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Unraveling The Impact of Feed Protein Content on Catfish (*Clarias* sp.) Growth, Survival, Meat Quality and Gastrointestinal Histology

Unraveling The Impact of Feed Protein Content on Catfish (*Clarias* sp.) Growth, Survival, Meat Quality and Gastrointestinal Histology

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Abstract

Catfish is a highly favored source of protein in Indonesia. Catfish farming can be carried out using various types of feed. This research aims to determine the effect of fish feed with high (24.724%) and low (4.368%) protein content on catfish cultivated using pond/bucket. 50 catfish fingerlings were placed into 5 buckets for each feed treatment. Cultivation lasted for 4 weeks with weekly measurements of physicochemical parameters, morphometrics, and weight. Survival number were assessed at the beginning and end. Flesh samples were taken for proximate testing, and gastrointestinal samples for histological analysis using paraffin method. Data were analyzed using IBM SPSS 20 software. The results show that the TDS and pH values in the high-protein feed are higher than the other group, while the temperature fluctuates. There is a significant difference in the morphometry of catfish fed with high-protein feed. The difference in body weight of catfish fed with high-protein feed is also significantly higher (119.58±16.72 g) compared to the other group (52.20±4.80 g). The average number of surviving catfish fed with high-protein feed is lower (27.60±8.23 fish) compared to the other group (44.00±2.55 fish). Proximate testing indicates that catfish meat with high-protein feed also has higher protein, fat, and carbohydrate content. Histological analysis shows that catfish with high-protein feed have longer villi and higher number of goblet cells, while the gastric pits length is lower. This research shows that high protein feed is important for catfish quality, but it must be accompanied by regular water changes to reduce mortality.

Keywords: *Catfish; Growth; Histology; Protein; Survival.*

Introduction

Catfish cultivation in Indonesia is highly favored by the community due to its promising prospects in terms of demand and selling prices (Hasrah *et al*., 2016). The high interest of the public in catfish as a commodity has driven cultivators to maximize their production. Efforts that can be made by optimizing the quality and efficiency of feed (Ahmadi *et al*., 2012). The nutrition in fish feed must be well maintained. According to research conducted by Muntafiah (2020), the protein content in the feed significantly affects the growth of catfish. If the feed has low protein content, catfish growth is inhibited, and their body weight remains low. According to research by Anggraeni and Rahmiati (2016), in addition to protein, carbohydrates and fats are also necessary for the growth of catfish larvae. The level of protein in the feed is influenced by non-protein energy content from carbohydrates and fats. If the levels of carbohydrates and fats are sufficient, the protein in the catfish is used only sparingly as an energy source, with the remainder being used for growth (Anggraeni and Rahmiati, 2016). If the feed fat content is high, it can accelerate fish growth and efficiency.

Another challenge in catfish cultivation is fish mortality. Mortality can occur when fish experience starvation due to insufficient energy for growth and mobility because of inadequate nutrition in the feed. This indicates that the protein, fat, and carbohydrate content are crucial for fish, and if these three components are well met, catfish survival rate increases (Anggraeni and Rahmiati, 2016). In addition to macronutrients, fish feed also needs to contain micronutrients such as minerals. The measurement of total minerals in a feed ingredient is known as the ash content. Good catfish feed should have a maximum ash content of 13%.

The fish's digestive system starts from the mouth, pharynx, esophagus, stomach, intestines, and anus (Irfandi *et al*., 2019). The walls of the digestive organs in fish are composed of longitudinal and circular muscle layers. The stomach of catfish functions as the site for chemical food digestion assisted by enzymes. The epithelial lamina of the catfish stomach consists of single-ciliated columnar epithelium with nuclei in the basal region that covers the entire outermost layer of the mucosa, mucus substances (Irfandi *et al*., 2019). The intestine organ in catfish serves as the primary location for the digestion and absorption of nutrients. The mucosal tunica of the catfish intestine forms protrusions towards the lumen (villi) that function to expand the nutrient absorption area, thereby increasing absorption efficiency (Sari *et al*., 2016). Based on research by Cahyadi *et al*. (2020), nutrient levels in the feed provided to catfish affect the length of villi during growth. Feeding with high-protein levels such as worms results in longer villi measurements compared to other feed treatments. The length of the villi affects the nutrient absorption capability that occurs in the digestive tract (Qiyou *et al*., 2014). Feed supplemented with enzymes can also improve absorption capacity by increasing the size of intestinal villi, which will suppress bile acid deconjugation earlier (Cahyadi *et al*., 2020).

Research conducted by Prihatini and Febrianto (2021) examined the effect of different protein percentages in catfish feed on survival and growth of catfish. The results showed that feeding catfish with 30% protein content resulted in the highest survival rate, reaching 95.67%. Catfish survival in treatments with lower protein content in the feed provided lower results. This research indicates that feed with different protein percentages has varying effects on fish survival. The protein content in the feed can provide high survival or survival, but if not eaten, it can become toxic to the fish. High protein feed that remains uneaten in the water can turn into toxins like NH3 and NO2-, which can cause fish mortality (Prihatini and Febrianto, 2021).

