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Application of Cellulase Enzyme in Feed on the Growth of Siamese Catfish (*Pangasius hypophthalamus*)

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Abstract: Siamese catfish (*Pangasius hypophthalamus*) is one of the most economically importantfreshwater commodities in Indonesian aquaculture. A major challenge during the growth-out phase, is the limited ability to digest plant-based diets due to high cellulose and crude fiber content, which reduce feed digestibility. Supplementation of cellulase in feed has been proposed as a strategy to improve nutrient utilization. Therefore the present study investigated the effect of dietary celulase supplementation on feed digestibility and growth performance in Seamese catfish. Juvenile catfish with an average initial length of 6cm were reared for 30 days in floating net in a cement pond. A complete reandomized design was employed with four treatments, two control, and four replciates. Treatments included cellulase derived from mivrobes sources T1 = Plant feed + WT, T2 = Plant feed + (WT + SKIK), T3 = Plant feed + Mutant, T4 = Plant feed + (mutant + SKIK). The control consisted of C1 = Commercial feed and C2 = Plant-based feed without enzyme supplementation. The results showed that C1 showed the best results for the growth of fish. However, T2, T3 and T4 showed the best results when compared to C2 and T1 which used 100% vegetable protein feed. Therefore, it can be concluded that the addition of cellulase enzymes to the feed has an effect on increasing the growth of Siamese catfish.

Keywords: Cellulase enzyme; Feed; *Pangasius hypophthalamus*.; Growth

Introduction

Aquaculture plays a crucial role in ensuring food security and providing nutritious food (Hu et al., 2024; Jaiswal et al., 2023). Indonesia, with a water area almost three times larger than its land area, has significant potential in the fisheries sector, both capture fisheries and aquaculture. Based on data from the Ministry of Maritime Affairs and Fisheries (Antaranews.com, 2021), the potential for sustainable production (Maximum Sustainable Yield/MSY) Indonesia's fisheries production is 67 million tons per year. Of this figure, the potential catch from the sea and inland waters is 10.2 million tons per year, while the remaining 56.8 million tons per year is from aquaculture. This certainly provides potential economic opportunities for the community through the utilization of existing natural resources, one of which is the fisheries sector.

Global catfish production has significant potential for both domestic and export markets. Vietnam dominated the global supply of patin fish and saw significant growth of 47.74% over the past two years, reaching a value of 2.26 billion USD. Meanwhile, Indonesia has the potential to increase its production and exports. The nutritional content of patin fish per 100 grams contains 132 calories of energy, 17 g of protein, 6.6 g of fat, 1.1 g of carbohydrates, 74.4 g of water, and 1.6 g of iron (Efendy, 2019). The head contains 2.28% of the fatty acid β-3, while the meat contains 2.28% of the vitamin C .belly flap (stomach meat) 2.11%, and stomach contents 1.45% (Hastarini et al., 2012). According to Ayu et al. (2019), the belly fat of catfish contains --3 of 1.89% and --6 of 21.84%. The composition of unsaturated fatty acids in belly fat of catfish is dominated by oleic acid of 40.14% while saturated fatty acids are in the form of palmitic acid of 26.22%.

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One of the important elements in cultivation activities that supports growth and the survival of fish is feed. Quality feed and balanced nutrition are essential for fish for optimal growth in quality and quantity in a short time (Djonu et al., 2020). Through the metabolic process, feed is absorbed by the body and becomes energy for fish in reproduction, growth, and other activities (Septiana et al., 2017). Fish feed for cultivation activities, especially for optimal growth, requires a protein content of around 30% and other nutrients such as fat, carbohydrates, vitamins, and minerals (Cahyoko et al., 2011). Craig & Helfrich (2002) said that the protein content for cultivationcatfishgenerally 28-32%. Feed is a source of material and energy to support the survival and growth of fish.

