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FEED EFFICIENCY AND GROWTH OF CATFISH (*Clarias* sp.) FED WITH THE ADDITION OF IMMUNE-BOOSTING FERMENTED EARTHWORMS

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ABSTRACT

A fermented earthworm (FEW) is reported to be an alternative to an immune-modulator feed additive in catfish. However, the effects on growth and feed efficiency have not been reported yet. The present study aimed to evaluate the effects of fermented earthworms (FEW) on the growth and feed efficiency of catfish (*Clarias* sp.). A feeding trial was conducted in a completely randomized design with five treatments of diet in quadruplicate. The trial was conducted for 78 days. The observed parameters comprised of survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), and total biomass. The results revealed that the FEW at up to 5% did not affect ($P>0.05$) the growth rate of catfish, but FEW gave a negative effect on the growth rate ($P>0.05$) at the addition rate higher than 5%. The highest growth rate was found at the addition rate of 2.5%. FEW also did not affect the survival rate (SR), FCR, PER, and total biomass ($P>0.05$). This study successfully confirmed that FEW could be used as an alternative to immuno-modulator ingredient without any negative impact on the growth of catfish when FEW was added to the feed at as high as 5%. These findings give a new perpective in utilizing FEW as a fuctional aqua-feed ingredient to increase immune response without alteration of the fish growth.

KEYWORDS: protein efficiency ratio; aquafeed; fermented worms; catfish; feed conversion ratio

INTRODUCTION

Catfish (*Clarias* sp.) is among the most cultivated freshwater fish worldwide, particularly in Southeast Asia and Africa. In 2020, the global production of catfishes reached 6,005,608 tons (FAO, 2022). Catfish production has contributed a significant portion to global aquaculture production. However, the main constraint of catfish aquaculture is the high cost of the feed. Feed in intensive aquaculture, including intensive catfish culture, expenses 70-80 % of the total business costs (Kawamoto *et al*., 2019).

Earthworms are considered among the feed ingredients with the highest content of protein (up to 70%); worm also contains carbohydrates, fat, and ash contents of 17%, 4.5%, and 1.5%, respectively (Taris *et al.,* 2018). When juxtaposed to each other, the Essential Amino Acids Indexes (EAAIs) of fish meal and unprocessed earthworms are 58.67% and 21.23%, respectively (Istiqomah *et al.,* 2009). Being rich in nutritional contents, earthworm meal has been used to replace fishmeal as the source of protein in feed for ornamental fish, consumption fish, or shrimps (Boaru *et al.,* 2016; Lourdumary *et al*., 2013). However, high content of protein is not the sole factor of a good feed to support fish growth. The types and proportions of amino acids also play significant roles.

A fermentation process can decompose complex nutrient compounds into simple ones. The process allows the nutrients to be easily digested and absorbed by the fish's digestive tracts. The working principle of fermentation is to break down hard-to-digest components, such as complex protein, into simple amino acids with the help of microorganisms (Sanjukta & Kumar, 2016). In similar process, fermented earthworm does not only contain highly digestible nutrients. It also able to increase the non-specific immune response of catfish through the improvement of respiratory burst, hematocrit, phago-

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cytic activity, phagocytic index, superoxide dismutase, and leucocyte differentiation (Nugraha *et al*., 2022). . Despite the advantage of FEWs as an immune-modulator improvement in catfish, earthworms were reported to gave a negative impact on fish growth. The use of earthworms (*Eisenia fetida*) as an aquafeed ingredient might decrease the growth rate of fish. This effect is due to chitin and foul-smelling coelom fluid in earthworms that lower the digestibility and palatability of feed when mixed with it (Musyoka *et al*., 2019). The feed formulation of earthworm meal, soybean waste, and chicken gut at a ratio of 25%:25%: 5.95% provides the optimum protein content. However, the effect of feed supplemented with fermented earthworms on fish growth rate has not been reported particularly for catfish. Therefore, it is urgent to study the utilization of fermented earthworms for an aquafeed ingredient to improve the fish growth. This study intends to analyze the effects of FEW addition in feed on the survival rate (SR), specific growth rate (SGR), feed conversion rate (FCR), feed efficiency (FE), and protein efficiency ratio (PER).

