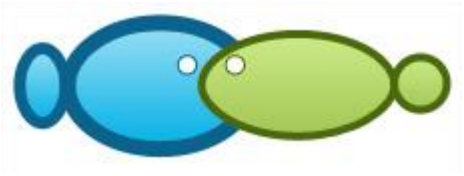

Impact of varied grotop booster doses in feed on the growth and survival of *Osteochilus hasselti* in a recirculation system

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Abstract. Grotop booster is a supplement that increases fish's appetite and immune system and stimulates fish digestive enzymes, which can accelerate growth. This study aimed to determine the best dose of grotop booster added to feed for increasing the growth rate and survival rate of *Osteochillus hasselti*. The method used is an experimental method using a completely randomised design with five treatments and three replications. The treatments used were: P_0 without grotop, P_1 with 15 g grotop kg^{-1} feed, P_2 with 20 g grotops kg^{-1} feed, P_3 with 25 g grotops kg^{-1} feed and P_4 with 30 g grotop kg^{-1} feed, for a rearing period of 50 days. The study indicated that the best treatment was P_4 (30 g grotop kg^{-1} feed), which resulted in an average final weight growth of 6.01 g, an average length growth of 6.76 cm, a specific growth rate of 2.85%, a survival rate of 100%, a feed efficiency of 56.76% and a feed conversion ratio of 1.76.
Key Words: protease enzyme, feed efficiency, feed conversion ratio, stocking density.

Introduction. *Osteochilus hasselti* is a freshwater fish native to Indonesian waters and one of the most commonly consumed fish in Sumatra, Java, and Kalimantan. *O. hasselti* is in high demand because of its delicious taste and meat that is not too bony (Syamsuri et al 2017). Currently, *O. hasselti* is exploited not only for its meat consumption, but also for its eggs. In addition to being used as a food fish, *O. hasselti* can be developed into ornamental fish. The high demand for *O. hasselti* has led to an increase in demand, but supply is still reliant on capture from the wild. *O. hasselti* is one of the freshwater fish commodities that has not been widely cultivated. This is because *O. hasselti* has a slow genetic growth rate, and it takes 5-6 months to reach consumption size (Anggani et al 2021).

One solution to accelerate the growth of *O. hasselti* is by providing high-quality feed. If the quality of the feed provided is good during the rearing period, the nutrients for the fish's growth will be optimally met. Improving the quality of feed can be done by adding probiotics or supplements to the feed. This is done to stimulate the fish's growth in a relatively short period. Grotop booster is an alternative for adding supplements to the feed, processed from various ingredients (animal and plant-based). It contains inositol, L-Lysine, DI-Methionine, vitamin C, vitamin B1, vitamin B2, and enzymes such as protease, amylase, cellulase, and lactase (Gea 2023). The benefits of this booster include increasing appetite, enhancing immune system resilience, stimulating digestive enzymes, and accelerating growth. According to research conducted by Yudiananda et al (2020), the use of grotop booster in the feed for catfish (*Hemibagrus nemurus*) raised in a recirculating system resulted in a specific growth rate of 1.62%.

Success in aquaculture activities is not only influenced by feed but also by the management of water quality as the fish's living medium. One effort to improve water quality is through the application of Recirculating Aquaculture System (RAS). RAS is a cultivation method conducted in limited land, integrating cultivation with minimal location utilization and minimizing water usage. This technology can create stable water parameters and quality, ensuring optimal conditions throughout the cultivation process

(Nurcahyono et al 2022). This research aimed to determine the optimal booster dose in feed to enhance protease enzyme activity, growth performance (absolute weight growth rate, total length growth rate, specific growth rate), feed efficiency, feed conversion, and survival rate of *O. hasselti*.

Material and Method

Time and place. This research was carried out from July to September 2022, for 50 days, at the Technology Laboratory Cultivation, Faculty of Fisheries and Marine Affairs, Riau University, Pekanbaru.

Materials and equipment. The material used in this research consisted of 225 *O. hasselti*, measuring 4 to 5 cm. Commercial pellets,with a content of 38% protein, at least 2% fat, a rough maximum of 3% fibre, and a rough maximum of 13% ash, were used as test feed. The other ingredients are the adhesive (progol) and the grotop booster, with code PF-800 production from PT. Charoen Pokphand, adhesive (Progol) and grotop booster.

