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Addition of *Gryllus bimaculatus* flour in commercial feeds to retention of protein and energy of *Oreochromis* Sp.

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ABSTRACT

The popularity of red nile tilapia (*Oreochromis* sp.) in aquaculture commodities does not compensate forthe cost of its feed. Therefore, the provision of alternative artificial feed that utilizes the availability of local ingredients, effective and efficientis needed. This study aimed to determine the potential effect of two-spotted cricket (*Gryllus bimaculatus*) flour on commercial feed to increase energy and protein retention of red nile tilapia (*Oreochromis* sp.). This was an experimental study with a completely randomized design, consisting of five treatments and four replications. The addition of two-spotted cricket flour was given in several commercial feeds with different doses; P0 (0%), P1 (2% cricket flour), P2 (4% cricket flour), P3 (6% cricket flour), and P4 (8% cricket flour). The parameters observed were protein retention and energy retention. The data were analyzed usingvariance analysis (ANOVA) and Duncan's Multiple Distance Test. The results showed the addition of two-spotted cricket on feed for 30 days had a significant effect ($P<0.05$) on protein rand energy retention of red nile tilapia (*Oreochromis* sp.). The highest protein retention was in P4 (83.03%) and the lowest was in P0 (57.04%). Whereas, the highest energy retention was in P4 (5.5174%) and the lowest was in P1 (2.1590%). The addition of two-spotted cricket (*Gryllus bimaculatus*) on commercial feed with a dose of 8% can increase protein retention and energy for red nile tilapia (*Oreochromis* sp.)

Key words : *Fish feed addition, Two-spotted cricket (Gryllus bimaculatus), Red nile tilapia (Oreochromis sp), Protein retention, Energy retention*

Introduction

The commodity of red nile tilapia (*Oreochromis* sp.) is one of the most famous aquaculture consumption commodities, due to its taste, affordable price, and as a source of animal protein (Siddiqui and Al-Harbi, 1995; Zonneveld and Fadholi, 1991). In addition, tilapia also has the advantage of easy to breed, fast growth, and tolerant of environmental conditions (Soegianto *et al.*, 2017; Zainuddin *et al.*, 2017). The popularity of *Oreochromis* sp. is also quite significant particularly in Indonesia. The production of *Oreochromis* sp. aquaculture in Indonesia has increased significantly every year, with volume of 567,078 tons in 2011 to 695,063 tons in 2012 and 909,016 tons in 2013 (Budidaya, 2014). One of the activities that support the intensive cultivation of *Oreochromis* sp. is the feeding. Fish farming is strongly influenced by the availability of sufficient food, in quantity and quality, to support its maximum quality (Goddard, 1996). Feed costs in fish farming can reach a high ratio of the total production costs (Shepherd and Jackson, 2013). Therefore, effective and efficient feed management is needed,

to provide alternative artificial feed that utilizes the availability of local ingredients, which are expected to have good nutritional quality.

The nutritional requirement in fish feed, that is enough to accomodate the energy and protein needs of the fish, can be obtained based on calculations of its protein and energy retention. Since the cost of feed for tilapia is quite expensive, insects flour is considered to be a source of protein and energy that can be used as an addition for making feed to reduce the cost. Insects have high potential as a alternative protein source in regards to fish diet since they are natural feed for freshwater and marine fish (Howe *et al.*, 2014) that can also be cultured under different environmental conditions (Premalatha *et al.*, 2011) also require less maintenance and particular large area (Rumpold and Schlüter, 2013). The previous study has reported that the level of essential amino acid (EAA) of five insects were quite similar with soybean protein (Yi *et al.*, 2013). Another study that investigated five species insects has found that crickets might seems superior from a nutritional perspective compared to conventional animal meats and and chicken eggs (Ghosh *et al.*, 2017). The popularity of insects in the use of fish diet has also developed significantly such as in the diet of juvenile fish and crustacean (Riddick, 2014), therefore the use of insects in fish diet can be implemented to reduce high cost production and as significantly alternative protein in addition use specifically two-spotted cricket (*Gryllus bimaculatus).*

Two-spotted cricket is type of insect that is easily cultivated and has the potential development, so that it can be used as a fish feed ingredient (Taufek *et al.*, 2018). Cricket can be used as fodder for birds and fish, in the form of fresh or flour due to its high levels of protein and fat found in cricket, each of which ranges between 55-73% and 11-12% (Barroso *et al.*, 2014; Ghosh *et al.*, 2017; Taufek *et al.*, 2018). Previous studies have investigated crickets as a formulated diet of fish in *African catfish fingerling* (Taufek *et al.,* 2018). However, the number of studies that uses crickets in formulated diet for fish is still minimal eventhough these insects give promising results. Hence, this study is aimed to determine the potential addition of two-spotted cricket (*Gryllus bimaculatus*) flour in the commercial feed on protein and energy retention of red nile tilapia (*Oreochromis* sp.).

