

Impact of Diet Variation on Growth Performance, Feeding Efficiency, Health Condition and Water Quality of Juvenile Short-finned Eel (*Anguilla bicolor*).

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Abstract This study aimed to assess the effects of three different types of feed on the growth and feeding performance, water quality and health condition of short-finned eel (*Anguilla bicolor*) juvenile. This experiment was conducted for 31 days and involved a total of 180 fish with initials of body weight of 202.89 ± 3.80 g and total length of 44.33 ± 0.6 cm. The fish were divided into three groups, with each group were fed with different types of diets: 1) Japfa dough feed (JDF), 2) dough feed made of from grinded marine pellet (GDF) and 3) pelleted feed (PF). Each treatment was prepared in triplicate. The findings revealed that the pellet-type feed (PF) significantly improved the overall growth performance including survival rate (100.00%) and specific growth rate (3.176 ± 1.08 %/day) with excellent feeding performance recorded with lower feed conversion ratio (2.15 ± 0.9) and high protein efficiency ratio (1.15 ± 0.45). Histological examination of the intestine and liver showed no notable differences in condition of fish in all treatments. In conclusion, this study demonstrated that PF is an effective feed and beneficial for improving growth and feeding performance while maintaining health condition of short-finned eel juveniles hence it suggests the potential for developing a promising feed in the future for eel aquaculture industry.

Keywords: Short-finned eel, feeds, growth performance, nutritional composition, health condition.

Introduction

Eel (*Anguilla* sp) belonging to the *Anguilla* genus is an economically important species owing to their high nutritional value [49], [13], [52], [43]. They are known for their excellent organoleptic quality and medicinal benefits, in which they are often labelled as water ginseng [43], [31]. Due to its peculiar food culture particularly in the Asian countries, eels are highly sought after in which has turned them as targeted aquaculture species [52].

In 2016, world eel production peaked at 293,000 tonnes but still insufficient to meet the global demand at 350,000 tonnes [10]. In fact, the Indonesia's projection was set at 120,000 tonnes production but only 8.33% were produced [28]. Interestingly, despite Indonesia's strategic advantage of being surrounded by vast seas, which serve as key waters for the migration and distribution of tropical anguillid eels, several challenges have arisen, including climate change, fluctuating water quality, overfishing, low aquaculture productivity, and slow growth rates that hampered the harvest and production of tropical eels.

As aquaculture seeks to lessen reliance on wild-caught eel, it encounters challenges, particularly with slow growth during the juvenile stage [52] Judging from the increasing eel consumption demands as seen in Korea (15,000 tons/year), Taiwan (5,000 tons/year) and Japan (300,000 tons/year) (KKP, 2011),

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establishing an effective culture method especially enhancing feeding management is crucial. The common feeding practice in eel aquaculture is by using dough type feed but it posing challenges including high cost, uncertain supply, unknown nutrition with lower feed efficiency and impacting water quality process [23], [47], [44].

Currently, eels are exclusively fed with dough-like feed, and no significant improvements have been made to overcome its drawbacks. Under these circumstances, replacing dough type of feed with pelleted feed seems a viable alternative to offers eel with enhanced growth performance through improved feeding performance and health condition [44]. Various studies have documented the substitution of dough feed with pelleted feed for various freshwater and seawater fish species [49], [23], [31] including Japanese eel, *A. japonica*. To date, no studies have evaluated the effectiveness of pelleted feed for juvenile short-finned eels. Therefore, this study aims to compare the effects of dough feed versus pelleted feed on the growth, feeding performance, water quality, and health condition of short-finned eels juvenile.

Materials and Methods

Experimental Diets

Three experimental feeds were tested including: 1) Japfa dough feed (JDF), 2) dough feed made of from grinded marine pellet (GDF) and 3) pelleted feed (PF). Pelleted feed for feed (2) and (3) are manufactured by Suri Tani Pemuka JAPFA Group. Proximate compositions of the experimental feed are shown in Table 1. The JDF was prepared with mixing water and pellet at 1:13 ratio to form dough, while GDF was prepared using finely crushed commercial marine pellets, which were then sieved. The mixture consisted of 80% GDF from marine pellets and 20% JDF. This mixture was combined with water in a 1:1 ratio until the desired consistency and shape were achieved. Table 1 shows the proximate composition of each experimental feed.