Research methods. This research used a Completely Randomized Design (CRD). The research factor consisted of different doses of grotop booster with five treatment levels and three replications. This selection method of the treatment levels refers to Utami (2018). The treatment levels determined in this study were P_0 (without grotop booster), P_1 (a grotop booster dose of 15 g kg^{-1} feed), P_2 (a grotop booster dose of 20 g kg^{-1} feed), P_3 (a grotop booster dose of 25 g kg^{-1} feed), and P_4 (a grotop booster dose of 30 g kg^{-1} feed).

Observed parameters. The parameters measured in this study were: 1) the content of protease enzymes that refers to the Standard Operating Procedure for enzyme analysis at the Laboratory of Nutrition and Feed Technology, Bogor Agricultural University, based on a modified Bergmeyer & Grasi method (1983); 2) the growth performance of *O. hasselti*, which includes: survival rates (Effendi 1986), absolute growth weight and length growth, referring to Effendi (1997); 3) the specific growth rate (Crane et al 2019), feed efficiency according to Zonneveld et al (1991); and 4) the feed conversion, according to the National Research Council (NRC 2011). The water quality parameters (temperature, pH, dissolved oxygen (DO) and ammonia), were measured every week during the study, according to Effendi (2003).

Data analysis. Data obtained from measurements of protease enzyme content, absolute weight growth, absolute length growth, specific growth rate, feed efficiency, feed conversion, survival and water quality were processed using the analysis of variance (ANOVA) to see if the treatments show a significant difference where $P<0.05$; further, a Student Newman-Keuls range test was carried out.

Results and Discussion

Protease enzyme content. The results of the protease enzyme content analysis in *O. hasselti'*s intestines, in all treatments, can be seen in Table 1. Table 1 shows that giving booster grotop in feed can increase the protease enzyme content. The treatment with the highest increase is P_4 (with a dose of booster grotop of 30 g kg^{-1} feed or 0.036 IU mL^{-1}). On the other hand, the treatment with the lowest protease enzyme content is P_0 (without added grotop booster). One way to increase the growth of *O. hasselti* is by optimising the physiological function of the *O. hasselti'*s body organs, in particular the digestive tract. An important organ that plays a role in the digestive tract is the intestine because it is closely related to the activity of digestive enzymes in the fish's body (Rojtinnakorn et al 2012) A high protein content will require a higher concentration of protease enzymes to help degrade the protein in the feed. According to Amalia et al (2013), enzymes in artificial feed can help speed up the digestion, so that nutrients are sufficient for fish

growth. A higher level of enzymes added to the meal stimulates the protein hydrolysis into amino acids, thereby increasing the fish's feed digestibility.

Table 1

Measurement of protease enzyme content in the digestive tract of *Osteochilis hasselti*

Treatment	*Observation time (mg L^{-1})*		*Percentage improvement (%)*
	Beginning	*End*	
P_0	0.013	0.016 ± 0.00^{a}	0.002
P_1	0.013	0.019 ± 0.00^{b}	0.005
P_2	0.013	0.022 ± 0.00^{c}	0.008
P_3	0.013	0.024 ± 0.00^{c}	0.010
P_4	0.013	0.036 ± 0.00^{d}	0.022

The values listed are the average ± standard deviation. Different letters on the row indicate significant differences ($p<0.05$).

Student Newman Keuls' follow-up test found differences in digestive protease enzyme activity between treatments. Adding a grotop booster to the feed significantly affected the protease enzyme activity of *O. hasselti* ($P<0.05$). The content of protease enzymes in the gut of *O. hasselti* is higher when fed with booster grotop supplementation (P_1, P_2, P_3, P_4) than without booster grotop supplementation (P_0). Studies of digestive enzyme activity with various sizes of fish show that growth and metabolic complexity cause differences in the enzyme activity related to the feed digestion process.

Enzymes are proteins which influences reaction kinetics by catalysing the reaction (they speed up chemical reactions by lowering the activation energy). One important enzyme is the protease, a proteolytic enzyme that works to break down proteins into amino acids. The enzymes activity in the digestive tract of *O. hasselti* is directly proportional to the activity of the enzymes contained in the feed. Also, the digestive enzymes' activity increases for feeds with a high nutritional value, due to a higher amount of nutrients which have to be broken down. However, a high nutrient content will not optimise the endogenous enzymes' activity without the support of exogenous enzymes obtained from outside the body, such as the protease enzyme found in the grotop booster, which is added to the feed to help breaking down nutrients.

