Papain and bromelain crude enzyme extract in commercial feed, effectiveness toward pisciculture production of striped catfish (*Pangasianodon hypophthalmus*) in aquaculture facility

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Abstract. Striped catfish (*Pangasianodon hipophthalmus*) is one of economic important fish species which has slower growth rate compared to other freshwater fish. The addition of enzymes to artificial fish feed is aimed to maximize the protein utilization for fish growth. Papain and bromelain enzymes are exogenous enzymes which can aid in the process of protein hydrolysis. This study aims to determine the effect of papain and bromelain enzyme combination toward daily growth rate (DGR) and feed utilization efficiency (FE). The research was conducted in March - June 2017 at Floating Fish Cage (FFC) of Fisheries and Ornamental Fish Conservation Centre of Cirata Reservoir - West Java, Indonesia. The materials used were 270 individuals of striped catfish with average weight of 20 g and 8-12 cm length. The method used was Completely Randomized Design (CRD) consisting of 6 treatments: A (without enzyme addition), B (5% papain), C (3.75% papain and 1.25% bromelain), D (2.5% papain and 2.5% bromelain), E (1.25% papain and 3.75% bromelain) and F (5% bromelain) with 3 replications each. Data analysis was performed by using F test analysis and continued with Duncan Multiple Range Test. The results showed that the combination of papain and bromelain enzymes can increase the rate of DGR and FUE. Treatment E gave the highest DGR and FUE values by 2.22% and 4.45% respectively.
Key Words: papain, bromelain, production, *Pangasianodon hypophthalamus*.

Introduction. Striped catfish (*Pangasianodon hypophthalmus*) is one of freshwater commodities which has high economic value and is easy to cultivate. This fish is omnivorous and is also relatively tolerant to low dissolved oxygen level (Ananda et al 2015). Catfish farming activities are closely related to the needs of fish feed. Fish farming efficiency can be fixed through feed costs (Oyakhilomen et al 2016). The addition of enzymes in fish feed can enhance the protein utilization for fish growth. Several enzymes, contained in fruit such as pineapple and unripe papaya, have proteolytic properties that can simplify the protein into amino acids that can be ingested by fish. It carries proteolytic activity and belongs to cysteine proteinase family (Brocklehurst et al 1981 in Paul et al 2013). This encourages the utilization of enzymes in the fruit for fish feed production.

The proteolytic activity of papain has been well described in the literature, including the degradation of elastin and proteoglycans (Johanson 1972 in Paul et al 2013). According to Paul et al (2013) papain possesses a very powerful digestive action, superior to pepsin and pancreatin. Papain is a single-chained polypeptide with three disulfide bridges and a sulfhydryl group that are highly essential for the activity of the enzyme. Papain is expressed as an inactive precursor, prepropapain. For the formulation of an active papain requires several cleavage steps that include an initial cleavage of the 18 amino acid preregion followed by further cleavage of the glycosylated 114 amino acid proregion (Vernet 1991 in Paul et al 2013). This proregion serves as an intrinsic inhibitor and folding template (Chau 1983, Taylor 1992 in Paul et al 2013).

Bromelain is a complex mixture of proteinases which typically derived from pineapple stem. Similar proteinases are also present in pineapple fruit. Previous *in-vivo* studies using bromelain have been limited by the lack of assays to control for potential differences in the composition and proteolytic activity of this naturally derived proteinase mixture (Hale et al 2005). Crude bromelain is a mixture of a cystein proteases that catalyses the hydrolytic cleavage of the internal peptide bonds of the protein substrate. Bromelain was isolated and purified from crown leaf and pineapple fruit (Ramalingam et al 2012).

Bromelain can help dissolving the mucus formation and speed up fat removal through kidneys. Bromelain also contains citric and malic acids that are important and necessary to improve the removal process of fat and manganese and also become an important component for certain enzymes in the metabolism of proteins and carbohydrates (Winastia 2011). Bromelain is a complex mixture of proteinases typically derived from pineapple stem. Similar proteinases are also present in pineapple fruit. Beneficial therapeutic effects of bromelain have been suggested or proven in several human inflammatory diseases and animal models of inflammation, including arthritis and inflammatory bowel disease (Hale et al 2005). Bromelain is a combination of dissimilar thiol endopeptidases and other components like phosphatases, glucosidase, peroxidases, cellulases, glycoproteins, carbohydrates, and several protease inhibitors (Bhattacharyya (2008) in Hale et al (2005)). Winarno (1995) also states that papain is more resistant to high temperatures than bromelain. To convert more plant-based protein into amino acids requires exogenous enzymes, whereas endogenous enzymes in fish can alter their own animal protein. Optimal absorption of plant and animal protein by catfish will affect in the growth rate (Haetami 2016). The combination of papain and bromelain enzymes is expected to complement each other, and this combination is expected to optimize nutrient absorption by striped catfish (*Pangasianodon hypophthalmus*) especially in protein absorption. This study aims to determine the effect of papain and bromelain enzyme combination toward daily growth rate (DGR) and feed efficiency (FE) in striped catfish fed papain and bromelain crude enzyme extract in commercial feed, along with the effectiveness toward pisciculture production in aquaculture facility.

