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Effect of Silk Worms (*Tubifex* sp.) Feed On Production Performance, Nutrition, And Gills Histology of Striped Catfish Fingerlings (*Pangasionodon hypophthalmus*)

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Abstract

Natural feed has emerged as an alternative to support production performance and the nutrients contained in fish meat. This study aimed to determine the effect of feeding silk worms (*Tubifex* sp.) in improving production performance, nutritional content, and gill histology of striped catfish fingerlings (*Pangasionodon hypopthalmus*). Silkworms were sourced from different origins, namely Group P1 (silkworms with dry food media), Group P2 (commercial silkworms), and Group P3 (wild silkworms). Silkworms were reared for 55 days and fed to catfish seed for 25 days ad libitum. Feeding had no significant effect on absolute length growth, total biomass, and feed conversion with worm feeding treatment (P1) with values respectively 2.3 ± 0.10%, 6.0 ± 0.38%, and 4.2 ± 0.47%. Likewise, the results of growth rate, weight, and length, group P1 had better results compared to treatments (P2) and (P3) but were not significantly different. The best protein efficiency ratio resulted from treatment (P1) of 0.42 ± 0.25%. Gill histology changes were shown in groups P2 and P3, namely hemorrhage and congestion. Based on these results, silkworms reared in dry media can be an effective natural food for striped catfish fingerlings.

INTRODUCTION

Aquaculture experiences the developments that can help food security and livelihoods (Ashry *et al*., 2023; Garlock *et al*., 2022). A report from Food and Agriculture (FAO) reveals that there is still an urgent need to increase productivity by implementing biosecurity strategies and new feed technologies (Oboh, 2022). The biodiversity of finfish species is highly recommended for aquaculture practices to

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meet sustainable food goals (Gosh *et al*., 2022; Kim *et al*., 2022).

Striped catfish (*Pangasianodon hypophthalmus*) is the most widely cultivated fish in the world. In this case, several countries have introduced Striped catfish into the aquaculture industry as a preferred finfish species (Allam *et al*., 2020). Several countries in Southeast Asia that carry out Striped catfish cultivation activities include Indonesia, Thailand, Cambodia, Vietnam, and Laos (Fawzy *et al*., 2017). The increase in fish production is because of its nutritional value and limited availability in both domestic and international markets (Ali *et al*., 2018; Ruan *et al*., 2022). Striped catfish is famous for its white skin color, low-fat content, and delicious taste. This fish can also reach a size that can be commercialized quickly, making it a competitor for other freshwater aquaculture commodities (De Silva and Phuong, 2011).

However, reducing feed prices can increase the sustainability of the Striped catfish farming industry in the long term (Xuan *et al*., 2022). Feed that contains essential macronutrients is necessary for the development and health of Striped catfish. A balanced feed must include macronutrients (minerals and vitamins) as well as important nutrients (protein, lipids, and carbohydrates) (Dawood, 2021). According to research from Exstrada *et al*. (2020), one of the problems encountered in raising striped catfish is the high death rate of fish seed after passing the egg yolk phase as a result of a decrease in food reserves.

Silkworms are known as *Tubifex* sp., a name for mud worms that live in freshwater conditions and have a high protein content. This natural food based on silkworms has been developed as fish food because it can provide the nutrients needed to improve health and development. Previous research reported that silkworm-based feed was able to increase fish growth, survival, and resistance to *Myxobolus cerebalis* infection (Oplinger *et al*., 2011). Snakehead fish *(Channa striata*) seeds that were given feed made from silkworms with a 50:50 during the 40-day trial period showed increased growth, feed conversion ratio, protein efficiency, and survival (Syamsunarno and Sunarno, 2022). Juveniles of *Carassius auratus* fish given a diet made from silkworms for 28 days during the trial period showed a survival rate of 88%, absolute growth, body weight, and protein content (15.28%), which were higher than those given commercial fish food (Mathew *et al*., 2022). A combination of *Tubifex* sp. flour with carrots has been proven to increase the color brightness of the scales of Botia ornamental fish, *Chromobotia macracanthus* (Andriani *et al*., 2020).

