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Anchovies (*Stolephorus* sp.) By-product Material as a Fish-feed Ingredient of Seurukan Fish (*Osteochilus vittatus*): Effect on Growth Performance and Gut Morphology

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Abstract

Fish meal is an important part of fish feed ingredient. However, the use of fish meal is considered unsustainable, competes with human beings and is expensive. Anchovy (*Stolephorus* sp.) by-product contains high protein and potential to be used to replace fish meal. The present study aimed to determine the utilization of anchovy by-products raw materials as a feed ingredient for seurukan fish (*Osteochilus vittatus*) and evaluate its effect on the growth performance and intestinal morphology. In total 250 seurukan fish (initial weight 0.67 ± 0.01 g) were randomly divided into four replicates groups: control group, and experimental diets which had different inclusion levels of anchovy by-products meal: 50%, 35%, and 20%. Fish were fed a different diet at 8% of body weight three times a day for 28 days. Present results showed that the inclusion of anchovy by-product meal as much as 50% can produce a higher value of weight gain (0.47 ± 0.02 g), length gain (0.69 ± 0.09 cm), SGR (2.20 ± 0.51%) and feed efficiency (77.89 ± 3.71%) compared to control feed ($p<0.05$). There was no significant difference in the survival rate and gut length ratio of seurukan fish among groups. Meanwhile, the average villi length and villi width of the seurukan fish fed experimental groups increased significantly compared to the control group ($p<0.05$). In conclusion, the 50% anchovy by-products meal inclusion in the diet of seurukan fish is beneficial to enhancing growth, feed efficiency, and improved feed absorption as indicated by histological analysis.

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INTRODUCTION

The utilization of fish meal as the primary raw material for fish feed is no longer considered relevant due to not being economical, scarcity of stock, and not environmentally friendly (Arriaga-Hernández *et al*., 2021; Amer *et al*., 2020). Therefore, various efforts to find alternative/by-products materials as fish feed formulations tend to be increasing (Zulfahmi *et al*., 2019; Adéyèmi *et al.*, 2020). Anchovy (*Stolephorus* sp.) by-products are potential raw materials to be used in fish feed formulations. Indonesia is an archipelagic country with high marine resources, including anchovies (Irnawati *et al.,* 2018; Mulya *et al*., 2021). Aside from being sold fresh, this fish is also salted to extend their storage time. The fish body is a major part usually consumed, while the fish head tends to be an underutilized by-product. About 15% of the anchovy resources are estimated to be by-products of material and waste (Ali *et al.,* 2015; Ali *et al.,* 2018).

The anchovy by-product contains 44,43% protein, 13.68% carbohydrates, and 6.62% ash (Ali *et al*., 2018). In addition, Gormaz *et al.* (2014) reported that anchovy by-products are also abundant in amino acids, fatty acids, and iodine. To date, the utilization of anchovy by-products as fish feed raw materials has been demonstrated on several fish, for instance, tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus*) (Ali *et al*., 2015; Ali *et al.,* 2018). The results showed that the substitution of anchovy by-product meal in the feed formulation could increase the growth rate of fish such as control/commercial feeds.

Seurukan fish (*Osteochilus vittatus*) is one of Indonesia's native fish species encouraged to be domesticated (Rostika *et al*., 2017; Eriani *et al*., 2017). In addition to having a high market price (up to IDR 35,000/Kg), this fish also has a delicious taste as well as high nutritional value (38.83% protein). Currently, the fulfillment of seurukan fish for consumption, especially in Aceh Province, still comes from direct fishing using natural bait. However, the natural catch of seurukan periodically continues to decline due to habitat destruction and overfishing (Mahendra and Supriadi, 2019; Maulidyasari and Djumanto, 2020; Zulfahmi *et al*., 2021). Therefore, the domestication effort of seurukan fish is attracting attention. Several studies related to the domestication effort of seurukan fish have been conducted in recent years, including alternative feed formulations (Yudhistira *et al*., 2015; Firdaus *et al*., 2017; Said *et al*., 2021;), the addition of supplements and enzymes in the feed (Yusnita *et al*., 2019; Rijal *et al*., 2020; Zakiah *et al*., 2020), reproductive performance and spawning (Muchlisin *et al*., 2014; Tarigan *et al*., 2020; Madihah *et al*., 2021), and optimization of stocking density and feeding time (Aryani *et al*., 2017; Syandri *et al*.,2015).