Material and Method. The research was conducted in March - June 2017 at Floating Fish Cage (FFC) of Fisheries and Ornamental Fish Conservation Centre of Cirata Reservoir - West Java, Indonesia. The identification of enzyme activity was carried out in Organic-Chemical Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The materials used in this study were striped catfish (*P. hypophthalmus*), with average weight 20 gr and length 8-12 cm (fingerling), obtained from BPBIPL Cijengkol - Subang, Indonesia. The treatment was conducted in Floating Fish Cage with a dimension of 1m x 1m x 1m with a stocking density of 15 individual for every treatment. There were 6 (six) treatments in total with 3 repetition each, so in total there were 270 striped catfish used in this research. This refers to SNI No. 01-6483.2-2000 which states that striped catfish at the size of 8-12 cm (fingerling) has a stocking density of 15-25 individual m^{-3}. The feed used in this study was commercial feed (ø 5 mm) with moisture content (12%), crude protein (24-26%), crude fat (5%), crude fibre (6%), ash (10%), calcium (2.5%), and phosphorus (1.5%). Amino acid content in papain and bromelain enzyme activity is presented in Table 1.

Six treatments were performed in 3 repetition, namely: A) artificial feed without additional crude enzyme extract; B) artificial feed with addition of crude papain (5%) enzyme extract; C) artificial feed with addition of crude papain (3.75%) and bromelain (1.25%) enzyme extract; D) artificial feed with addition of crude papain (2.5%) and bromelain (2.5%) enzyme extract; E) artificial feed with addition of crude papain (1.25%) and bromelain (3.75%) enzyme extract; and F) artificial feed with addition of crude bromelain (5%) enzyme extract. The data collected included daily growth rate (DGR) and feed efficiency (FE) measurement.

Table 1

The complete amino acid composition of papain* and bromelain**

Amino acid	*Papain (%)*	*Bromelain (%)*
Lysine	10	4.6
Histidine	2	-
Arginine	12	4.6
Aspartic acid	7	5.5
Asparagine	12	5.5
Glutamic acid	8	6.7
Glutamine	12	3.1
Threonine	8	5.2
Serine	13	8.0
Proline	10	3.7
Glycine	28	8.9
Alanine	14	8.3
Valine	18	5.8
Isoleucine	12	6.1
Leusine	11	3.1
Tyrosine	19	5.5
Phenylalanine	4	4.9
Tryptophan	5	3.1
Cysteine	1	1.8

Source: *Paul et al (2013); ** Ramli et al (2018).

DGR was calculated according to Steffens (1989):

$$DGR = \frac{lnWt - lnWo}{t} \times 100\%$$

Where: DGR = daily growth rate;
W0 = weight of fish biomasss d-0 (g);
Wt = weight of fish biomasss d-t (g);
t = research period (day).

FE was calculated according to Tacon (1987):

$$FE = \frac{Wt - Wo}{f} \times 100\%$$

Where: FE = feed efficiency (%);
W0 = weight of fish biomasss d-0 (g);
Wt = weight of fish biomasss d-t (g);
F = feed consumed during research (g).

Results and Discussion

Daily growth rate. Growth is a change in both the weight and length for a certain period. Physically, growth is a change in body length, weight and width. While from a chemical point of view, the change is seen from the increase in protein, fat, carbohydrates, ash content in water and water content in the fish body (Halver & Hardy 2002). The results of observation showed that different treatment in the feed gave various results. Crude enzyme extracts in feed gave a good response toward the growth of catfish. This can be seen from the increase in the average weight of catfish during sampling (10 days/sampling). The average weight of catfish for each treatment tend to increase with the increasing time (Figure 1).