One of the problems faced in aquaculture activities is the low digestibility of feed, especially feed formulations using vegetable protein with a high crude fiber content. This is because fish have limited ability to digest crude fiber, so the maximum crude fiber content in feed is recommended to be only 8% (Djajasewaka, 1985). According to Nainggolan & Adimunca (2005), crude fiber is a plant fiber that is insoluble in water and consists of three types: cellulose, hemicellulose, and lignin. Cellulose is the most abundant biomolecule found in nature and is the main component of plants (Koolman, 2001). Cellulose is not easily degraded either chemically or mechanically, which impacts growth performance, reduces digestibility, nutrient utilization, and triggers intestinal dysfunction in fish, especially carnivorous fish (Hussain et al., 2024; Xue et al., 2023). Most fish can tolerate crude fiber content up to 8% for growth, but crude fiber content higher than 8% can suppress growth (NRC, 1993).

One method for degrading cellulose in feed is the addition of enzymes to the feed. Enzymes function as catalysts, accelerating the reaction rate without being involved in the reaction (Robinson, 2015). There are many different types of enzymes, one of which is cellulase. Cellulase is one type that plays a crucial role in the bioconversion process of organic materials (Deka et al., 2013). Cellulase is an extracellular enzyme consisting of the endo-β-1,4-gluconase complex (CMCase, Cx cellulase, endocellulase or carboxymethyl cellulase), exo-β-1,4-gluconase complex (avicellase, cellobiohydrolase, C1 cellulase), and β-1,4-glucosidase or cellobiase (Crueger & ACrueger, 1984).

Enzymes from microorganisms are more widely used than those from plants or animals because microorganisms can reproduce quickly, their growth is relatively easy to control, they are more stable, and they produce high levels of enzymes, making them economical for industrial use (Yusak, 2004). Cellulase can be produced by cellulase-producing microbes from the bacteria, actinomycetes, and fungi groups. Some bacteria known to produce cellulase include: *Clostridium thermocellum* (Howard et al., 2003); *Salmonella typhimurium* (Yoo et al., 2004) and *Cytophaga hutchinsonii* (Louime et al., 2006). Cellulase produced by bacteria is the main choice, because bacteria have fast growth and are therefore more efficient in terms of time (Sangrila Sadhu & Maiti, 2013).

Cellulase enzyme production can be increased by strain improvement through genetic modification of microbial strains through advanced techniques (Kumari & Kayastha, 2011). In addition, molecular cloning of cellulase genes or increasing the copy number also has a major impact on the degradation of cellulosic materials and the production of high-quality value-added products (Bhardwaj et al., 2017). Strain wild type (Wild Type) or normal may have some special characteristics and find potential applications in industry. To further exploit the potential of wild-type microorganisms, strain improvements are needed that can modify or eliminate certain properties (Derkx et al., 2020). Commercial production can be increased with Recombinant DNA technology (Zhou et al., 2018), Site-Directed Mutagenesis (Koeller & Wong, 2001), Recombinant Protoplast Fusion (Moreno-Bondi et al., 2018), and Random Mutagenesis (Xu et al., 2017).

Supplementation of cellulase enzymes obtained from genetic modification through Microbial strains are expected to increase cellulase production and significantly impact the degradation of complex molecules like cellulose into simpler carbohydrates like glucose, before being fed to fish. Therefore, the addition of cellulase enzymes to feed is expected to increase feed digestibility, thereby increasing the absorption of nutrients, which supports fish growth.

Method

Research Materials

The materials used in this study included distilled water, spirtus, 70% alcohol, LB (Luria Bertani), SB (Super Broth), PBS (Phosphate Buffer Saline), ice, plastic wrap, cotton swab, *E. coli* BL21 (DE3), Siamese catfish fry. The main ingredients for feed formulations consisted of soybean flour, duckweed flour, corn flour, bran, starch, yeast, promix, probiotic, molasses, fish oil, commercial pellets, bamboo.