To date, the alternative raw materials that have been tested as fish feed ingredient for seurukan fish, including *Pistia stratiotes, Aspergillus niger,* and *Lemna* sp. (Yudhistira *et al*., 2015; Firdaus *et al.*, 2017; Said *et al*., 2021). Firdaus *et al*. (2017) reported that the substitution of fermented *A. niger* meal in fish feed as much as 30% produced a not significantly different daily growth rate and feed conversion ratio compared to the control/commercial feed. Similar results by Said *et al.,* 2021 reveal that *Lemna* sp. meal substitution by 50% in the fish feed affects the higher growth than control/commercial feed. However, the utilization of anchovy by-products materials as a feed ingredient of seurukan fish has not been explored. Therefore, this study aims to evaluate the utilization of anchovy by-products materials as feed ingredient for seurukan fish (*O. vittatus*) and investigate its effect on growth performance and intestinal morphology.

METHODOLOGY

Place and Time

The research was conducted from May to July 2021. The production of feed and feeding trials were carried out at the Fish Seed Center (BBI) Lukup Badak, Takengon, Aceh Tengah. The proximate analysis of the experimental diets was conducted at the Research Center for Industrial Standardization (Baristand) of Banda Aceh, while histology preparation was done at the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh.

Research Materials

The materials used in the research include supporting tools for feed formulation and production, proximate test equipment, water quality measurement tools, fish rearing, as well as supporting tools for histology preparation and observation. In detail, tools to support the formulation were pellet printing machine, oven, analytical balance, and measuring cup. The tools used for the proximate test are heater, 300 ml beaker, volumetric flask, filter paper, soxtec, electric furnace, burette, distillation, aluminum cup, and Erlenmeyer flask. A thermometer, DO meter (Lutron YK-2005WA; Taiwan) and pH meter (HANNA HI-83214; United States of America) were used to measure the water quality of the fish rearing media. A 70x30x40 cm aquarium was used as the fish rearing media. Meanwhile, the tools used for histology preparation and observation were measuring cups, sample bottles, minor surgery sets, tissue cassettes, block molds, staining jars, ovens, embedding processors, microtome, microtome knife, object-glass, cover glass, label paper, 37°C slide warmer, water bath, and a microscope equipped with a camera (Olympus CX31; Japan).

Feed formulation materials, materials for proximate analysis and materials for histological preparation had also been prepared. The ingredients used for the feed formulation were anchovy by-products meal, soybean meal, rice bran, wheat flour, fish oil and vitamin-mineral premix. In the meantime, the materials used for the proximate analysis were distilled water, hydrochloric acid (HCl), calcium sulfate (K_3SO_4), magnesium sulfate ($MgSO_4$), sodium hydroxide (NaOH), benzoic acid (H_3BO_4), ether, benzene, methylene red, bromocresol green and acetone. The materials used during the feeding trial were commercial feed CPP 888-2, alternative feed, *seurukan* fish with an average length of 3.75 cm, freshwaters, masks, and gloves. Meanwhile, the materials used for histological observations were 10% Neutral Buffered Formalin (NBF), aquadest, xylol, alcohol, paraffin, Hematoxylin Eosin (HE), and adhesives.

Research Design

The experimental design of the present study consisted of three experimental groups and a control (commercial feed) with triplicates. Specifically, the control feed used was commercial feed CPP888-2 (28% protein, 6% fat, and 12%), whereas the experimental diets had different inclusion levels of anchovy by-products meal, namely 50% anchovy by-products meal for Diet 1, 35% anchovy by-products meal for Diet 2, and 20% anchovy by-products meal for Diet 3 (Table 1).

Table 1. Dietary ingredient and proximate composition of the control and experimental diets.