Table 1. Proximate composition of experimental feed

Parameter	Unit	Diet		
		JDF	GDF	PF
Moisture	%	7.93±1.26	6.63±1.22	6.04±0.89
Ash	%	14.10±2.55	10.87±2.39	11.14±2.21
Lipid	%	5.64±1.46	12.44±2.98	14.01±2.67
Protein	%	52.40±4.56	45.86±3.56	44.00±7.89
Fiber	%	0.44±0.01	0.83±0.02	0.93±0.07
Phosphorus	%	2.25±0.07	1.71±0.07	1.65±0.09

Experimental Fish and Feeding Trials

Feeding trial was conducted at Aquaculture Research Center (ARC) of PT. Suri Tani Pemuka, JAPFA, Indonesia. A total of 180 eels (initial body weight: 202.89±3.8 g and initial total length: 44.33±0.6 cm) were used in this study. Prior to experiment, fish were acclimated, conditioned, and fed dough feed for 3 weeks, following the practices used at ARC. A 24-hour fasting period preceded their transfer to nine units of 1-tonne circular fiberglass tanks, each equipped with an aeration system. Stocking density was set at 20 fish per tank and all treatments were prepared in triplicate group. Fish were fed until satiation stage and feed weight was recorded. In each treatment, fish were fed twice daily with all three different feeds at 2% body weight. Daily water quality monitoring was conducted using a YSI ProODO device to measure dissolved oxygen, pH, and temperature, and a Merck test kit to assess ammonia, nitrite, and nitrate, in order to maintain optimal conditions and water exchanged was done at 20% daily.

Sample Collection and Analysis

Growth performance (survival rate (%), total length (cm), body weight (g) and specific growth rate (%/day) were measured in weekly basis. Following are formulas:

$$\text{Survival rate (SR, \%)} = (\text{final number of fish} / \text{initial number of fish}) \times 100,$$

$$\text{Specific growth rate (SGR)} = [(\ln W_x - \ln W_i)] / t \times 100$$

where W_x is the final body weight (g), W_i is the initial body weight (g) and t is the duration of the experiment (days)

Prior to measurement, all fish were subjected to 24 hours fasting period to stabilize their physiological state and minimize stress. As for feeding performance analysis, amount of feed given to fish were calculated daily and data were used to calculate total feed intake, protein efficiency ratio and feed conversion ratio (FCR).

$$\text{Feed intake} = \text{Total feed intake}/\text{days}$$

$$\text{Protein efficiency ratio} = (\text{Weight gained (g)})/(\text{Total protein intake (g)})$$

$$\text{Feed conversion ratio (FCR)} = (\text{Feed intake (g)})/(\text{Wet weight gain (g)})$$

To assess the health condition of the fish, histological analysis was carried out on three fish from each treatment group. The process involved sacrificing the fish, followed by the fixation in Davidson's solution, dehydration, paraffin embedding, and 6- μm sectioning of their intestines and livers. These samples were then stained with hematoxylin-eosin according to the methodology outlined by [42] to examine tissue structures, fatty degeneration density, goblet cell counts, and the overall health of the fish.

Statistical Analysis

All data collected were subjected to statistical analysis by using completely randomized design through ANOVA. The difference among the treatment means were compared by using Duncan's Multiple Range (DMR) Test (Duncan, 1995). Treatment effects were considered at $p < 0.05$ level of significance.

Results and Discussion

Growth Performance

All short-finned eel juvenile survived in the end of experiment despite being fed with different types of feeds and they exhibited equally excellent condition factor as seen in Table 2. Similar to other eels, short-finned eels are resilient and have a broad range of feeding preferences, allowing them to accept various types of diets including both dough and pelleted feeds without complications [30].

The highest final body weight was recorded for short-finned eels fed with PF, reaching 223.40 ± 11.14 g, though this difference was not significant compared to those fed with JDF (217.57 ± 3.11 g) but those fed with GDF were found to significantly lower final body weight (204.22 ± 8.3 g). Additionally, the highest final total length was also observed in short-finned eels fed with PF, measuring 46.61 ± 1.41 cm, with no significant differences noted between those fed with GDF and JDF. Condition factor remained not significantly different across all treatments.

Short-finned eel juvenile fed with PF also attained significantly higher ($p < 0.05$) specific growth rate at 3.17 ± 1.08 %/day compared to GDF which was only 0.445 ± 1.02 %/day. Meanwhile, no significant differences in specific growth rate were detected when comparing JDF with both conventional DF and PF (Table 2).

