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Effect of additional types of different probiotics on feed on the consumption rate, feed conversion ratio, protein, and fat retention *Cyprinus carpio*

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ABSTRACT

Cyprinus carpio is a freshwater fish in great demand by the public because of several advantages, including its high protein content and affordable price. Carp (*C. carpio*) cultivation requires artificial feed as nutrients to support its growth. One aspect of stunted growth is low feed utilization. This is related to feed protein digestibility that is not optimal. One way to increase the digestibility of feed is by adding probiotics. This study aimed to examine the addition of probiotics to artificial feed on the feed consumption level, the feed conversion ratio value, the retention of protein in meat, and the retention of fat in the fish of *Cyprinus carpio*. This research was conducted in May-June 2022 at the School of Health and Natural Sciences Laboratory. The results found that adding different types of probiotics to the feed can affect the level of consumption, feed conversion ratio, protein retention, and fat retention of Cyprinus carpio fish. This study used an experimental method with a completely randomized design, five treatments, and four replications. Observations on each treatment showed different results. The results showed that probiotics affected ($p<0.05$) TKP, RKP, RP, and RL. The best use of probiotics was found in P4 with probiotic bacteria content, namely *Lactobacillus* sp., *Acetobacter* sp., and yeast. The results were obtained in the P4 treatment with a total value of 105.93 grams of feed consumption, 1.40 feed conversion ratio value, 65.68% meat protein retention value, and 187.59% meat fat retention value.

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1. Introduction

Cyprinus carpio is a freshwater fish that is quite developed in Indonesia. The demand for goldfish products is quite high. Based on data from the Directorate General of Aquaculture (2018), there was an increase in production of 33,954 tons from 2010 to 2017. Carp has an advantage in high productivity compared to other types of freshwater fish because carp (Cyprinus carpio) is abundant. in demand by the public. This is due to carp's advantages, including having a fairly high protein content and an affordable price (Sulasi et al., 2018). Feed availability strongly influences carp cultivation, and feed is one of the determinants of production costs 60-70% of the common problem that often occurs in carp cultivation is slow growth and the use of feed that has not been maximized (Rachmawati et al., 2017). According to research by Ridwantara et al. (2019), obtaining 1 kg of carp meat requires up to 5.5 kg of feed. Utilization of feed that has not been maximized will inhibit carp growth. This is related to the low absorption of feed nutrients due to the digestibility of feed protein absorbed by the fish's body is not optimal (Sulasi et al., 2018).

The factors that can affect the digestibility and absorption of feed nutrients by the fish body are the quality of the feed or nutrients in the feed, digestibility bacteria, and the amount of feed given (Aslamyah et al., 2009), therefore to increase the absorption of feed nutrients by the fish body, and digestibility of feed can be added by adding a feed additive in the form of probiotics. Several studies have stated that probiotics in feed can affect the survival and growth of snakehead fish (*Channa striata*) fry (Jayadi et al., 2021), the efficiency of using tilapia (*Oreochromis niloticus*) feed (Shofura et al., 2017), conversion and growth rates catfish (*Pangasius hypophthalmus*) seeds (Jusadi et al., 2014).

Freshwater fish seeds generally have a simple digestive system and still require an external supply of enzymes (Rimalia, 2016). This is what causes the importance of using feed, so research needs to be done to improve the nutritional value of feed and the level of feed digestibility by adding probiotics. Many probiotic products are on the market with various compositions of their constituent bacteria. Some examples of bacteria found in commercial probiotics include; the bacteria *Lactobacillus bulgaricus*, *Sacchoramyces* sp., *Lactobacillus cassei*, *Lactobacillus bulgaricus*, *Bacillus subtilis*, *Bacillus megaterium*, *Lactobacillus* sp., *Acetobacter* sp., it is necessary to add different types of probiotics in the feed to the level of consumption, feed conversion ratio, protein retention, and fat retention of Cyprinus carpio meat.