The study by Prihatini and Febrianto (2021) also showed that high-protein feed resulted in better fish growth. High-protein feed can enhance growth because protein is responsible for building body cells and repairing damaged tissue. The lowest absolute growth is found in fish that received the lowest protein content in the feed (Prihatini and Febrianto, 2021). Observations of the digestive organs of catfish have shown that an acidic environment in the intestines increases the secretion of proteolytic enzymes and affects the rate of protein breakdown in fish feed. Protein that has been modified by probiotics is used for fish growth, which is characterized by increased weight and body length. However, an increase in protein content in feed after fermentation does not show significant results in improving the specific growth rate of fish. This happens because when there is not enough energy for deamination and excretion of excess amino acids absorbed from excessive feed breakdown, a high protein content in the feed (Prihatini and Febrianto, 2021).

Several studies have been conducted on the influence of protein content on the growth and survival of catfish. Based on the data provided by Anggraeni and Rahmiati (2016), fish feed with protein content of 42.01% and 43.05% exhibited catfish weights of 3.85 grams and 3.70 grams, respectively, at the age of 4 weeks. Another study conducted by Mir *et al*. (2020) demonstrated that a protein content of 55% in the feed had a significant impact on survival rates exceeding 70%. Although several studies have explored the effects of protein content in catfish feed on specific aspects, there is still a lack of comprehensive research on the overall impact of feed composition, especially protein, on multiple concurrent effects, including growth, survival, meat quality, as well as gastrointestinal histology. This research urgently endeavors to comprehensively examine the impact of protein, fat, and carbohydrate levels in the feed utilized by the community on the growth, morphological characteristic, survival rate, and meat quality, as well as gastrointestinal histology of catfish, with the goal of providing practical insights for improving guidance in feed management in catfish culture.

Materials and methods

Materials

The tools used in this research include 70-liter bucket, aerator, water quality testing kit, measuring tape, digital scale, stationary, dissecting kit, oven, microtome, microscope, flacon, beaker glass, Optilab software, IBM SPSS 20 software, and Image J software. The materials used in this research include catfish fingerlings (*Clarias* sp.), water, Hi-profit brand fish feed, fish powder, milk powder, fish oil, ice pack, aquades, Bouin's solution, ethanol (70%, 80%, 90%, 96%, and 100%), toluene, paraffin, hematoxylin and eosin (H&E), Mallory Acid Fuchsin (MAF), microscope slides, and microscope coverslips.

Methods

1. Fish maintenance and physicochemical measurements.

This methods is referring to Mujiono *et al.* (2020) with modification. Fish maintenance in a 70-liter bucket involves the following steps: The bucket is initially cleaned with running water, then filled with clean water that has been settled for a minimum of 24 hours. Water quality monitoring is conducted once a week, measuring physicochemical parameters such as Total Dissolved Solids (TDS), pH, and temperature. An aerator is used to maintain the water quality for the catfish. Biofloc is added in the forst week. Both high-protein and low-protein feeds were given to 50 catfish in each buckets. Feeding is performed in the morning and evening using high-protein commercial feed (5 replicates/bucket) and low-protein artificial feed (5 replicates/bucket). Water replacement is carried out every week following the water quality assessment. Fish maintenance is conducted for a duration of 4 weeks**.**

2. Morphometric measurements

Morphometric measurements of catfish are conducted weekly. Morphometric measurements include body length, head width, and body weight on 3 catfish samples for each replicate (total of 2x5x3 = 30 individuals). Body length and head width are measured using a measuring tape, while body weight is measured using a digital scale.

3. Catfish survival number measurements

Survival number measurements of catfish are conducted at the beginning and the end of observation. At the beginning, a total of 50 catfish fingerlings were noted in each bucket, while at the end, the total number of surviving catfish in each bucket (a total of 10 buckets) was counted.

4. Proximate testing of feed and catfish meat

The artificial feed is created with optimization, taking into account the composition and basic texture of fish feed. The composition of the artificial feed used is as follows: fish powder 40 g, milk powder 10 g, water 10 ml, and half a teaspoon of fish oil. As for the store-bought feed from a brand called Hi-Profit. The protein content of the artificial feed and store-bought feed is measured through proximate testing at the Chem-Mix Pratama Laboratory in Bantul, Yogyakarta. Proximate testing covers the determination of water content, ash content, protein, fat, crude fiber, carbohydrates, and energy. Following the proximate testing, it is determined that the artificial feed is low in protein but high in fat, while the store-bought feed is high in protein and low in fat. There is no significant difference in carbohydrate content between the store-bought and artificial feed. The results of the proximate testing for both types of feed are presented in Table 1.