The recombinant bacterium *E. coli* BL21 (DE3) carried the plasmid pET22b which had been genetically engineered in previous research. The strains consisted of *wild type* and genetically modified strains optimized to improve protein production as well as cellulase activity. The recombinant plasmid pET-22b-Cell E carried the cellulase gene (celE) from *Ruminococcus flavefaciens,* which had undergone codon optimization for expression in *E. coli* and was then inserted into the pET-

22b vector. In this study, only culturing and enzyme extraction were required. The production of cellulase enzyme was induced using lactose monohydrate, which functioned as the inducer to stimulate microorganisms to produce higher levels of cellulase enzyme.

Experimental Design

This study used a single-factor Completely Randomized Design. The treatment observed in this study was the application of cellulase enzymes to feed. The treatment plan included 4 treatments and 2 controls, each with 4 replications. The treatments applied in this study were C1 (Commercial feed), C2 (100% vegetable protein formulation feed (corn, soybeans, bran, duckwwe), T1 (Enzyme (WT) + 100% vegetable protein formulation feed), T2 (Enzyme (WT + SKIK) + 100% vegetable protein formulation feed), T3 (Enzyme (Mutan) + 100% vegetable protein formulation feed), and T4 (Enzyme (Mutan + SKIK) + 100% vegetable protein formulation feed).

Cellulase Enzyme Production

Pre-Culture

Pre-culture aims to grow bacteria *E. coli* recombinant in small amounts to ensure that the bacteria are active and ready for use in primary culture (main culture), the steps are to select a single colony from the agar plate (which has previously been prepared with *E. coli* BL21 (DE3)) containing the plasmid pET22b. Next, it was inoculated into 4 mL of LB medium containing selective antibiotics (1µL/ml). Then, it was incubated at 37^{o}C with shaking speed (Incubator shaker) 150-200 rpm for 16-18 hours. The goal is to produce a sufficient bacterial culture in stationary phase for use in the next step.

Glycerol Stock Preparation

Glycerol stock preparation aims to make glycerol stock from recombinant bacteria so that it can be stored for long-term use. Inoculation for glycerol stock: At the same time as pre-culture, inoculate the bacteria into LB liquid media (with antibiotics) in another tube. Next, incubate overnight at 37^{o}C with shaking (incubator shaker). Preparation of glycerol stock: After incubation, mix 500 µL of bacterial culture with 500 µL of sterile glycerol (50% v/v) in a cryogenic tube, then store in a freezer at -80^{o}C for long-term use.

Cellulase Expression

1 mL of preculture (initial culture result) was inoculated into 100 mL of Super Broth media as the main culture medium (containing 4 mL/L glycerol, 0.05% glucose, 0.2% lactose monohydrate, and 100µg/mL ampicillin) in an Erlenmeyer flask. The culture was then incubated at 37^{o}C with constant shaking (incubator shaker) at a speed of 150-200 rpm to ensure optimal bacterial growth for 3-4 hours, then the temperature was lowered to 20°C and incubation was continued for 72 hours to allow autoinduction by lactose.

Harvesting

The completed induced culture was centrifuged at 9000 rpm for 30 minutes at 4°C. After the centrifugation process, the supernatant (culture medium) was discarded and the bacterial cell pellet was used for the extraction process.

Cellulase Extraction

Aims to release proteins expressed in cells. The steps are as follows: suspend cells in PBS: take a centrifuged cell pellet from a 1 mL culture. Resuspend the cell pellet in 100 µL PBS to obtain a solid cell suspension. Then, sonication is carried out to break down bacterial cells using ultrasonic sonication, so that proteins localized in the cytoplasm can be released. The steps are to place the cell suspension on ice (to prevent excessive heat). Sonicate for 10 minutes with a cycle of 1 sonication / 30 seconds followed by a pause to cool.

Next, confirm the effectiveness of the sonication, aiming to ensure the sonication was successful by checking the turbidity (cloudiness) of the cell solution. The steps are to take a small amount of suspension (a few microliters) and dilute it 10-fold with PBS. If the sonication is successful, the solution will appear less cloudy (the cells have ruptured).