2. Material and methods

This research was carried out for 40 days, starting in May-June 2022, at the Wet Laboratory of the School of Health and Natural Sciences, Airlangga University in Banyuwangi.
The following are some of the tools and materials used during the research;

2.1 Material

There was Potassium permanganate for aquarium sterilization and PBS (Phosphate Buffer Saline) for probiotic retailing. The carp used in this study was 8-10 cm in size and weighed 9-11 grams. The feed used in this study is a commercial feed made in a factory with the HI PROVIT-1 brand with about 30% protein. The probiotics used in this study were four types of probiotics, namely probiotic A (EM-4) containing *Lactobacillus bulgaricus., sacchoramyces* sp., and *yeast*; probiotic B (Boster-Biolacto) contains the bacteria *Lactobacillus cassei, Lactobacillus bulgaricus*.; probiotic C (Minapro) contains *Bacillus subtilis, Bacillus megaterium*, probiotic D (King catfish) contains *Lactobacillus* sp., *Acetobacter* sp., and *yeast.* This study used a container in the form of an aquarium with a size of 40 x 30 x 30 cm, with as many as 20 pieces. The media used are fresh water and supporting tools for aerator stones, siphon hoses, aeration stones, spray bottles, analytical scales (ABS 220-4), trays, basins, stationery, rulers, cleaning sponges, sectio tools, erlenmeyer, glass funnels, volume pipette, thermometer, pH test, ammonia test kit and DO test kit (Hebei Norvka).

2.2 Method

Completely Randomized Design (CRD) using five treatments and four replications. The treatment given in this study was as follows:
P0: feed without the addition of probiotics (control); P1: feed with probiotic A 15 ml/kg feed; P2: feed with probiotic B 15 ml/kg feed; P3: feed with probiotic C 15 ml/kg feed; P4: feed with probiotic D 15 ml/kg feed.

2.2.1 Research Container Preparation

Aquarium sterilization was done using soap, then soaked in potassium permanganate ($KMnO_4$) at a dose of 2.5 ppm for 24 hours and aerated. This aims to remove the remaining $KMnO_4$ that is still in the aquarium. After sterilization, the aquarium was filled with a height of 20 cm.

2.2.2 Test Animal Preparation

Goldfish measuring 8-10 cm with a weight of 9-11 grams, as many as 120 tails were acclimatized in the tub for 15 minutes. Then the fish were reared and adapted for seven days by feeding three times a day at 08.00, 12.00, and 16.00 WIB with a protein content of 30%. Before treatment, fish have fasted for 24 hours.

2.2.3 Test feed preparation

The feed used in this study was commercial feed with a protein content of 30% which would be mixed with 15ml/kg Probiotics by spraying it onto the feed. Before being sprayed onto the feed, the bacterial content in the probiotics was diluted using PBS (Phosphate Buffer Saline). PBS is a physiological solution that can be used for sample dilution. This solution is isotonic and non-toxic to cells and aims to maintain pH levels and cell osmolarity (Sari, 2015). Then after the feed was sprayed with probiotics, the feed was dried for 30 minutes so that the probiotics were absorbed in the feed, spraying the probiotics every 3 days.

2.2.4 Pisciculture

Fish rearing was carried out for 40 days in the aquarium. Each aquarium was filled with ten fish with a stocking density of 1 fish/liter and an 8-10 cm seed size. Feed for carp was given ad satiation thrice daily at 08.00, 12.00, and 16.00 WIB. To maintain the quality of the water, it was carried out once every three days, and the addition of water. Siphoning was done by changing the water as much as 25% of the water in each aquarium every 2-3 days.

2.2.5 Fish Sampling

Fish sampling was carried out every ten days by weighing the weight of the fish to determine the amount of feed to be given. After 40 days of rearing, all treatments were harvested, and surgery was performed to separate the fish flesh from other organs. The separated Fish meat was used as a proximate test sample for the protein content of 10 grams of fish meat and a proximate test for the fat content of 25 grams of fish meat (Hafiluddin *et al.*, 2014).

