, having good nutrient. Independent feed is very helpful, particularly for the breeder at a small scale because the cost for purchasing feed is rather high, at 60-70% of total production cost. It aims to improve efficiency in the production cost through efficiency improvement in the feed funding of a fish cultivation business. One of the feed raw materials frequently found is cassava (*Manihot esculenta*). According to Nazriati *et al*. (2021), this type of commodity has a considerable potency to be developed into a carbohydrate-based food commodity. The abundance of cassava needs to be made use of into a more durable product with a higher sale value. One of the efforts which can be made is the making of cassava flour.

Some researches showed that biological modification (fermentation) can change the characteristic of natural flour. Based on the result of a research by Mokoginta *et al*. (2003), it turned out that cassava flour can substitute the wheat flour at 50% as an ingredient for making fish feed. Cassava flour is a kind of carbohydrate or starch substance, from the vegetables. Carbohydrate level in fish feed is between 10-50% (Sutikno, 2022). The concoction of sour eggplant and fragrant ginger extracts is known to have an excellent benefit for goldfish, catfish, and tilapia fish. Besides being an anti-bacterial substance (Hardi *et al*., 2018[a]) and immunostimulant (Hardi *et al*., 2018[b]), it improves growth and survival (Hardi *et al*., 2020). Sour eggplant and fragrant ginger extracts are packaged in Biomun® product with a distribution license from Ministry of Marine Affairs and Fisheries of the Republic of Indonesia No. D 2112573 HBC, while the use has been tested on the freshwater fishes. Additional plant extract in the feed is expected to improve quality of the feed itself.

Catfish is a species of freshwater fish which is favored by the people and has been an icon. This commodity can be raised at a high density on a limited (space-saving) area in a marginalized region and the efficient use of water Based on these references, a research needs to be conducted to identify the use of the fermented cassava (*Manihot esculenta* C.) flour as a substitution of wheat flour on the growth of catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

Juvenile catfish preparation

Juvenile catfishes used were from the fish hatchery owned by fish farmer with weight of 10-11 g and length of 10-12 cm. This research used 300 juvenile catfishes as the samples, in which they have passed the acclimatization process for 14 days. Acclimatization was carried out in the fiber tub with commercial feed given three times a day. One aquarium was for 10 fishes or 30 fishes were in every treatment.

Feed Making

Feed was made by substituting cornstarch with the fermented cassava flour and by adding sour eggplant and fragrant ginger extracts (BIOIMUN®) (Hardi et al., 2022) as the immunostimulant feed supplement. Feed composition is as follows:

P1: 0 g of cornstarch + 200 g of cassava flour;
P2: 100 g of cornstarch + 100 g of cassava flour;
P3: 75 g of cornstarch + 125 g of cassava flour;
P4: 50 g of cornstarch + 150 g of cassava flour;
P5: 25 g of cornstarch + 175 g of cassava flour;
P6: Fishes were fed with commercial feed (F99).

Feeding was carried out three times a day 2 with 5% of fish weight/day. Before feed was given to these fishes, phytochemical test was conducted first, to identity protein, carbohydrate, fat, moisture content, ash level, and metabolic content from BIOIMUN® addition.

Experiment

Fishes were raised by giving different feed formulation for 60 days with stocking density of 10 fishes per aquarium. To evaluate effectiveness in the use of the fermented cassava flour and BIOIMUN® as catfish feed which can improve growth and immunity, measurement was carried out on survival, growth, and non-specific immunity of these fishes.

Survival

Survival Rate (SR) is a comparison of the living fishes from beginning to end of research, in which the survival can be measured using formula of Wirabakti (2006) as follows:

$$SR = \frac{Nt}{No} \times 100\%$$

SR, Survival Rate (%); Nt, amount of fishes in the end of observation; No, amount of fishes in the beginning of observation.

Absolute weight gain

Absolute weight gain was calculated using a formula based on Zonneveld *et al*. (1991) as follows:

$$W = Wt - Wo$$

W, absolute weight (g); Wt, fish weight in the end of observation (g); Wo, fish weight in the beginning of observation (g).

Absolute length growth

Absolute length growth was calculated using a formula based on Zonneveld *et al*. (1991) as follows:

$$L = Lt - Lo$$

L, Absolute length growth (cm); Lt, fish length in the end of observation (cm); Lo, fish length in the beginning of observation (cm).

Feed conversion

Feed conversion can be calculated using a formula according to Tacon (1988) as follows:

$$FCR = \frac{F}{Wt - Wo}$$

FCR, feed conversion ratio; F, total feed given (g); Wt, fish weight in the end of observation (g); Wo, fish weight in the beginning of observation (g).

Daily growth rate

Daily growth rate can be identified with calculation using a formula of Steffens (1989) as follows:

$$SGR = \frac{(LnWt) - (LnWo)}{T} \times 100$$

SGR, specific growth rate; T, fish breeding period (in day) ; Wo, fish weight on day-0 (g); Wt, fish weight on day-t (g).

Non-specific immunity of fishes

Some parameters observed on the fish immunity were total leukocyte, total erythrocyte, hematocrit level, and phagocytosis index (Hardi *et al*., 2018[a]).

Water quality

Parameters of the water quality observed every three days were temperature, DO, pH, and ammonia.

Statistical analysis

Data obtained from the result of research were statistically analyzed using the F test variance analysis or Analysis of Variance (ANOVA) with a level of confidence at 95%, besides the descriptive analysis in form of table and graphic. Before variance analysis, model fit test was conducted in form of normality test and variance homogeneity test.

RESULTS AND DISCUSSION

Feed proximate test

Fish feed formulation by adding the fermented cassava flour and BIOIMUN® can improve feed protein as shown in Table 1. Addition of the fermented cassava flour and BIOIMUN® at 200 g had higher protein content, compared with cornstarch only.

