The inclusion of moringa leaf extract fermentation in commercial feed to enhance feed conversion ratio and specific growth rate of tambaqui fish, *Colossoma macropomum*.

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Abstract. Probiotics could be used as an alternative substitute for Antibiotic Growth Promoter (AGP). One of the bacteria that have the potential as a probiotic was *Lactobacillus acidophilus* which was able to secrete digestive enzymes that could help digestion and absorption of feed nutrients. The activity of probiotic bacteria could be stimulated by giving Moringa leaf extract through the process of breaking down oligosaccharides into lactic acid. The purpose of this study was to determine the effect of giving Moringa leaf extract, *L. acidophilus* probiotic, and the interaction between Moringa leaf extract and *L. acidophilus* probiotic in commercial feed on the specific growth rate and feed conversion ratio of Tambaqui fish. The results showed that administration of Moringa leaf extract and administration of *L. acidophilus* probiotics to commercial feed with different doses did not have a significant effect (p> 0.05), but the interaction of the combination of Moringa leaf extract and *L. acidophilus* probiotics gave a significant effect (p <0.05) on the specific growth rate and feed conversion ratio of Tambaqui fish, namely the A1B2 treatment (4.03% per day) for the specific growth rate and A2B1 treatment (0.92) for the feed conversion ratio.

1. Introduction
Tambaqui fish *Colossoma macropomum* had great potential for aquaculture [1]. One aspect of aquaculture that played an important role in supporting the growth and survival of fish was feed [2]. Feed was the largest cost component in aquaculture activities with a range of 50-80% of the total production cost [3, 4]. The high production costs was resulted in many fish farmers using the addition of feed additives to produce optimal growth and feed efficiency, increase growth performance, nutrient digestibility, and reduce production costs [5].

Feed additives were substances that could be added to fish feed in small quantities to improve feed quality so as to improve growth performance [6]. This feed additive had become an alternative to antibiotics [7]. The global ban on the use of Antibiotic Growth Promoter (AGP) in feed had resulted in researchers looking for alternatives to other growth promoters. Alternative growth promoters that were often used in feed were probiotics, prebiotics, enzymes, acidifiers, antioxidants and phytogetic feed additives [8].
Probiotics were live microbial feed supplements that were useful for influencing host animals by increasing the balance of their intestinal microbes [9]. Probiotics were also able to secrete enzymes that help the process of digestion of food [10,11, 12]. One candidate for bacteria that was good for use as probiotics was \textit{L. acidophilus}. \textit{L. acidophilus} was one of the lactic acid bacteria (LAB) which had the ability to inhibit the growth of pathogenic microbes because it produced several chemicals including lactic acid, acetic acid, hydrogen peroxide and bacteriocins [13].

The activity of probiotic bacteria could be stimulated by giving Moringa leaf extract through a fermentation process in the feed. The content of oligosaccharides contained in Moringa leaf extract was used by probiotic bacteria to produce energy through the process of breaking down sugar into lactic acid [14]. Moringa leaf extract also had the main active ingredients, namely saponins, tannins, and flavonoids [15]. Saponins function as antimicrobials that could increase immunity so that they were resistant to disease and smooth the digestive system. Flavonoids as antioxidants and maintained the body's immune system. Tannin had antiseptic properties so that it had a good effect in the digestive tract [15]. Research on the use of phytogenic feed additives in the form of Moringa leaf extract in fish is still very limited, but it had been widely applied in livestock, as reported by Trisna \textit{et al.} [15] stated that the used of 5% Moringa leaf extract mixed in drinking water could significantly increase the efficiency of using 2–6 week old broiler chicken rations. Feed additives in the form of a combination of probiotic \textit{L. acidophilus} and Moringa leaf extract in commercial feed were expected to optimize the absorption of nutrients in the digestion of Tambau fish so as to increase the specific growth rate and decrease the feed conversion ratio.

2. Material and methods

2.1. Material

This research activity was carried out from October 2019 to March 2020 at the Laboratory of Aquaculture and Anatomy, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. Moringa leaf extraction activities were carried out at the Laboratory of Chemistry and Analysis, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, while for proximate feed analysis was carried out at the Laboratory of Feed Analysis, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya.

