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The Effectiveness of Experimental Diet with Varying Levels of Papain on The Growth Performance, Survival Rate and Feed Utilization of *Keureling* Fish (*Tor tambra*)

✉**Zainal A. Muchlisin[1], Fardin Afrido[1], Tanzil Murda[1], Nur Fadli[1], Abdullah A. Muhammadar[1], Zulkarnain Jalil[1], Cut Yulvizar[2]**

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[1]Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University Banda Aceh, Indonesia
[2]Department of Biology, Faculty of Sciences, Syiah Kuala University Banda Aceh, Indonesia

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Abstract

The objective of present study was to determine the optimum level of papain in the diet of *keureling* fish (*Tor tambra*). The complete random design was utilized in this study. Six levels of papain dosage were tested in triplicates, i.e. 0 (control); 17.5 mg kg^{-1}, 20.0 mg kg^{-1}, 22.5 mg kg^{-1}, 25.0 mg kg^{-1} and 27.5 mg kg^{-1} of feed. The experimental fish were fed the experimental diet two times a day at 8 AM and 5 PM at feeding level of 5% body weight for 90 days. The Anova test result showed that papain enzyme gave a significant effect on the weight gain, daily growth rate, specific growth rate, survival rate, feed conversion ratio and feed efficiency ($P<0.05$). The Duncan multi-rage test result showed that the higher values for all measured parameters were obtained at the dosage of 27.5 mg kg^{-1}. Therefore, it is concluded that the optimum dosage of papain enzyme for *keureling* fish was 27.5 mg kg^{-1} of feed.

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✉ Correspondence Author:
Jl. Teuku Nyak Arief, Kopelma Darussalam, Syiah Kuala, Aceh, Indonesia
E-mail: muchlisinza@unsyiah.ac.id

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INTRODUCTION

There are at least three species of Tor, or locally names as *keureling* fish, i.e. *T. tambroides*, *T. soro* and *T. tambra*, where the last species is widely distributed in inland waters of Aceh (Muchlisin et al., 2009). However, the population of wild *keureling* fish has been decreasing over the years (Personal communication with local fishermen) and it was probably due to unfriendly fishing practices and habitat degradation. *Keureling* fish is the higher economic value of freshwater fish in Indonesia and it has potency as a target species for aquaculture industry (Muchlisin et al., 2015b); unfortunately, the culture technology especially a commercial breeding has not been well developed. In Aceh Province, *Tor tambra* has been initially cultured and the larvae are harvested from the wild. However, presently this busines is not profitable yet, because the growth performance of *keureling* fish in captive condition was lower. However, Muchlisin et al., (2013b) reported that the growth performance of *keureling* fish (*T. tambra*) in the pond was higher when the fish fed with a commercial feed contains 30% crude protein. The tasks to boost the growth performance of *keureling* fish has been conducted by application of vitamin E in the diet (Muchlisin et al., 2016). However, in general, their growth performance is still relatively low compared to other species, and likely due to the digestibility of feed has not been processed optimally.

The quality of feed is strongly depending on materials used. Therefore, the provision of high quality raw materials is very crucial. In addition, the feed digestibility rate also depends on the availability of digestive enzymes and its activity in the alimentary tract, and one of the important enzyme is proteases. Papain is a hydrolase cysteine protease enzyme contained in papaya (*Carica papaya* and *Vasconcellea cundinamarcensis).* Presently, papain has been extracted commercially and available in the market in dried form with the reasonable price. This enzyme has an important role in protein hydrolysis into amino acids which can be absorbed by the intestine (Muchtadi et al., 1992). Therefore, the addition of the papain into the diet is one of the ways to improve the digestibility of protein in fish feed of *keureling* fish (*T. tambra*).

Application of papain in fish feed has been successfully in increasing of growth performance and feed utilization in several fish species, for example in gourami, *Osphronemus gourami* (Hasan, 2000) and African catfish *Clarias gariepinus* (Amalia et al., 2013). However, the application of papain enzyme into the diet of *keureling* fish (*T. tambra*) has never been conducted. Presently, the main raw materials in the *keureling* fish diet are rice bran, soybean meal, and fishmeal. Rice bran and soybean meal are the plant materials and difficult to digest by fish. Therefore, the study on application of papain into the diet with the important rationale to increase the feed digestibility is crucially needed. Hence, the objectives of the present study were to evaluate the effect of papain enzyme in the diet on growth performance and feed utilization of *keureling* fish and to determine the optimum dosage of papain in the diet for *keureling* fish.