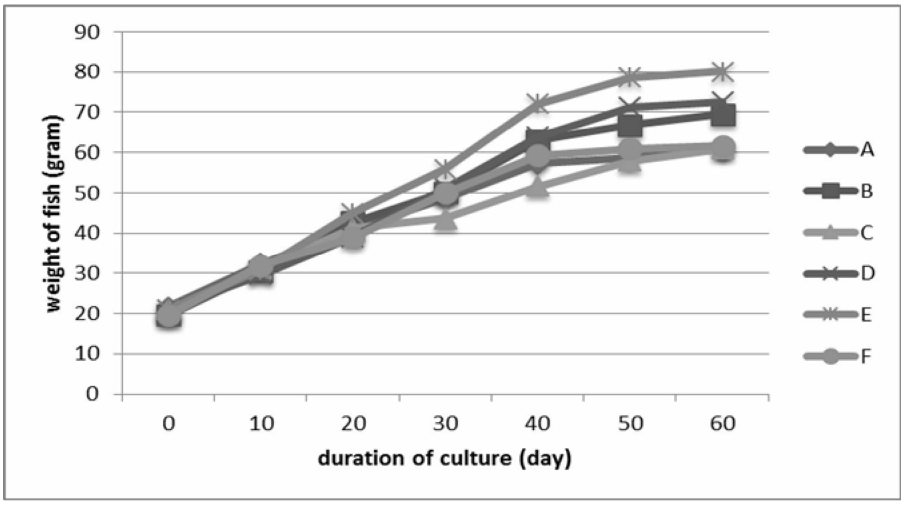

Figure 1. Weight of striped catfish during research period.

Figure 1 shows a rapid increase in DGR. However, the increase in growth starts to slow down in days 50-60. This is allegedly due to fish harvesting activities in double layer net. In day 60, the highest average weight was acquired in treatment E (80.09 g ind^{-1}), followed by treatment D (72.69 g ind^{-1}), treatment B (69.45 g ind^{-1}), treatment F (61.59 g ind^{-1}), treatment C (60.86 g ind^{-1}) and treatment A (60.81 g ind^{-1}).

In Figure 2, it can be seen that the highest daily growth rate of catfish was acquired in treatment E with an average DGR of 2.22%, followed by treatment B of 2.10%, treatment D of 2.05%, treatment F of 1.92%, treatment C of 1.78% and treatment A with the lowest DGR value of 1.72%.

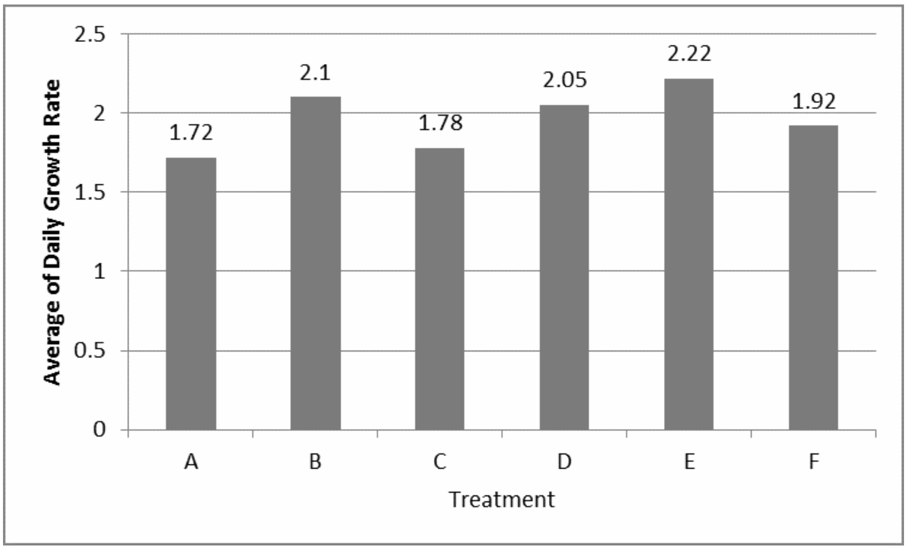

Figure 2. Daily growth rate graphic of striped catfish.

DGR is categorised as good if the growth rate is > 1% (Retnosari 2007). This can be expected since the fish used were in fingerling size with complete digestive tract. According to Effendie (1997), the length of intestine is linear with the growth rate. Therefore, the absorption of protein and nutrients in intestines will also be increased upon given the feed containing of enzymes. The result of analysis of variance showed a significant difference between treatments on catfish growth rate. Duncan Multiple Range Test results showed that catfish which is given the feed mixed with papain and bromelain crude enzyme extract has the highest DGR on treatment E, as well as D and B. Papain and bromelain addition on fish feed is recommended as it gives a positive effect toward DGR. This can be proven by the significant difference ($p < 0.05$) between control and treatment provided with the addition of exogenous enzymes. A study on common carp

(*Cyprinus carpio*) fed diets containing papain and soybean residue resulted in higher growth rates than fish fed with soybean or fish protein alone (Wong et al (1996) in Mo et al (2016)). More recently, it has been observed that adding papain to feed (containing fish meal 10%, soybean oil cake 25%, mustard oil cake 25%, and rice bran 45%) enhanced the growth of *Labeo rohita* (a major Indian carp species) fingerlings (Khati et al (2015) in Mo et al (2016)). The addition of papain and bromelain crude enzyme extracts to fish feed is highly recommended and it gives positive influence on daily growth rate. This has been proven from the significant difference found between the control treatment and the treatments which add certain amounts of exogenous enzyme (Table 2).