MATERIALS AND METHODS

Fermentation of Earthworm

Earthworms used in the study were purchased locally in Bantul Regency, Yogyakarta, Indonesia. They were then cleaned from the soil and organic fertilizer which were used as the culture media. One kilogram of the earthworms was processed using a blender (10,000 rpm for 5 min) to obtain juice before being placed into 2 liters fermentation bottle. The next process was adding 45 ml of molasses and 5 g of a bacterial mixture containing *Bacillus* sp. (T2A), *Bacillus* sp. (T3PI), and *Lactococcus raffinolactis* (JALI) with a total density of 9×10^4 CFU/g. The bacteria have been proven as non-pathogenic bacteria, and produce useful enzymes such as proteolytic, cellulolytic, and lipolytic enzymes (Nugraha *et al.*, 2022). The container was then closed tightly and incubated using a shaker at room temperature for four days.

Feeding Experiment

A total of 1,000 catfish fingerlings (*Clarias* sp.) sized 8-10 cm in total length was obtained commercially from a hatchery in Sidoluhur, Godean, Sleman Regency, Yogyakarta, Indonesia. First, the fingerlings were acclimatized in the cultivation container for a week. Then, the fingerlings were placed in 20 fiberglass containers sized 50 cm x 50 cm x 60 cm at a stocking density of 50 fish/container previously filled with aerated clean water set at 40 cm of water depth. The fingerlings were reared for 78 days with a daily feeding rate of 5% of total weight. The length and weight of fingerlings as well as water quality parameters were measured every 26 days. The fish length was measured using a 30 cm ruler, while the fish weight was measured using the Ohaus digital Scale (NNVT1601/3) with 0.01 g accuracy.

Research Design

The study's experimental design used a Completely Randomized Design containing five treatments with quadruplicates. The treatments were: T1 = Control treatment (without fermented earthworm).

T2 = 2.5% addition of fermented earthworm
T3 = 5.0% addition of fermented earthworm
T4 = 7.5% addition of fermented earthworm
T5 = 10% addition of fermented earthworm

Feed Composition/Experimental Feed

The experimental diets were composed based on the report of Nugraha *et al*. (2022). The complete ingredient mixtures were processed using a pellet machine to produce pellets of 4-6 mm length and 2-3 mm diameter. The feed pellets were dried in an oven at 60°C for 24 hours. The feed was then analyzed proximately andstored in a refrigerator until use.

Proximate Analysis

Dietary protein, ash, crude fiber, and fat contents were determined using the Micro Kjeldahl method, ashing method in a muffle furnace (Furnace), hydrolysis method of acid and strong base, and Soxhlet method, respectively (AOAC, 2005). The proximate composition of experimental diets is presented in Table 1.

Parameters observed and Data Analysis

Specific Growth Rate (SGR)

A formula by Zonneveld *et al*. (1991) was employed to calculate the parameter of the fish-specific growth rate.

The specific growth of length was calculated using the following formula:

$$SGR = \frac{\ln Lt - \ln Lo}{t} \times 100\%$$

Moreover, the specific growth of weight was calculated using the following formula:

$$SGR = \frac{\ln Wt - \ln Wo}{t} \times 100\%$$

Table 1. Proximate composition and energy content of the experimental diet with the addition of FEW at various dosages (% DM)

Proximate composition of experimental feeds	Percentage (%)				
	T1 (0%)	T2 (2.5%)	T3 (5%)	T4 (7.5%)	T5 (10%)
Ash (%)	13.581	13.316	13.218	14.180	17.902
Protein (%)	27.320	26.569	26.309	26.007	25.118
Fat (%)	8.942	8.246	8.217	9.174	10.137
Crude Fibers (%)	2.209	2.784	3.047	7.649	7.485
Carbohydrates (%)	47.948	49.084	49.209	42.991	39.358
Calories (cal/100 g diet)	381.55	376.826	376.025	358.558	349.137

* Calories were calculated by multiplying protein and carbohydrate contents by 4 cal/g, and fat contentby 9 cal/g.