Composition	Control	Diet 1	Diet 2	Diet 3
Formulation (%)				
Anchovy by-products meal		50	35	20
Soybean meal	Commercial	20	35	50
Wheat flour	Feed	9	9	9
Bran meal	CPP888-2	20	20	20
Fish oil		0.5	0.5	0.5
Vitamin and Mineral Premix		0.5	0.5	0.5
Proximate Composition (%)				
Moisture	12.00	10.12	13.11	13.48
Ash	13.00	17.66	12.57	8.34
Protein	28.00	23.86	28.86	29.07
Lipid	6.00	2.35	2.34	2.32
Carbohydrates	20.00	18.36	22.52	18.90
Price (Rp/kg)	20.000	11.000	13.000	17.000

*The proximate content to control/commercial fish was obtained from the packaging.

Work Procedure

Feed Formulation and Proximate Test

Raw materials in the form of processed by-products of anchovy were obtained from Paya Ilang Market and Kelaping Market, Takengon, Aceh Tengah, Indonesia. Before use, anchovy by-products material was dried and pounded into a homogeneous meal by using a flour mill machine. Table 1 shows a description of the ingredients of the experimental diet. The diet was formed into pellets shaped 1–1.2 mm using a manual pellet press machine. The feed was dried under the sun for one day. The proximate content of the control and experimental diet, including moisture, ash, protein, lipid, and carbohydrates, were measured. Moisture and ash content were measured using the gravimetric method (AOAC, 1990), protein content was measured by using the Kjeldahl method (Persson and Wennerholm, 2008), lipid content was measured with Soxhletasi method (Christie, 2003), and carbohydrates were measured with Luff Schoorl method (Ofori *et al.*, 2019).

Feeding Trial

A total of 250 seurukan fish from the same broodstock with an average length of 3.75 ± 0.09 cm and an average total weight of 0.67 ± 0.01 g were collected from BBI Lukup Badak Takengon and transported to the laboratory. Fish were acclimatized for seven days in a fiber of 200x100x40 cm with a volume of 500 L of water and equipped with aeration. During acclimatization, the fish were fed with commercial feed CPP 888-2 three times a day (08.00, 12:00, and 17.00 WIB) with ad libitum level. Before the test, all fish were starved for 24 h. The fish were maintained in a 70x30x40 cm aquarium with a volume of 40 L of water. Each aquarium contained as many as 20 seurukan fish. The feeding trial lasted for 28 days. The feeding frequency was three times a day at 08:00, 12:00, and 17:00 WIB, according to 8% of body weight. Fish feces were stripped every day using the siphon method. The water was completely changed every 14 days to avoid bacterial growth and accumulation of ammonia. The water source used came from local springs that have been aerated for 24 hours before being used.

Growth Performance, Feed Efficacy and Water Quality

The parameters observed in this research consist of survival rate (SR), absolute weight growth, absolute length growth, survival growth rate (SGR), and feed efficiency, refer to the protocol developed by Effendie *et al*. (1997). The measurements of these parameters were undertaken every seven days, both for control and experimental groups. The gut length ratio (GLR) was calculated at the end of the feeding trial period using the following formula (Berumen *et al*., 2011):

$$\mathrm{GLR} = \frac{\text{gut length (cm)}}{\text{total body length (cm)}}$$

Water quality such as temperature, pH, and dissolved oxygen was monitored at the beginning and the end of the feeding trial.

Histological Preparation

Intestinal histology preparation refers to histotechnique, including sampling, fixation, dehydration, paraffin infiltration, cutting preparations, and staining (Humason, 1979). At the end of the feeding trial period, three fish from the control and experimental groups were randomly selected for dissection. The fish intestines were then placed in a container that had been added with buffered neutral formalin (BNF) for preservative purposes. The water removal process was performed by soaking the tissue in a graded alcohol solution of 80%, 90 %, and 95% to absolute alcohol. Moreover, paraffin infiltration in the tissue was performed using an embedding center device. The sectioning of paraffin blocks was done by using a microtome with a thickness of five microns. The staining process was done by soaking the preparations with hematoxylin for seven minutes, followed by eosin for three minutes. The intestinal tissue structure was then observed with a microscope equipped with a digital camera. The average villi height and villi width among groups were measured by using the following equation (German and Horn, 2006):