Table 2

Daily growth rate of striped catfish during the research period

Treatment	*Daily growth rate*
A (0%)	1.72^{b}
B (papain 5%)	2.10^{a}
C (papain 3.75% and bromelain 1.25%)	1.79^{b}
D (papain 2.5% and bromelain 2.5%)	2.05^{a}
E (papain 1.25% and bromelain 3.75%)	2.22^{a}
F (bromelain 5%)	1.92^{b}

Notes: Value followed by different superscript indicates a significant difference ($p < 0.05$).

DGR value is influenced by the activity of enzymes in fish intestine. According to Sugiyanto (2016), enzyme activity in intestine between treatment A (feed without enzyme addition) and treatment B (1.5% of crude papain enzyme extract) in tilapia showed a lower amount of protease enzyme (4.82 units of activity/mg protein on treatment A, whereas treatment B showed a higher to 5.77 units activity/mg protein). The beneficial effects of enzymes including papain and bromelain were further confirmed forculturing grass carp (*Ctenopharyngodon idella*), using food waste based pellets (mainly consisted of fruits, vegetables and cereals). Results showed better growth and immunity (reflected by total protein, total immunoglobulin and nitroblue tetrazolium activity of fish blood) of fish when enzymes were incorporated into the feeds, than their counterparts fed with commercial pellets, without addition of papain (Choi et al (2016) in Mo et al (2016)).

Feed efficiency. Feed efficiency is a major goal for aquaculture sustainability, and selecting fish to genetically enhance this trait would be highly valuable (Daule et al 2014). FE shows how much feed is used by the fish body, which is the ratio between the weights of feed consumed (Tacon 1987). The result of FE values on 6 (six) treatments can be seen in Table 3. Based on Table 3, the highest FE value in this research is acquired by treatment E by 4.45%, followed by treatment D by 4.32%, treatment B by 4.21%, treatment F by 3.81%, treatment C by 3.69% and treatment A by 3.59%. The addition of papain and bromelain crude enzyme extracts can increase the value of FE which is proven by the lowest FE level on treatment A (without enzyme addition). This is caused by the help of exogenous enzymes in the hydrolysis of proteins, producing more amino acids that will be directly digested by the fish body. In this research, papain and bromelain crude enzymes act as exogenous enzymes.

According to Hastuti (2001), hydrolysis that occurs with proteolytic enzyme is the separation of peptide bond from the substrate bond. Hydrolysis of proteins is carried out and assisted by exogenous enzymes. With papain and bromelain enzyme extract as an exogenous enzyme, the consumed feed can be utilized more efficiently. According to Huet & Timmermans (1970), the high level of feed utilization efficiency shows the efficient utilization of feed that only a few substances are used for energy and the rest are used for growth.

Table 3

Feed efficiency in every treatment

Treatment	Average of feed efficiency (%)
A (0%)	3.59[b]
B (papain 5%)	4.21[a]
C (papain 3.75% and bromelain 1.25%)	3.69[b]
D (papain 2.5% and bromelain 2.5%)	4.32[a]
E (papain 1.25% and bromelain 3.75%)	4.45[a]
F (bromelain 5%)	3.81[b]

The result showed that the highest FE values were acquired by treatment E, D and B respectively. The addition of papain crude enzyme extracts in three treatments gave the optimum enzyme performance compared to other treatments. This is presumably due to the addition of papain and bromelain enzymes which complement each other, giving a significant effect. According to Charley (1982), bromelain enzyme is more active against collagen and can also convert collagen into gelatine. Gelatine usually comes from bone and skin collagen which categorised as animal protein. Meanwhile, papain enzyme is more active in vegetable protein. The highest FE value in treatment E is presumably due to the contribution of bromelain enzyme, which is higher in concentration (3.75%), combined with papain enzyme (1.25%) which complement each other. The combination gives a significant influence on the value of FE. The completeness of essential amino acids in the enzyme may also affect the FE value. Taqwadasbriliani et al (2013) stated that 75% papain and 25% bromelain treatment appear to be the best FU (45.862±0.444%), PER (97.579±0,946%), SGR (1.210±0.037%/day), SR (100%) for 10 grams *E. fuscoguttatus* fry.

Conclusions. Papain and bromelain enzyme combination in fish feed can increase daily growth rate (DGR) and feed utilization efficiency (FUE). The highest daily growth rate of striped catfish in this research is obtained at treatment E by 2.22%, followed by treatment B by 2.10% and D by 2.05%. The best feed utilization efficiency in this research is obtained at treatment E with FUE value of 4.45%, followed by treatment D by 4.32% and B by 4.21%.

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