Table 1. Result of the feed proximate test.

Treatment	Result					
	Level (%)					
	Protein	Fat	Carbohydrate	Crude Fiber	Water	Ash
P1	56.60	19.10	4.68	0.75	4.76	14.11
P2	55.77	22.75	0.18	1.29	7.11	12.92
P3	55.28	22.28	1.66	1.02	8.42	11.36
P4	55.39	21.11	1.29	0.67	8.44	13.10
P5	55.54	18.52	4.91	0.68	6.81	14.50
P6	56.73	14.01	4.09	0.84	8.61	15.73

Feed phytochemical test

In this research, compounds tested were alkaloid, flavonoid, and steroid as shown in Table 2 below.

Table 2. Result of phytochemical test.

Treatment	Chemical Composition		
	Alkaloid	Flavonoid	Steroid
P1	(+)	(-)	(-)
P2	(+)	(-)	(-)
P3	(+)	(-)	(-)
P4	(+)	(-)	(-)
P5	(+)	(-)	(-)
P6	(+)	(-)	(-)

Survival and growth

Variance analysis (ANOVA) of survival rate, absolute weight, daily growth rate, and P6 feed conversion ratio had insignificant effect on all treatments (P>0.05), while the variance analysis on the absolute length showed that P6 treatment had insignificant effect on treatments P1, P2, P3, and P5 (P>0.05) and significant effect on treatment P4 (P<0.05).

Catfish immunity

Parameters of the catfish immunity observed during the research included hematocrit level, total erythrocyte, total leukocyte, phagocytosis activity, and total bacteria.

Heavy metal contamination test

Result of the heavy metal test on the water of breeding media is as follows:

Table 5. Result of the heavy metal test.

Treatment	Results (mg/L)		
	Iron (Fe)	Copper (Cu)	Lead (Pb)
P1	0.042	0.014	0.009
P2	0.036	0.014	0.009
P3	0.071	0.014	0.009
P4	0.039	0.014	0.009
P5	0.082	0.014	0.009
P6	0.056	0.014	0.009

Float test and palatability

Result of the float test and palatability of feed is as follows:

Table 6. Result of float test and response of fish feed

Treatment	Buoyancy (second)	Feed response (second)
P1	3.33	5.82
P2	3.00	5.68
P3	3.67	3.13
P4	3.67	3.73
P5	4.00	7.38
P6	4.67	3.42

Table 3. Result of observation on survival and growth of catfishes.

Treatment	Parameter				
	SR (%)	AW (g)	AL (cm)	SGR (%/day)	FCR
P1(200 g)	100.00 ± 0.00^{a}	92.33 ± 28.29^{ab}	2.97 ± 0.57^{abc}	1.11 ± 0.22^{ab}	2.05 ± 0.32^{a}
P2(100 g)	100.00 ± 0.00^{a}	78.33 ± 9.71^{a}	2.07 ± 0.25^{a}	0.96 ± 0.09^{b}	2.29 ± 0.20^{a}
P3(125 g)	100.00 ± 0.00^{a}	132.67 ± 8.33^{b}	3.43 ± 0.25^{bc}	1.41 ± 0.28^{b}	1.76 ± 0.10^{a}
P4(150 g)	93.33 ± 11.55^{a}	101.33 ± 30.66^{ab}	3.63 ± 0.21^{c}	1.21 ± 0.18^{ab}	2.17 ± 0.70^{a}
P5(175 g)	100.00 ± 0.00^{a}	106.00 ± 18.52^{ab}	3.13 ± 0.68^{bc}	1.20 ± 0.18^{ab}	1.91 ± 0.21^{a}
P6(0 g)	100.00 ± 0.00^{a}	127.00 ± 24.52^{b}	2.53 ± 0.72^{ab}	1.27 ± 0.21^{ab}	2.09 ± 0.27^{a}

Explanation: SR (survival), AW (absolute weight), AL (absolute length), SGR (daily growth rate), and FCR (feed conversion ratio).

Table 4. Result of observation on the catfish immunity.

Treatment	Parameter				
	He (%)	TE (x10^6 cell/mm^3)	TL (x10^6 cell/mm^3)	PA (%)	TB (x10^5 cfu/ml)
P1(200 g)	23	0.56	36.20	22.64	1.70
P2(100 g)	25	0.59	57.85	31.44	4.00
P3(125 g)	28	1.05	47.70	23.90	2.10
P4(150 g)	30	1.25	56.70	27.23	3.10
P5(175 g)	20	0.45	26.10	17.98	0.21
P6(0 g)	10	0.38	27.90	22.11	0.23

Explanation: He (hematocrit), TE (total erythrocyte), TL (total leukocyte), PA (phagocytosis activities).

Water quality

Result of the measurement on some parameters of water quality for 60 days of breeding is shown in Table 7 below.

Table 7. Results of water quality measurement.

Treatment	Temperature (°C)	DO (mg/L)	pH	Ammonia (mg/L)
P1	26.63	3.49	7.01	0.41
P2	26.59	3.35	7.02	0.41
P3	26.59	3.73	7.08	0.41
P4	26.65	3.75	7.07	0.42
P5	26.62	3.73	7.04	0.42
P6	26.64	3.97	7.12	0.41

Economic analysis

Estimate of the cost for artificial feed with cassava flour, compared with the commercial feed (F99), for a fish-breeding period of three months is as follows:

Feed proximate test

Table 1 shows the protein level in feed between 55.28-56.73%. This result shows that the protein level in feed has been capable of meeting the need of catfishes which require the protein at 35-45% to live (Sari, 2021).

