Effect of Supplementation of Pineapple Extract in Fish Feed on The Growth of Catfish *(Clarias batrachus)*

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Abstract
The study of commercial feed with combination of pineapple extract for 90 of catfish cultivation has been executed during 40 days. The catfish has been taken randomly and were given feed with three different dosage. The combination treatment of pineapple extract with 33% protein of commercial feed was given. The pineapple extract with dose of 0.75%/kg (P1), 1.5%/kg (P2) and 2.25%/kg (P3) were mixed with commercial feed for catfish. The result showedthat significantly higher weight of 20.56 gram and length of 15.53 cm was obtained in P2, compare with P1 and P3 was lower of body weight and body length.The survival rate of fingerlings was also found higher in P2 with value of 80% compared with P1 and P3. This conclude that the combination of feed and pineapple extract of 1.5% was better compare of 0.75% and 2.25% for catfish cultivation.

Keywords: Catfish (*Clariasbatrachus*), Pineapple Extract, *Ananas* sp., Commercial Feed

Introduction

The freshwater of aquaculture fishery sector in Indonesia has been a potential to develop through extensification and intensification. According to data from the Fisheries and Maritime Affairs Office, it shows that freshwater fish cultivation commodities such as catfish have a high demand, reaching 124,94 tons/year in the domestic market. Catfish *(Clarias batrachus*) is much favoured by the community, by having a good taste, and high protein content (Andini *et al.,* 2017). Therefore farmers have to meet the high demands of catfish for local consumption.

Catfish are often classified as omnivore and are known to be greedy due to having wide mouth, which is able to eat a large amount of natural and artificial foods such as pellets. In the cultivation pond, catfish are willing to accept all kinds of food that are given. The intestines of catfish are shorter than their body length; meanwhile the stomach is relatively large and long (Habibi, 2016). Catfish is very tolerant of high water temperatures (20^{o}-35^{o}C) and can live in low water quality environment. They can survive in very minimal oxygen content due to having an additional breathing apparatus called the *arborescent* organ. In wild, catfish prefer slow flowing water,

and Wardhani (2014) also mentioned that catfish do not like fast-flow water.

Pineapple contains protein digestive enzymes called bromelain, which are composed of proteolytic enzymes. Studies on the bromelain enzyme show that the bromelain enzyme derived from pineapple extract contains various proteinase enzymes. During pineapple processing, the crown and stem are cut off before peeling and the core is then removed fo further processing. The waste portions such as the peel, core, stem, and crown represent 29%–40%, 9%–10%, 2%–5%, and 2%–4% (w/w) respectively (Ketnawa *et al*., 2012). Bromelain enzyme is a proteolytic enzyme, which has the property of hydrolyzing protein into its constituent elements. The hydrolysis that occurs with proteolytic enzymes is the breaking of the peptide bonds from the substrate bonds, where the proteolytic enzymes act as catalysts in the cell. Protein hydrolysis is carried out by endogenous enzymes and is assisted by exogenous enzymes bromelin enzymes can act as exogenous enzymes. Bromelain enzymes can also dissolve collagen contained in collagen proteins by hydrolyzing the protein (Pratama *et al.,* 2017), thus making the flesh not hard. It also functions in increasing activities of digestive enzymes (Lin *et al.,* 2007), and improving the histological structure and the health of the fish intestines (Castillo & Gatlin 2015; Mathlouthi *et al*., 2002). Dietary protease can compensate for deficiencies in endogenous enzymes, especially in young animals, and assist in the breakdown of macromolecular proteins that are difficult to digest.

Setiyani *et al.,* (2017) mentioned that feed is the main aspect in aquaculture activities. This is because 40-70% of the production costs are used for feed, so that efforts are needed to increase the growth of catfish fingerlings through combining pineapple extracts in commercial feed. One of the enzymes that can be used to increase the growth of catfish fingerlings is the bromelain enzyme, which can be found in pineapple extract. The more enzymes added to the feed, the more protein would be hydrolyzed into amino acids, thus increasing the growth and digestibility of the fish. However, if it has passed the optimum point, it can have a negative effect, thus inhibiting its growth. This happens because excess amino acids will have an impact on the digestibility of fish protein, so that the protein that has been hydrolyzed into amino acids is not used for growth but will be used as energy (Delima *et al.,* 2017). Wulandhari *et al.,* (2017) also mentioned that the addition of the right dose affects growth of fishes.