METHODS

Experimental design

The study was conducted in Blang Adei Jaya Hatchery, Meunasah Krueng Village, Nagan Raya district, Aceh Province, Indonesia, from Juni to December 2014. The non-factorial of the complete random design was used in this study; the tested factor is the differences dosage of papain in the diet. The previous studies by Amalia et al., (2013) and Hasan (2000) in gourami and catfish, respectively, showed that the best dosage of for these species is 22.5 mg kg^{-1}. Therefore, the tested dosage of papain in this study was control (0 mg kg^{-1}), 17.5 mg kg^{-1}, 20.0 mg kg^{-1}, 22.5 mg kg^{-1}, 25.0 mg kg^{-1} and 27.5 mg kg^{-1}, every dosage was done in triplicates.

Pond and feed preparation

A ground pond (10m × 26m × 1.2m) was used in this study, the pond was sundried for one week prior filled with the water of 1 meter depth, then a total of 18 cagenets (1m × 1m × 1m) were settled up parallelly in the pond. The papain powders were purchased from the local market in Jakarta.

The experimental diet contains 30% crude protein was formulated using several raw materials as presented in Table 1. All materials were analyzed for proximate composition especially crude protein, crude lipid and ash prior to used. The materials including the papain were mixed homogeneously then the corn oil and water were added into the mixed materials gradually to form the dough and then pelleted using an extruder machine. The diet was sun-dried for 12 hours and the dried diet was kept in a freezer prior to use in the experiment.

Feeding

The fingerling *keureling* fish (*T. tambra*) was

purchased from farmer in Beutong village; the fish sample has average weight of 0.30 g and 3.5 cm of average length. The fish was acclimatized for 2 hours in the pond before stocking into the cagenets at stocking density 15 fish/ hapa. The fish was fed two times a day on 08.00 and 17.00 at feeding level of 5% body weight per day. The weight gain was monitored 10 days interval for 90 days.

Measured parameters

Weight gain

The weight gain was calculated as follow Wg = Wt – Wo, where Wg= weight gain during the study (g), Wo= weight of fish at the start of the study (g), Wt= weight of fish at the end of the study (g).

Daily growth rate

The daily growth rate was calculated as follow: DGR= Wg/t, where Wg= weight gain during the study (g), t= duration time of the study (day)

Specific growth rate

The daily growth rate was calculated as follow, SGR = (Ln W_t – Ln W_o) / t x 100%, where Wo= weight of fish at start of study (g), Wt= weight of fish at the end of study (g), t= duration time of the study (day), Ln= logarithmic of natural.

Survival rate

The survival rate was calculated using the formula as follow, SR= (No-Nt)/No , where SR= survival rate (%), Nt= total fish died during of the experiment, No= total fish at the start of the experiment.

Feed conversion ratio and feed efficiency

Feed conversion ratio and feed efficiency were calculated as follow: FCR= F/Wg, where FCR= feed conversion ratio, F= total of feed intake (g), Wg= weight gain during the study (g). The feed efficiency (FE)= (1/FCR) x 100.

Water quality

Chemical and physical water qualities i.e. water temperature (°C), dissolved oxygen (mg L^{-1}) and pH were measured during the study.

Data analysis

All data were subjected to one-way Anova and followed by the Duncan multi-range test at 95% of confidence level using SPSS ver. 17.0 (Zar, 1984).

RESULTS AND DISCUSSION

The results showed that the weight gain ranged between 0.63 g to 1.97 g, the daily growth rate was from 0.008 g day^{-1} to 0.024 g day^{-1}, specific growth rate ranged from 1.17 % day^{-1} to 2.19% day^{-1} and survival rate ranged from 66.67% to 91.11% (Table 2). The ANOVA test revealed that the papain in the diets gave significant effects on the weight gain, daily growth rate, specific growth rate and survival rate of *keureling* fish ($P<0.05$), where the higher weight gain, daily survival rate, specific growth rate and survival rate were recorded at dosage of 27.5 mg kg^{-1}, these values were significantly different to others dosage. The results also revealed that the feed con-