$$\mathrm{TRV} = \frac{T_{Vlg} + T_{Vrg} + T_{Vug} + T_{Vbg}}{4}$$

$$\mathrm{LRV} = \frac{L_{Vlg} + L_{Vrg} + L_{Vug} + L_{Vbg}}{4}$$

Where:
TRV = average length of intestinal villi (µm)
LRV = average width of intestinal villi (µm)
T_{Vlg} = length of the left intestinal villi (µm)
T_{Vrg} = length of the right intestinal villi (µm)
T_{Vug} = length of the upper intestinal villi (µm)
T_{Vbg} = length of bottom intestinal villi (µm)
L_{Vlg} = width of the left intestinal villi (µm)
L_{Vrg} = width of the right intestinal villi (µm)
L_{Vug} = width of the upper intestinal villi (µm)
L_{Vbg} = width of the bottom intestinal villi (µm)

Data Analysis

The data were presented in range, mean and standard deviation. Significant values of parameters between groups including initial weight, final weight, absolute weight, initial length, final length, absolute length, specific growth rate, survival rate, feed efficiency, gut length ratio, villi height, and villi width were analyzed using one-way ANOVA followed by least significant difference test. Values of $p<0.05$ were considered statistically significant. Intestinal morphology between control and experimental groups was analyzed descriptively. Statistical analysis was performed using SPSS software version 22 program (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Macintosh.

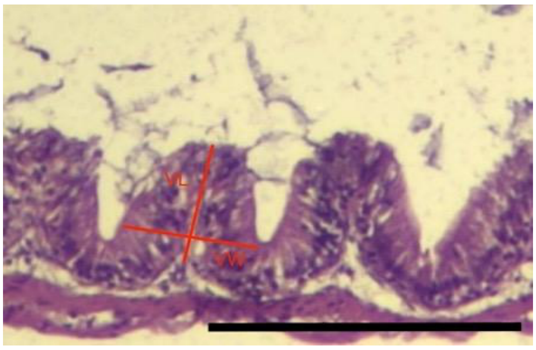

Figure 1. The technique of measurement the length and width of villi. VL: Villi length (μm), VW: Villi width (μm). Scale bar: 100 μm.

RESULTS AND DISCUSSION

Growth Performance and Feed Efficacy

This research is part of various efforts to determine the sustainable and economical material for fish feed formulations to support seurukan fish farming. Currently, fish feed still becomes the major substantial component of fish farming (reaching 80% of total operational costs) (Zulfahmi *et al.*, 2019). Therefore, the utilization of low-cost and easy to obtain raw materials as fish feed ingredients is increasing. Several raw materials that have been tested as the feed ingredient of seurukan fish were *P. stratiotes*, *A. niger,* and *Lemna* sp. (Yudhistira *et al.*, 2015; Firdaus *et al.*, 2017; Said *et al.*, 2021). The results of growth performance and feed utilization are presented below.

Table 2. Survival rate, growth performance, and feed utilization of seurukan fish (*O. vittatus*) fed the control/commercial and experimental at the end of the 28 days feeding trial.

Parameters	Control	Diet 1	Diet 2	Diet 3
Survival rate (%)	98.33±2.89	100.00±0.00	100.00±0.00	100.00±0.00
Initial weight (g)	0.69±0.08	0.67±0.01	0.63±0.05	0.72±0.07
Initial length (cm)	3.74±0.06	3.76±0.04	3.75±0.09	3.75±0.09
Final weight (g)	1.02±0.06	1.14±0.03	1.04±0.04	1.06±0.07
Final length (cm)	4.25±0.14	4.45±0.08	4.29±0.02	4.33±0.12
Weight gain (g)	0.33 ± 0.04^{a}	0.47 ± 0.02^{c}	0.41 ± 0.04^{bc}	0.34 ± 0.01^{ab}
Length gain (cm)	0.51 ± 0.09^{a}	0.69 ± 0.09^{b}	0.54 ± 0.08^{ab}	0.58 ± 0.06^{ab}
Specific growth rate (%/day)	1.57 ± 0.16^{ab}	2.20 ± 0.51^{c}	1.81 ± 0.25^{bc}	1.36 ± 0.14^{a}
Feed efficiency (%)	57.69 ± 8.75^{a}	77.89 ± 3.71^{b}	50.71 ± 8.46^{a}	58.50 ± 5.07^{a}