After that, Post-Sonication centrifugation aims to separate the dissolved protein (soluble fraction) from the insoluble cell components (insoluble fraction). The stages are centrifugation of the sonicated solution at 4^{o}C, 15000 rpm, for 10 minutes. Supernatant fraction: contains soluble protein. Pellet fraction: contains insoluble protein. Finally, Separation and Storage of Fractions, Soluble fraction: Take the supernatant and store it at -20^{o}C. This is the protein dissolved in the cytoplasm. Insoluble fraction: Resuspend the pellet in 100 µL PBS to facilitate further analysis.

Feed Preparation

The feed used in this study was made independently using 100% plant-based protein as the main raw materials, such as soybean meal, duckweed meal, corn meal, and bran. The feed formulation used the Pearson's Square method, which calculates the composition of the raw materials based on the basal protein content and supplemental protein content. The results of the protein and fiber content tests for the plant-based feed ingredients are shown in Table 1, the results of the proximate test for the plant-based and commercial feeds can be seen in Table 2.

Table 1. Protein and crude fiber content of plant-based feed ingredients

Main Ingredient	Crude Fiber (%)	Crude Protein (%)
Bran	29.7802	6.9252
Corn	8.2351	8.4199
Soya bean	16.3751	31.9417
Duckweed	19.4183	22.8782

Table 2. Proximate test results of commercial feed and plant-based feed

Feed	Water (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude Protein (%)	Carbohydrate (%)
Commercial Feed	9. 8406	8.0396	4.0425	3.2135	28.201	46.8728
Plant Feed	10.7396	3.5592	10.3339	9.6148	29.5112	26.8561

In addition to the basic raw materials and supplements, additional ingredients such as starch as an adhesive, fish oil, and a vitamin mix were also prepared. A total of 5 kg of plant-based feed was prepared. The stages of fish feed production begin with raw material selection, flouring, sieving, weighing, mixing, molding, and drying (Maftuch et al., 2020). In addition to feed containing 100% plant-based protein sources, a commercial feed was also prepared as Control 1. While the composition of the ingredients for making the plant-based feed is presented in Table 3.

Table 3. Composition of ingredients for making plant-based feed

Composition	Σ (%)	Σ (gr/ 5 kg)
Soybean Flour	44.787	2.060
Duckweed Flour	44.787	2.060
Corn Flour	5.212	239
Brain Flour	5.212	239
Tapioca Flour	7	350
Vitamin Mix	0.3	15
Fish oil	0.7	35
Total	100%	5

Addition of Enzymes to Feed

The pelleted feed was then weighed as much as 1 kg each. The enzyme was prepared with a dose of 0.5 ml using a 3 ml syringe. The enzyme was placed in a spray bottle and then mixed with 5% distilled water from the feed until evenly distributed. The feed was placed in a container and then the enzyme that had been mixed with distilled water was sprayed onto the feed until evenly distributed.

Preparation of Test Animals and Maintenance Media

The fish used in this study were Siamese catfish with an average initial length of 6.13±0.12 cm and an average initial weight of 1.96±0.03 gr. Catfish were obtained from the Lingsar Fish Seed Center. The fish were first acclimatized to the maintenance media by not being fed for 1 day. The maintenance media was in the form of fixed nets measuring 60 × 60 × 60 cm each as many as 24 fixed nets. The density of catfish was 30 fish.

Research Parameters

Absolute Weight Gain

The difference between fish weight data at the beginning of the maintenance period and weight data at the end of the maintenance period is known as absolute weight gain which can be calculated using the formula:

$$W = Wt - Wo \quad (1)$$

Description:

W : Absolute weight growth of fish (gr)
Wt : Average final weight of fish (gr)
W0 : Average initial weight of fish (gr)

Specific Growth Rate (SGR)

SGR is a specific growth rate measured in units (%/day). SGR is a calculation to determine the daily weight growth rate. Calculating SGR can be done using the formula:

$$SGR = \frac{(\ln Wt - \ln W0)}{t} X\ 100\ \% \quad (2)$$

Description:

SGR : Specific Growth Rate (%/day)
Wo : Initial average weight (gr)
Wt : Final average weight (gr)
T : Maintenance period (days)

Survival Rate (SR)

The survival rate was observed at the end of the study to determine the percentage of larvae that were still alive. The calculation of the Survival Rate was carried out using the formula:

$$SR = \left(\frac{Nt}{No}\right) x\ 100\% \quad (3)$$

Description:

SR : Survival Rate (%)
Nt : Number of fish alive at the end (tail)
No : Number of fish alive at the beginning (tail)

Water Quality

The water quality parameters measured in this study were temperature, pH, DO, and amonia.