Based on the research, the proximate test showed fat level which was quite high, between 14.01-22.75%, while catfishes require fat at 4-18% to meet their need. The fat excess in feed can affect the capability of fishes to digest and assimilate fatty acid (Ruyter *et al*., 2000), thus leading to the decrease in protein absorption and slow growth in fishes (Lin & Shiau, 2003).

The need for carbohydrate in every fish is different, while the optimum carbohydrate level in omnivorous fishes is between 20-40%. Meanwhile, the result of feed proximate test in this research showed carbohydrate level between 0.18-4.68%. Carbohydrate serves as a raw material for metabolism process, as required in growth, namely non-essential amino acids and nucleic acid (NRC, 1993).

Crude fiber in general is from vegetables. The result of proximate test analysis in feed showed the crude fiber between 0.67-1.29%. Ingerlev (2014) stated that crude fiber plays an important role in fishes as prebiotics to improve the intestinal microbiota and digestion, absorption, and assimilation in fishes' digestive system.

In terms of feed quality, moisture content must be in accordance with the need. Moisture content in fish feed is between 70-90%, while wet weight of the quality feed has moisture content <12%. In the research, moisture content in feed was between 4.76-8.61%, so that it can be said that feed still met the standard (Indonesian National Standard, 2006).

Ash in feed belongs to the inorganic component which cannot be consumed (Zaenuri *et al*., 2014). Ash level represents mineral level in the feed itself, in which ash level in this research was between 11.36-15.73%. It showed that ash level was above the standard, <12% (Indonesian National Standard, 2006).

Feed Phytochemical Test

Phytochemicals can improve several activities, such as growth, feed consumption, while acting as tonic in immunostimulation, anti-stress, and the improvement of antimicrobial trait in fishes (Citarasu, 2010). Based on its chemical structure, phytochemicals in principle can be categorized into alkaloid, flavonoid, pigment, phenolates, terpenoid, steroid, and essential oil (Chakraborty *et al*., 2013). In this research, compounds tested were alkaloid, flavonoid, and steroid.

Alkaloid is a hetero-cyclic organic compound from plants, usually with basic chemical trait, containing nitrogen in negative oxidation condition (Hesse, 2002). In this research, reactor used was HCl + Dragendorff, showing alkaloid positive (+). Alkaloid positive means that the color changes into orange and forms the sediment which is orange and even brownish yellow, when the Dragendroff reactor is added (Hammado & Illing, 2013). Alkaloid is beneficial for improving antioxidant activities, anti-angiogenesis, and heart health (Chakraborty & Hancz, 2011).

Flavonoid is one of the secondary metabolite classes which are the most typical in plant (Cao *et al*., 1997). In this research, reactor used was NaOH 1% + HCl 1%, showing flavonoid negative (-), as marked with the color change into transparent (Nurmila *et al*., 2019). Flavonoid has been proven as strong antioxidants, capable of reducing hydroxyl radical, superoxide anion, lipid peroxide radical, having antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-virus, anti-neoplastic, anti-thrombotic effects, as well as vasodilation (Yao *et al*., 2004).

Steroid is the lipid terpenoid known to have four rings of carbon base frame united. This research showed steroid negative (-), indicating that feed did not have the compounds. Synthetic steroid is usually used for genital manipulation and to make fishes more fertile. However, because of its danger potency, the use of phytochemical is a potential alternative to be explored (Chakraborty *et al*.,

Table 8. Cost of catfish feed during the breeding.

Treatment	Breeding Period for Three Months			
	Amount of feed/kg	Cost of feed/kg (IDR)	Total price (IDR)	Difference with P6 (IDR)
P1 (200 g/kg)	240	10,820	2,596,800	1,003,200
P2 (100 g/kg)	240	11,820	2,836,800	763,,200
P3 (125 g/kg)	240	11,570	2,776,800	823,200
P4 (150 g/kg)	240	11,320	2,716,800	883,200
P5 (175 g/kg)	240	11,070	2,656,800	943,200
P6 (commercial feed)	240	15,000	3,600,000	0

Explanation: Amount of feed during the breeding period from BBI Bontang.

2013).

Survival and growth
Survival rate of catfishes in all treatments was between 93.33-100.00%. This result is in accordance with a research by Olurin *et al*. (2006), in which the administration of cassava flour at a dose of 25-100% had a good survival rate, between 85.72-100.00%, compared with control (0%) at 71.43% in juvenile African catfish. This result is still in a decent range, referring to the opinion of Andriyan *et al*. (2018) in which a decent survival rate in fishes is averagely between 73.5-86.0%.

Survival is affected by biotic and abiotic factors. Biotic factors include age and the capability of fishes to adapt with the environment, while the abiotic factors are food availability and quality of water as the living media (Subandiyono *et al*., 2014). Food availability in this research was supposedly adequate to meet the need of catfishes to survive. Besides, the quality of food was decent to support the improvement of survival in catfishes.

Absolute weight gain
Feed is an important aspect because it can affect activities and growth of fishes, so that the provided feed must meet the nutrient standard, with a complete nutrient composition. NRC (1993), stated that fish feed is considered good, not only in terms of its components, but also about how much the components in feed can be absorbed and used by fishes.

Absolute weight gain is the increase of weight in fishes during the breeding period. Catfishes raised for 60 days had different score, in which the highest score was in treatment P3 (125 g/kg) at 132.67±8.33 g, followed with treatment P6 (pellet F99) at 127.00±24.52 g, treatment P5 (175 g/kg) at 106.00±18.52 g, P4 (150 g/kg), treatment P1 (200 g/kg), while the lowest score was in treatment P2 (100 g/kg).

Treatment P3 had a score higher than (P6), while treatments P1, P2, P4 showed score not better than control treatment (P6), so that cassava flour administration in feed can increase absolute weight gain at certain dose. It is in accordance with a research by Abu *et al*. (2021), in which cassava flour administration was capable of increasing fish weight at doses of 50%, 75%, and 100%, by 39, 32-40, 72 g, but it decreased at a dose of 25% by 36.07 g, compared with a dose of 0% at 38.75 g in juvenile African catfish (*Clarias gariepinus*).