Note: different superscript letters in the same row indicate significant difference ($p<0.05$).

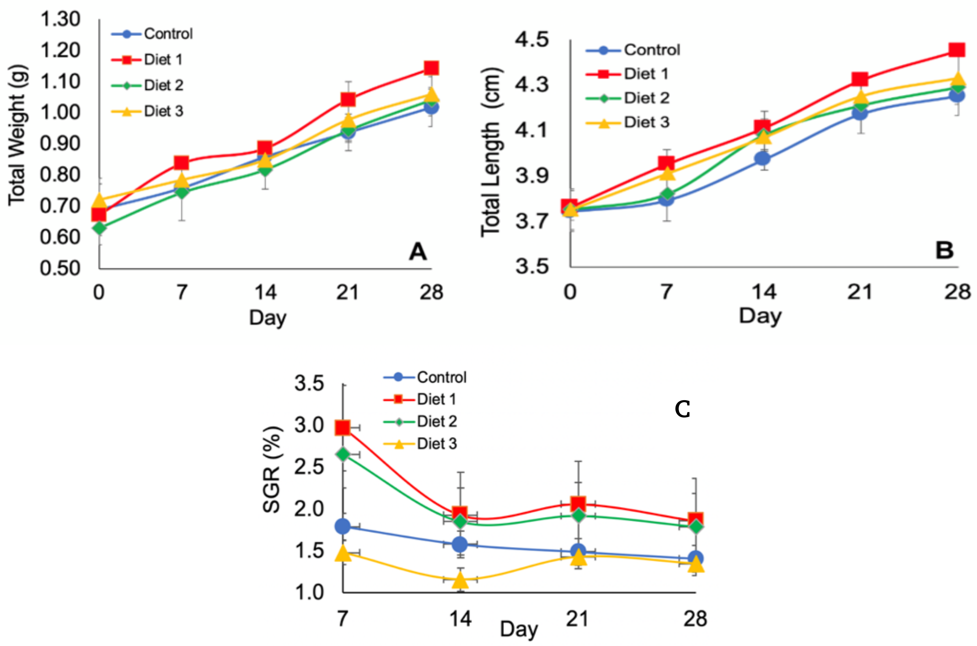

Figure 2. Average trend in total weight (A), total length (B), and specific growth rate (C) of seurukan fish during the feeding trial period in each experimental group.

This study revealed that the inclusion of anchovy by-products in the feed formulation of seurukan fish did not cause toxic effects that might impact death. There was no significant difference in the survival rate of seurukan fish between the control and experimental groups (p>0.05). Similar results were also shown in seurukan fish fed with a fermented diet containing *P. stratiotes*, *A. niger,* and *Lemna* sp. (Yudhistira *et al*., 2015; Firdaus *et al*., 2017; Said *et al*., 2021). The use of inclusive raw materials to substitute the fish meal usually uses sources that are not potentially toxic to fish. In addition, the free contaminants of rearing media also affect the high survival rate of fish in each experimental group.

Furthermore, the application of experimental diets resulted in higher fish growth than the control feed (p>0.05). Moreover, the inclusion of anchovy by-product meal in the feed by 50% (Diet 1) can produce a higher value of weight gain, length gain, SGR and feed efficiency compared to control feed (p<0.05), namely 0.47±0.02 g, 0.69±0.09 cm, 2.20±0.51%, and 77.89±3.71%, respectively (Table 2). During the feeding trial, the increased growth of seurukan fish was closely related to the nutrients contained in the feed. The results of the proximate test showed that Diet 1 had lower protein, moisture, and carbohydrate content than the control feed and other experimental diets, which were 23.86%, 10.12%, and 18.36%, respectively. Otherwise, Diet 1 has a higher ash content of 17.66% (Table 1).