Where:
SGR = Specific growth Rate
Wo = Initial weight of fish (g)
Lo = Initial length of fish (cm)
Wt = Final weight of fish (g)
Lt = Final length of fish (cm)
t = Feeding experiment period (day)

Survival Rate (SR)

The fish survival rate was calculated based on the numbers of alive fish at the end of the rearing period compared to the initial numbers of fish stocked at the beginning of the experiment. The following formula was used:

$$SR = \frac{Nt}{No} \times 100\%$$

Where:
SR = Fish survival rate (%)
N = Total fish at the end of the raising period (g)
No = Total fish at the beginning of raising period

Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated using the formula by Zonneveld *et al*. (1991).

$$FCR = \frac{F}{(Wt + D) - Wo}$$

Where:
FCR = Feed conversion ratio
F = Amount of feed given during research (g)
D = Total weight of dead fish during the research (g)
Wt = Total final biomass of fish (g)
Wo = Total initial biomass of fish (g)

Protein Efficiency Ratio (PER)

The formula by Zonneveld *et al*. (1991) was applied to calculate the protein efficiency ratio.

$$PER = \frac{Wt - Wo}{pi} \times 100\%$$

Where:
PER = Protein efficiency ratio (%)
Wt = Total weight of fish at the end of raising period (g)
W0 = Total weight of fish at the beginning of raising period (g)
Pi = Weight of protein in the feed consumed by the fish (g)

Statistical Analysis

A One-Way analysis of variance (ANOVA) was employed to analyze the effect of FEW feed additive on survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), the protein efficiency ratio (PER), and total biomass. A post-hoc of the Tukey test was carried out.

RESULTS AND DISCUSSION

ANOVA results highlighted that the addition of FEW did not affect ($P>0.05$) the growth of total fish length. On the other hand, the addition of FEW yielded a significant influence ($P<0.05$) on the fish weight growth. Feed treatment containing up to 5% of FEW had no effect on the weight growth of the treated fish, but the addition of FEW at higher levels to the feed decreased the weight growth (Figure 1). The previous report on the same diet indicates that the FEW levels of at least 2.5% improved the immune status of catfish (Nugraha *et al*., 2022). These results indicated that the addition of FEW up to 2.5% is the most effective diet composition to improve immune status without any negative impact on the catfish growth. These results are also supported by the FCR exhibiting the lowest FCR at this level (Table 2).