With cassava flour administration in feed of all treatments, the feed itself cannot provide the weight gain, but still capable of meeting the need for catfishes, as proven with survival rate reaching 93.33-100%. In this research, it was supposedly caused by fat content which was extremely high, thus leading to the growth disturbance. The fat excess in feed can affect the capability of fishes to digest and assimilate fatty acid (Ruyter *et al*., 2000), thus leading to the decrease in protein absorption and slow growth in fishes (Lin & Shiau, 2003).

Absolute length growth
Based on the result of observation, absolute length growth in fishes was 2.97±0.57 cm in treatment P1, 2.07±0.25 cm in treatment P2, 3.43±0.25 cm in treatment P3, 3.63±0.21 cm in treatment P4, 3.13±0.68 cm in treatment P5, and 2.53±0.72 cm in treatment P6.

Treatments P1, P3, P4, and P5 had a better length between 2.97 and 3.63 cm, but they decreased in P2 by 2.07 cm, compared with control (P6) with a size of 2.53 cm. This result was in line with a research by Radampola *et al*. (2021) stating that cassava flour administration with the highest dose (25 g) had a better final length, at 9.36±0.67 cm, compared with a lower dose administration (17 g) at 9.31 ±0.29. Besides, absolute length growth which were more prominent in catfishes was found in treatment P4 with average length of 3.63±0.21 cm. This was supposedly caused by the capability of fishes to make use of carbohydrate level in feed more optimally.

Daily growth rate
Growth is the increase of volume and weight at certain time (Handajani & Widodo, 2010). Figure 4 shows the highest daily growth in treatment P3 at 1.41±0.06 %/day, followed by treatment P6 at 1.27±0.21 %/day, treatment P4 at 1.21±0.28 %/day, treatment P1 at 1.11±0.22 g/%, while the lowest was in P2 with a value of 0.96±0.09 %/day. The daily growth rate in treatments P1, P2, P4, P5 was between 0.96-1.21 %/day, in which this result was still below control treatment (P6) at 1.27±0.21 %/day, but a dose of 125 g/kg (P3) had the best score at 1.41±0,06 %/day. This result is in line with a research by Abu *et al*. (2010) in which cassava flour administration in feed had a value better than the control (0%) in hybrid catfish (hetero x *Clarias*).

Low carbohydrate level caused a non-optimum growth. In this research, the non-optimum growth of fishes can be caused by the size of catfish at 10-11 cm. According to Umar *et al*. (2007), small fishes will grow relatively faster, while the older ones grow more slowly. Adult fishes eat feed, more frequently used for body metabolism. If the feed is sufficient only for body maintenance, the weight of fishes will remain the same (Halver, 1972).

Feed conversion ratio
Feed conversion was (2.05±0.32) in P1, (2.29±0.20) in P2, (1.76±0.10) in P3, (2.17±0.72) in P4, (1.91±0.21) in P5, and (2.09±0.27) in P6. A research by Abu *et al*. (2021) showed that cassava flour administration at a dose of 100% had the best feed conversion ratio at 1.19±0.01, compared with a dose of 0% at 1.32±0.04 in Dumbo catfish (*Clarias gariepinus*).

This research had feed conversion ratio/FCR at 1.76 - 2.29. High FCR value was supposedly caused by low carbohydrate level in feed, thus slowing the growth. On the contrary, low feed conversion ratio showed optimum capability of fishes in digesting and absorbing feed provided during the breeding period, thus optimally converting feed into meat (Mardhiana *et al*., 2017).

Catfish immunity
Hematocrit level
Hematocrit level in all treatments given with cassava flour had better score approximately at 20-30%, compared with control (P6) at 10%. It was in line with a research by Bamidele *et al*. (2018), in which the cassava flour administration was capable of increasing hema-

tocrit level by 31.00% at 25% dose and by 30.00% at 100% dose, compared with the control treatment (0%) with an increase by 26% in Dumbo catfish (*Clarias gariepinus*). Hematocrit level can be an indicator of stress in fish, in which low hematocrit level can indicate that fishes suffer from anemia, causing them to stop eating because of stress or disease (Taipur & Ikhwanuddin, 2013).

Total erythrocyte

Erythrocyte is red blood cell with the largest amount in the blood component structure of fishes. The observation on total erythrocyte in catfish fed with cassava flour during the breeding period had different values, indicating that total erythrocyte in catfish fed with additional cassava flour had better score, compared with control (0%). A research by Oliveira *et al*. (2016) showed that treatment with 30% of cassava flour had the best score at 2.32x10^6 cell/mm^3, while additional 15% dose at (1.96x10^6 cell/mm^3) and 45% dose at (1.95x10^6 cell/mm^3) were not better, compared with control (0%) at 2.11x10^6 cell/mm^3 in tambaqui fish (*Colossoma macropomum*). Low amount of erythrocyte is an indicator of anemia, while high amount indicated that fishes are in stress (Wedemeyer & Yatsuke, 1977).

Total leukocyte

Leukocyte is the only cell having nucleus and organelle, without hemoglobin, at 1% of the whole blood volume, but it plays an important role in the immunity system (Akers, 2013). The observation on total leukocyte showed the result that the highest value was in treatment P2, followed with P4, P3, P6 (commercial feed) at 27.90x10^4 cell/mm^3, while the lowest was in treatment P5. This means that the cassava flour can increase total leukocyte in catfish at certain dose, as supported with a research by Bamidele *et al*. (2016) in which cassava flour administration at a dose of 50% can increase total leukocyte in catfish at 21.20x10^4 cell/mm^3, but the doses of 25, 75, and 100% had a lower value, between 15.30 - 18.80x10^4 cell/mm^3 compared with control (0%) at 19.60x10^4 cell/mm^3. The increase of leukocyte production in catfish showed the response of body resistance against the foreign substance causing the disease (Martin *et al*., 2004).