The tools used in the study were 27 aquariums with a size of 45 × 40 × 50 cm³ for fish aquaculture, aquaculture racks, aerated hoses and stones, aerators, reservoir tanks, sieve, siphon tools, analytical scales, material scales, trays, syringes 5 mL, blender, rotary evaporator, 1000 mL Erlenmeyer, orbital shaker, 1000 mL measuring cup, 600 mL beaker glass, 1000 mL Erlenmeyer Buchner, Buchner funnel, vacuum pump, spatula, glass spatula, electric aspirator, water bath, spoon, compressor, oven, refrigerator, baking sheet, crusstang, 10 mL vial bottle, petri dish, plastic clips, sample pots, label paper, basin, sectio set, millimeter paper, thermometer, pH paper, DO meter, and ammonia test kit.

The research materials used included Tambau fish measuring 5-6 cm with an average weight of 4.5 g/ind, Moringa leaf extract, \textit{L. acidophilus} probiotic (collection from Lokapirnasari), and commercial feed type PF 1000 which has a crude protein content of 32.31%, fat 4.97%, crude fiber 3.56%, ash 9.73%, nitrogen-free extract (NFE)43.25%.

2.2. Methods

The research method used was an experimental method using a completely randomized design (CRD) factorial pattern consisting of 3×3 treatments with 3 replications so that there were 27 experimental units. The treatment of this study could be seen in Table 1.

2.2.1. Moringa leaf extraction

The fresh Moringa leaves were dried without sun exposure, then the Moringa leaves were ground using a blender to make them a smaller size or powdered. Furthermore, the maceration process was carried out by soaking 200 grams of Moringa leaf powder with 1000 mL of 96% ethanol as the solvent. The ratio of Moringa leaf flour to the solvent used was 1: 5. The maceration process was
carried out for $1 \times 24$ hours with a closed container and protected from sunlight. The macerate results were then filtered with Whatman filter paper no. 42 and concentrated using a rotary vacuum evaporator at $60^\circ$C with a speed of 65 rpm for approximately 60 minutes to obtain Moringa leaf extract. Moringa leaf extract was stored in an oven at $35^\circ$C to evaporate the remaining ethanol from the extraction process for 24 hours, in order to obtain concentrated Moringa leaf extract (paste).

### Table 1. Treatment research

| Probiotics $L. \text{acidophilus}$ (B) | Moringa Leaf Extract (A)       |
|--------------------------------------|-------------------------------|
|                                      | 0% (A₀) | 1% (A₁) | 2% (A₂) |
| 0% (B₀)                              | A₀B₀    | A₁B₀    | A₂B₀    |
| 1% (B₁)                              | A₀B₁    | A₁B₁    | A₂B₁    |
| 2% (B₂)                              | A₀B₂    | A₁B₂    | A₂B₂    |

#### 2.2.2. Treatment feed making

Preparation of the treated feed began with diluting 1 gram of Moringa leaf extract in 100 mL distilled water. The extract solution was then mixed with probiotics in a vial bottle according to the dose of each treatment, then left for 24 hours and then mixed in the commercial feed. The application of moringa leaf extract and $L. \text{acidophilus}$ probiotic to commercial feed was done by spraying according to the predetermined treatment dose, then fermentation for 7 days by inserting the feed into plastic which was tightly tied to make it airtight. The compositions of the treated feed used were as follows.

- A₀B₀: Commercial Feed 100 grams
- A₀B₁: Commercial feed 100 grams + 1 mL probiotic
- A₀B₂: Commercial feed 100 grams + 2 mL probiotics
- A₁B₀: 100 gram Commercial Feed + 1 mL Moringa leaf extract
- A₁B₁: Commercial feed 100 grams + 1 mL Moringa leaf extract + 1 mL probiotic
- A₁B₂: Commercial feed 100 grams + 1 mL Moringa leaf extract + 2 mL probiotics
- A₂B₀: 100 gram Commercial Feed + 2 mL Moringa leaf extract
- A₂B₁: Commercial feed 100 grams + 2 mL Moringa leaf extract + 1 mL probiotic
- A₂B₂: Commercial feed 100 grams + 2 mL Moringa leaf extract + 2 mL probiotic

#### 2.2.3. Preparation of equipments and experimental fish

Preparation of the equipments by cleaning them that would be used for raising Tambaqui fish. The equipments were a reared aquarium that were washed using soap and rinsed with clean water, then sterilized using chlorine as a disinfectant to avoid bacteria and disease and soaked for 24 hours, after which the aquarium was dried. The dry aquarium was filled with fresh water that had been deposited for 3 days in a water reservoir. Each aquarium was filled with 10 liters of water and given an aerator that was turned on for 24 hours to increase dissolved oxygen and eliminate chemical odors that remain in the aquarium. Tambaqui fish were acclimatized first in a fiber bath for 30 minutes before being put in the aquarium maintenance media, then continued with Tambaqui fish for 24 hours to eliminate the effect of the previously given feed.