Data Analysis

SPSS Statistics was used for homogeneity and normality tests, the aim was to ensure that the data was normally distributed and to obtain the assumption that the data came from the same conditions. One-Way ANOVA test can be performed if the data meets the assumptions of normality and homogeneity. Analysis of variance (ANOVA) aims to determine whether there is a significant difference due to the provision of different treatments. If a difference is found, the Duncan's Multiple Range Test is performed with an α level of 0.05 or a 95% confidence level to determine the treatment that produces the highest and lowest values.

Result and Discussion

Absolute Weight Growth

The highest absolute weight growth value of Siamese catfish was obtained in C1 with an average value of 7.94±0.03 gr, then the second highest was in treatment T4 with an average value of 7.00±0.51 gr, then T3 with an average value of 6.96±0.63 gr, T2 with an average value of 6.85±0.06 gr, T1 with an average value of 5.93±0.08 gr, and the lowest absolute weight growth in Siamese catfish was in C2 with an average value of 5.85±0.11 gr. The results of observations of absolute weight growth of catfish are presented in full as in Figure 1.

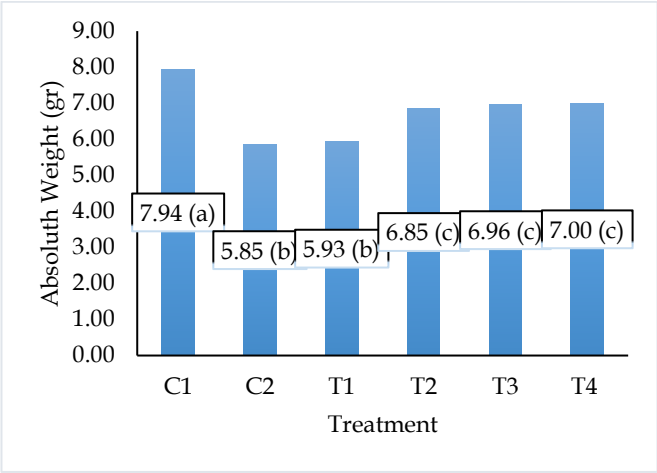

Figure 1. Absolute Weight Growth

The high absolute weight gain in C1 compared to other treatments is likely due to the commercial feed used in C1, which is of better and more stable quality compared to the feed used in C2, T1, T2, T3, and T4, which use homemade feed made from 100% vegetable protein. According to Supriyadi (2010), feed quality is determined by the quality of the raw materials, formulation, and feed manufacturing process. Giri et al. (2009) also stated that feed formulas for fish must contain sufficient energy sources and essential amino acids, essential fatty acids, specific vitamins, and minerals to stimulate growth. According to Rasidi & Haryadi (2016), it is necessary to know the nutrient composition of feed raw materials, which consists of protein and amino acids, lipids and fatty acids, carbohydrates, vitamins, and minerals. This is why protein quality is important in fish nutrition. Making fish feed requires skill in measuring the nutritional composition to meet the standard needs of fish.