Table 1. Proximate Testing of High-Protein and Low-Protein Feeds

Analysis	Water (%)	Ash (%)	Fat (%)	Crude fiber (%)	Carbohydrate (%)	Protein (%)	Energy (cal/100 g)
High-protein feed	10.649	8.680	4.219	8.554	43.174	**24.724**	309.970
Low-protein feed	7.469	12.895	15.864	23.196	36.195	**4.368**	301.632

At the end of the observation, two sample fish, one with low-protein feed and one with high-protein feed, were placed in an ice pack and sent to the Chem-Mix Pratama Laboratory in Bantul, Yogyakarta, for the testing of their meat content using proximate testing. Proximate testing was conducted in triplicate and included measurements for water content, ash content, fat, crude fiber, carbohydrates, protein, and energy.

5. Preparation of histological specimens

This methods is referencing from Layton *et al.* (2018) and Wolfe (2019). The preparation of histological specimens for the catfish's intestines and stomach using the paraffin method is as follows: The intestines and stomach of the catfish are cut and then fixed in Bouin's solution for 24 hours. After fixation, the organ sections are washed with 70% ethanol. Subsequently, dehydration is carried out using a series of ethanol concentrations (70%, 80%, 90%, 96%, and 100%) to remove water. Clearing is performed using toluene as an ethanol clearing agent because paraffin, which will be used, cannot mix with ethanol. Infiltration is done within the paraffin placed in an oven at 65°C. Then, tissue sections in the paraffin block are left to cool and harden. Once hardened, the paraffin block is cut into 5µm thick sections using microtome, and they are stained using hematoxylin and eosin (H&E) and Mallory Acid Fuchsin (MAF) dyes to reveal different tissue structures in microscop slides covered by coverslips. Histological specimen being placed in microscope and then being captured with Optilab with three types of magnification; 4x, 10x, and 40x.

6. Data analysis

Five datasets were obtained from this study. Water quality data, including TDS, pH, and temperature, are presented in graphical form. Morphometric data for catfish are presented in both graphical and tabular formats. The morphometric data, which includes body length, head width, and body weight, were analyzed for normality using the Shapiro-Wilk statistical test and then tested for differences using the Mann-Whitney U test. Catfish survival data were also tested for normality using the Shapiro-Wilk test and then followed by an *Independent Sample T*-test for significant differences. The results of the proximate testing of catfish meat are presented in tabular form. Data from the histological analysis of the intestines and stomach, including the calculation of villi length and gastric pit, as well as the number of goblet cells, are presented in the form of bar charts.

Results and Discussion

1. Physicochemical parameters

Several physicochemical parameters were observed, including total dissolved solids (TDS), pH, and temperature of the catfish cultivation bucket on a weekly basis. TDS represents solid substances in water, such as organic ions, colloids, or dissolved compounds. It's known that the higher the ionized TDS in water, the greater the conductivity of the solution (Zamora *et al*., 2015). Elevated levels of dissolved solids or TDS will also lead to water turbidity, which can result in pollution (Kustiyaningsih and Irawanto, 2020).