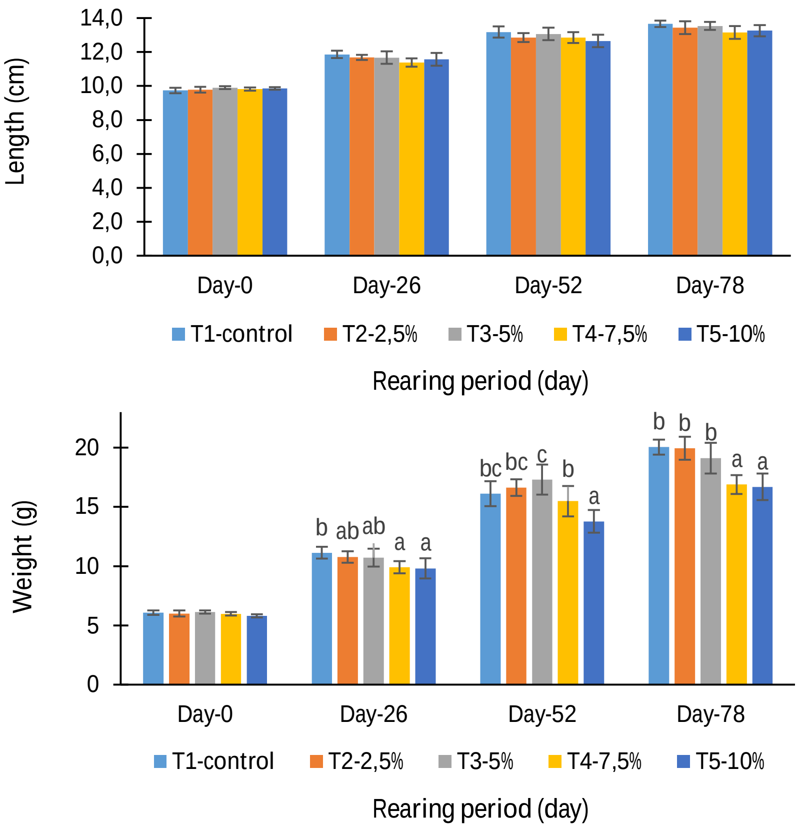

Figure 1. Length and weight growth of the catfish fed with experimental diet at various addition rates of FEW.

Although total biomass was not significantly different among treatment diets, the highest biomass was also reached by the diet with a 2.5% of addition level. In accordance with the FCR, the 2.5% addition level also did not affect the Protein Efficiency Ratio (PER) significantly. The protein absorbed by the fish is essential for the basic metabolism process, maintaining the cells, and promoting growth (Maryam *et al*., 2019). According to Halver & Hardy (2002), protein is the source of energy in fish. Therefore the energy balance by the content of fat and carbohydrate is essential for the PER in fish. The highest growth rate of the weight of catfish fed with 2.5% FEW addition is supposed that the FEW increase the absorption and utilization of nutrients.

The analysis of variance (ANOVA) indicated that the application of FEW did not affect the specific growth rate of length ($P>0.05$). The highest specific length growth was exhibited by the T1 treatment (the

Table 2. The survival rate, FCR, biomass, feed efficiency, and protein efficiency ratio of catfish feeding with various addition rates of FEW ($P>0.05$)

Treatment	SR[1]	FCR[2]	Biomass[3]	Per[4]
T1 (Control diet))	68.5±12.8[a]	1.14±0.09[a]	685.2±119.9[a]	2.24±0.62[a]
T2 (2.5 %)	74.0±7.1[a]	1.07±0.04[a]	736.4±63.3[a]	2.64±0.39[a]
T3 (5.0%)	71.0±10.4[a]	1.35±0.19[a]	677.9±108.7[a]	1.95±0.58[a]
T4 (7.5%)	81.0±14.3[a]	1.32±0.30[a]	687.0±143.5[a]	2.54±0.84[a]
T5 (10%)	72.0±10.7[a]	1.07±0.06[a]	602.790±111.9	2.38±0.76[a]

[1] Survival rate (%)
[2] Feed conversion ratio (%)
[3] Biomass (g)
[4] Protein efficiency ratio (%)

control treatment), while the T4 treatment (addition of 7.5% FEW) showed the lowest specific length growth. In contrast, the specific growth rate of weight was significantly affected by the addition of FEW ($P<0.05$). This growth rate ranged from 1.330 ± 0.081 - 1.538 ± 0.059 %/day at the end of the experiment (Figure 2). The addition of FEW at 5% did not decrease the SGR of weight, but the addition at higher levels gave a negative effect on the SGR of weight (Figure 2).

The survival rate of the fish ranged from 68.5 ± 12.79 to 81.0 ± 14.28%. The ANOVA test indicated that the addition of FEW did not affect the survival rate ($P>0.05$). The addition of FEW at 7.5% gave the highest SR, while the lowest one was exhibited by the control treatment (Table 2).

Meanwhile, the feed conversion ratio (FCR) in this study showed no significant difference among the treatments ($P>0.05$). The lowest FCR of 1.07 ± 0.04% was obtained by T2 treatment (2.5% addition of FEW), while the highest FCR of 1.35 ± 0.19% was exhibited by T3 treatment (5% addition of FEW) (Table 2). The feed conversion ratio (FCR) refers to the correlation between growth rate and feed efficiency. Lower FCR indicates higher efficiency and increases growth. Inline, Mohanta *et al*. (2016) reported almost similar FCR on the use of earthworms (*Eisenia foetida*) as a source of protein for Rohu (*Labeo rohita*).