The efforts to result in the balanced feed requirements to support the nutrition and production performance of Striped catfish are still needed. The purpose of this study was to assess the effect of feeding *Tubifex* sp. on nutritional content, production performance, and health as seen from the gill histomorphometric features of Striped catfish fingerlings.

METHODOLOGY

Ethical Approval

Test animals were not treated improperly during the research. Test animals in this study were given proper treatment under research rules. The implementation of the research has adjusted to the regulations set by the Study of Fisheries Resources Utilization of the Postgraduate Program of the Jakarta Fisheries Business Expert Polytechnic through feasibility tests and seminars.

Place and Time

The research was carried out at the Aquaculture Laboratory, Fisheries Business Expert Polytechnic, Pasar Minggu, Jakarta in May – July 2022. The cultivation period for Striped catfish fingerlings used in this research was 25 days. Analysis of the water quality of the rearing media was carried out at the Sukamandi Freshwater Fisheries Research Institute Assay Laboratory.

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Research Materials

The tools used in this study include an aquarium with a volume of 49.7 L (Nikita, ID), a Thermometer (IPEC, ID), a pH meter (Hanna Instruments, USA), a Nitrite and Nitrate tester (Hanna Instrument, USA), Dissolved Oxygen (DO) meter (Hanna Instrument, USA), spectrophotometer (KJ-2097, Germany), Microtome machine (Fisher Scientific, USA), H&E solution (Merck, USA). The materials used include 0.5 cm Siamese catfish and silkworms (Cultivation Lab. Politeknik AUP Jakarta), Commercial fish feed (Japfa, ID), MBF solution (Merck, USA), 70-100% alcohol (Merck, USA), Xylene (Merck, USA), Liquid paraffin (Merck, USA), Aquades (Merck, USA).

Research Design

This research is included in laboratory experimental research using a completely randomized design (CRD) that includes 3 treatment groups with 4 repetitions. The treatment groups in this experiment consist of Group P1 (silkworms with dry food media), Group P2 (commercial silkworms), and Group P3 (wild silkworms).

The container used for rearing silk worms made from High-Density Polyethylene (HDPE) measures 100 cm × 39 cm × 20 cm with a water volume of 31 L. Each rearing container was filled with 1,140 silkworms. Silkworms were kept in each container for 55 days and the silkworms were dried and then fed to Striped catfish seed. The Striped catfish seeds used in this research were 0.5 cm in size and weighed 0.4 grams for 10 fish/L. Striped catfish fingerlings were put into each aquarium (a total of 15 aquariums) measuring 59 cm × 49 cm × 17 cm with a volume of 50 L. The aquarium was equipped with aeration and feeding made from silkworm flour was given *ad libitum*. Feeding made from silkworms was carried out for 25 days (Umidayati *et al*., 2020).

Work Procedure

Production Performance of Striped Catfish Fingerlings

Production performance measurements were carried out to determine the effectiveness of feeding silk worms on several test parameters, including specific growth rate (length and weight), weight and length gain, survival rate, feed conversion, protein efficiency, and retention. Measurements were carried out by taking samples of Striped catfish from the rearing container at the end of the experimental period, measuring the length of the fish using an analytical ruler and measuring the weight of the fish using a digital scale.

Specific Growth Rate

The specific growth rate (length and weight) of fish is calculated using the following equation (Umidayati *et al*., 2020):

$$\text{L} - \text{SGR}(\%) = \frac{\text{LN}(\frac{\text{Final Length}}{\text{Initial Length}})}{\text{Treatment Day}} \text{x}100\%$$

$$\text{W} - \text{SGR}(\%) = \frac{\text{LN}(\frac{\text{Final Weight}}{\text{Initial Weight}})}{\text{Treatment Day}} \text{x}100\%$$

Absolute Length

The increase in absolute length of striped catfish seeds is calculated using the following equation (Umidayati *et al*., 2020):

Length (L) = initial length (L0) - final length (L1)