Table 2. Specific growth rate (%/day), survival (%) and condition factor of short-finned eel fed with different feed type

Parameter	Unit	Value		
		Japfa Dough Feed (JDF)	Grinded Dough Feed (GDF)	Pellet Feed (PF)
Specific Growth Rate (SGR)	%/day	1.92 ± 0.67^{ab}	0.445 ± 1.02^a	3.176 ± 1.08^b
Survival Rate (SR)	%	100 ± 0.00^a	100 ± 0.00^a	100 ± 0.00^a
Condition Factor		1.051 ± 0.10^a	1.005 ± 0.10^a	1.010 ± 0.11^a
Final Body Weight	g	217.57 ± 3.11^{ab}	204.22 ± 8.3^a	223.40 ± 11.14^b
Final Total Length	cm	46.29 ± 0.11^a	45.78 ± 0.17^a	46.61 ± 1.41^a

Enhance growth performance seen in short-finned eel fed with PF agrees with studies conducted by [23], [26], [31], [15] which had reported pelleted feed enhances the growth performance of most *Anguilla* sp. as it offers advantages such as improved digestibility through gelatinization during extrusion cooking, increased palatability, and greater feed stability.

In this study, PF exhibits superior palatability as similarly reported by [44] compared to dough feed due to uniform texture, reduced leaching leading to lower water stability and effective nutrient retention. The PF has demonstrated significant advantages over dough feed, particularly in terms of higher acceptance and improved growth performance, which can lead to enhanced production. The industry should take decisive steps to transition away from the conventional practice of using dough feed.

Feeding Performance

Feed utilization of short-finned eel fed with different feeds is shown in Table 3. Specifically, those fed PF showed significantly better performance ($p < 0.05$) and experienced a significant improvement in total feed intake (TFI) at 41.07 ± 3.92 g/fish compared to JDF and GDF. A high total feed intake indicates a high preference of the feed, reflecting the fish acceptance to consume the diet provided. This high level of consumption is often a positive sign, suggesting that the feed is not only palatable but also meets the nutritional needs of the fish, encouraging them to ingest more. As a result, this can lead to improved growth rates, better overall health, and enhanced production efficiency. In an aquaculture setting, achieving high feed intake is crucial, as it directly correlates with the success of the feeding strategy and the overall productivity of the operation.

Meanwhile, feed conversion ratio (FCR) result also showed a similar findings whereby short-finned eel fed PF achieved significantly lower FCR at 2.15 ± 0.9 , compared to JDF and GDF. This suggests that they were able to achieve strong growth while consuming less feed, highlighting the feed's effectiveness. In contrast, higher FCR in dough feed groups (JDF and GDF) in the present study could be due to high leaching properties of feed before the ingestion of feed by fish [31]. [23] reported that the decline in feed quality during the preparation of dough feed could adversely affect its acceptance by fish.

In addition to TFI and FCR, protein efficiency ratio (PER) also underlined the advantages of PF for shortfin eels when they attained significant higher (1.15 ± 0.45) compared to JDF and GDF) indicating that the high quality of feed formulation with easily digestible protein sources. According to [40], in pelleting, the feed is extruded, and heat treated, which can improve its digestibility and stability. Hence contributing to the high PER in the present study.

Table 3. Feeding performance of short-finned eel juvenile fed with different feed type

Parameter	Unit	Value		
		Japfa Dough Feed (JDF)	Grinded Dough Feed (GDF)	Pellet Feed (PF)
Total Feed Intake	g/fish	70.30 ± 1.17^a	71.18 ± 0.26^a	41.07 ± 3.92^b
FCR		6.21 ± 2.63^a	14.74 ± 5.68^b	2.15 ± 0.9^c
PER		0.34 ± 0.12^a	0.17 ± 0.09^a	1.15 ± 0.45^b

The process of making dough type of feed often involves steps that may lead to nutrient loss or changes in texture and palatability, which can reduce the feed's appeal to fish. The leaching rate of PF was significantly low (5.59 ± 1.23) compared to JDF (51.4 ± 7.6) and GDF (61.34 ± 8.90) (Table 4), which not only induces poor water quality and reduces visibility but also deteriorates feed quality. This deterioration in quality may result in lower consumption rates, potentially impacting the overall effectiveness of the feeding strategy and the growth performance of the fish. As a result, maintaining feed quality is more feasible for pelleted feed compared to dough feed, which is crucial throughout the preparation process not only to ensure high acceptance and optimal nutritional benefits for the fish but also to maintain good water quality.