The biomass of fish harvested from each tank varied ranging from 602.8 ± 111.9 g to 736.4 ± 63.3g (Table 2). The highest and the lowest biomass values were at 2.5% and 10% of FEW, respectively. However, the ANOVA resulted in no significant difference among the treatment ($P>0.05$).

The protein efficiency ratio (PER) in this study was not affected ($P>0.05$) by the treatment diet with a range of 1.95 ± 0.58-2.64 ± 0.39. This finding is relatively high in comparison to the PER reported by Olele

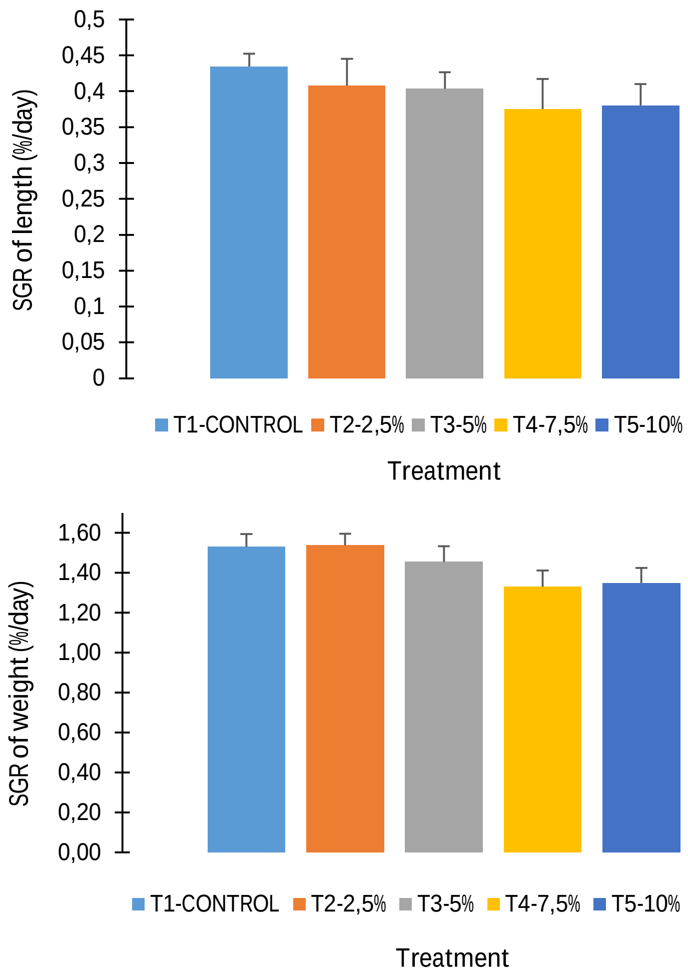

Figure 2. Length and weight-specific growth of the catfish fed with experimental diet at various addition rates of FEW for 78 days of feeding period.

(2011) in *Heteroclarias* fingerlings fed with worm meal at a 50% substitution rate to fish meal resulting PER value of 0.604. Moreover, Mohanta *et al*. (2016) reported PER of 1.26 in rohu fish (*Labeo rohita*) fed with earthworms as the main protein source in the diet. Meanwhile, PER of 1.29 ± 0.08 was obtained by Vital *et al.* (2016) by substituting 50% of fish meal with earthworm meal for *Parachanna obscura* fingerling. However, by using worm meal and maggot meal in a 2:5 ratio for *Clarias gariepinus* fingerling, Djissou *et al*. (2016) reported an almost similar PER to that obtained in this study, viz. 2.47 ± 0.06. Such varied findings indicate that FEW protein is able to increase fish growth and feed digestibility.

CONCLUSIONS

The addition of FEW up to 5% did not affect the fish-specific growth rate, weigth gain and feed efficiency. However, the higher addition level of FEW decrases the weight gain and SGR of weight. The addition of 2.5% FEW is considered as the most appropriate dosage to promote fish growth and the feed efficiency.

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest/competing interest

Ethical Approval Statement

All guidelines for the care and use of animals/fish were followed

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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