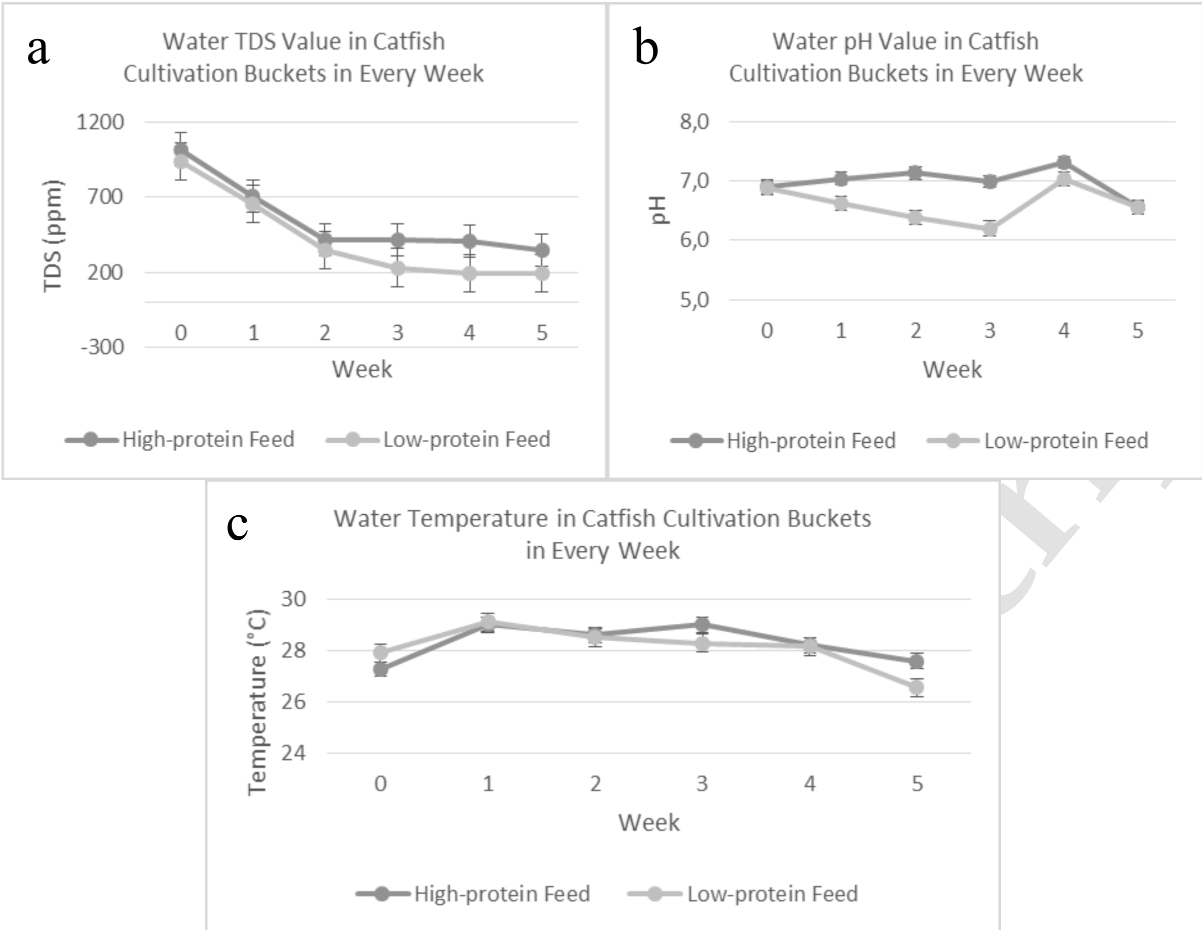


Figure 1. Physicochemical water parameters in buckets with high and low protein feed treatment. (a) TDS; (b) pH; (c) Temperature

Based on the observation of physicochemical parameters (Figure 1), it's evident that the TDS values in the catfish cultivation bucket experienced a drastic decrease from week zero to the second week. Low-protein feed continued to decrease until it stabilized in the fifth week. On the other hand, high-protein feed maintained stable TDS values until the fourth week before experiencing a decrease in the fifth week. It's known that TDS values in fish cultivation can vary depending on the cultivation method and the food given to the catfish. The initial high TDS values may have occurred due to the application of biofloc treatment in the cultivation bucket.

An increase in TDS values can also be interpreted as an increase in mineral content. This increase in mineral content will raise the pH of the water (Sulistiyarto, 2016). In the low-protein feed, it's evident that there was a significant decrease in pH from week zero to the third week. However, there was a significant increase in pH in the fourth week before dropping again in the fifth week. On the other hand, the pH in the high-protein feed bucket initially experienced a mild increase until the second week. In the third week of observation, there was a slight decrease in pH before it rose again in the fourth week and decreased significantly in the fifth week. Temperature observations showed fluctuating results in the high-protein feed bucket until the third week. After that, there was a significant drop in temperature until the fifth week. On the other hand, temperature observations in the low-protein feed bucket showed an increase in temperature in the first week. Subsequently, the temperature observations continuously decreased until the fifth week.

2. Morphometric Analysis

Figure 2 displays the measurements of catfish body length, head width, and body weight with high and low-protein feed treatments.