The characteristic of PF is considered excellent compared to dough feed as [33] investigated that protein breakdown in the majority of feed components in pelleted feed may aid in the process of food digestion by releasing peptides and free amino acids from polypeptide bonds. Similar findings also reported by [19] that dough type was not well digested and absorbed in the intestine of eel. According to [9] through a decrease in protease activity and digestibility, flounder fed a diet containing 5% CMC concentration experienced slower growth rates [51].

This study also revealed water usage is significant low for in maintaining better water quality for short-finned eel fed with PF (3601.22 ± 503.13 m³) compared to extensive use of water for those fed with JDF (5633.34 ± 701.2 m³) and GDF (6040.29 ± 137.6 m³) respectively. Water pollution by fish feeding is caused largely by increasing biological oxygen demand, as well as nitrogen and phosphorus loading through unconsumed feed and feces [23].

Table 4. Leaching rate (%) and water stability (%) of experimental feeds and water usage (m³)

Parameter	Unit	Value		
		Japfa Dough Feed (JDF)	Grinded Dough Feed (GDF)	Pellet Feed (PF)
Water stability	%	57.7±2.4 ^a	36.75±6.5 ^b	91.53±3.3 ^c
Leaching rate	%	51.4±7.6 ^a	61.34±8.90 ^b	5.59±1.23 ^c
Water usage	m ³	5633.34±701.2 ^a	6040.29±137.6 ^a	3601.22±503.1 ^b

Water Quality and Water Volume

Water quality parameter including DO, pH, ammonia, nitrite and nitrate are presented in figure 1 Overall performance of water quality parameter recorded to be more favourable for the groups of PF compared to FDF and GDF. In the present study, the dough group have the higher ammonia and nitrite level rather than PF. Ammonia can be produced naturally from the breakdown of organic matter and is excreted by fish as a nitrogenous waste product [4].

The use of dough-based with high leaching rate as seen in this study can lead to a deterioration of water quality [31], primarily due to its tendency to increase ammonia levels in the aquatic environment. Elevated ammonia concentrations are particularly hazardous for fish including eels, especially when they are under stress, as it can compromise their health and lead to poor growth outcomes [41]. This adverse impact on water quality may be a significant factor contributing to the suboptimal growth performance as observed in this study.