On the other hand, the analysis results showed that the T2, T3, and T3 treatments were better than C2, indicating that the addition of cellulase enzymes to the feed had a positive impact on increasing the absolute weight growth of Siamese catfish. This is because cellulase enzymes are a type of enzyme that plays a crucial role in the bioconversion process of organic material (Deka et al., 2013). The ability of cellulase enzymes to degrade cellulose in plant-based feed ingredients undergoes a nutritional improvement process so that the nutrients contained in the treated feed are simpler and more easily digested, which are then absorbed and can be utilized by the fish. These simpler nutrients are because they have been broken down by fiber-degrading enzymes, namely cellulase. This is in line with research conducted by Masriah et al. (2018) reported that the process of hydrolysis of commercial fish feed with rumen fluid, in which the most active enzyme is the cellulase enzyme, at a concentration of 80 mL/100 g of feed can increase the protein content of the feed and reduce the fiber content of the feed with a protein value of 30% to 30.630±0.360% and crude fiber of 6% to 1.999±0.039%.

In addition, enzymes can accelerate a reaction process up to 108 to 1011 times faster than without a catalyst. Supplementation of enzymes including cellulase with feed can cause weight gain and improve fish health. Digestion and absorption of feed ingredients are improved by adding enzymes including cellulase to the feed, thus making the digestive process very good and helping fish become healthy with weight gain (Bhat, 2000; Karmakar & Ray, 2011; Shrivastava et al., 2011). In addition, the specificity and yield of cellulase are improved by recombinant DNA technology to insert cellulase-producing genes into targeted microbial strains (Uddin et al., 2025). Cellulase enzyme production is induced using lactose monohydrate, which functions as an inducer to stimulate microorganisms to produce cellulase enzymes in higher levels also affecting enzyme activity.

Specific Growth Rate (SGR)

Specific Growth Rate (SGR) is the percentage of fish weight gain per day expressed in units (%/day). Based on the results of the study conducted for 30 days, data on the specific growth rate of catfish was obtained which can be seen in Figure 2. The highest specific growth rate of Siamese catfish was in C1 with an average value of 5.44±0.03%/day, then the second highest was in treatment T3 with an average value of 5.06±0.22%/day, then T4 with an average value of 5.03±0.22%/day, T2 with an average value of 4.98±0.04%/day, T1 with an average value of 4.63±0.04%/day, and the lowest specific growth rate in Siamese catfish was in C2 with an average value of 4.61±0.07%/day.

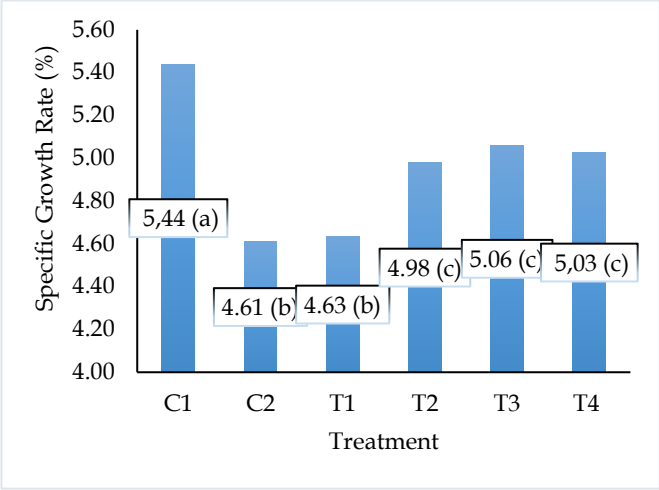

Figure 2. Specific Growth Rate

Specific growth rate is supported by feed containing optimal nutrition (Nuansa et al., 2018). Fish growth is influenced by several factors including feed, cultivation container, temperature, salinity, season, and physical activity. Fish growth is also supported by the availability of sufficient feed. High protein levels in feed will affect the body's metabolic processes in digesting and absorbing feed optimally for use in metabolic processes and growth, in this case, in particular, can increase the SGR value (Fissabela et al., 2017). According to Isnawati et al. (2015), high growth rates are related to high feed efficiency. High feed efficiency indicates efficient feed use, so that only a small amount of food is broken down to meet energy needs and the rest is used for growth.