2.3 Analysis Data

Total feed consumption, feed conversion ratio, protein retention, and fat retention of carp meat obtained from this study were analyzed using Analysis of variance (ANOVA) statistically using the SPSS 20 IMB software at a 95% confidence interval (α = 0.05) to determine whether or not the treatment carried out on the results of the study. The results that had a significant effect were further tested by Duncan's multiple range test (DMRT) at a 95% confidence interval. Water quality data (temperature, DO, pH, nitrate, nitrite, and ammonia) were analyzed descriptively.

3. Results and Discussion

3.1 Consumption Rate

The total consumption of carp (*Cypinus carpio*) feed that has been fed with the addition of different types of probiotics can be seen in Table 1.

Table 1. Average Calculation of Total Feed Consumption of Carp (*Cypinus carpio)*

Different superscripts showed significant differences ($p<0.05$).

Treatment	Consumption Rate (gram) ± SD
P0 (Control)	$92.66^{d} \pm 4.42$
P1 (15mL)	$110.10^{bc} \pm 1.03$
P2 (15mL)	$115.28^{b} \pm 3.64$
P3 (15mL)	$123.24^{a} \pm 6.17$
P4 (15mL)	$105.93^{c} \pm 2.04$

Description :P0: Feed without the addition of probiotics (Control); P1: Feed with the addition of probiotic A; P2: Feed with the addition of probiotic B; P3: Feed with the addition of probiotic C; P4: Feed with the addition of probiotic D.

The results of the Analysis of variance (ANOVA) showed a significant difference ($p<0.05$) in the total consumption of carp feed. Duncan's further test results showed that P3 (Feed with probiotic C) was significantly different from P4 (Feed with probiotic D), P2 (Feed with probiotic B), P1 (Feed with probiotic A), and P0 (control), but P1 (Feed with probiotics B). with probiotic A) was not significantly different from P2 (Feed with probiotic B), and P4 (Feed with probiotic D). The highest total feed consumption value was P3 (Feed with probiotic C), while the lowest was P0 (control).

Based on the study's results, it was shown that the addition of different probiotics to the feed had a significant difference ($p<0.05$) in the level of consumption of carp feed. Based on the results of ANOVA calculations showed that the addition of probiotics containing different bacteria in each treatment had a significant effect ($p<0.05$) on the level of feed consumption (TKP) of carp. The highest total feed consumption value was at P3 of 123.24 grams, and the lowest amount of feed was at P0 of 92.66 grams, but P3 feed did not show a good level of feed use because P3 showed high FCR results, and the retention value of meat and protein. Meat fat is low, and it can be said that the increased total feed consumption in P3 cannot be used efficiently by fish. This is supported by Sunarto and Sabariah (2012), who state that the low value of feed consumption indicates a higher efficiency level in utilizing food for growth.

The high value of feed consumption indicates that the efficiency level is lower in utilizing food for growth. The best feed use was P4, 105.93 g because it can reduce the FCR with the lowest value and increase the high protein and fat retention value. This was presumably because the P4 fodder contains *Lactobacillus* sp., *Acetobacter* sp., and yeast. Probiotics contain bacteria from the genus. Lactobacillus is a lactic acid bacteria because it can convert carbohydrates into lactic acid. Lactic acid can create an acidic atmosphere in the digestive tract, and acidic conditions can increase the secretion of enzymes in digestion, namely protease, lipase, and amylase enzymes. These enzymes include digestive tract enzymes that help hydrolyze feed nutrients (Putra, 2010).

3.2 Feed Conversion Ratio

The feed conversion ratio of carp (*Cypinus carpio*) that has been fed with the addition of different types of probiotics can be seen in Table 2

Table 2. Average Calculation of Feed Conversion Ratio for Carp *(Cypinus carpio)*
Different superscripts showed significant differences (p<0.05).

Treatment	Feed Conversion Ratio (gram) ± SD
P0 (Control)	$2.24^{a} \pm 0.26$
P1 (15mL)	$1.44^{c} \pm 0.16$
P2 (15mL)	$1.55^{bc} \pm 0.17$
P3 (15mL)	$1.81^{b} \pm 0.26$
P4 (15mL)	$1.40^{c} \pm 0.10$

Description :P0: Feed without the addition of probiotics (Control); P1: Feed with the addition of probiotic A; P2: Feed with the addition of probiotic B; P3: Feed with the addition of probiotic C; P4: Feed with the addition of probiotic D.