Given the important role of bromelain in the process of protein digestion, it is necessary to conduct research on the effect of pineapple extract on feed protein utilization rates and fish growth (Anugraha*et al.,* 2014). Therefore, in this study we are looking at the effect of supplementation of pineapple extract to fish feed on the growth of catfish under cultivation.

Methodology

This research was conducted from July to September 2021 which located at Jalan Flamboyan Raya Gg. Flamboyan Mas No. B23, Medan. The pineapple extract prodution by using AOAC (1990) method and was done at the Palm Oil Research Center Laboratory, Medan.

To get the optimum parameters for the growth of catfish fingerlings, treatment of combination of commercial feed with pineapple extract was carried out, whereby the function is to facilitate the sdigestion process.

The experimental design used was a completely randomized design with a single treatment and the treatment was repeated 3 times. The aquarium for each replication is placed randomly (random). The treatments carried out are as follows:

Treatment P1: Test feed with the addition of pineapple extract at a dose of 0.75% / kg.
Treatment P2: Test feed with the addition of pineapple extract at a dose of 1.5% / kg
Treatment P3: Test feed with the addition of pineapple extract at a dose of 2.25% / kg

Cultivation of fish

In this study, 9 aquariums (30 cm x 25 cm x 17 cm) was used as container for catfish farming. The fish used in this study were catfish fingerlings*(Clarias batrachus)* measuringabout 8 cm length and weight of ± 3 g, which comes from a catfish farmer in Medan Tuntungan. The catfish were spread in an aquarium containing 10 liters of water, 1 head/L of watereach. The number of fingerlings for each treatment was 10 and the total number of fishes used was 90.

Extraction of pineapple extract

The process of making pineapple extract was carried out at the Palm Oil Research Center Laboratory, Medan. Determination of pineapple extract dosage was modified from Anugrah *et al.,* (2014). The dosage of pineapple extract were mixed with commercial feed of 7.5 g, 15 g and 22.5 g of pineapple extract for 1 kg of feed. After the pineapple extract was weighed based on the dosage of each treatment, than dissolved of100 ml water and blended until homogeneous. After being evenly mixed, puted in sprayer bottle than sprayed slowly on the commercial feed. The feed were used in this study was of 33% protein, 5% fat, 6% fiber, 13% ash, and 13% moisture content. The feeding was given 3 times a day that is morning, afternoon, and evening. The maintenance of catfish fingerlings was carried out for 40 days, and the data was collected once every ten days by measuring of length, weight, weight gain, survival rate of fish and water quality.

Length growth

Length measurement from mouth to tail According to Effendie (1997), fish length growth is calculated using the formula:

$$L = L_t - L_o$$

Information:
L = growth length (cm)
L_t = average length at the end of the study (cm)
L_o = average length at the start of the study (cm)

Weight growth
The analysis data of weight were used by Weatherley (1972) with formula of:

$$\Delta W = Wt - W_o$$

Information:
ΔW = absolute growth (g)
W_t = Final weight (g)
W_o = initial weight (g)

Survival rate
The survival rate of fish were used formalation by Effendi (1979), the formula is:

$$SR = \frac{N_t}{N_o} x100\%$$

Information:
SR = fish survival (%)
N_t = Number of fish at the end of the study (tail)
N_o = Number of fish in the initial growth performance

Water quality measurement
The parameter of water quality measured are the temperature, pH, and dissoved oxygen (DO). Temperature measurements was carried out every day during the study. DO and pH measurements were carried out every 10 days during these study. All the measurement done was based on Karbassi *et al.,* (2011).

Data analysis
The data obtained from the results of observations during the study were analyzed using Microsoft Excel and the results of the experimental data were tabulated statistically using the analysis of variance (ANOVA). The analysis data were using the SPSS computer program. If there is a real effect, a further test will be carried out with the F test. Furthermore, the data will be presented in tables and graphics.

Results and Discussion

Length growth of catfish
The increase in length of catfish for 40 days of maintenance showed that in each treatment, the highest average length measurement was in treatment P2 of 7.2 cm, then followed by P1 treatment (5.2 cm) and lastly P3 treatment (4.1 cm) (Table 1).