Phagocytosis activities

Phagocytosis activity is the process of phagocytosis cells in eating foreign objects or microorganism penetrating the body. Table 3 shows the best phagocytosis activities in treatment P2 (31.44%), P4 (27.23%), P3 (23.90%), P1 (22.64%), P6 (22.11%), while the lowest was P5 (17.98%). It means that cassava flour administration in feed was capable of improving phagocytosis activities in catfish, compared with control (P6).

Phagocytosis activity is one of the indicators to determine pathogenicity of the phagocytized bacteria (Wulansari, 2009). Phagocytosis activity identifies the presence of bacterial infection. Lower index in phagocytosis activities indicates that phagocytic cells work harder to phagocytize bacteria (Lusiastuti *et al*., 2013).

Total microbial content test

Total microbe is a parameter showing the amount of microbe in a product. Administration of cassava flour at different doses in cat fish had different result as shown in Table 3. Total bacteria in all treatments were between 0.21 – 4.00 x10^5 cfu/ml. This result showed total bacteria in normal range. According to National Standardization Agency (2006), maximum total bacteria is 5 x10^5 cfu/ml. Amount of bacteria in fresh fishes is different from the non-fresh ones. Body defense system in the living fishes can suppress bacteria in the meat, but after the mechanism in fish body stops, the bacteria multiply and penetrate the meat (Lakollo *et al*., 2020). The increasing microbial activities in fish can cause the change of odor, appearance, and texture (Susanto, 2003).

Heavy metal contamination test

Heavy metal is one of the aspects contaminating the water, and its existence needs to be aware of. According to Supriatno (2009), heavy metal contamination in freshwater is easier to occur. Heavy metal contamination is from the human activities, whether industrial or household waste, and the nature (Fardiaz, 1995).

Iron (Fe) is an essential heavy metal which is highly required at certain amount by the living organism, but it can cause the toxic effect in excessive amount (Supriyanti, 2015). Result of heavy metal analysis showed iron level (Fe) at 0.042 - 0.056 mg/L, meaning that iron (Fe) was in good range. Standard of the iron content in clean water based on the Regulation of the Ministry of Health of the Republic of Indonesia No. 32 Year 2021 is not higher than 1 mg/L.

Copper (Cu) is found in water naturally and because of the result of human activities (Harteman, 2011). The copper (Cu) test showed the result at 0.014 mg/L, meaning that the water was feasible for pisciculture. High concentration of copper in fish can damage gill, heart, kidney, and nervous system in fishes (Palar, 2004).

Table 5 shows the lead (Pb) level in the water of breeding media at 0.009 mg/L. This result indicates that Pb level is not dangerous for the life of catfish based on the verdict of Ministry of Environment No. 51 Year 2004 stating that Pb metal allowed is $\leq$ 0.02 mg/L in the cultivation environment and $\leq$ 0.008 mg/L for biota life.

Float test and palatability

Buoyancy

Buoyancy is a benchmark in determining the feed quality. This research showed the buoyancy of fish feed between 3.00-4.67 second. Fish feed was made submerged in a short time because of the density of feed was higher than that of water. Mudjiman (2008) stated that the buoyancy of feed is related to the density. Higher density in feed, compared with water density, means that feed will quickly submerge and vice versa.

Palatability

Palatability is a factor which can determine the consumption level in feed. The feed palatability is determined by favour, odor, and color as caused by physical and chemical factors in the feed itself (Parakkasi, 1990). This research showed the response of fishes on feed at 3.13-7.38. Although the result was rather low, the palatability can be considered good, as proven with high consumption and survival at 93.33-100%.

Water quality

Temperature plays important role in the metabolism process, such as growth, food intake, and oxygen solubility in water. The result of water temperature observation during the research in the breeding media of catfish was between 26.59-26.65 °C. Based on the result of measurement, the temperature was in the normal limit, as stated by Fadillah *et al*. (2019) optimum temperature in catfish cultivation is at 26-32 °C.

Dissolved oxygen (DO) in the breeding media during the research was between 3.35-3.97 mg/L. Based on the result, DO/dissolved oxygen was below the optimum range, in which ideal DO for growth and survival of catfish is 4.4-4.6 mg/L (Augusta, 2016). However, the DO does not interfere with the survival of catfish. It is in accordance with a statement from Stickney (2005) in which the dissolved oxygen for catfish must not be lower than 3 mg/L, as supported with Indonesian National Standard (2014) in which minimum requirement of the dissolved oxygen for catfish cultivation is 3 mg/L.

Acidity degree is a measure of the hydrogen ion concentration showing acid or base environment of water. Acidity degree (pH) in the breeding media during the research was between 7.01-7.12. Based on the result, pH was still in the normal range based on a statement by Hermansyah *et al*. (2017) in which catfish has the pH tolerance level at the range of pH 5.5-7.5.

Ammonia is a toxic compound which can bring negative effect to the health of fishes (Levit, 2010). Ammonia level in the breeding media during the research was between 0.41-0.42 mg/L. This showed that the ammonia level of fish was still in the normal range in which the maximum limit of ammonia level in cultivation of freshwater, according to the Government Regulation No. 82 Year 2001, is 0.50 mg/L. High ammonia level can lead to listless, sickness, and death in fishes.

Economic analysis

The role of artificial feed in the fish cultivation is highly dominant, particularly in an intensively managed cultivation. The process of making feed should be based on the consideration of the need for nutrient in fish, quality of the raw material, and economic value.