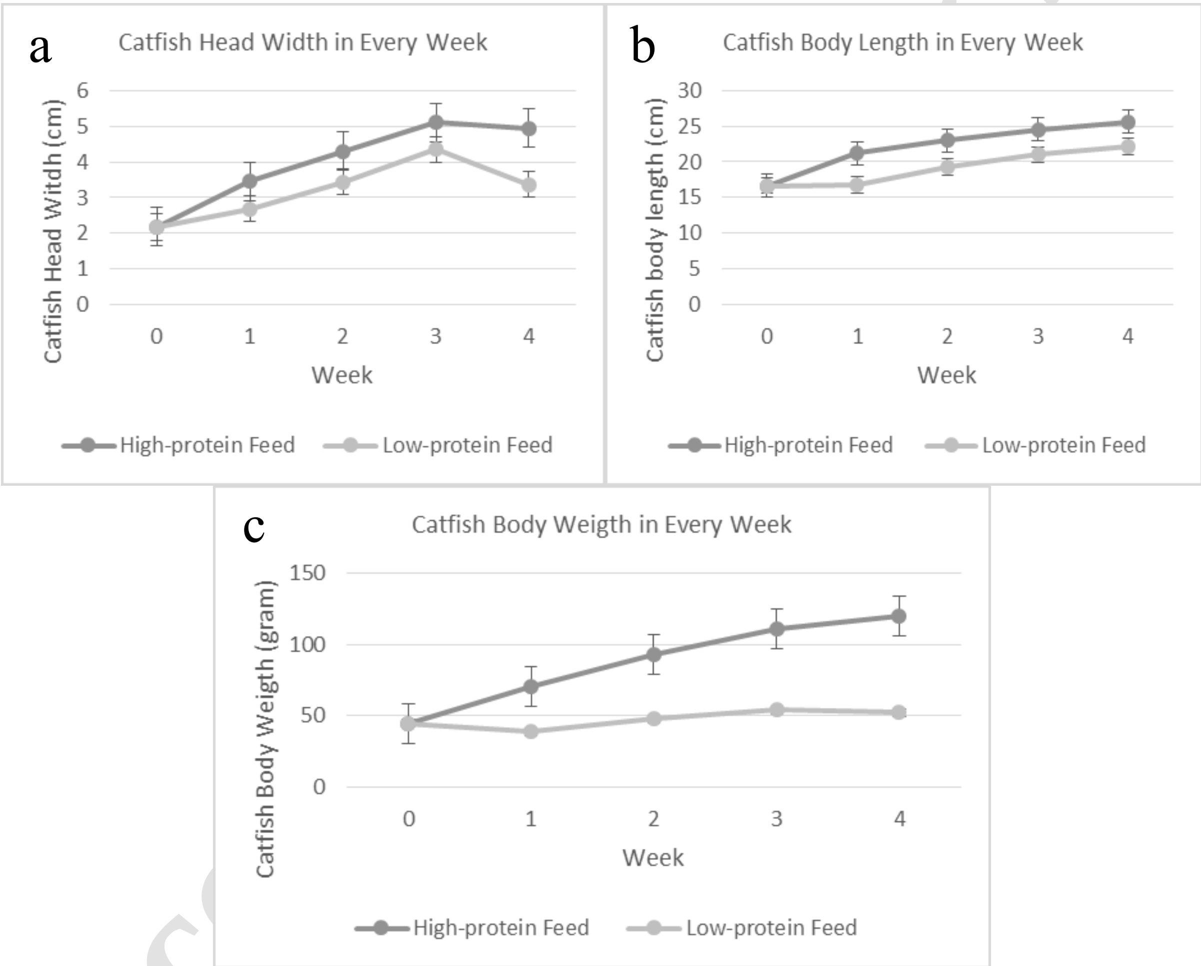


Figure 2. Observations of catfish morphometry under high and low-protein feed treatments in every week. (a) body length; (b) head width; (c) body weight.

In Figure 2, it can be observed that there is an overall increase in the three morphometric characteristics (head width, body length, and body weight) of catfish in both high and low-protein feed treatments from week 0 to week 4. Catfish fed with high-protein feed exhibit larger morphometric characteristics. A notable disparity is evident in the body weight of catfish, with those fed high-protein feed experiencing a drastic increase from less than 50 grams at week 0 to over 100 grams by week 4, whereas catfish fed low-protein feed do not undergo significant changes. The data for these three morphometric characteristics are subsequently analyzed using the Mann-Whitney U Test to assess the presence of significant differences.

Table 2. Catfish Morphometry with High and Low-Protein Feed Treatments (*Mann Whitney U*, *$P<0.05$*)

Week	Morphometry	High-protein Feed	Low-protein Feed	*p*
		***Mean* ± ES**	***Mean* ± ES**	
	BW	70.33±5.82	38.80±7.27	0.003*
1	HW	3.46±0.13	2.69±0.16	0.002*
	BL	21.17±0.44	16.77±1.01	0.005*
	BW	93.00±9.25	47.73±3.38	0.000*
2	HW	4.29±0.15	3.44±0.14	0.001*
	BL	22.98±0.65	19.27±0.43	0.00*
	BW	110.07±17.51	54.27±5.94	0.022*
3	HW	5.09±0.32	4.34±0.26	0.153
	BL	24.53±1.34	21.01±0.95	0.057
	BW	119.58±16.72	52.20±4.80	0.000*
4	HW	4.93±0.32	3.37±0.13	0.000*
	BL	25.62±1.18	22.13±1.11	0.019*

Notes: BW (Body Weight), HW (Head Width), BL (Body Length), ES (Error standard), *P (Significance),* * Asterisk indicates significant difference ($p<0.05$)

Since the *Shapiro-Wilk* test indicated that the morphometric data were not normally distributed, the *Mann Whitney U* test was used to assess significant differences in three fish morphometrics under high and low protein feeding treatments over a 4-week observation period. Data are expressed as mean ± ES. Statistical significance was set at $P < 0.05$. Based on the results of the *Mann-Whitney U* test in Table 2, it can be observed that there is a significant difference where catfish with high-protein feed treatment have significantly higher body weight (BW), head width (HW), and body length (BL) compared to catfish with low-protein feed in all weeks ($p<0.05$), except in the third week. In the observations of the third week, body length and head width between catfish with high and low-protein feed were not significantly different ($p>0.05$). From these results, it can be inferred that the body weight (BW) of catfish with high-protein feed is significantly higher in all weeks. Other research conducted by Ullah *et al*. (2021) also indicates an increase in the body weight of fish parallel to the increase in protein content in the feed. High protein content alone does not cause an increase in body weight. However, a high protein content can stimulate muscle growth, including in animals. Increased weight or body weight is also significantly influenced by the consumed energy.