The results of the Analysis of variance (ANOVA) showed that there was a significant difference (p<0.05) in the feed conversion ratio of carp. Duncan's further test results showed that P4 (Feed with probiotic D) was significantly different from P3 (Feed with probiotic C) and P0 (Control), but P4 (Feed with probiotic D) was not significantly different from P2 (Feed with probiotic B), and P1 (Feed with probiotic A), besides that P2 (Feed with probiotic B), was also not significantly different from P1 (Feed with probiotic A) The highest feed conversion ratio values were at P0 (Control) and). The lowest feed conversion ratio value was found in P4 (Feed with probiotic D).

The feed conversion ratio (RKP) value in the treatment with different types of probiotics decreased higher than in the treatment without the addition of probiotics. This shows that adding different probiotics to the feed can reduce the value of the conversion ratio of carp feed than the treatment without adding different probiotics or controls. The average value of the highest feed conversion ratio (FCR) was P0 (Feed without the addition of probiotics) with a feed conversion ratio value of 2.24, and the lowest value was found in P1 and P4 with a feed conversion ratio value of 1.4, the high feed conversion ratio at P0 indicates fish are not appropriately using the amount of feed given during the maintenance period, this is following research according to Ardita et al., (2015) the high FCR indicates that the feed given cannot be absorbed and digested correctly by fish.

The low FCR at P1 and P4 was thought to be because the treatment contained bacteria that can help digest feed nutrients. The bacteria Saccharomyces sp. are bacteria that can produce amylase enzymes (Sumardiyani et al., 2020). This amylase enzyme can break down carbohydrates into glucose (Sumardiyani et al., 2020). So that it can help absorb feed nutrients so that feeding can be used efficiently.

3.3 Protein Retention

Protein retention of carp (*Cypinus carpio*) meat that has been fed with the addition of different types of probiotics can be seen in Table 3. The value of protein retention in the treatment with different probiotics in the feed increased higher than in the treatment without the addition of probiotics. The highest average value of protein retention in carp meat was at P4, 65.68%, and the lowest value at P0 was 35.40%. This is presumably because probiotic P1 contains *Lactobacillus bulgaricus*, sacchoramyces sp, and yeast, while probiotic P4 includes *Lactobacillus* sp., *Acetobacter* sp., and *yeast,* then the results were not significantly different between P4 and P1. This is also suspected because the two treatments had a bacterial composition of more than one different type of bacteria, one of which was from the genus Lactobacillus which can create an acidic atmosphere in the digestive tract of fish which can cause digestive problems, making the secretion of digestive enzymes faster increasing the level of digestibility of feed (Sakinah, 2015).

One of the digestive enzymes, namely protease enzymes, can hydrolyze feed proteins into simpler compounds, namely amino acids that fish can absorb (Tondais et al., 2020). Acetobacter sp. are acetic acid bacteria that can inhibit the growth of pathogens because acetic acid is an antimicrobial compound, which can inhibit the growth of pathogenic bacteria so that the performance of good bacteria in the digestive tract can work optimally (Nurainy and Rizal, 2018). The bacteria

Saccharomyces sp. are bacteria that can produce amylase enzymes (Sumardiyani et al., 2020). This amylase enzyme can break down carbohydrates into glucose (Sumardiyani et al., 2020). In addition, probiotics P1 and P4 also contain yeast. The presence of yeast in probiotics can optimize the performance of probiotic bacteria and accelerate fish growth and help absorb feed nutrients. This is because yeast functions to control and kill various kinds of microflora in the digestive tract that can interfere with the digestive process of fish. (Wulandari, 2008).

Table 3. Average Calculation of Protein Retention of Carp Meat (*Cypinus carpio*)

Different superscripts showed significant differences ($p<0.05$).