Table 1: Length of fish (cm) during 40 days of cultivation

Treatment	Growth Length (cm)				
Pineapple Extraction	H0	H10	H20	H30	H40
P1	8.333[a] ±0.202	9.300[a] ±0.057	10.366[a] ±0.055	12.300[c] ±0.152	13.566[b] ±0.120
P2	8.366[a] ±0.185	9.500[a] ±0.11	11.233[b] ±0.145	13.233[b] ±0.145	15.533[c] ±0.176
P3	8.166 [a] ±0.120	9.366[a] ±0.088	10.400[a] ±0.225	11.300[a] ±0.152	12.300[a] ±0.115

[a,b and c], The difference in letter notation indicates that there is a significant difference

Furthermore, to determine the effect of each pineapple extract treatment on fish length, it was followed by the ANOVA test where the result was presented in Table 2. The total of length was significantly different of (p≤ 0.01), and have shown every 10 day was increase the length.

Table 2: Analysis of variance of length (cm) of catfish during 40 day.

Source of variance	df	Sum of Squares	Mean Square	F	Sig
Pineapple extract	2	17,853	8,927	159,089	0,00**
Total	2				

** (Significantly different) (p≤ 0.05) * (Significant) (p≤ 0.01)

Based on observations for 40 days of cultivation, catfish fingerlings in P2 treatment with the addition of 1.5% pineapple extract produced the highest length growth. This shows that the effect of bromelain enzyme in feed has a higher ability to digest the feed. It is could be due to the feed with a combination of pineapple extract of 1.5% gave optimal utilization to the catfish. According to Delima*et al.,* (2017), enzyme concentration is one of the factors that affect the protein breakdown process, so it will increase the digestibility of fish to feed. The growth rate in fish is influenced by the absorption of feed nutrients given. Feed affects the growth and development of fish, as well as a source of energy, movement, and reproduction. The food eaten by fish will be processed in the body and the nutritional elements or nutrients will be absorbed and used to build tissue so that growth occurs.

It can be concluded that pineapple extract at different treatments increased the length of catfish. The most effective pineapple extract to increase fish length was found in P2 treatment (15.53 cm), which was significant compared to the other treatment.

Weight growth of Catfish

The increase in weight of catfish fingerlings for 40 days of maintenance showed an increase in the average weight obtained from each treatment, the highest increase in weight was obtained in P2 treatment (17.15 g), followed by P1 treatment (13.21 g) and lastly P3 treatment (11.27 g) (Table 3).

Table 3: Mean and standard errors of weight gain (g) of catfish during 40 day

Treatment	Increased weight (g)				
Pineapple extract	H0	H10	H20	H30	H40
P1	3.106 [a] ±0.014	5.260[a] ±0.073	8.353[a] ±0.060	13.293[b] ±0.129	16.320[b] ±0.105
P2	3.226[b] ±0.034	6.895[b] ±0.182	11.263[b] ±0.084	15.566[c] ±0.118	20.373[c] ±0.151
P3	3.286 [b] ±0.053	5.696[a] ±0.178	8.436[a] ±0.063	10.296[a] ±0.095	14.560[a] ±0.166

[a,b,c] The difference in letter notation indicates that there is a significant difference

The most effective pineapple extract to increase fish weight was P2 treatment with highest significant increase in weight (20.373 g). This could be due to the amount of pineapple extract given at a dose of 1.5% was optimum feed. The need for amino acids in fish is necessary in increasing body weight in fish. The increase in catfish weight is also thought to be due to the combination effect of commercial feed and pineapple extracts. The pineapple extract in feed being utilized by the catfish to gain weight and growth optimally. According to Wulandhari *et al.,* (2017), utilization of protein in fish can be increased by using feed supplements such as the enzyme bromelain. Bromelain enzyme contained in pineapple extract can hydrolyze feed protein into simpler elements. The addition of the right dose affects growth of fish studied.

Table 4: Analysis of variance of weight (gr) of catfish during 40

Source of variance	df	Sum of Squares	Mean Square	F	Sig
Pineapple extract	2	83,409	41,704	1082,13	0,00**
Total	2				

** (Significantly different) (p≤ 0.05) * (Significant different) (p≤ 0.01)

It can be seen that the significant value of the source of variance of pineapple extract is p (≤ 0.01). This shows that all these sources of variance have a very significant effect on fish weight. Analysis of Variance (ANOVA) weight gain of catfish fingerling showed significant difference to the weight gain of catfish fingerling.