3. Catfish survival number

At the beginning of the catfish cultivation, all treatments, whether high-protein or low-protein feed, were provided to the catfish, with a total of 50 fish per cultivation bucket and a total of 5 buckets per treatment. During the course of cultivation, many catfish died, especially in the high-protein feed treatment. However, based on the results of the *Independent Sample T*-test with a significance level of 0.05 (P<0.05) using data on all catfish farming buckets (Table 3), it can be concluded that at the end of the observation period, the average number of live catfish with high protein feed (27.60±18.41 fish) was not significantly different from the low-protein feed treatment (44.00±5.70 fish) with a significance value of 0.94 (P> 0.05).

Table 3. The *Independent Sample T*-Test Results for Catfish Survival

Feed Treatment	Number of Surviving Catfish	
	Beginning of Observation (mean±ES)	End of Observation (mean±ES)
High-protein	50.00±00	27.60±8.23[a]
Low-protein	50.00±00	44.00±2.55[a]

Note: ES (Error Standard), [a] (The same superscript letter indicates no significant difference).

Feeds containing high protein do result in significantly higher catfish weights. However, when looking at survival number, the number of surviving catfish is lower in the cultivation buckets with high-protein feed. This can be attributed to feed contaminating the water, which is also related to TDS and pH values. Fish feed can affect the quality of feces and water. Easily soluble feed can reduce water quality and clarity. High-protein feed can increase nitrate levels in the aquarium water, leading to higher toxicity, especially in freshwater fish. High nitrate and ammonia levels are also associated with increased fish mortality. Research conducted by Isaza *et al*. (2021) shows that increasing the frequency and duration of nitrate pollution in fresh water causes fish to experience hypoxia, which is a state of low levels of dissolved oxygen. Without immediate treatment, this condition can cause fish death. In this study, high protein feed tended to dissolve more easily in water, causing the TDS value to also increase. Therefore, the use of high-protein feed should be accompanied by frequent water changes in catfish cultivation buckets to ensure clear water and minimize fish toxicity.

4. Catfish meat proximate testing

The results of the proximate testing of catfish meat with high and low-protein feed can be seen in Table 4. There are six types of analyses: water, ash, protein, fat, carbohydrates, and energy. Catfish meat with high-protein feed has higher amount of protein content (15.130%), fat (8.655%), carbohydrate (1.708%) and energy (149.507%). However, catfish meat with low-protein feed has higher amount of water (77.200%) and ash (4.613%).

Table 4. Result of Catfish Meat Proximate Testing

Analysis	Catfish meat fed with high-protein feed	Catfish meat fed with low-protein feed
Water (%)	72.200	77.200
Ash (%)	2.307	4.613
Protein (%)	15.130	14.374
Fat (%)	8.655	5.639
Carbohydrate (%)	1.708	0.673
Energy (cal/100 g)	149.507	115.005

The protein and fat content of catfish meat with high-protein feed is indeed higher. This is in line with other research conducted by Shadieva *et al*. (2020), which shows that high-protein and high-fat feed also result in significantly higher protein and fat content in catfish meat, although in this study, low-protein feed has higher fat content. The high-protein feed treatment indicates fish meat with a higher protein content and significantly higher energy levels compared to other treatments. This suggests that the protein content of the feed is well absorbed by fish with efficient metabolism.

5. Histology of catfish intestine and gastric

At the end of the observation, the stomachs and intestines of the catfish from the high and low protein feed treatment were taken to determine their histological structure (Figure 3).