Growth of O. hasselti. The results of measurements of absolute weight growth, absolute length growth and specific growth rates of *O. hasselti* during the study are presented in Table 2.

Table 2

Absolute weight growth, absolute length growth and specific growth rates of *Osteochilis hasselti*

Treatment	*Absolute weight growth rate (g)*	*Total length growth rate (cm)*	*Specific growth rate (% day^{-1})*
P_0	3.08 ± 0.08^{a}	1.70 ± 0.06^{a}	2.31 ± 0.04^{a}
P_1	3.35 ± 0.37^{ab}	1.88 ± 0.09^{ab}	2.36 ± 0.40^{a}
P_2	3.58 ± 0.10^{b}	2.10 ± 0.12^{b}	2.51 ± 0.11^{ab}
P_3	3.72 ± 0.08^{b}	2.33 ± 0.17^{c}	2.61 ± 0.04^{ab}
P_4	4.57 ± 0.12^{c}	2.49 ± 0.11^{c}	2.85 ± 0.07^{b}

Superscript letters appear in columns that indicate significant differences in all treatments ($P<0.05$).

The observations in Table 2 show that giving a grotop booster dose of 30 g kg^{-1} feed resulted in the highest absolute weight growth of the *O. hasselti*, namely 4.57 g. In contrast, the control treatment resulted in the lowest absolute weight growth of the *O. hasselti*, namely 3.08 g. Based on the results of the Analysis of Variation test (ANOVA), at $P<0.05$, continued with the Student Newman Keuls test, the treatments with different added doses of grotop booster had significantly different effects on the absolute weight

growth. Grotop booster has several compositions, such as multivitamins, complex amino acids and protease enzymes. These enzymes will hydrolyse feed nutrients (complex molecules); thus, proteins will be decomposed into simpler molecules, which will facilitate the digestion and absorption processes in the digestive tract of *O. hasselti*. According to Kurniasih et al (2013), administering the bacterial protease enzyme *Bacillus cereus* increased feed efficiency and fish growth significantly.

The highest absolute length increase of *O. hasselti* was found at P_4, namely 2.49 cm, while the lowest length growth was at P_0, namely 1.70 cm. Based on the results of the Analysis of Variance Test (ANOVA; $P<0.05$; Table 2) different doses of grotop booster had significantly different effects on the total length growth of *O. hasselti* reared in a recirculation system. Table 2 shows that the fish experienced growth during the entire rearing period, and the results of the specific growth rate of P_4 were higher compared to other treatments, namely 2.85%. This is because the feed meets the nutritional needs and can be utilized well by the fish, resulting in a good growth (Yolanda, 2013). The increase in weight and length of fish indicates that the energy content in the feed consumed exceeds the energy requirements needed for maintenance and body activity so that fish growth is optimal (Cortes et al 2005).

Feed efficiency, feed conversion ratio, and survival of O. hasselti. The results of feed efficiency, feed conversion ratio and survival of the *O. hasselti* during the 50 days of research can be seen in Table 3.

Table 3

Feed efficiency, feed conversion ratio, and survival of the *Osteochilis hasselti*

Treatment	*Feed efficiency (%)*	*Feed conversion ratio*	*Survival rate %*
P_0	44.94 ± 1.27^{a}	2.23 ± 0.06^{c}	88.89
P_1	47.39 ± 2.53^{ab}	2.11 ± 0.11^{bc}	95.55
P_2	50.31 ± 1.58^{b}	1.99 ± 0.06^{b}	95.55
P_3	50.63 ± 0.71^{b}	1.98 ± 0.03^{b}	100.00
P_4	56.76 ± 1.13^{c}	1.76 ± 0.03^{a}	100.00

Feed conversion ratio (FCR) is the ratio between the weight of feed given and the weight of fish produced. Taufik et al (2017) stated that food is a source of energy for fish to move, grow and defend against disease. The research showed that the highest feed conversion ratio was in the P_0, namely 2.23, while the lowest FCR value was in P_4 of 1.76. A good feed conversion ratio in aquaculture is at the lowest FCR value. A low feed conversion means a higher efficiency of the feed and, conversely, a higher feed conversion value indicated a lower efficiency (Fran & Akbar 2013).