Survival Rates

The survival rates of striped catfish seeds are determined using the following equation (Wiradana *et al*., 2019):

$$\text{SR}(\%) = \frac{\text{Nt}}{\text{N0}} \text{x}100\%$$

Description:

SR = survival rate (%)
Nt = number of fish at the end of maintenance
N0 = number of fish at the initial of maintenance

Feed Conversion Ratio (FCR)

Feed Conversion Ratio (FCR) is carried out using the following equation (Andrino *et al*., 2014):

$$\text{FCR} = \frac{\text{Total feed is given (g)}}{\text{Total final biomass (g)}}$$

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Average Daily Growth (ADG)

The growth coefficient in question is the growth in size, length, and weight achieved in a certain period. The growth coefficient is expressed in the formula (Umidayati *et al*., 2020):

$$\text{ADG} = \frac{\text{Lt} - \text{L0}}{\text{h}}$$

Description:
Lt = Final length (cm)
Lo = Initial length (cm)
h = Maintenance length

Nutritional analysis of striped catfish meat

The water, lipid, crude protein, and ash contents of Striped catfish seed were analyzed based on standard methods from AOAC. Water content was analyzed using an oven at a temperature of 105°C for 24 hours, crude protein was measured via N × 6.25 using the Kjeldahl method, lipid content using an extraction method using a Soxtec instrument, and ash content through a combustion process at a temperature of 550°C for 24 hours (AOAC, 1995).

Histological examination

The histology of the gills of the Striped catfish seed was analyzed to observe the nature of possible toxicity resulting from feeding silkworm-based feed. The stage of making histological preparations starts with a necropsy of the gills of Striped catfish and then fixation using clove solution. The samples were then preserved using MBF solution to maintain the morphological quality of the tissue for 24 hours. Dehydration of gill tissue was carried out by soaking the samples in graded alcohol solutions (70 – 100% alcohol) for each 2 hours. After that, the tissue was soaked in Xylen solution 3 times, each for 30 minutes. The next stage is the paraffinization of tissue samples for 2 hours.

The gill samples were then dehydrated into cassettes and labeled. The cassette containing the tissue sample was then soaked in distilled water for 1 minute. Next, proceed with making blocks using a stainless steel mold to facilitate the cutting process using a 4 µm microtome. The tissue that had been cut into ribbons was placed above a 50% alcohol solution. Staining of histology samples using H&E solution. Before staining, the slides were filled with tissue; they were stored in an oven at 40°C to increase adhesion to the surface of the preparations. The stained preparations were then observed using a digital binocular microscope. Observations were made by observing tissue changes in each treatment group. Gill histology data were displayed descriptively (Nikjoo *et al*., 2023; Rahardjo *et al*., 2022).

Water quality measurement

Water quality monitoring consists of temperature, dissolved oxygen, ammonia, nitrite, and pH values. Temperature measurements used a thermometer, dissolved oxygen levels used a DO meter, ammonia and nitrite measurements used a spectrophotometer, and pH values used a pH meter. Water quality measurements carried out in situ include temperature, dissolved oxygen, and pH value, while ammonia and nitrite levels were carried out ex-situ at the end of the maintenance period at the Sukamandi Research and Development Laboratory. Water quality checks were carried out every day (morning and evening) for 25 days. Maintenance water cleaning was carried out to remove food residue and fish feces. Water changes were carried out every 2 days, as much as 25% of the total amount in the maintenance container (Marwiyah *et al*., 2019).

Data Analysis

Research data were tabulated using Excel software (Ms. Word, USA). The results of water quality data and gill histology were displayed descriptively. Production performance data from Striped catfish fingerlings given a diet made from silkworms were analyzed quantitatively using One Way ANOVA (Analysis of Variance) at a 95% confidence level. Next, Duncan's test was carried out to determine significant differences ($p \leq 0.05$) between treatment groups in this study.