It takes 3 months or 90 days for raising juvenile fishes, until they can be harvested (Suwato et al., 2018). The cost for making artificial feed by adding cassava flour (P1-P5) was between IDR 10,820 - 11,820 per kg of feed, in which this price was more affordable, compared with commercial feed (F99) at IDR 15,000/kg. Besides, if the amount of feed required in the sowing process until harvest is 240 kg, the cost required is IDR 2,596,800 - 2,836,800 for artificial feed (P1-P5) and IDR 3,600,000 for commercial feed. Considering the aforementioned comparison, it can be identified that the difference of margin on the need for feed is IDR 763,200 - 1,003,200. Thus, the use of alternative feed is capable of saving production cost on feed at approximately 21.56-27.87%

CONCLUSION AND SUGGESTION

Conclusion

The feed for Sangkuriang catfish is made of a formulation of the fermented cassava flour substitution, having the phytochemical content namely alkaloid (+), flavonoid negative (-), and steroid negative (-) The research on some treatments showed that feed can affect the growth of catfish, with insignificant effect (P>0.05). In terms of immunity, the hematocrit level in all treatments had a better score between 20-30%, compared with control (P6) at 10%. Besides, the feed can affect survival rate (SR) at 93.33-100%. The use of alternative feed was capable of saving production cost of feed at approximately 21.56-27.87% Thus, the fermented cassava flour can be mixed with the feed for Sangkuriang catfish using the right formulation, P3 (125 g/kg).

Suggestion

Based on the conducted research, the suggestion for this research is that the water quality must be preserved to ensure the survival and to support the fish growth. Besides, the raw material for feed and the process of making feed prioritize quality, so that the resulted feed can support the growth of fish, without disturbing their health.

ACKNOWLEDGEMENT

The writers express their gratitude to the Mulawarman University, State Vocational High School 2 Bontang, and all parties supporting this research.

AUTHORS' CONTRIBUTIONS

SA is doing research and data analyze; and EHH & FF is doing manuscript preparation and written manuscript.

REFERENCES

Abu, O.M.G., T. Lazarus, O. Onisowurun & C.S. Obiyom. 2021. Effect of graded level of whole cassava root meal as a replacement for maize on growth performance of *Clarias gariepinus* fingerlings. Journal of Agriculture and Veterinary Science (IOSR-JAVS). 14 (2): 9-13.

Abu, O.M.G., U.U. Gabrieland, & O.A. Akinrotimi. 2010. Performance and survival of hybrid catfish (hetero X *clarias*) fed with whole cassava root meal as a replacement for maize. Journal of Tropical Agriculture, Food, Environment and Extension. 9 (3): 176-183.

Akers, R.M., & D.M. Denbow. 2013. Anatomy and Physiology of Domestic Animals (Second edition). Wiley Blackwell.

Andriyan, M.F., S. Rahmaningsih & U. Firmani. 2018. The effect of salinity on survival rate and blood profile of tilapia fish (*Oreochromis niloticus*) given with feed and cheese fruit (*Morinda citriffolia* L.). Journal of Pantura Fishery. 1 (1): 1.

Augusta, T.S. 2016. Dynamic of the water quality change on the growth of dumbo catfish (*Clarias gariepinus*) raised in ground pool. Journal of Tropica Animal Science. 5 (1): 41-44.

Bamidele, N.A., S.O. Obasa, I.O. Taiwo, I. Abdulraheem, O.S. Oladoyin & I.J. Ovie. 2018. Utilization of enzyme supplemented fermented cassava root tuber flour based diets of *Clarias gariepinus* fingerlings. International Journal of Fisheries and Aquatic Studies. 6 (2): 117-122.

Cao, G., E. Sofic & R.L. Prior. 1997. Antioxidant and prooxidant behavior of flavonoids: Structure–activity relationships. Free Radical Biology and Medicine. 22 (5): 749-760. https://doi.org/10.1016/S0891-5849(96)00351-6

Chakraborty, S.B & C. Hancz. 2011. Application of phytochemicals as immunostimulant, anti-pathogenic and anti-stress agents in finfish culture. Reviews in Aquaculture. 3 (3): 103-119. https://doi.org/10.1111/j.1753-5131.2011.01048.x

Chakraborty, S.B., P. Horn & C. Hancz. 2013. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture. 6 (1): 1–19. https://doi.org/10.1111/raq.12021

Citarasu, T. 2010. Herbal Biomedicines: A new opportunity for aquaculture industry. Aquaculture International. 18: 403-414. https://doi.org/10.1007/s10499-009-9253-7

Craig, S. 2017. Understanding Fish Nutrition, Feeds, and Feeding. Communications and Marketing, College of Agriculture and Life Sciences. Virginia Tech. 420-256.

Fadillah, A., A.T. Hanuranto & N. Bogi. 2019. Implementation of the monitoring system on water quality of catfish pool based on the wireless sensor network. E Proceeding of Engineering. 6 (2).

Fardiaz, S. 1995. Water and Air Pollution. Yogyakarta: Kanisius.

Government Regulation. 2021. Government Regulation No. 82 Year 2001 concerning Water Quality Management and Water Contamination Control. Indonesian Ministry of Maritime Affairs and Fisheries

Halver, J.E. 1972. London, NewYork: Fish Nutrition. Academic Press, 713.

Hammado, N & I. Illing. 2013. Identification of the alkaloid active compound in lahuna (*Eupatorium odouratum*). Dinamika Journal. 4 (2): 1-18.