Methods

Preparation of *Oreochromis* sp. and rearing conditions

The experimental animals used in the study were *Oreochromis* sp. with size of 7-10 cm long obtained from the Technical Implementation Unit for the Development of Umbulan Freshwater, Pasuruan, Indonesia. The maintenance media used in this study is PDAM (local water company) water. The feed used was commercial feed mixed with *Gryllus bimaculatus* flour obtained from cultivators in Kediri, Indonesia, and tapioca flour as an adhesive. Red nile tilapia (*Oreochromis* sp.) was acclimatized in advance. Each aquarium was filled with 40 liters of water with a density of 10 *Oreochromis* sp. and given with aeration.

Preparation of experimental diets

This study was an experimental method with a completely randomized design (CRD). The variables in this study were: independent variables, namely the administration of *Gryllus bimaculatus* flour in commercial feed with doses of 0%, 2%, 4%, 6%, and 8%, dependent variables, which were protein retention and energy retention in red nile tilapia; and control variables, which were red nile tilapia, age of red nile tilapia, aquarium size, temperature, ammonia, DO and pH. This study used 5 types of treatments with 4 replications. The treatments were:

Treatment P0: 100% commercial feed

Treatment P1: 98% commercial feed + 2% *Gryllus bimaculatus* flour

Treatment P2: 96% commercial feed + 4% *Gryllus bimaculatus*flour

Treatment P3: 94% commercial feed + 6% *Gryllus bimaculatus* flour

Treatment P4: 92% commercial feed + 8% *Gryllus bimaculatus* flour

Each treatment was added with 1% tapioca flour which has a function as a binder or adhesive. Nutritional content of experimental feed ingredients can be seen at Table 1.

Water Quality

The value of the temperature during the study ranged from 25.4-33.1 °C (Kungvakij and Pudadera, 1984) while the water acidity (pH) during research showed the value of 7. The water acidity (pH) that

Table 1. Nutritional content of experimental feed ingredients

Feed Ingredients	Dry Ingredients	Crude Protein (%)	Fat (%)	Crude Fiber (%)	Ash (%)	BETN (%)
Commercial feed	89.5180	32.228	8.1361	0.5809	0.1596	86.4676
Gryllus bimaculatus	88.0487	47.6103	13.7992	14.9628	5.1242	6.5522
Tapioca flour	89.5180	1.7361	0.5738	0.5809	0.1596	86.4676

suitable for *Oreochromis sp.* is 5.5-7.5 (Yildiz *et al.*, 2017). The results of Dissolved Oxygen (DO) measurement during the study ranged from 4.05-5.35 mg/L (Yildiz *et al.*, 2017). The concentration of ammonia in water is 0-1 mg/L.

Analytical Procedure

The groups of *Oreochromis* sp. were maintained in aquariums with the size of 50x30x40 cm^3 as many as 10 fish per aquarium for 30 days. Fish feeding was carried out twice a day at 08.00 and 16.00 with feeding rates as much as 5% of the average body weight of*Oreochromis* sp. Protein retention and energy retention were obtained from the proximate analysis of *Oreochromis* sp.meat after a maintenance period of 35 days. Protein retention (PR) and energy retention (ER) are calculated using the formula (Tung and Shiau 1991):

$$RP = \frac{(\text{Final body protein weight} - \text{Initial body proten weight})g}{(\text{Total feed protein given})\ g} \times 100\%$$

$$RE = \frac{(\text{Final body energy} - \text{Initial body energy})\ kcal}{(\text{Total feed energy given})\ kcal} \times 100\%$$

Statistical Analysis

The data were analyzed using Variant Analysis (ANOVA) and Duncan's distance test with a confidence level of 0.05.

Results

The results of protein and energy retention in *Oreochromis* sp. for 30 days can be seen in Table 2 and Figure 1. The addition of *Gryllus bimaculatus* flour in the commercial feed had significant differences ($p < 0.05$) between each treatment. The mean retention of *Oreochromis* sp. protein of P4 was significantly different with P0, P1, P2, and P3. While P0 was not significantly different with P1, P2 and P3, but it had significant differences to P4 using an 8% dose of *Gryllus bimaculatus* flour. Whereas, the results of variance analysis (ANOVA) showed a significant difference ($p > 0.05$) between each of the *Gryllus bimaculatus* flour addition treatment on commercial feed. The results of the mean energy reten-

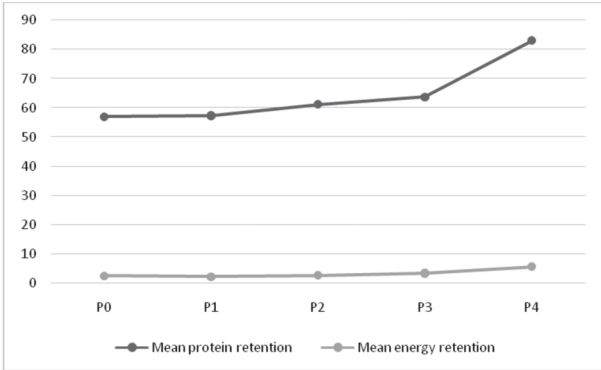

Fig. 1. Mean protein and energy retention results

Table 2. Mean results of protein and energy retention in *Oreochromis* sp.