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RESULTS AND DISCUSSIONS

There was no significant difference in the production performance of Striped catfish fingerlings among different silkworm-based diet treatments. The results of the study showed that the P1 group in terms of survival parameters obtained better results compared to the other two treatment groups. The survival rate of Striped catfish fingerlings in group P1 was 50.3 ± 6.34%, respectively, higher when compared to group P2 (46.6 ± 7.98%) and group P3 (36.3 ± 11.7%). The feed ratio values in the P1 group showed more effective and efficient values when compared to treatments P2 and P3 (Table 1).

In the treatment of feeding silk worms caught by P3, absolute length growth results were 1.54 ± 0.20 cm or higher when compared to P1 worm feed and worms using P2 tofu dregs media. While the feed conversion rate (FCR) in treatment P2 obtained more effective and efficient results of 7.1 ± 0.70 compared to the other two groups (Table 1). The values of specific weight growth rate (W-SGR) and specific length growth rate (L-SGR) in this study did not show significant differences between treatment groups ($p \geq 0.05$) (Table 1).

Table 1. Production performance of Striped catfish fingerlings given a silk worm-based diet using various media.

	Treatments Group [1]			
Production Parameter	(P_1)	(P_2)	(P_3)	p-value
ADG[2]	0.066 ± 0.005	0.060 ± 0.01	0.068 ± 0.0047	0.218
Final length (cm)[3]	2.1 ± 0.10	2.3 ± 0.20	2.3 ± 14	0.383
Absolute length growth (cm)	1.60 ± 0.81	1.54 ± 0.20	1.67 ± 0.14	0.427
FCR	7.6 ± 0.557	7.1 ± 0.70	8.5 ± 0.97	0.064
Life sustainability (%)	50.3 ± 6.34	48.6 ± 7.45	36.3 ± 11.7	0.056
Population (fish)	240 ± 37.5	251 ± 31.7	181 ± 58.9	0.610
W-SGR[4]	0.12 ± 0.07	0.11 ± 0.11	0.12 ± 0.14	0.966
L-SGR[5]	5.5 ± 0.15	5.4 ± 0.39	5.6 ± 0.28	0.488

[1]Treatment P1 (best results research worms), P2 (worms using tofu dregs media), P3 (naturally wild silkworms).
[2] Average fish weight at the end of the study
[3] Average fish length at the end of the study
[4] W-SGR: specific weight growth rate
[5] L-SGR: specific length growth rate
P value ≤ 0.05 indicates a significant difference between treatment groups.

Growth rate, final biomass weight addition, and survival are some important factors in measuring the optimization of fishery commodity production performance. Fish growth, in simple terms, can be interpreted as an increase in weight or length over a while due to the provision of certain food sources. Previous research showed that the effectiveness of providing feed containing *Agaricus bisporus* polysaccharide levels in cultivating catfish (*Ictalurus punctatus*) was able to increase body weight and specific growth rate of fish, with FCR values decreasing, especially when the feed was added at a dose of 250 mg/kg (Yang *et al*., 2023).

A diet supplemented with *Zingiber officinale* flour at a dose of 1.5% for 90 days in Striped catfish cultivated outdoors was recently reported to be able to increase growth performance, reduce feed conversion ratio, and have high survival (Ashry *et al*., 2023). In this regard, this research report that used silkworm meal as a natural feed for Striped catfish seeds can result in increased growth performance, although further research is still needed to assess the effectiveness of feed in increasing fish resistance to biotic and abiotic stressors

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(Akter *et al*., 2016; Shawky *et al*., 2023; Sutriana *et al*., 2018).

Growth is a biological process with many factors, among others the factors from within the fish's body and environmental factors. It may be the first information reporting the effectiveness of natural feed made from silkworms with various media in increasing Striped catfish seed production performance. The increase in production performance can be explained by the activation of digestive enzymes in Striped catfish fingerlings by silkworm-based feed because it contains proteinase that is involved in the digestive system and the absorption of amino acids (Gao *et al*., 2022). Silkworms may have potential as antibacterial agents that can reduce the colonization of harmful microorganisms in the intestine so that intestinal absorption is more optimal (Ringø *et al*., 2016); thus increasing the utilization of the feed provided to produce high growth and body weight (Görelşahin *et al*., 2018; Sarkar *et al*., 2006). The relatively short rearing period for Striped catfish in this study can be the reason that no significant differences were found from feeding silkworms in this study.