#### 2.2.4. Fish aquaculture and feeding

The experimental fish were selected based on healthy fish conditions and weighed to determine the wet weight of the fish at the beginning of maintenance. The selected test fish were then put into each aquarium with stocking density of 1 individual / L [16]. Feeding was done twice a day, namely morning and evening by ad satiation. The feed to be given to the fish was weighed every week, to determine the total feed during aquaculture.

#### 2.2.5. Water quality

Water quality parameters observed were temperature, pH, dissolved oxygen (DO), and ammonia. Temperature, pH, and DO checks were carried out every day, while ammonia checks was carried out once a week. Measurement of temperature and dissolved oxygen by a DO meter, pH measurement by
a pH paper and ammonia measurement by an ammonia test kit. Cleaning the aquarium was carried out every two days in the afternoon before feeding by changing the water as much as 50% of the volume of the maintenance medium, and siphoning done every day in the afternoon.

2.2.6. Observation parameters

The parameters observed during the study were the specific growth rate and feed conversion ratio (FCR). Measurement of the specific growth rate and feed conversion ratio was carried out by measuring the length and wet weight of the fish at the beginning and end of aquaculture, as well as weighing the feed given to the fish during aquaculture using analytical scales. The formula for calculating the specific growth rate was as follows:

\[ SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\% \]

Information :
SGR : Specific growth rate (%)
Wt : Biomass of test animals at the end of aquaculture (gr)
W0 : Biomass of test animals at the beginning of aquaculture (gr)
t : Length of maintenance time (days)

The feed conversion ratio could be determined by calculating the quotient between the amount of feed (gr) consumed and body weight gain (gr). According to Rozi et al. [17] calculation of the feed conversion ratio (FCR) could be calculated using the following formula:

\[ FCR = \frac{F}{Wt + D - W_0} \]

Information :
FCR : Feed conversion ratio
F : Weight of feed given (gr)
Wt : Biomass of test animals at the end of aquaculture (gr)
D : Weight of dead fish (gr)
W0 : Biomass of test animals at the beginning of aquaculture (gr)

2.2.7. Data analysis

Observations data, namely specific growth rates and feed conversion ratios, were statistically analyzed using the Analysis of Variance (ANOVA) test to determine the effect of the treatment given. If the results of the analysis show a real effect, the calculation was continued with Duncan's Multiple Range Test [18].

3. Result and discussion

3.1. Results

3.1.1. Specific growth rate (SGR)

Based on the combination study of Moringa leaf extract and \textit{L. acidophilus} probiotics in commercial feed, the average specific growth rate for Tambaqui fish could be seen in Table 2.

The results of ANOVA statistical analysis showed that the combination of Moringa leaf extract and \textit{Lactobacillus acidophilus} probiotic in commercial feed was known to have a significant difference (p < 0.05) on the specific growth rate of Tambaqui fish. The specific growth rate was known to have no significant effect (p > 0.05) from giving Moringa leaf extract (factor A) and giving probiotics (factor B). However, there was a significant difference (p < 0.05) from the interaction factor (extract* probiotics) so that it could be continued with Duncan's continued test and the best value was obtained
in the A1B2 treatment with a dose of 1% Moringa leaf extract and 2% probiotic, which was 4.03% / day which was not significantly different from A0B2, A0B0, A2B1.

Table 2. Average specific growth rate (SGR) of Tambaqui fish

| Probiotics L. acidophilus (B) | Moringa leaf extract (A) | Average |
|-------------------------------|--------------------------|---------|
| 0% (B0)                       | 3.76±0.23                | 3.39±0.25|
| 1% (B1)                       | 3.33±0.16                | 3.49±0.20|
| 2% (B2)                       | 3.81±0.20                | 3.77±0.47|
| Average                       | 3.36±0.19                |         |

Note: The values shown were the mean ± standard deviation. Different superscripts showed significantly different effects between treatments (P <0.05).

3.1.2. Feed conversion ratio (FCR)
The results obtained from the combination study of Moringa leaf extract and L. acidophilus probiotic in commercial feed in the form of the average feed conversion ratio (FCR) value of tambaqui fish were presented in Table 3.

