



## Additional of EM4 and molasses in feeds on the growth and survival rate of snakehead (*Channa striata*)

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### ABSTRACT

This study aims to determine the effect of giving EM4 probiotics on the growth and survival rate of snakehead (*Channa striata*). This research was carried out at the Fish Hatchery Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala. This study was conducted using a Complete Randomized Design (CRD) method with 4 treatments used, namely A (control), B (EM4 6 ml/kg of feed), C (EM4 8 ml/kg of feed), and D (EM4 10 ml/kg of feed). The results showed that the addition of 10 ml/kg of feed of EM4 probiotic resulted in an absolute length growth of  $1.60 \pm 0.17$  cm, an absolute weight growth of  $1.85 \pm 0.18$  gram, a specific growth rate of  $1.97 \pm 0.10$  % per day, a feed conversion ratio of  $1.56 \pm 0.03$ , feed efficiency  $64.19 \pm 1.41$  % and survival rate  $97.78 \pm 3.85$  %. The results of this study can be concluded that the addition of probiotic EM4 10 ml/kg of feed can increase the growth and survival rate of snakehead (*Channa striata*).

### Introduction

Snakehead (*Channa striata*) is a river fish or freshwater fish. Snakehead is one of the fishery commodities favored by the community to meet the needs of animal protein. In addition to the problem of high protein, the main trigger for the high exploitation of snakehead fish is the albumin content which is used to accelerate postoperative wound healing.

Public demand for snakehead continues to increase every year. This has resulted in the level of exploitation of snakehead fishing in nature increasing, so that the sustainability of snakehead in nature can be disrupted (Prakoso *et al.*, 2018). One solution that can be done to overcome the reduced population of snakehead in nature is the effort to cultivate snakehead (Trisna *et al.*, 2013). However, cultivators currently face problems in maintaining snakehead germs, such as the price of expensive feed and a lack of appetite for fish, which inhibits growth and causes a high rate of fish mortality at the stage of

germs maintenance (Hartini *et al.*, 2013). One of the steps that can be taken to overcome this is to add probiotics to fish feed.

Probiotics are an alternative that can be used as additional ingredients (supplements) in aquaculture fish feed (Mansyur and Tangkok, 2008). Probiotics are food additives (supplements) in the form of living microorganism cells that have a beneficial effect on host animals that consume them through balancing the intestinal microorganism flora in the digestive tract (Fadri *et al.*, 2016). Several studies have stated that the application of probiotics has a role in improving water quality, increasing biosecurity, increasing productivity, increasing feed efficiency and reducing production costs through reducing feed costs (Mansyur and Tangkok, 2008; Trisna *et al.*, 2013; Lu *et al.*, 2019).

Commercial probiotics that are commonly used in aquaculture and are obtainable are EM4. This probiotic contains *Lactobacillus* sp., *Acetobacter* sp.,

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*Streptomyces* sp., and yeast bacteria. Anis and Hariani (2019) reported that commercial feeding with the addition of EM4 was able to increase the growth rate of catfish. Probiotics are able to increase growth, non-specific immune responses and are susceptible to disease also fish survival rate because EM4 probiotics contains *Lactobacillus* bacteria, where these bacteria are able to increase protein levels of feed which are good for fish growth (Simamora et al., 2021). The utilization of EM4 has been carried out by Augusta (2017) on sangkuriang catfish, Syahrizal et al. (2018) on catfish and Khotimah et al. (2017) on catfish. Molasses may be added so that probiotic bacteria can maximize their work and increase their population.

Molasses which is a source of nutrition for probiotic bacteria is expected to increase the population of probiotic bacteria so that it can maximize the work of probiotic bacteria as a bioremediation agent (Sartika et al., 2012). Molasses (cane drops) is a by-product of the sugar processing industry which still contains quite high sugar (Aderolu et al., 2013)

Molasses is a source of nutrition for probiotic bacteria and can increase the growth of probiotic bacteria. Provision of probiotic bacteria into culture media so that beneficial bacteria can be used to grow more dominantly (Sartika et al., 2012). Based on the above, this study was conducted to determine the effect of giving EM4 and molasses on the best frequency of probiotic administration for growth, survival rate, feed conversion, and feed efficiency in snakehead (*Channa striata*).

## Materials and Methods

### Location and time of research

The research was conducted from August to September 2021. The research was conducted at the Fish Hatchery Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala.

### Experimental fish and research container

The test fish used in the form of fry snakehead came from the Fish Showroom, Ingin Jaya District, Aceh Besar with a weight ranging from 1-2 gram as many as 180 tails. The selected fish were adapted before being treated for approximately 1 week. During acclimatization the fish were given commercial feed with a protein content of 30%.