Table 1. The composition of raw materials used in the diet

Materials (g kg^{-1})	Experimental dosage of papain in fish diets					
	0 mg kg^{-1} (control)	17.5 mg kg^{-1}	20 mg kg^{-1}	22.5 mg kg^{-1}	25 mg kg^{-1}	27.5 mg kg^{-1}
Fishmeal	210	210	210	210	210	210
Soybean meal	200	200	200	200	200	200
Cornmeal	40	40	40	40	40	40
Rice bran	280	262.5	260	257.5	255	252.5
Ebi shrimps flour	205	205	205	205	205	205
Cassava flour	15	15	15	15	15	15
Vitamins mix	15	15	15	15	15	15
Minerals mix	15	15	15	15	15	15
Corn oil	20	20	20	20	20	20
Papain	0	17.5	20	22.5	25	27.5
Total	1000	1000	1000	1000	1000	1000

Table 2. The weight gain, daily growth rate and specific growth rate of *keureling* fish (*Tor tambra*) fed the experimental diet for 80 days. The mean values (±SD) at the same column with similar superscripts are not significantly different (P<0.05).

Papain dosage (mg kg^{-1})	Weight gain (g)	Specific growth rate (% day^{-1})	Daily growth rate (g day^{-1})
0	0.63 ± 0.05^{a}	1.17 ± 0.07^{a}	0.008 ± 0.001^{a}
17.5	0.97 ± 0.01^{b}	1.60 ± 0.04^{b}	0.012 ± *0.*000^{b}
20.0	1.08 ± 0.07^{bc}	1.72 ± *0.*05^{c}	0.013 ± 0.001^{bc}
22.5	1.15 ± 0.04^{c}	1.77 ± 0.03^{c}	0.014 ± 0.001^{c}
25.0	1.55 ± 0.04^{d}	2.04 ± 0.05^{d}	0.019 ± *0.001*^{d}
27.5	1.97 ± 0.15^{e}	2.19 ± 0.01^{e}	0.024 ± *0.002*^{e}

Table 3. The survival rate, feed efficiency, feed conversion ration of *keureling* fish (*Tor tambra*) fed the experimental diet contains papain for 80 days. The mean values (±SD) at the same column with similar superscripts are not significantly different (P<0.05).

Papain dosage (mg kg^{-1})	Survival rate (%)	Feed efficiency (%)	Feed conversion ratio	Protein in the feces (%)
0	66.67 ± 13.33^{a}	28.87 ± 3.70^{a}	3.90 ± 0.42^{c}	16.67
17.5	64.44 ± *10.18*^{a}	37.74 ± 5.34^{b}	2.68 ± 0.40^{b}	16.54
20.0	71.11 ± 13.88^{ab}	38.90 ± 2.07^{b}	2.58 ± 0.13^{b}	12.19
22.5	88.88 ± 13.88^{b}	38.90 ± 1.16^{b}	2.57 ± 0.08^{b}	7.59
25.0	84.44 ± 3.85^{ab}	48.82 ± 4.93^{c}	2.06 ± 0.21^{a}	7.30
27.5	91.11 ± 10.18^{b}	53.44 ± 2.05^{c}	1.87 ± 0.07^{a}	3.98

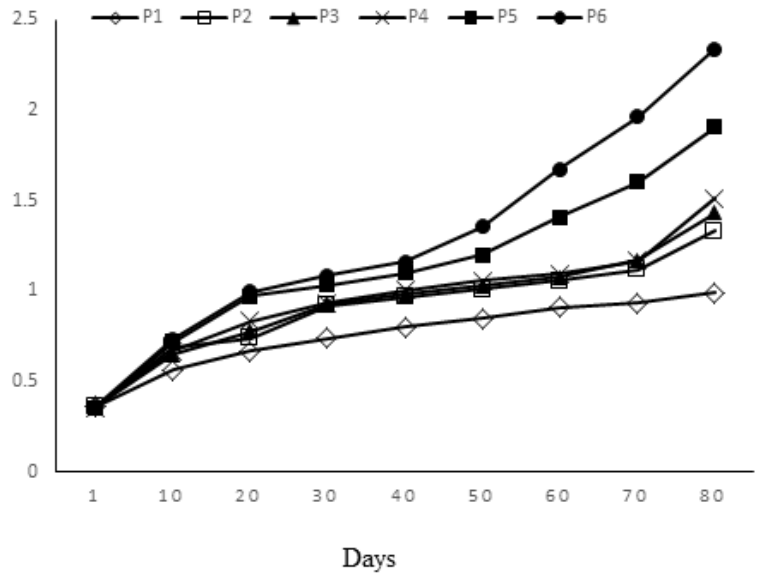

Figure 1. The average of weight of the fish during the experiment. P1= control, P2 = 17.5 mg kg^{-1}, P3 = 20.0 mg kg^{-1}, P4 = 22.5 mg kg^{-1}, P5= 25.0 mg kg^{-1}, P6= 27.5 mg kg^{-1}