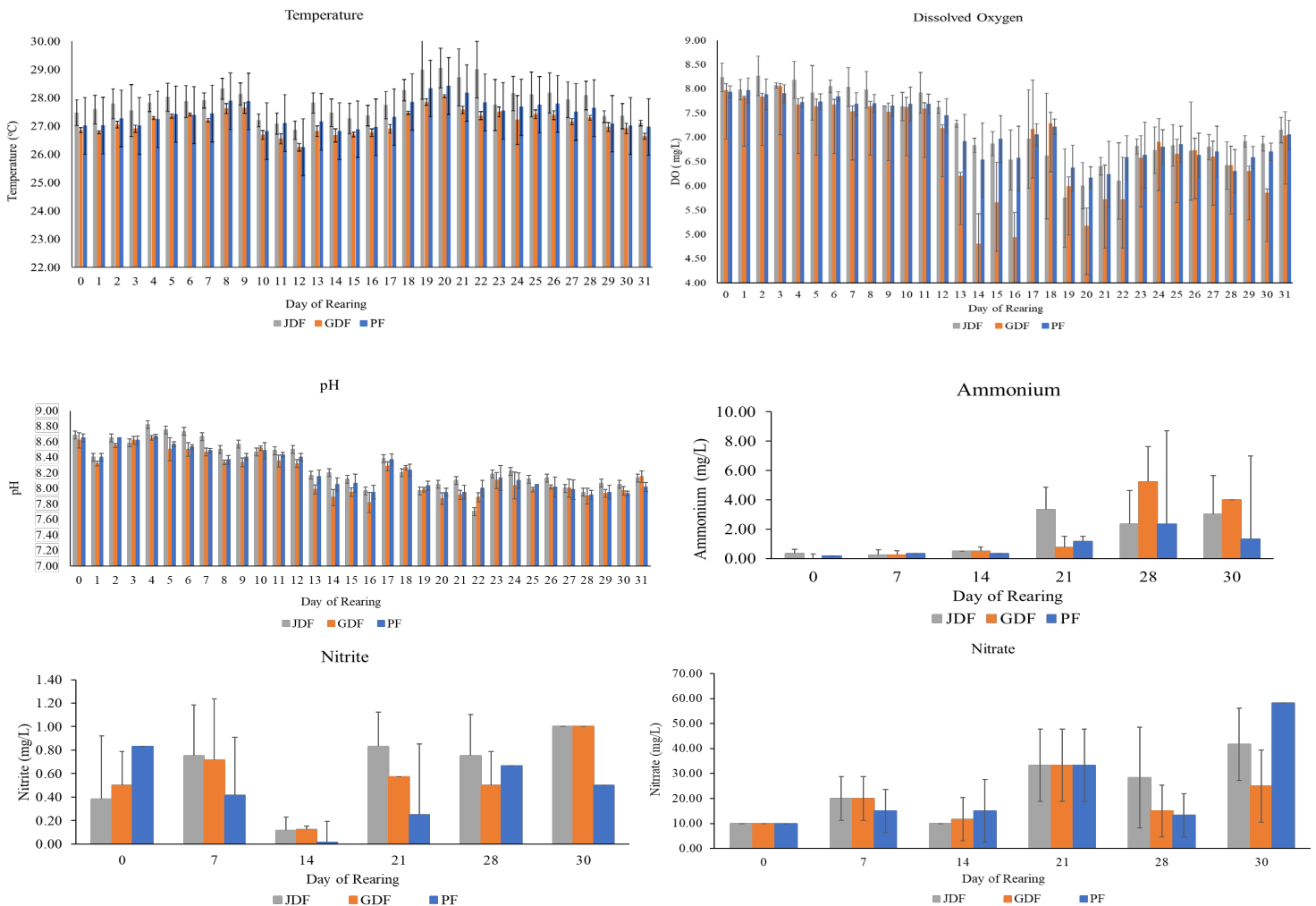


Figure 1. Water quality of the short-finned eel fed with different types of feed

Health Condition

Intestinal and liver histopathological analyses showed no significant visible differences between the all treatments of PF, JDF and GDF. Figure 2 shows the histological of the liver and highlights elements such fatty degeneration and necrosis. It is important to highlight that short-finned eels fed with PF displayed milder and better hepatic tissue health than those fed dough-type feeds. The observed decrease in liver tissue necrosis and fatty degeneration in eels fed pellet feed highlights the potential advantages of this feed in supporting liver health and general wellbeing.

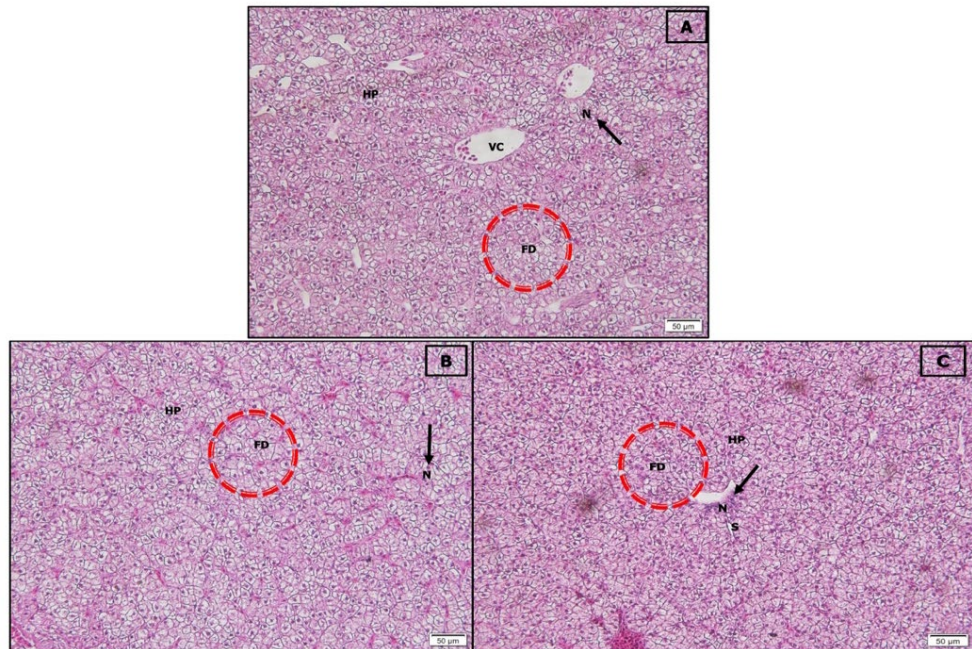


Figure 2. Liver of short-finned eel fed with different types of feed for 31 days. A: Japda dough feed (JDF); B: Dough feed made of from grinded marine pellet (GDF); C: Pelleted feed (PF). Circle indicate Fatty degeneration (FD) and arrow indicate necrosis (N), while others are sinusoid (S), hepatocyte (HP), vena central (VC)