Based on Table 3, the highest feed efficiency was found in P_4, namely 56.76%. In contrast, the lowest feed efficiency was found in the control treatment, namely 44.94%. The Analysis of Variation Test (ANOVA) carried out showed that the treatments with different grotop booster doses (in the *O. hasselti* rearing media with the system recirculation) had significantly different effects on the fish feed efficiency *O. hasselti* ($p<0.05$), which was corroborated with the results of the Student Newman Keuls. The best treatment is P_4 because the dose of booster grotop was higher than in other treatments, significantly increasing the performance of digestive enzymes such as protease, amylase and lipase contained in the feed.

The highest number of live *O. hasselti* was found in treatments P_3 (with a booster grotop dose of 25 g kg^{-1} feed) and P_4 (with a booster grotop dose of 30 g kg^{-1} feed), with a total survival rate of 100%. The results of the Student Newman Keuls test showed that the effects of all treatments on the survival rate of *O. hasselti* were not significantly different. The survival rate of *O. hasselti* during the rearing ranged from 88.89 to 100%. The survival value of *O. hasselti* is classified as good. Andrila et al (2019) stated that a survival rate >50% is considered good, 30-50% is moderate, and less than 30% is not good.

Table 4

Water quality during research period

Treatment	*Temperature (°C)*	*pH*	*DO (mg L^{-1})*	*Ammonia (mg L^{-1})*
P_0	28.70-29.00	5.70-6.87	4.05-5.80	0.001081
P_1	28.72-29.74	5.34-6.90	4.10-5.70	0.001723
P_2	27.61-29.01	5.25-6.83	4.0-5.70	0.000791
P_3	28.30-29.37	5.25-6.83	4.10-5.80	0.001839
P_4	28.55-29.11	5.39-6.88	4.11-5.80	0.002455

Table 4 shows that the water quality conditions in the rearing media during the research period were almost the same, and their values were quite good for the growth and survival of the *O. hasselti*. This is because all treatments occurred at the same location, under the influence of the same environmental factors, and also due to the influence of the recirculation system, which maintained the oxygen supply in the container. The average value of the temperature measurement in the rearing media for the 50 days period was around 27.61–29°C, which is still suitable for rearing *O. hasselti*. Simanjuntak (2010) stated that the optimum water temperature for *O. hasselti* ranges from 18 to 28°C. According to Ridwantara et al (2019), an appropriate temperature improves the metabolism, which has a good impact on fish growth and weight gain. Wicaksono (2005) stated that the optimum pH for water ranges between 6.5 and 9. During the rearing period, it ranged from 5.25 to 6.90. This shows that the average pH value found in the rearing media was not optimal, but *O. hasselti* still tolerated it during the research.

Dissolved oxygen (DO) measurements during the study ranged from 4.05 to 5.80 mg L^{-1}. The results show that the DO contained in the rearing media during the research was quite good. Wijayanti et al (1995) stated that the DO requirements for *O. hasselti* range from 5 to 7 mg L^{-1}. According to Swingle (1968), the minimum oxygen content in water is 2 mg L^{-1}, which is sufficient to support normal aquatic organisms.

The results of measuring the ammonia content at the end of the study ranged from 0.000791 mg. L^{-1} to 0.002455 mg L^{-1}. The ammonia content is still within the tolerable range for *O. hasselti*, according to Chervinsky (1982), who stated that appropriate ammonia concentrations for fish life are less than 2.4 mg L^{-1}. The main source of ammonia is fish excretion; the increase in fish waste at the bottom of the aquarium is related to feeding and to the protein levels in the feed.

Conclusions. Feeding with added booster grotop affects the average weight growth, average length growth, specific growth rate, feed efficiency and feed conversion ratio. The best treatment consisted of administering 30 g kg^{-1} of grotop booster, which caused significantly different effects ($P<0.05$), except for the survival rate, compared to the other treatments, namely: an absolute weight growth of 4.57 g, a total length growth of 2.49 cm, and a specific growth rate of 2.85%.

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Conflict of interest. The authors declare no conflict of interest.

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