version ratio ranged from 1.87 to 3.49 and feed efficiency ranged from 28.87% to 53.44% (Table 3). The Anova test showed that papain enzyme in the diet have significant effect on feed conversion ration (FCR) and feed efficiency (FE) of *keureling* fish (P<0.05) where the best FCR and FE were recorded at dosage of 27.5 mg kg^{-1}, however, these values were not significantly different with dosage of 27.5 mg kg^{-1}.

The growth trends of the fish during the

study showed that the growth rates at day-1 to day-30 were slow, and the growth rates were sharply increased from day-40 to day-80 where the best growth performance was recorded at the dosage of 27.5 mg kg^{-1} (Figure 1). The dissolved oxygen ranged from 6.2-6.8 mg $L^{-1,}$ water temperature ranged from 28.2 to 28.8 ^{O}C and the average pH was 6.4, indicates the physical and chemical water parameters during the study were suitable for fish.

The study revealed that the growth performance, survival rate and feed utilization were increased with increases of papain dosage treatment in the diet where 27.5 mg kg^{-1} was the best dosage for all measured parameters, it indicates that the feed digestibility was optimal at this dosage. The higher feed digestibility can be detected by the protein content in the feces where lower protein content was recorded at the dosage of 27.5 mg kg^{-1} (Table 3). This indicates that most of the protein can be digested by the fish at this dosage. Steffens (1989) stated that the higher the digestibility of a feed mean more nutrients was utilized by the fish. A similar result was also reported in goramy (*O. gourami*) where the lower protein feces were recorded at papain dosage of 27.5 mg kg^{-1} (Hasan, 2000). The higher protein digestibility was probably due to to the presence of papain enzyme (protease group) at the suitable dosage, it causes the protein hydrolysis into amino acids form can be occurred optimally.

Based on data, it showed that the higher survival rate was also recorded at fish fed on experimental diet with 27.5 mg kg^{-1} papain (91.11%), but this value was not significantly different with 20.0 to 25.0 mg kg^{-1}. We speculated that the survival rate of the *keureling* fish in this experiment was not directly associated with feeding, but it was strongly correlated to parasite infested. Our direct observation on dead fish showed that most of the fish have been infected by *Lernea* sp. dan *Argulus* sp. The infestation of *Lernea* sp., *Argulus* sp. and tapeworm, *Bothriocephalus acheilognathi* on *keureling* fish have been reported by Muchlisin et al., (2014) and Muchlisin et al., (2015a), unfortunately until now no treatment was suggested to overcome this problem. In general, the survival rate is affected by internal and external factors such as sex, age, reproductive activity, diseases, water quality, stocking density and nutrition (Hepher, 1988).

The similar trends were also recorded for feed efficiency and feed conversion ratio where the best results were recorded at papain dosage of 27.5 mg kg^{-1} while the lower result was found at fish fed on control diet. The higher feed efficiency and lower feed conversion ratio indicate that the application of papain enzyme in the diet was successfully improving the feed utilization of *keureling* fish. According to Steffens (1989) feed efficiency is an indication of feed utilization by fishes, where high efficiency and low feed conversion ratio indicating the feed has digested and absorbed optimally by the fish for their growth. The study indicated that the application of papain at the dosage of 2.75 mg kg^{-1} can reduce feed conversion ratio from 3.90 to 1.87, it means that the feed intake can be declined approximately 52%.

CONCLUSION

The study revealed that application of papain in the diet has the significant effect on growth performance, feed utilization and survival rate of *keureling* fish (*T. tambra*) where the values of all parameters except the feed conversion ratio were increased with increases of papain dosage in the diet and the best dosage of papain for *keureling* fish was 27.50 mg kg^{-1}.

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