The results of the nutritional analysis of Striped catfish fingerlings meat after feeding with silk worm-based feed showed that the P1 treatment group showed significantly higher levels of protein, fat, and ash than the P2 and P3 groups. However, the water content in group P1 was significantly lower when compared with groups P2 and P3 in this study (Table 2). Based on the results of this study, it was confirmed that silkworms with dry food media were able to increase the crude protein content in the meat of Striped catfish seeds in the P1 group, which was 77.89% higher when compared to groups P2 (60.12%) and P3 (58.74%). Previous research stated that protein was the main requirement to support the growth rate of Siamese catfish up to 37.7% and the specific growth rate value was 1.60 – 1.63% and protein efficiency was 3.40% (Jayant *et al.*, 2018). Increasing the protein content in feed had a significant effect on the protein content in the fish's body as well as increasing its protein retention value (Katongole and Yan, 2020; Sankian *et al*., 2017).

Table 2. Proximate analysis results of Striped catfish meat given silkworm-based feed.

Chemical parameters (%, dry weight)	Treatment[1] (P_1)	(P_2)	(P_3)
Protein	77.89[a]	60.12[b]	58.74[c]
Fat	9.1[a]	5.59[b]	6.10[b]
Ash	22[a]	5.30[b]	5.34[b]
Water	80.75[a]	85.34[b]	84.75[b]

Note: [1]Treatment P1 (Silkworms, the best results based on previous study); P2 (silk worms using tofu dregs as media); and P3 (wild silkworms). Notations with different letters in the same column show significant differences between treatment groups based on the results of the Duncan test with a value of $p \leq 0.05$.

The lipid content in catfish meat from group P1 was significantly higher ($p \leq 0.05$) when compared with the other two groups (P2 and P3) in this study. Further research is still needed to determine the lipid accumulation found in Striped catfish fingerlings because several types of diet can result in lipid accumulation that disrupts lipid and glucose metabolism in the fish's body (Chen *et al*., 2020; Ling *et al*., 2019). However, lipids act as an important energy source in aquatic animals and are crucial for fulfilling fish nutrition (Chen *et al*., 2023).

Histological observations were carried out to determine changes in organ tissue in the fish body after being given a diet made from silkworms. The results of observations on gill histology showed that there were

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changes in the structure of the gill lamella in all treatment groups. Figure 1 showed that the gill lamella had hyperplasia, desquamation, and congestion that caused morphological changes in the tissue. Hyperplasia is an increase in the size of a tissue due to an increase in the number of cells because of an inflammatory response and the beginning of necrosis. The cause of hyperplasia is an increase in the number of chloride cells, which is a form of adaptation to the metsulfuron methyl reaction to maintain electrolyte balance in the body. Metsulfuron methyl will cause mucus production in fish as a protective response to the parts that are reduced by toxic particles so that they do not suffer damage. However, it was seen that the P1 and P3 treatment groups had primary and secondary lamella shapes that still looked good when compared to group P2, which showed hemorrhage due to increased secondary lamella hyperplasia.