Treatment	Protein Retention (%) ± SD
P0 (Control)	35.40^{c} ± 5.24
P1 (15mL)	59.90^{a} ± 5.28
P2 (15mL)	44.59^{b} ± 4.03
P3 (15mL)	41.03^{bc} ± 4.70
P4 (15mL)	65.68^{a} ± 3.03

Description :P0: Feed without the addition of probiotics (Control); P1: Feed with the addition of probiotic A; P2: Feed with the addition of probiotic B; P3: Feed with the addition of probiotic C; P4: Feed with the addition of probiotic D.

One of the digestive enzymes, namely protease enzymes, can hydrolyze feed proteins into simpler compounds, namely amino acids that fish can absorb (Tondais et al., 2020). Acetobacter sp. are acetic acid bacteria that can inhibit the growth of pathogens because acetic acid is an antimicrobial compound, which can inhibit the growth of pathogenic bacteria so that the performance of good bacteria in the digestive tract can work optimally (Nurainy and Rizal, 2018). The bacteria Saccharomyces sp. are bacteria that can produce amylase enzymes (Sumardiyani et al., 2020). This amylase enzyme can break down carbohydrates into glucose (Sumardiyani et al., 2020). In addition, probiotics P1 and P4 also contain yeast. The presence of yeast in probiotics can optimize the performance of probiotic bacteria and accelerate fish growth and help absorb feed nutrients. This is because yeast functions to control and kill various kinds of microflora in the digestive tract that can interfere with the digestive process of fish. (Wulandari, 2008).

In addition, the crude fiber content of the feed also affects the protein retention value of fish meat. The crude fiber content of the feed in this study was 5.88%-6.89%. While the crude fiber content of good feed in carp feed ranges from 4-5% Silvianti et al., (2016). According to research conducted by Soedibya (2013), high crude fiber content will accelerate the rate of food travel in the digestive tract, decreasing the opportunity to digest feed mutient substances, thus causing the level of feed digestibility.

3.4 Fat Retention

Fat retention of carp (*Cypinus carpio*) meat that has been fed with the addition of different types of probiotics can be seen in Table 3.

Table 3. Average Calculation of Fat Retention of Carp Meat (*Cypinus carpio*)

Different superscripts showed significant differences ($p<0.05$).

Treatment	Fat Retention (%) ± SD
P0 (Control)	136,07c ± 8.58
P1 (15mL)	179,76a ± 13.13
P2 (15mL)	159,75b ± 12.27
P3 (15mL)	157,36b ± 18.02
P4 (15mL)	$187{,}59^{a}$ ± 6.99

Description :P0: Feed without the addition of probiotics (Control); P1: Feed with the addition of probiotic A; P2: Feed with the addition of probiotic B; P3: Feed with the addition of probiotic C; P4: Feed with the addition of probiotic D.

The value of fat retention in the treatment with the addition of different probiotics experienced a higher increase than in the treatment without added probiotics. The highest average value of carp meat fat retention was at P1 and P4, 179.76% and 187.59%, and the lowest was at P0, 136.07%. The high fat influences the feed's fat retention value (Mokoginta et al., 2004). Fat was used as energy in fish rather than the amount of fat that can be absorbed in the fish's body. This follows the research of Dewi and Tahapri (2017), which says that fat is also used as a sparing effect, which is used as a substitute for the function of the protein as an energy source, thereby optimizing the role of protein in the body for fish growth. The high-fat retention in P1 and P4 was thought to be because the P1 feed contains *Lactobacillus bulgaricus, saccharomyces* sp., and *yeast*, while the P4 feed contains Lactobacillus sp., *Acetobacter* sp., and yeast.