Handajani and Widodo (2010) stated that seurukan fish included herbivorous fish, which generally requires feed with low protein content, around 20-25%. Khan *et al*. (2013) also affirm that herbivorous fish can utilize lower protein to grow optimally. The nutritional content of Diet 1 is also thought to have influenced the taste, durability, and texture of feed preferred by seurukan fish. Therefore, the feed's appropriate taste, durability, and texture can increase the fish's appetite and affect the high value of feed utilization and growth performance (Witoko and Nursandi, 2014). High carbohydrate content in the Control diet, Diet 2 and Diet 3 can lead to a long-lasting feeling of fullness and reduce fish appetite, reducing feed consumption and growth. Yanti *et al.* (2013) revealed that the high carbohydrate content in feed would affect cellulose levels, resulting in low protein digestibility.

Figure 2 showed that the mean value of the total weight and length of seurukan fish in both the control and the experimental groups continue to increase as long as rearing time (Figure 2A and 2B). Otherwise, SGR tends to decrease on day 14 compared to day 7, rising again on day 21 before declining again on day 28 (Figure 2D). The high value of SGR in the early period of feeding trial might be related to the amount of energy allocated for the somatic growth of seurukan fish. Poletto *et al*. (2018) and Esin *et al*. (2018) suggest that the initial period of rearing is crucial for the somatic growth of fish, especially in the larval and pre adult stages. In this period, the bones of the fish skeleton will develop rapidly so that the weight and length of the fish will increase significantly. In this study, the somatic growth of seurukan fish was significantly observed in the first two weeks (14 days). The high SGR value of seurukan fish fed with Diet 1 and Diet 2 indicated better appropriateness of feed than control feed and Diet 3.

Histometric and Histology of Intestine

Intestinal morphology is closely related to digestive performance, and the absorption rate of nutrients from the feed consumed (Zulfahmi *et al*., 2019). The statistical analysis showed no significant difference in gut length ratio parameter among groups. Meanwhile, the average villi length and villi width of the seurukan fish in the experimental group increased significantly compared to the control ($p<0.05$) (Figure 3). The average value of the gut length ratio ranges from 5.14±0.34 to 5.42±0.08. The value of villi width of seurukan fish in the control group was 30.72±1.82 μm, whereas in the experimental diet group (Diet 1, Diet 2 and Diet 3) increased significantly to 48.10±7.77 μm, 51.51±13.29 μm and 49.40±9.73 μm, respectively. A similar pattern was observed in the value of villi length of seurukan fish, wherein the control group was 36.24±5.30 μm. In contrast, the experimental diet group (Diet 1, Diet 2 and Diet 3) increased significantly to 66.93±7.41 μm, 70.81±17.09 μm and 71.09±23.08 μm, respectively. These results indicate that the inclusion of anchovy by-product meal in the feed formulation of seurukan fish has a positive effect on the histometric parameters resulting in higher absorption rate of nutrients and growth performance.

To date, the histometric value of the fish intestinal system (including the length and width of the gut villi) has been considered an essential indicator to assess the nutrient absorption rate of formulated feed and link it with the fish growth performance. Several studies have revealed a positive correlation between the increase of the length and width of the gut villi and the higher fish growth. On the other hand, lower villi length and width values were closely associated with decreased growth performance. The study from Pirarat *et al*. (2015) revealed that the inclusion of activated charcoal by 1-2% in the feed has been proved to increase the value of the villi length, which positively impacts the nutrient uptake and growth of Nile tilapia (*O. niloticus*). Furthermore, the finding of Yu *et al*. (2020) reported that the inclusion of oxidized fish oil in the feed ingredient was responsible for the lowered length and width of the intestinal villi of tilapia, impacting the low absorption of nutrients.