Hardi, E.H, R.A. Nugroho, R. Rostika, C.M. Mardliyaha, K. Sukarti, W. Rahayu, A. Supriansyah & G. Saptiani. 2022. Synbiotic application for improving growth, body endurance, and disease resistance on the bacterial infection in catfish (*Clarias gariepinus*). Aquaculture. 549. https://doi.org/10.1016/j.aquaculture.2021.737794

Hardi, E.H., G. Saptiani, I.W. Kusuma, R.A. Nugroho, W. Suwinarti, R. Anjani & A. Aziza. 2020. Prevention methods against *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection in Tilapia. BIOTROPIA. 27 (3): 209 – 221.

Hardi, E.H., G. Saptiani, N. Nurkadina, I. W. Kusuma & W. Suwinarti. 2018[b]. In vitro test of concoction plant extracts of *Boesenbergia pandurata*, *Solanum Ferox*, *Zingimber zerumbet*. Veteriner Journal. 19 (1): 35.

Hardi, E.H., G. Saptiani, Nurkadina, I.W. Kusuma & W. Suwinarti. 2018[a]. In vitro test on the concoction of *Boesenbergia pandurata*, *Solanum ferox*, *Zingimber zerumbet* Extracts on the pathogenic bacteria in tilapia fish. Veteriner Journal. 19 (1): 35. https://doi.org/10.19087/jveteriner.2018.19.1.35

Hermansyah, E., D. Derdian & W.F.T. Pontia. 2017. Water pH controlling design for catfish cultivation based on atmega16 micro-controller. Journal of Electrical Engineering, Tanjungpura University. 2 (1).

Hesse, M. 2002. Alkaloids: Nature's Curse or Blessing? Verlag Helvetica Chimica Acta, Zurich, Switzerland €. Wiley-VCH, Weinheim, Federal Republic of Germany.

Indonesian National Standard. 2006. Fish Feed. Indonesian National Standard 01-4087-2006. Jakarta: National Standardization Agency of Indonesia.

Indonesian National Standard. 2014. Indonesian National Standard. Dumbo Catfish (*Clarias* sp.). National Standardization Agency of Indonesia. Jakarta. Indonesian National Standard 6484.3.

Ingerslev, H.C., M.L. Strube, L. Jørgensen, G. Von, I. Dalsgaard, M. Boye & L. Madsen. 2014. Diet type dictates the gut microbiota and the immune response against *Yersinia Ruckeri* in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 40 (2): 624-633. https://doi.org/10.1016/j.fsi.2014.08.021

Irfak, K. 2013. Optimal Design in the Processing of Biogas Solid Sludge as a Raw Material for Catfish Feed. in Magetan, East Java. Undergraduate Thesis. Malang: Faculty of Agriculture, Brawijaya University.

Jusuf, D.D., O.R. Pinonton & R.H. Akili. 2021. Analysis of lead (Pb) and zinc (Zn) content in water and fish. in the Fish Pond of Remboken District, Minahasa Regency in 2021. KESMAS Journal. 10 (6): 82-92.

Lakollo, E & M.N. Mailoa. 2020. Management technique and microbe contamination in the fresh flying fishes in traditional market of Ambon City. Journal of Indonesian Fishery Product Processing. 23 (1): 103-111.

Levit, S.M. 2010. A Literature Review of Effects of Ammonia on Fish. The Nature Conservancy, Centre for Science in Public Participation, Bozeman, Montana.

Lin, Y.H & S.Y. Shiau. 2003. Dietary lipid requirement of grouper, *Epinephelus malabaricus*, and effects on immune responses. Aquaculture. 225 (1-4): 243-250. https://doi.org/10.1016/S0044-8486(03)00293-X

Lusiastuti, A.M., T. Sumiati & W. Hadie. 2013. *Bacillus firmus* probiotics for controlling *Aeromonas hydrophilla* disease in the cultivation of dumbo catfish (*Clarias gariepinus*). Journal of Aquaculture Research. 8 (2).

Mahyuddin, K. 2011. A Complete Guide on the Catfish Agribusiness. Jakarta: Penebar Swadaya, 170.

Mardhiana, A., I.D. Buwono, Y. Andriani & I. Iskandar. 2017. Supplementation of the commercial probiotics in artificial feed for induction of sangkuriang catfish growth (*Clarias gariepinus*). Journal of Fishery and Marine Sciences. 8 (2): 133-139.

Martin M.L, D.T. Namura, D.M. Miyazaki, F. Pilarsky, K. Ribero, M.P. De Castro & C.M.D. Campos. 2004. Physiological and hematological response of *Oreochromis niloticus* exposed to single and consecutive stress of capture. Animal Science. 26: 449-456.

Ministry of Marine Affairs and Fishery. 2022. Steps of Ministry of Marine Affairs and Fisheries in Improving the Use of Independent Feed for the Fish Cultivator. https://kkp.go.id/djpb/artikel/25172

Mokoginta, N.P. A.D. Utomo, A. Akbar & M. Setiawati. 2003. The use of cassava flour as substitution of wheat flour in the feed of goldfish, *Cyprinus carpio*. Journal of Indonesian Aquaculture. 2 (2): 79-83.

Mujiman, A. 1984. Fish Feed. Jakarta. Penebar Swadaya.

National Standardization Agency of Indonesia. 2006. Fresh Fish - Part 1. Indonesian National Standard 01- 2729-1- 2006. Jakarta (ID): National Standardization Agency of Indonesia.

Nazriati, E., S. Wahyuni, H. Herisiswanto & R. Rofika. 2021. The Making of mocaf flour as an effort of optimization in the use of cassava in the farmer group in Pekanbaru. COMSEP- Journal of Social Service. 2 (3): 305-310.

NRC. 1993. Nutrient Requirement of Fish. USA: National Academy of Science. National Press, 39-53.