The research container consisted of 12 plastic containers with a size of 22 liters at the Fish Hatchery Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala.

### Preparation of EM4 and Molasses mixed into test feed

The probiotics used in this study were trademarked Effective Microorganisms-4 (EM4) produced by PT Songgolangit Persada while molasses was obtained from industrial products that process granulated sugar. Before EM4 and molasses are mixed into the feed, first, molasses and water are mixed in a ratio of 1:2, 500 ml of molasses and 1 liter of water are then added with 1 liter of EM4 then put into a bottle and left overnight. Furthermore, the EM4 culture was mixed into the feed according to the treatment, namely without EM4, 6 ml/kg, 8 ml/kg and 10 ml/kg by spraying evenly and then air drying for 30 minutes and avoiding direct sunlight.

### Experimental design and fish rearing

The research method used was a Complete Randomized Design (CRD) consisting of four treatments with three replications. The manipulated factor was EM4 cultured with addition of molasses. After the culture is then sprayed into commercial feed. The dose given in this study refers to the research that has been done according to Simanjuntak et al. (2020) on catfish, with the best dose being 8 ml/kg of feed. The treatments given to fry snakehead were, A = 0 ml/kg of feed, B = EM4 6 ml/kg of feed + Molasses, C = EM4 8 ml/kg of feed + Molasses and D = EM4 10 ml/kg of feed + molasses.

Before being put into the rearing container, fry snakehead were counted, weighed and recorded as initial research data. During rearing the fish were fed 2 times a day, in the morning and evening as much as 7% of the body weight of the fish seeds with a maintenance period of 40 days.

Collecting growth data by calculating the weight of the fish and the length of the fish. During the sampling period, the number of fish was calculated to calculate survival and overall weight (biomass) that lived and died in each period to calculate feed efficiency.

Data were collected once every 10 days, on days 0, 10, 20, 30 and 40 by weighing the biomass from each research container. Parameters observed were absolute length gain, absolute weight gain, specific growth rate, feed conversion ratio, feed efficiency, and survival. Then the observations of water quality observed in this study including temperature, pH and dissolved oxygen. These parameters were measured every day during the study. If there is a change in water quality, 25% of the total water in each container is changed.

### Test parameters

Growth performance parameters including absolute length gain and absolute weight growth according to [Effendi \(1979\)](#), Specific Growth Rate (SGR) and Food Conversion Ratio (FCR) according to [Jin et al. \(2018\)](#), feed use efficiency (EP) according to [Tacon \(1987\)](#), and survival rate (SR) according to [Wang et al. \(2018\)](#). The equation is as follows:

$$PPM = Lt - L0$$

$$W = Wt - Wo$$

$$SGR = (\ln Wt - \ln Wo) / t \times 100\%$$

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

$$FCR = F / Wg \times 100$$

$$EP = (1 / FCR) \times 100 \%$$

Note: PPM = Absolute length growth (cm); Lt = The average length of the end of the study (cm); Lo : The average length of the start of the study (cm); W = Weight gain (gram); Wt = Weight of biomass at the end of the study (gram); Wo : Weight of biomass at the beginning of the study (gram); t = Time (maintenance period); F = Amount of feed consumed (gram); Nt = Number of live fish at the end of rearing; N0 = Number of fish that live at the beginning of maintenance.

### Data analysis

The research data were analyzed by one-way analysis of variance (ANOVA). Significant results of ANOVA were followed up with Duncan's multiple distance test ( $P < 0.05$ ) ([Stell et al., 1996](#)).

### Results

The results of the ANOVA test showed that the addition of EM4 had a significant effect ( $P < 0.05$ ) on absolute length gain, absolute weight growth, specific growth rate and survival rate, and had no significant effect ( $P > 0.05$ ) on feed conversion ratio and feed efficiency ([Table 1](#)).

Duncan's further results showed that there was a significant difference between treatments on the parameter absolute length gain, absolute weight growth, specific growth rate, and survival rate.

The water quality parameters measured during 40 days of snakehead rearing are described in [Table 2](#). The temperature parameters measured are 30-31°C. The degree of acidity (pH) ranged from 6.1-7.4 and the dissolved oxygen content (DO) ranged from 4-5.5 mg/L. The results of water quality measurements

during the study tend to be good for the growth of snakehead.

**Table 1.** Measurement results of absolute length gain, absolute weight growth, specific growth rate, survival, feed conversion ratio, and feed efficiency.