Survival Rate of Catfish

The survival rate of catfish was studied from a total of 90 individuals, which was maintained for 40 days. The treatment showed the highest survival rate of catfish was was observed in treatment P2 (80% survival rate), followed by treatment P1 (73.33% survival rate), and the lowest was found in treatment P3 (76.67% survival rate).

Table 5: Analysis of Variance on the Survival of Catfish during 40 day

Source of variance	df	Mean Square	F	Sig
Pineapple extract	2	22.222	0.800	0.472^{ns}
Total	2			

^{ns}(Not significantly different)

From the analysis, the value of 0.472 was not significant for the sources of variance in pineapple extract (Table 5). This shows that the source of the variance does not have a significant effect on the survival of catfish fingerling during 40 days, may be could be due to very small samples or other factors.

Death that occurred during 40 days of rearing was thought to be due to stress due to the sampling activities, because some fish died after 10 days of sampling. During the sampling process, catfish was removed from the aquarium and transferred to a digital scale for weighing, placed on a millimeter block of paper to measure the length of the fish, which causes the catfish to experience stress due to lack of oxygen. Fish that are stressed will experience a decrease in their immune power, so that the fish are easily infected with diseases and cause death. According to Anugraha *et al.,* (2014), the factors that can affect the level of survival are internal and external factors. Internal factors come from the catfish itself, which experience stress because of careless treatment so that the mortality is high. External factors that influence include environmental conditions such as high ammonia and laboratory conditions that are less supportive of maintenance.

Based on the analysis of catfish survival, it does not have a significant effect on the treatment given. It is suspected that the fish mortality that occurred was not caused by pineapple extract, but by the sampling process factor at the time of the study. Water quality during the study in all treatments was feasible and optimal for catfish life so that mortality was not significantly different. According to Pratama *et al.,* (2017), stressed fish will experience a decrease in immune power in their body, so that fish are easily infected with diseases. Fish survival is very dependent on the adaptability of fish to food and the environment, fish health status, stocking density, and adequate water quality to support growth.

Water quality

The survival and growth of catfish is greatly influenced by water quality. Water quality parameters measured during the study were temperature, pH and DO. The data obtained is relatively stable because the maintenance is carried out in a controlled manner. During the observation of catfish water quality, the temperature was 26.3 - 27.5°C, pH 7.1 - 7.7 and DO 5.1 - 5.8 mg/l (Table 6).

Table 6: Water quality measurement (T, pH and DO) during fish cultivation

Treatment	Temperature (°C) Min-Max	pH Min-Max	DO (mg/l) Min-Max
P1	26.3 – 27.5	7.1 – 7.7	5.1 – 5.7
P2	26.5 – 27.3	7.1 – 7.4	5.2 – 5.8
P3	26.4 – 27.3	7.1 – 7.6	5.4 – 5.8

It can be seen that the water quality scores are almost the same (Table 6). This is because each treatment is at the same location and gets the similar environmental factors.

Water quality measurements carried out during the study period were temperature, pH, and DO. The water was syphoned out once a day with a volume of 10% of the total water available and cleaning the remaining feed remains on the bottom of the test medium. The source of water used is well water that has been deposited for ± 48 hours or more and then aerated to supply oxygen to the maintenance medium.

Water quality has an important role in aquaculture because it is needed as a medium for fish growth. Good water quality can support the growth and survival of the fish. In this study, the average pH was in the range of 7.0-7.6 and that temperature range was in the range of normal temperature. According to Nuraeni (2018), the degree of acidity (pH) has a major effect on the life of the aquatic organism where the quality standard value is 6.5 - 8.5. pH. Therefore the pH of water is used as a parameter to indicate whether a water quality is good or bad.

The condition of water quality at the time of the research was good with dissolved oxygen (DO) between 5.0 - 5.8 mg/L. Based on the results of the water quality parameter values, it can be concluded that the water quality during the study can support the growth and survival of the catfish. This is in accordance with Permana (2015), which states that dissolved oxygen in water is ideal for catfish life and growth is 5 mg / L.

Conclusion

From this study, it can be concluded that, the moderate pineapple extract supplemention with a dose of 1.5% /kg and commercial feed with 33% of protein was provide a significant effect on the growth of catfish. This can serves as the base study for further enhancement of fish growth, which could increase the aquaculture industry in Indonesia.

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