Nurmila, N., H. Sinay & T. Watuguly. 2019. Identification and analysis of flavonoid level in angsana (*Pterocarpus indicus* Willd) extract in Wanath Village, Leihitu Regency, Central Maluku Regency. Biopendix. 5 (2): 65-71. https://dx.doi.org/10.30598/biopendixvol5issue2page65-71

Olurin, K.B., E.A.A Olojo & O. Olukoya. 2006. Growth of african catfish *Clarias gariepinus* fingerlings, fed different levels of cassava. International Digital Organization for Scientific Information. 1 (1): 54-56.

Palar, H. 2004. Contamination and Toxicology of Heavy Metal. Rineka Cipta. Jakarta.

Parakkasi A. 1990. Nutritional Science and Monogastric Livestock Feed. Jakarta. UI-Press, 230.

Pasca, B.D., J. Muhandri, D. Hunaefi & B. Nurtama. 2021. Food Science Study Prog, Postgraduate School, Bogor Agricultural University, Bogor, Department of Food Science and Technology, Faculty of Agricultural Technology.

Pilay, T.V.R. 2004. Aquaculture & The Environmental, second Edition. Blackwell Publishing, United Kingdom.

Regulation of the Minister of Environment. 2004. No. 51 Year 2004 concerning the Policy and Institution of Environment, Seawater Quality for Sea Biota.

Ruyter, B., C. Røsjø, O. Einen & M.S. Thomassen. 2000. Essential fatty acids in atlantic salmon: Effects of increasing dietary doses of n-6 and n-3 fatty acids on growth, survival and fatty acid composition of liver, blood and carcass. Aquaculture Nutrition. 6: 119–127.

Sahwan, M.F. 2003. Fish and Shrimp Feed. Jakarta: Penebar Swadaya.

Sari, L.A. 2021. Effect of the Use of Feed Management Technology in Breeding Pearl Catfish (*Clarias gariepinus*). Unair News. https://iopscience.iop.org/article/10.1088/1755-1315/718/1/012030.

Steffens, W. 1989. Principle of Fish Nutrition. England. Ellis Horwood Limited.

Stickney, R.R. 2005. Aquaculture: An Introductory Text. Oxford: CABI Publishing. 265.

Subandiyono, S., A.S. Airmawati & P. Pinondoyo. 2014. The effect of vitamin C in artificial feed on the feed consumption level and the growth of red tilapia fish (*Oreochromis niloticus*). Journal of Aquaculture Management and Technology. 3 (4): 191-198.

Supriatno, S & L. Lelifajri. 2009. An analysis of heavy metal, Pb and Cd, in fish and shellfish samples in spectrophotometry of atom absorption. Journal of Chemical and Environmental Engineering. 7 (1): 5-8.

Susanto, J.P & N. Sopiah. 2003. The Effect of metal and substrate concentration on growth and activity of proteolytic bacteria in the deproteination of crab shell. Journal of Environmental Technology. 4 (1): 40-45.

Sutikno, E. 2017. Instruction for Technique in Making Affordable Feed with Simple Technology. Jepara, Central Java. Main Station for Brackish Water Aquaculture.

Suwarto, E., K. Utomo, P. Paryono & A. Suwondo. 2018. Catfish farming as a business for improving economy and independence of Takmir in Diponegoro Mosque II of Tembalang, Semarang. National Seminar on Research Result and Community Service. 1: 433-445.

Tacon, A.E.J. 1987. The Nutrition and Feeding Formed Fish and Shrimp. A Training Manual Food and Agriculture of United Nation Brazilling, Brazil.

Talpur, A.D & M. Ikhwanuddin. 2013. *Azadirachta indica* (neem) Leaf dietary effects on the immunity response and disease resistance of asian seabass, *Lates Calcarifer* challenged with *Vibrio Harveyi*. Fish Shellfish Immunol. 34 (1): 254-264. https://doi.org/10.1016/j.fsi.2012.11.003

Umar, M.T., S. Suwarni, R. Salam & S.B.A. Omar. 2007. A study on the growth of bonti-bonti fish (*Paratherina striata Aurich*, 1935) in Towuti Lake, South Sulawesi. Faculty of Fisheries and Marine Science, Hasanuddin University. National Seminar on Fishery. 3(2): 1-9.

Wedemeyer, G.A & W.T. Yatsuke. 1977. Clinical Methods for the Assessment of the Effects of Environmental Stress or Fish Health. Technical Paper of the US. Fish and Wildlife Service: 11-18.

Wirabakti, C.M. 2006. Growth Rate of Red Tilapia Fish Raised in Calm Water in Fish Cage and Pool. http://google.com./jurnal.upr.ac.id.

Wulansari, W. 2009. The Effect of Water and Ethanol Alpinia spp Extracts on the Phagocytosis Activities and Capacity of Macrophage Cell Induced from the Epidermal *Stapilococcus Bacteria* In Vitro. Bogor: Biological Research Centre of National Institute of Science.

Yao, L.H., Y.M. Jiang, J. Shi, T.F.A. barberan, n. datta & r. singanusong. 2004. Flavonoids in food and their health benefits. Plant Foods for Human Nutrition. 59: 113-122. https://doi.org/10.1007/s11130-004-0049-7

Zaenuri, R., B. Suharto & A.T.S. Haji. 2014. Quality of the fish feed in form of pellet made of the agricultural waste. Journal of Natural Resource and Environment. 1 (1): 31-36.

Zonneveld, N., E.A., Huisman & J.H. Boon. 1991. Principles of Fish Cultivation. Gramedia Pustaka Utama. Jakarta.

How to cite this article Asih, S., E.H. Hardi & F. Fitriyana. 2024. The fermented cassava (*Manihot esculenta* C.) flour and BIOIMUN® as additional feed for the juvenile catfish (*Clarias gariepinus*) to improve growth, immunity, and survival. Jurnal Perikanan Universitas Gadjah Mada. 26 (1): 61-69. https://doi.org/10.22146/jfs.91538