Treatments	Protein retention ± SD	Protein retention transformation ± √y + 0.5	Energy retention ± SD	Energy retention transformation ± √y + 0.5
P0 (0%)	57.04^{a} ± 7.636	7.57 ± 0.508	2.4277^{ab} ± 0.240	2.055 ± 0.077
P1 (2%)	57.36^{a} ± 6.825	7.60 ± 0.452	2.1590^{a} ± 0.123	1.970 ± 0.042
P2 (4%)	61.23^{a} ± 4.076	7.85 ± 0.261	2.5624^{ab} ± 0.922	2.100 ± 0.027
P3 (6%)	63.69^{a} ± 9.415	8.00 ± 0.573	3.3015^{b} ± 0.726	2.307 ± 0.202
P4 (8%)	83.03^{b} ± 10.252	9.13 ± 0.727	5.5174^{c} ± 1.012	2.842 ± 0.221

Note: Different superscripts in the same column show significant differences ($p<0.05$)

tion of *Oreochromis* sp. indicated that P4 was significantly different from P0, P1, P2, and P3, while between treatments P0, P1, P2 and P3 there was no significant difference.

Discussion

Gryllus bimaculatus flour as feed addition to the energy and protein retention of *Oreochromis sp.* showed a significant difference (p> 0.05) Utilization of *Gryllus bimaculatus* flour as a feed addition for *Oreochromis* sp. can increase the value ofenergy and protein retention. The result showed that the groups with *Gryllus bimaculatus* flour as feed addition were better than the control group. This study revealed that the *Gryllus bimaculatus* flour can be utilized commercially as a provided feed for *Oreochromis* sp., so that theweight of fishes can increase because the fish can digest and absorb the feed optimally.

The result showed the highest mean of protein retention in *Oreochromis* sp. was in treatment P4, with 8% *Gryllus bimaculatus* flour as feed addition on the commercial feed with the amount of protein as much as 25.1436%. This study revealed that feeds with a protein content of 25.1436% in *Oreochromis* sp. had a protein retention value of 83.03%. It can be interpreted that every 25.1436% of protein, 0.208 g from total feeds given (0.8303 g) can be digested by *Oreochromis* sp. for its growth. The protein content in treatment P4, which is 25.1436%, showed thatthis treatment (8% *Gryluss bimaculatus flour*) was suitable for the needs of *Oreochromis* sp. so that it can be optimally digested by *Oreochromis* sp. The use of feed with a protein content that is suitable to the needs and with the optimum amount will promote the new tissue formation and increase the growth rate (Schulz *et al.*, 2008; Wang *et al.*, 2006). The lowest protein retention was in treatment P0, the feed without *Gryllus bimaculatus* flour had a protein content of 20.833%. If protein synthesis is low, it can reduce the protein content that is stored in the body. While non-protein energy and nutrient are not enough to accomodate the needs of the fish, the protein will be used as an energy source, as a result the function of protein as tissue repair will be disrupted. However, the protein content in the feed is considered too high that only a portion that will be absorbed and used to form or repair the damaged cells (Winfree and Stickney, 2015).

The energy use is influenced by the amount of feed that consumed by the fish. Energy is obtained from reforming chemical bonds through the process of oxidation reactions to feed components, which are proteins, fats and carbohydrates into simpler compounds (amino acids, fatty acids, and glucose) so that they can be absorbed by the body to be used or stored(FAO 1980). The results of mean value of energy retention ranged from 2.1590% to 5.5174%. The variant analysis (ANOVA) showed a significant difference (p<0.05) between each treatment for *Gryllus bimaculatus* flour in the feed. This showed the use of *Gryllus bimaculatus* flour as addition in commercial feed for *Oreochromis* sp. can increase the energy retention better than feed without any addition of *Gryllus bimaculatus* flour.

The highest energy retention in the treatment P4 of 5.5174% indicated that the 8% *Gryllus bimaculatus* flour was suitable for the needs of *Oreochromis* sp. and it could be optimally absorbed by the *Oreochromis* sp. Although the feeds with a proportional energy-protein ratio will increase the fish growth (Wang *et al.*, 2006), not all of the energy source can be digested and utilized for growth (Niagara *et al.*, 2018). The energy source will be used first for activity, metabolism, and growth needs. Then the rest will be used to be stored in the body.

Conclusion

The study has successfully demonstrated the 8% *Gryllus bimaculatus* flour doseas feed addition can increase energy and protein retention in *Oreochromis* sp.

Ethical clearance

The present studywas carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

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