Dosage Treatment EM4 (ml/kg feed)	absolute length growth (cm)	absolute Weight Growth (gram)	Specific Growth Rate (%/day)	Survival rate (%)	FCR	Feed Efficiency (%)
A (0)	0.90±0.07 <sup>a</sup>	0.92±0.14 <sup>a</sup>	1.31±0.19 <sup>a</sup>	84.44±3.85 <sup>a</sup>	1.89±0.13	3.06±3.5
B (6)	1.35±0.19 <sup>b</sup>	1.24±0.23 <sup>ab</sup>	1.56±0.23 <sup>ab</sup>	88.89±3.85 <sup>ab</sup>	1.75±0.22	7.65±7.0
C (8)	1.51±0.17 <sup>b</sup>	1.54±0.14 <sup>bc</sup>	1.74±0.04 <sup>bc</sup>	95.56±3.85 <sup>bc</sup>	1.62±0.10	1.76±3.7
D (10)	1.60±0.17 <sup>b</sup>	1.85±0.18 <sup>c</sup>	1.97±0.10 <sup>c</sup>	97.78±3.85 <sup>c</sup>	1.56±0.03	4.19±1.4

Note: different superscript letters in the same column show significant differences ( $P < 0.05$ )

**Table 2.** Water quality parameters were measured during the study.

Chemical physics parameters	Measurement data	Literature data	Source literature
Temperature (°C)	30-31	25-32	
pH	6.1-7.4	6.5-8.5	<a href="#">Kodri, 2013</a>
DO (mg/L)	4.0-5.5	3-7	

### Discussion

Absolute length growth, absolute weight growth, daily growth rate and survival rate of snakehead tended to increase with the increasing dose of EM4 ([Table 1](#)). The addition of EM4 and molasses to the feed gave better growth results than the feed that was not given the addition of EM4 and molasses. Duncan's further results showed that there were significant differences in the parameters of absolute length increase, absolute weight growth, specific growth rate, and survival rate.

The highest absolute length growth of snakehead was obtained in treatment D (EM4 10 ml/kg feed + molasses) which was 1.60±0.17 cm and the lowest was in treatment A (0 ml/kg of feed) which was 0.90±0.07cm. Based on the results of further tests, Duncan's showed that treatment D (EM4 10ml/kg of feed + molasses) was significantly different from treatment A (0 ml/kg of feed) but not significantly different from treatment B (EM4 6 ml/kg of feed + molasses) and C (EM4 8 ml/kg of feed + molasses). [Khotimah et al. \(2017\)](#), reported that the addition of EM4 as much as 30 ml/kg of feed was able to produce the highest absolute length and absolute weight in catfish of 11.42 cm and 13.25 gram, respectively. This is also reinforced by [Simanjuntak](#)

*et al. (2020)* stated that in sangkuriang catfish the addition of EM4 as much as 8 ml/kg of feed resulted in the highest absolute weight and absolute length of 7.56 gram and 3.51 cm. *Lisna and Insulistyowati (2015)* reported that fish growth increased due to the effect of adding probiotics in the feed so that probiotic bacteria worked to improve the digestive tract of fish.

The highest absolute weight growth was obtained in treatment D (EM4 10 ml/kg of feed + molasses) which was  $1.85 \pm 0.18$  gram and the lowest was in treatment A (0 ml/kg of feed) which was  $0.92 \pm 0.14$  gram. Duncan's further test results showed that treatment D (EM4 10 ml/kg of feed + molasses) was significantly different from treatment A (0 ml / kg of feed) and B (EM4 6 ml / kg of feed + molasses) but not significantly different from treatment C (EM4 8 ml/kg of feed + molasses). The highest specific growth rate was also obtained in treatment D (EM4 10 ml/kg of feed + molasses) which was  $1.97 \pm 0.10\%$  day and the lowest was in treatment A (0 ml/kg feed) which was  $1.31 \pm 0.19\%$  days. Based on Duncan's further test results showed that treatment D (EM4 10 ml/kg of feed + molasses) was significantly different from treatment A (0 ml/kg feed) and B (EM4 6 ml/kg of feed + molasses) but not significantly different from treatment C (EM4 8 ml/kg of feed + molasses).

The addition of EM4 and molasses into the feed is able to have an effect on the growth and survival rate of snakehead. Allegedly addition of EM4 can increase the presence of the number of bacteria that enter the digestive tract. EM4 contains *Lactobacillus* sp., *Acetobacter* sp., *Streptomyces* sp., and Yeast bacteria. These bacteria will secrete enzymes such as proteases and amylase in the digestive tract (*Setiawati et al., 2013*). Probiotics can increase the nutritional value of feed because it has a mechanism to produce several enzymes. The addition of probiotics to feed can help hydrolyze nutrients into simpler molecules that will facilitate digestion and absorption in the digestive tract of fish (*Permana et al., 2019*). The addition of molasses can also increase bacterial growth, molasses is a source of energy for bacteria because molasses contains amino acids, minerals and sugar content (*Modenti et al., 2021*)

*Simanjuntak et al. (2020)* reported that the addition of EM4 as much as 8 ml/kg of feed resulted in the highest specific growth of 4.64 %/day in sangkuriang catfish. Meanwhile *Rarassaria et al. (2021)* stated that the addition of EM4 as much as 15ml/kg of feed resulted in the highest specific growth of 6.6 %/day in pearl catfish. *Hariani and Purnomo (2017)* reported that the more probiotic

microorganisms, the more digestive enzymes produced, so that the feed degradation process during fermentation can be faster and thus increase the nutrients that are ready to be used by fish for the growth process.