Similar to the data analysis on absolute weight growth, the highest SGR value for Siamese catfish was obtained in C1, which used commercial feed. This is because commercial feed, in granular or pellet form, provides nutrients in a more stable and concentrated form, allowing the fish to eat efficiently and grow optimally. While the nutritional value of the feed may be a major issue, the most important factor is the fish's acceptance of the feed. Feed consumption is also thought to be related to the fish's preference for the feed, particularly in terms of color and aroma.

However, treatments T2, T3, and T4 showed significant differences compared to C2 and T1. These results indicate that the application of cellulase enzymes to artificial feed affects the SGR of Siamese catfish. These results align with research by Zamini et al. (2012) concluded that the use of Caspian salmon (Salmo trutta caspius) feed fed with complex enzymes with the trademark Natuzyme (protease, lipase, phytase, α amylase, cellulase, amyloglucosidase, β-glucanase, pentosonase, hemicellulase, xylanase, pectinase, acid phosphatase and acid phytase) and Hemicell (endo-β-mannanase, amylase, xylanase, cellulose and α-galactosidase) resulted in better SGR and FCR values compared to feed without added enzymes, namely 1.01±0.01%/day and 0.64±0.01.

The cellulase enzyme is a multienzyme system consisting of three components, namely endoglucanase, which breaks down cellulose polymers to produce oligodextrins with varying chain lengths, exglucanase which breaks down crude fiber (cellulose) from the reducing and non-reducing ends to produce short-bonded cellulose or cellobiose, and β-glucosidase which breaks down cellobiose to produce glucose. The cellulase enzyme will change crude fiber (cellulose) into simpler molecules so that it is no longer a polysaccharide. Cellulase is an enzyme needed to break down the beta-glycosidic bonds in cellulose, thus allowing cellulose to be digested and absorbed as a nutrient.

Survival Rate (SR)

Survival is the percentage of the total number of fish that survive at the end of maintenance from the total number of fish at the beginning of maintenance in a cultivation container. Based on the results of the study conducted for 30 days, data on the survival of catfish were obtained which can be seen in Figure 3. Based on the results of the research that has been done, it can be seen that the highest survival rate of Siamese catfish is found in C1 and T4 treatments with the same value of 98.33 ± 1.92%, then the second highest value is found in T3 treatment with a value of 97.50 ± 1.67%, after that, T2 and C2 with the same value of 96.67 ± 2.72%, and the lowest value is found in T1 treatment with a value of 95.83 ± 3.19%. However, there is no significant difference between each treatment and control on the percentage of survival of Siamese catfish kept for 30 days.

Based on the results of the research conducted, it can be seen that the survival rate of Siamese catfish did not differ significantly between each treatment and the control. Factors that influence survival are abiotic and biotic factors, including competitors, population

density, age, and the organism's ability to adapt to the environment. The survival rate of catfish species can reach 80-90% (Pasch & Palm, 2021). A survival rate of >50% is considered good, a survival rate of 30-50% is considered moderate, and <30% is considered poor (Latifah et al., 2022).

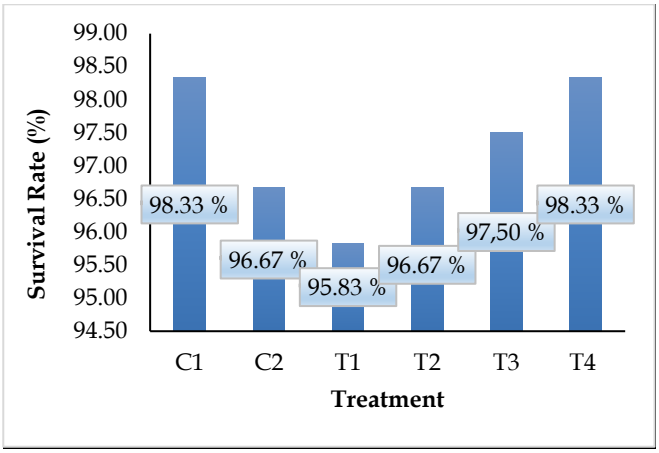

Figure 3. Survival rate

The high survival rate of catfish during the study was due to a supportive environment, such as water quality. Minggawati (2012) argue that water quality significantly influences the survival and growth of living organisms in the waters. A good, hygienic environment is essential for animal growth and survival. This aligns with Effendi (2004) statement that fish survival is strongly influenced by feed and environmental conditions. Providing adequate feed, quality and quantity of feed, and a suitable environment will increase the survival of the fish being raised. Conversely, a decrease in water quality can cause stress in fish, and if the decrease in water quality exceeds the limit, it can even result in fish death.