Treatment Group

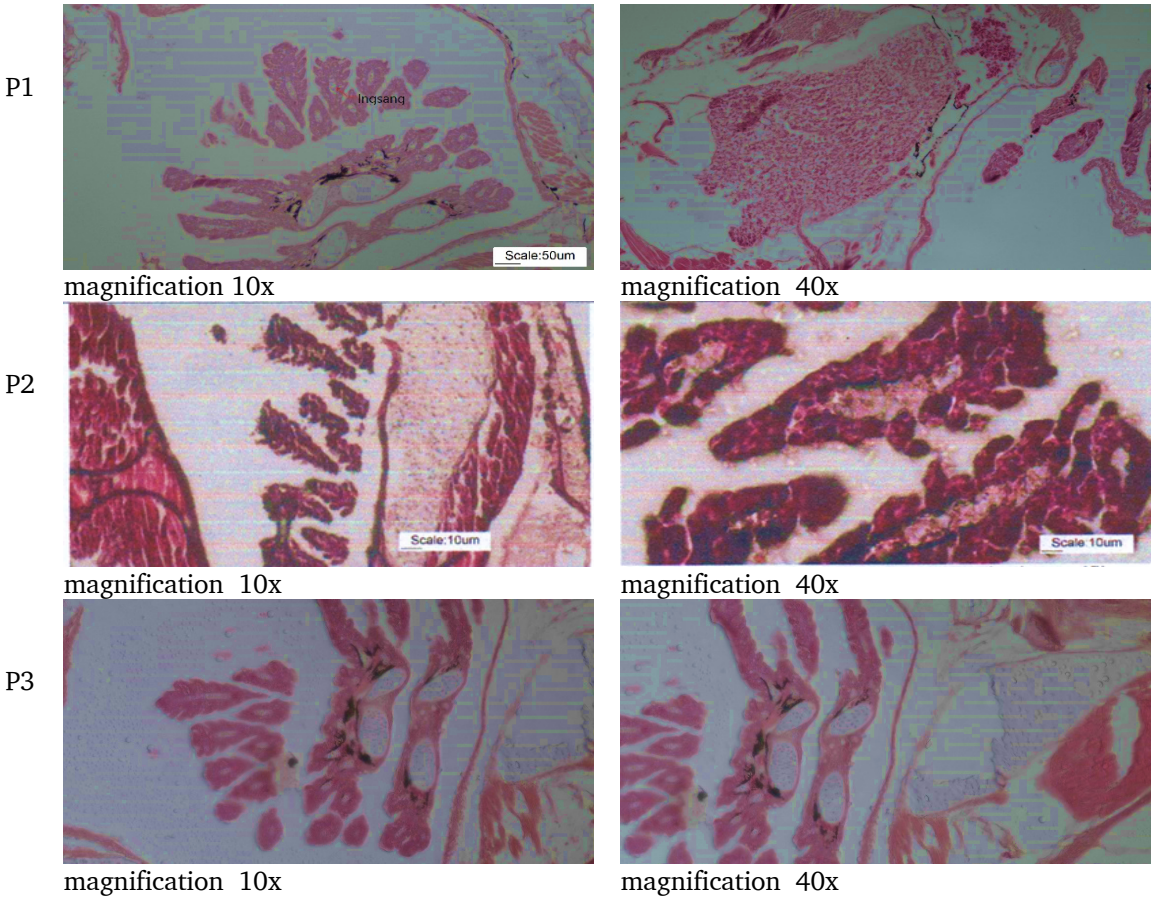

Figure 1. Histology of the gills of Striped catfish seed given a silk worm-based diet. P1: silk worms with dry food media, P2: commercial silkworms, and P3: wild silkworms. Description. The red arrow in the image indicates the occurrence of hemorrhage in the gills.

Internalization of silkworm flour components in the gill is probably caused by adsorption that occurs on the gill surface (Hao *et al*., 2013). It is possible to increase the absorption of food waste from the water by transport carriers in the gill epithelium (Giardina *et al*., 2009). Increased cell membrane permeability due to oxidative damage and lipid peroxidation may cause the deposition of food waste in the gill organs (Ma *et al*., 2013), and further research is needed in various other organs.

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CONCLUSION

The study concludes that feeding with dry food media-reared worms, as well as better protein efficiency and retention values so is highly recommended as a substitute feed. Further research is still needed to determine the need for the right amount of feeding to support the immune system of Striped catfish fingerlings.

CONFLICT OF INTEREST

The authors state that they have no conflict of interest in writing and publishing this article. All authors have authorized the work for publication, and it is not being submitted to any other journals.

AUTHOR CONTRIBUTION

YC developed the research idea collected data and performed most of the technical work; SR and MN designed, planned, and reviewed the experiments; PAW and PES performed data processing, data analysis, and manuscript preparation.

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