Furthermore, the highest snakehead fish survival was obtained in treatment D (EM4 10 ml/kg of feed + molasses) which was  $97.78 \pm 3.85\%$  and the lowest was in treatment A (0 ml/kg of feed) which was  $84.44 \pm 3.85\%$ . Duncan's further test results showed that treatment D (EM4 10 ml/kg of feed + molasses) was significantly different from treatment A (0 ml/kg of feed) and B (EM4 6 ml / kg feed + molasses), but not significantly different from treatment C (EM4 8 ml/kg feed + molasses). Fish survival is the percentage of the number of fish that live from the number of fish kept in one container, survival is indicated by mortality (*Iskandar and Elrifadah, 2015*). *Harmilia et al. (2019)* reported that the addition of EM4 of 11 ml/100 g of feed resulted in the highest survival of 88 % in pearl catfish. Meanwhile, *Rarassaria (2021)* stated that the addition of EM4 as much as 15ml/kg of feed resulted in the highest survival of 74.6 % in pearl catfish.

The greater the feed conversion value, the more feed is needed to obtain 1 kg of cultured fish meat. Meanwhile, the lower the feed ratio value, the better the quality of the feed provided (*Simanjuntak et al., 2020*). Based on the data obtained during the study, the highest feed conversion value (FCR) was in treatment A (control). Meanwhile, the lowest feed conversion value was in treatment D (EM4 10 ml/kg of feed + molasses). The results of this study are the same as *Aderolu et al. (2013)* which stated that feeding molasses to catfish (*Clarias gariepinus*) had a significant effect ( $P < 0.05$ ) on body weight growth and specific growth rate, but had no significant effect ( $P > 0, 05$ ) on feed conversion ratio and feed efficiency. *Rarassaria et al. (2021)* reported that the addition of EM4 as much as 15 ml/kg of feed resulted in the best feed conversion of 1.28 in pearl catfish. This was also reported by *Simanjuntak et al. (2020)* which stated that the addition of EM4 as much as 8 ml/kg of feed resulted in the highest conversion value of 0.99 in sangkuriang catfish. According to *Effendi (1979)*, factors that affect feed conversion include age of fish, type of fish, size of fish, genetic characteristics, odor and durability of feed in water.

Feed efficiency is closely related to feed conversion. The smaller the feed conversion value, the better the feed efficiency, on the contrary if the feed conversion value is high, the feed efficiency level is not good. Based on the data obtained during the

study, the highest feed efficiency was in treatment D (EM4 10 ml/kg of feed + molasses). Noviana et al. (2014) reported that the addition of 10 g/kg of probiotics resulted in the best feed efficiency of 78.29 % in tilapia. This is presumably because probiotics that enter the intestines of fish can help the digestive system so that food digestibility will increase. Increased digestibility will increase feed efficiency because the nutrients in the feed are absorbed optimally. Meanwhile, Feed efficiency that is less than optimal is caused by the lack of probiotic bacteria that reach the digestive tract, so it does not affect the digestive process and fish growth. This is in line with the statement of Irianto et al. (2006), which states that one of the factors that influence the success of probiotic products in increasing feed efficiency in fish is the presence of probiotic bacteria in the digestive tract of fish.

Water quality is a factor that can affect the environment of the maintenance media and can directly affect the growth and survival of snakehead fish. Based on the results of the study, the temperature measurement during the study was 30-31°C. The degree of acidity (pH) during the study was 6.1-7.4 and the dissolved oxygen content (DO) was 4-5.5 mg/l. The results of water quality measurements during the study tend to be good for the growth of snakehead fish. Good water quality for snakehead fish farming is the temperature ranges from 25-32°C, pH ranges from 6.5-8.5 and dissolved oxygen 3-7 (Kodri, 2013). According to Putra (2015) the addition of probiotic bacteria into the culture media can maintain the quality of the cultured water while increasing the growth of the cultivated biota because water quality is an important factor in supporting the growth of fish.

## Conclusion

Based on the results of the research that has been done, it can be concluded that the addition of EM4 and molasses to the feed has a significant effect on the growth and survival rate of snakehead. The addition of EM4 and molasses with the best dose for the growth and survival rate of snakehead is 10ml/kg of feed.

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