Histologically, the seurukan fish gut comprises several primary parts, namely submucosa, serosa, lamina propria, and columnar epithelium (Figure 4). The gut structure of seurukan fish fed with both control and experimental diet appeared to have an orderly lamina propria. The serosa appears to have a smooth shape resembling a thin layer, while the submucosa has a relatively dense shape and is located adjacent to the lamina propria. The morphology of the columnar epithelium from all groups looked like a column-shaped thin layer that covers the lamina propria.

Islam *et al*. (2021) stated that differences in the nutritional content of feed could affect the histology of the gut. Moreover, the finding of Zulfahmi *et al*. (2019) proved that milkfish fed with a high crude fiber content caused damage to the gut structure, including irregularities in the lamina propria accompanied by a thickening of the tunica muscular layer of the intestine. Besides, Haloi *et al*. (2013) reported that damage to the histological structure of the fish intestine could inhibit the process of absorption of nutrients from the feed consumed, resulting in lower growth rate.

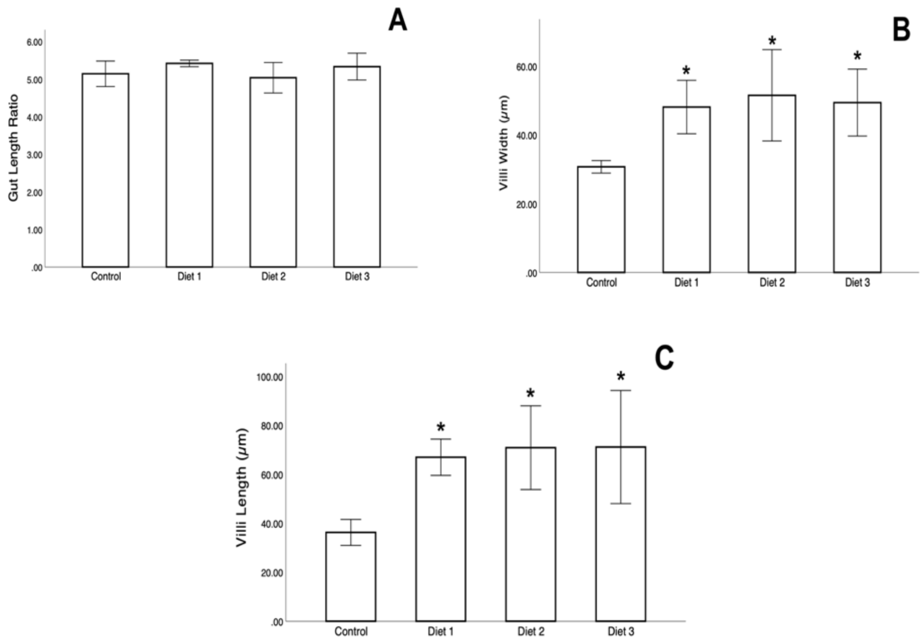

Figure 3. The average value of the gut length ratio (A), villi width (B), and villi length (D) of seurukan fish fed the control/commercial and experimental diet at the end of the 28 days feeding trial. Asterisks indicate significant differences ($p < 0.05$).