The content of different bacteria in each probiotic results in other meat fat retention values. This follows the statement of Saputra and Ibrahim (2021) that different bacterial compositions can work synergistically in the digestive tract to increase the digestibility of feed nutrients. The high-fat retention value of carp meat was thought to be due to an excess of nutrients in the form of carbohydrates. The proximate feed analysis, namely BETN (Syaputra et al., 2018), can show carbohydrate levels in feed. The content of BETN in feed in the study of Silvianti et al. (2016) was lower at around 39.92%-44.17% than the content of BETN in this study, which was 46.58%-49.75%. According to Sanjayasari (2010), the carbohydrate content in good feed for carp is around 38-40%. Carbohydrate content in BETN in this study can be seen in the table of feed proximate test results. The value of fat retention was influenced by fat in feed and carbohydrate content in feed as an energy source. According to Mokoginta et al. (2004), excess carbohydrates will increase the value of fat retention due to excess carbohydrates. Carbohydrates were converted to body fat. In addition, probiotics P1 and P4 also contain yeast. The presence of yeast in probiotics can optimize the performance of probiotic bacteria and accelerate fish growth and help absorb feed nutrients. This is because yeast functions to control and kill various kinds of microflora in the digestive tract that can interfere with the digestive process of fish. (Wulandari, 2008). This shows that adding probiotics with different contents can increase lipase enzyme activity in the digestive tract so that fish can absorb fat in feed optimally into the body.

3.5 Water Quality

The results of the calculation of the average water quality for 40 days of maintenance can be seen in Table 5.

Table 5. Calculation of the average value of water quality.

Parameter	**Result**					**SNI**
	P0	**P1**	**P2**	**P3**	**P4**	
Temperature (°C)	25	27	26	26	27	< 28
pH	6	7	6	6	7	6,5-8,5
DO (mg/L)	6	7	6	7	7	7,1
Ammonia (mg/L)	0,2	0,2	0	0,2	0,2	0-0,2
Nitrate (mg/L)	0,2	0,2	0	0,2	0,2	0-0,2
Nitrite (mg/L)	40	37	38	37	37	<40

The water quality parameters observed in this study were temperature, pH, dissolved oxygen (DO), ammonia (NH_4), nitrate (NO_2), and Nitrite (NO_3). The results of temperature measurements during maintenance in this study ranged from 26-27°C. The temperature is still within the tolerance limit in carp culture. According to Gusman and Muhammad (2014), the appropriate water temperature range for goldfish is 24-28°C. Temperature affects fish's metabolic processes, such as growth, food intake, and fish body activities. The optimal temperature will give the fish an optimal metabolism which has a good impact on the development and weight gain of the fish. Low temperatures will cause the metabolic rate of fish to be slow and cause the fish's appetite to decrease, and eventually, the fish will experience slow growth (Ridwantara et al., 2019). The result of measuring pH during maintenance in the study was 7. The pH was still within the tolerance limit in carp culture, according to Tahir et al. (2021). The optimal pH range for goldfish survival was 6.5-8.5. A high pH value (>9) will result in stunted fish growth, while a low pH (< 4.5 ± 6.5) causes water quality to be toxic to fish, small growth, and fish will become sensitive to bacteria and fungi. parasites (Sabrina et al, 2018).

The result of DO measurement during maintenance in the study was 6-7 mg/L. The DO is still within the tolerance limit in carp cultivation. According to Sulawesty et al. (2014), oxygen levels are still within normal levels for the maintenance of carp is 2.5-7.1 mg/L. suppose there is a decrease in dissolved oxygen content. In that case, the presence of oxygen is often unable to meet the oxygen needs of aquatic organisms to carry out metabolic and respiratory processes. This disrupts fish growth and survival (Sabrina et al., 2018). The result of the measurement of ammonia during maintenance in the study was 0.2 mg/L. According to Minggawati (2012), free ammonia levels exceeding 0.2 mg/L are toxic to several types of fish. The results of measurements of nitrite and nitrite, respectively, during maintenance in the study, were 0-0.2 mg/L. The result of nitrate measurement during maintenance in the study was 37-40 mg/L. Usually, the dissolved nitrite content in water is 0.2 mg/L, while the excellent nitrate content is 40 mg/L (Sihite et al., 2020). Excess nitrate and nitrite content will be toxic to fish and can cause fish death (Rejito, 2020).

4. Conclusion

Based on the results of the research that has been done, it can be concluded that the addition of different types of probiotics in the feed affects the growth rate of carp with the best feed consumption level of 105.93 g by reducing the feed conversion ratio to 1.40 and increasing meat protein retention by 65.68%, and increased fat retention of carp meat by 187.59%.

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