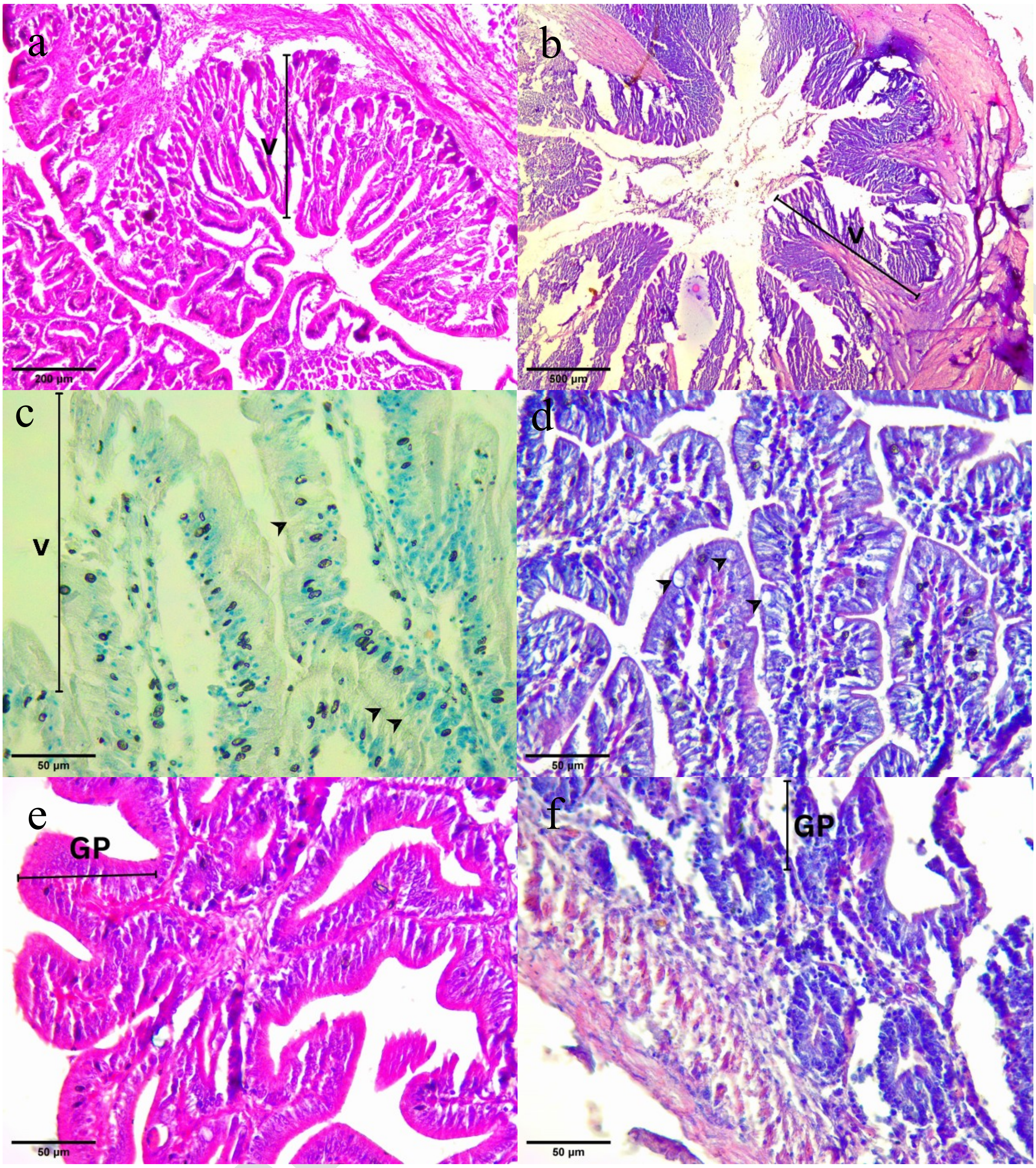
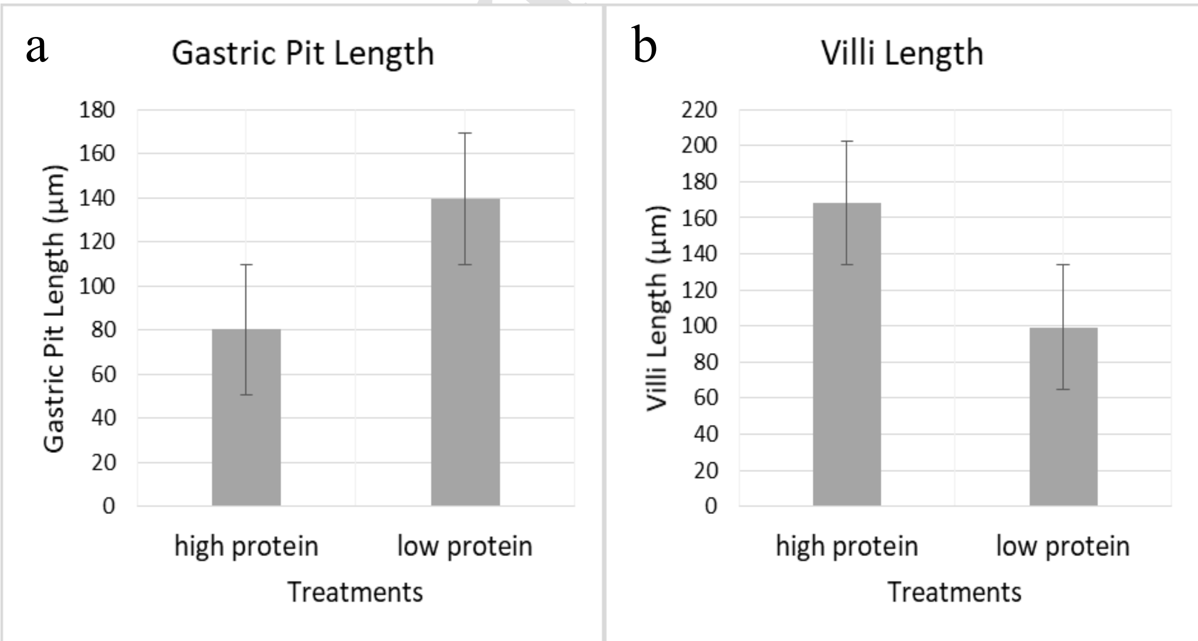


Figure 3. Histological structure of catfish intestines with (a; c) high-protein feed; (b; d) low-protein feed. Histological structure of catfish stomach with (e) high-protein feed; (f) low-protein feed. Both high-protein and low-protein treatments show normal histological architecture. V represent villi. GP represent gastric pit. Arrow heads indicate goblet cells in the intestine, while stomach doesn't have. Specimen stained using (c) Mallory Acid Fuchsin; (a; b; d; e, f) Hematoxylin-Eosin staining. Magnification: 100x (a), 40x (b), 400x (c, d, e, f).