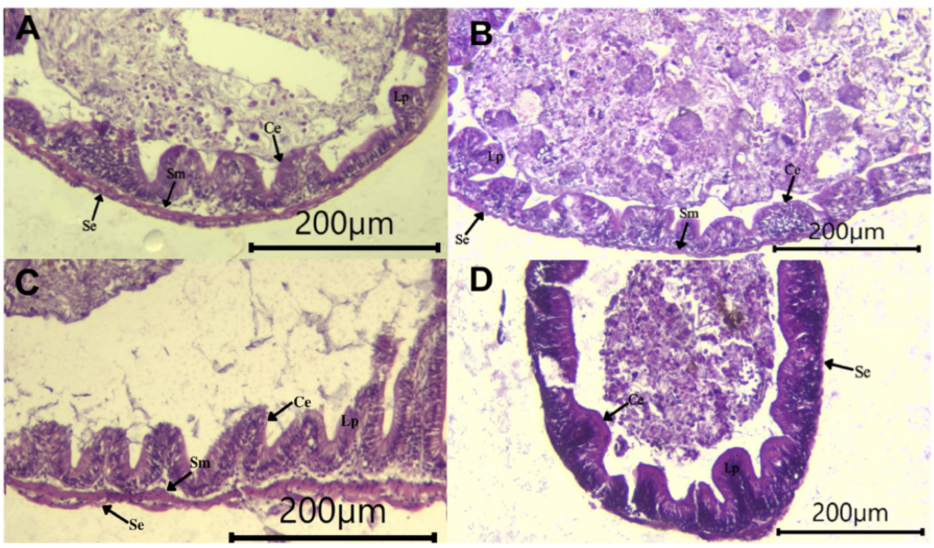

Figure 4. Histological structure of gut of seurukan fish in control/commercial feed (a), Diet 1 (b), Diet 2 (c) Diet 3 (d). Se: serosa layer, Sm: submucosa layer, Lp: lamina propia Ce: epithelium columnar. Scale bar = 200 μm, magnification 100×.

Water Quality Parameters

During the rearing period, the temperature value ranges from 22.1 to 24.2 °C, pH ranges from 7.0 to 8.3, and dissolved oxygen ranges from 7.6 to 8.6 mg/L. These value ranges are still in the optimum range to support the growth of seurukan fish. Susanto (2001) suggests that the optimum range of physical and chemical parameters for the survival and growth of seurukan fish are as follows: temperature of 18-28 °C, pH of 6.7-8.6, and dissolved oxygen is higher than 3 mg/L.

The physico-chemical parameters of water are essential factors for supporting the survival and growth of fish. Temperatures exceeding the maximum range can affect the performance of fish digestive enzymes. Nugraha *et al.* (2012) mentioned that fluctuating temperature under 20 °C or above 30 °C leads fish to suffer stress and reduce digestibility, impacting lower feed consumption. pH value below the optimum range can lead to metabolism disorder, lower growth, and vulnerability to infection by disease (Pramleonita *et al*., 2018). Putra (2015) stated that oxygen has a vital role in supporting the metabolic processes of the fish body, including converting glucose into energy, building the immune system, increasing food absorption and detoxification.

Table 3. Mean±SD range of physical-chemical parameters of water in rearing media during feeding trial period.

Group	Value	Parameters		
		Temperature (°C)	pH	Dissolved Oxygen (mg/L)
Control	Range	22.3-23.7	7.0-8.3	7.9-8.6
	Mean±SD	23.1±0.80	7.7±0.61	8.2±0.31
Diet 1	Range	22.3-24.2	7.0-8.3	7.8-8.3
	Mean±SD	23.1±0.92	7.6±0.80	8.1±0.16
Diet 2	Range	22.1-23.6	7.1-8.2	7.8-8.4
	Mean±SD	22.9±0.73	7.7±0.57	8.0±0.12
Diet 3	Range	22.2-23.9	7.0-8.1	7.6-8.3
	Mean±SD	23.1±0.92	7.6±0.52	7.9±0.21

CONCLUSION

The utilization of anchovy by-products material as fish-feed ingredients of seurukan fish has encouraged higher growth and gut performance than control/commercial feed. The highest value of growth parameters and feed utilization were observed in seurukan fish fed the Diet 1 (50% anchovy by-products meal). Villi length and width of seurukan fish fed the experimental diet increased significantly compared to the control group. The gut tissue structure of seurukan fish in both the control and experimental group appeared to have an orderly lamina propria, with a shape resembling a thin layer. In contrast, the submucosa has a relatively solid shape and is adjacent to the lamina propria. Meanwhile, the columnar epithelium has a shape resembling a column-shaped thin layer that covers the lamina propria. The inclusion of anchovy by-products material was able to increase the performance of gut of seurukan fish and appropriate taste, durability, and texture in feed supporting the fish growth performance and feed utilization. Further research related to the composition of amino acids and fatty acids in both experimental diet and seurukan fish is highly recommended.

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