The histopathological examination of liver tissue revealed no significant differences between all treatments, but indicated a potential advantage of PF in maintaining better liver health of short-finned eels. Liver is an important organ of fish and it serves several vital functions including various metabolic processes, detoxifying harmful substances in the body, storage of energy and immune function and more

Use of dough feed should be discontinued to avoid severe fatty liver in eel that will results in reduced growth and feed efficiency, impaired immune response and decreased nutritional quality as similar observed in the present study [48]. Quantitatively, short-finned eels feed in PF had a significantly lower number of adipose degeneration cells ($p < 0.05$) at approximately 125.93 ± 12.52 cells/mm².

Table 5. Average fatty degeneration (cells/mm) and average goblet cell number (per mm) of short, finned eel fed with different type of feeds

Parameter	Unit	Value		
		Japfa Dough Feed (JDF)	Grinded Dough Feed (GDF)	Pellet Feed (PF)
Fatty degeneration	cell/mm ²	399.07±15.80 ^a	313.89±29.40 ^b	125.93±12.52 ^c
Goblet cells	cell/mm ²	77.45±9.45 ^b	127.45±36.3 ^a	34.21±8.49 ^b

Fatty liver is a physiological condition due to an unbalanced diet and is reversible by a balanced diet [11]. It is believed that dough food, which is usually inconsistently prepared by mixing all ingredients into

a paste-like texture, might consists of unknown nutrients that cause rapid fatty liver according to [53]. Unknown nutritional composition of dough feed might consist of high level of free fatty acids and when these components taken too much by fish, it accumulated in the liver can causing fatty degeneration as similarly described by [46]. In the histopathological examination of the intestinal tissue, short-finned eels fed different diets showed similar trend in villi size and goblet cell number, as shown in Figure 2 and detailed in Table 5.

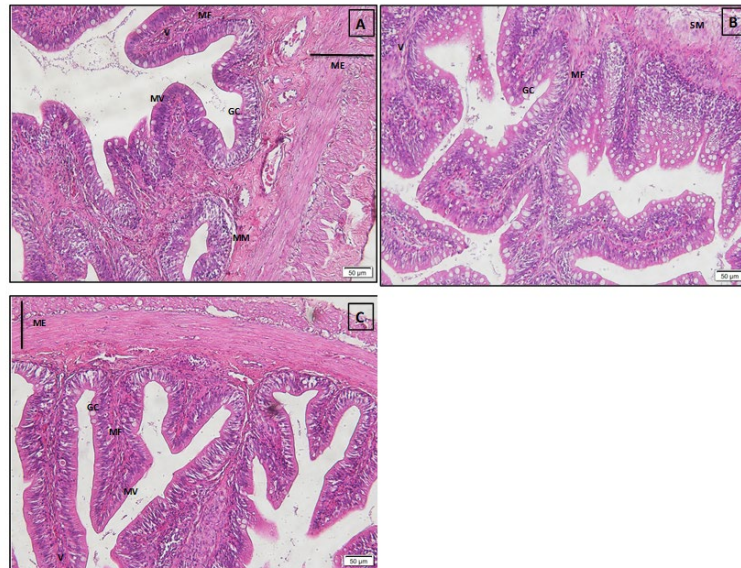


Figure 3. Intestine of short-finned eel fed with different feed type (H and E stain. x400) A: JDF; B: GDF; C: PF. Muscularis mucosae (MM), muscularis externa (ME), Submucosa (SM), Villi (v), Microvilli (MV) vessel (V), goblet cell (GC), mucosal fold (MF)

In this study, no pathomorphological changes were observed in the intestinal among the experimental feed. Increased goblet cells suggest adaptive responses to different types of feed [5], [22]. As an adaptation strategy of the guts to protect the re-absorptive epithelium from antinutritive factors, more goblet cells were generated to create higher content of intestinal mucus in response to higher intestine activity [14]. The similar finding was reported by [18], [24], [31], that different feeding does not affect histological of liver and intestine, the changes is only in the level of folding of intestinal epithelium which more prominent in the anterior and the abundance of goblet cell.

Conclusions

This study concludes pelleted feed does not significantly differ in growth performance of short-finned eel juveniles compared to dough-based feeds but it enhances feeding efficiency with higher feed intake and a lower feed conversion ratio (FCR). It also offers significantly water stability, lower leaching rates, reduced water usage which potential to reduce production cost, and improved health, as indicated by significantly lower fatty degeneration in the fish.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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