Based on histological observations of the intestines, it is known that both high-protein and low-protein feed treatments show normal architecture with circular and longitudinal muscles, serosa, and villi (Figure 3a and b). At medium power, the small intestine mucosa appears to be normal. Columnar surface eoithelium cells are organized on long fibrovascular cores to form a pattern of villi, which increases absorptive surface area. The villi represented by the letter "V" in Figure 3. In the epithelium, there is growing quantity of goblet cells that are mucin-secreting and pale. Goblet cells appear cup-shaped and are slightly stained in transparent blue in the stainingMallory Acid Stain (Figure 3c) and transparent purple in Hematoxylin-Eosin staining (Figure 3d). In addition, observations of the stomach in both treatments also show normal structures (Figure 3e and f). Unlike the intestine that shows a lot of goblet cells, stomach doesn’t have goblet cells.

Based on the observation of intestine and stomach histological architecture there are no significant difference between high and low-protein feed treatments. Research conduted by Aslaksen *et al*. (2007) using Atlantic Salmon treated with either whole or dehulled faba beans at a 20% inclusion level also show no histological alterations were seen in gastrointestinal tract. Unlike our results and Aslaksen *et al*. (2007), research conducted by Sahin and Gurkan (2022) using Ancistrus cirrhosus fish, a high-protein diet leads to intestinal damage. In line with the study conducted by Sahin and Gurkan (2022), Henish (2023) also found that a higher protein content in the diet leads to more pronounced histopathological changes. Research in the field of fish nutrition should pay attention to the histological condition of the intestine in the future. It will undoubtedly provide additional information about the effect of the treatment to this organ.



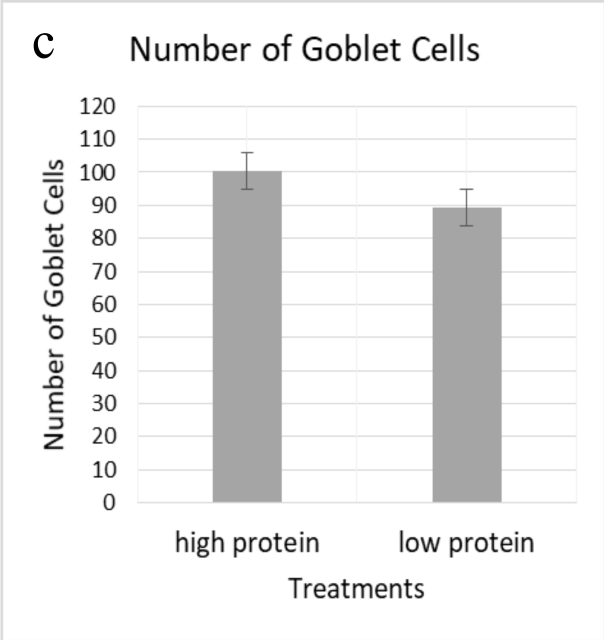


Figure 4. (a) The length of gastric pits in stomach, (b) the length of intestinal villi, and (c) number of goblet cells in the intestines of catfish with high-protein and low-protein feed treatments. Both observation of gastric pits and villi length show significant difference between the two treatments, while the number of goblet cells does not.

Based on the calculations of the length of intestinal villi in both treatments, it is known that they show a significant difference (P<0.05) (Figure 4). This is in line with the research conducted by Ozel *et al*. (2018). In their study, it was found that Salmo labrax had higher intestinal villi length when given higher protein. Additionally, the study conducted by Leduc *et al*. (2018) also showed that feeding with higher dry matter resulted in longer intestinal villi. On the other hand, the length of gastric pits in the stomach of both treatments showed a significant difference between each other (P<0.05). The length of gastric pits in the low-protein treatment yielded higher results compared to the high-protein treatment. Research indicates that a decrease in food intake leads to changes in epithelium architecture (Gilbert *et al*., 2018). This, in turn, affects the length of the gastric pits. Moreover, based on the calculation of the number of goblet cells, there is no significant difference between the two treatments (P<0.05). On the other hand, a study conducted by Lan *et al*. (2015) revealed that a high protein intake results in a low number of goblet cells on the surface of the colon epithelium and increased goblet cell activity in the ileum. Therefore, further research on the impact of protein intake levels on the length of intestinal villi, gastric pit length, and the number of intestinal goblet cells is essential to understand its effects.

Conclusions

The administration of high-protein feed (24%) to catfish results in greater weight, total length, and head width compared to low-protein feed (4%). The catfish meat produced from high-protein feeding also contains higher levels of protein, fat, and carbohydrates than low-protein feed. The structure of the intestines and stomach with high-protein feed does not cause damage or abnormalities in growth. In contrast, low-protein feeding leads to a reduction in gastric pit length due to changes in epithelial structure. Therefore, high-protein feeding remains essential in catfish cultivation in pond/bucket media, while still paying attention to water quality to reduce the number of catfish deaths.

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