Table 3. The average value of the feed conversion ratio (FCR) of tambaqui fish

| Probiotic L. acidophilus (B) | Moringa leaf extract (A) | Average |
|-------------------------------|--------------------------|---------|
| 0% (B0)                       | 0.98±0.04                | 1.036±0.05|
| 1% (B1)                       | 1.07±0.04                | 1.00±0.07|
| 2% (B2)                       | 0.95±0.05                | 1.02±0.19|
| Average                       | 1.00±0.04                | 1.06±0.09|

Note: The values shown were the mean ± standard deviation. Different superscripts showed significantly different effects between treatments (P <0.05).

ANOVA statistical analysis results showed that the administration of Moringa leaf extract (factor A) and the administration of L. acidophilus probiotics (factor B) did not have a significant effect (p> 0.05), but the interaction of the combination of Moringa leaf extract and L. acidophilus probiotics (factor AB) had a significant effect (p <0.05) on the feed conversion ratio of Tambau fish. The results of the best treatment interaction were A2B1 with the lowest feed conversion ratio value of 0.92 which was not significantly different from the A0B2, A1B2, A0B0, A1B0 and A1B1 treatments of 0.95; 0.96; 0.98; 1.00 and 1.02. Meanwhile, the highest feed conversion ratio value was in the A2B2 treatment of 1.15 which was not significantly different from the A2B0, A0B1 and A1B1 treatments of 1.13; 1.07 and 1.02.

3.2. Discussions
According to Anggraeni et al. [19], Growth was the percentage of fish increase every day. Growth was also defined as an increase in the number of mitotic cells which would cause a change in tissue size. According to Wiadnya et al. [20] stated that the nutritional content in feed could affect the value of its growth rate. The main raw material for the formation of cells and body tissues was protein, therefore protein was needed for growth [21].

The value of this specific growth rate was good because the food combined with Moringa leaf extract and probiotics was easy to digest food. This was also supported by Analysa [22], that Moringa leaf extract was a good feed additive for fish because it contained active substances that could improve
the performance of internal organs and prevent damage to internal organs such as pancreas which would give a good effect on increasing metabolism and absorption of nutrients (carbohydrates, fats and proteins) in the body of livestock. The high nutritional content in Moringa leaves had the potential as a supplement in feed to improve the productivity performance of Tambaqui fish.

*L. acidophilus* probiotics in feed could produce digestive enzymes that could remodel the macro nutrients that entered through the feed in the digestive tract of fish so that it helped the digestion process of feed [5]. *L. acidophilus* probiotics could also increase the length of the intestinal villi by activating cell mitosis and inducing the proliferation of intestinal epithelial cells. Increased villous height was beneficial for fish because increased surface area of the villi increased the absorption of nutrients in the digestive tract [23].

A good feed conversion ratio value was thought to be due to the high absorption of nutrients in the digestive tract due to the role of *L. acidophilus* bacteria activity stimulated by Moringa leaf extract. This was supported by Setiarto et al. [24] stated that Moringa leaf extract contained oligosaccharides which could stimulate the activity of probiotic *L. acidophilus* bacteria in producing energy through the process of breaking down oligosaccharides into lactic acid which would be used for growth, metabolism and activity of probiotic bacterial cells. *L. acidophilus* probiotics had the ability to lower pH through the process of breaking down oligosaccharides into lactic acid so that the environmental conditions in the digestive tract became acidic [25], which causes pathogenic bacteria to be eliminated and the process of absorption of nutrients could work optimally without being hindered by pathogenic bacteria [26].

The low feed conversion value caused by giving a combination of Moringa leaf extract and *L. acidophilus* probiotics could reduce feed consumption [27]. Low feed consumption was due to the adequate nutrition and energy needed by fish [28]. Lokapirmasari et al. [29] stated that a decrease in the value of the feed conversion ratio indicated an increased in feed efficiency. The higher the feed efficiency value, the better the fish response to the feed was indicated by the rapid growth of fish [30].

### 4. Conclusion

The combination of Moringa leaf extract and *L. acidophilus* probiotic added to this commercial feed had a high specific growth rate value of Tambaqui fish in A1B2 treatment and a low value of feed conversion ratio in A2B1 treatment due to sufficient nutritional and energy requirements of Tambaqui fish.

### 5. References

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