Water Quality

Water is a limiting factor in fish farming. Water quality in farming is used as a reference for carrying out the farming process. The water quality parameters measured are temperature, pH, dissolved oxygen and ammonia. Data from water quality measurements can be seen in Table 4.

Table 4. Water quality (Sinaga et al., 2020)

Parameters	Values range				Optimal levels
	Morning	Midday	Afternoon	Evening	
Temperature	29-30	31-33	31-32	30-31	25-30*)
pH	6-7	7	7	7	6.5-8.5*)
DO	2-5 mg/ L	14 mg/L	14 mg/L	5 mg/L	>4*)
Amonia	0.10-0.20 ppm	0.10 ppm	0.10 ppm	0.10 ppm	<0,2 ppm**)

In fish farming, water quality is crucial because it is the primary medium for fish survival. Suitable water conditions for cultivation must meet the quantity and quality standards required for fish survival. Water quality measurements during the study showed normal conditions and met the requirements for supporting the growth and survival of Siamese catfish. Several water quality parameters that are very important for cultivated fish include temperature, pH, and dissolved oxygen.

Temperature is a crucial factor in fish farming. Temperature measurements obtained during the study showed a range of 29°C-33°C. The optimal temperature for catfish is between 27-32°C. During the study, the average daytime and nighttime temperatures in the cultivation ponds were higher than the optimal limit for Siamese catfish. This is because the study was conducted in April, which coincided with the dry season, resulting in relatively higher temperatures. The pH of pond water significantly affects fish growth. At low pH, dissolved oxygen content decreases, resulting in decreased oxygen consumption, increased respiratory activity, and decreased appetite. The ideal pH for optimal catfish growth ranges from 6.5 to 8.5. Freshwater pond fish have an acidic dead point of 4.0 and a basic dead point of 11.0 (Syahrizal & Arifin, 2017).

A good dissolved oxygen level for fish growth is between 7.0 and 8.4 ppm, but a dissolved oxygen level of 5 ppm is still sufficient for fish life (Asnawi, 1983). The dissolved oxygen (DO) content obtained during the study ranged from 2-14 mg/L. This value can be said to be good for the cultivation of catfish because the good oxygen content for Siamese catfish is >3 mg/L. Siamese catfish is a type of fish that is resistant to conditions of oxygen deficiency. The ammonia content value during the study did not exceed 0.1 ppm. The increase in ammonia content occurred due to the accumulation of uneaten feed residues by the fish. As mentioned by Sinaga et al. (2020), maintaining ammonia levels below 0.2 mg/L is crucial to prevent toxicity that can affect fish health. Amonia is the end result of the protein decomposition process of metabolic products and food residues that settle in the water, and is toxic to the fish being raised (Teodósio et al., 2017).

Conclusion

Based on the results of the research that has been conducted, treatment C1 (commercial feed) showed the best results on the growth of Siamese catfish. However, T2, T3 and T4 showed the best results compared to C2 (without enzymes) and T1 which both used 100% vegetable protein feed made independently. This indicates a positive effect of the cellulase enzyme on the growth of Siamese catfish. Thus, it can be concluded that the addition of cellulase enzymes to the feed has an effect on increasing the growth of Siamese catfish.

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Author Contributions
L. D. A. S. A, M. A., S. F. A and M. A conceptualized the research idea, research method, data collection and analysis. A. T. M. and G. M guided the writing, review and editing, survey and validation of the instruments used in the study.

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Conflicts of Interest
The authors declare no conflict of interest.

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