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The Effect of Maggot and Silkworm on the Growth and Intestinal Histological Structure of Wader Pari Fish (*Rasbora lateristriata* Bleeker, 1854)

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ABSTRACT Wader pari is one of the autochtonous fish in Indonesian waters. One of the problems regarding fish farming is the problem of feed. This study aims to determine the effect of alternative feeding on the growth and histological structure of the Wader pari fish's intestine. The paraffin method was used in constructing intestinal histological preparations by Haematoxylin-Eosin (HE) and Periodic Acid Schiff-Alcian Blue (PAS-AB) staining. Observations were done using the DMRT test on various ANOVA, and the histological structure of the intestine was analyzed descriptively and quantitatively. The treatments used in this study were P1 (commercial feed), P2 (maggot feed), and P3 (mixed feed between maggot and silkworms). The study's results indicated that the higher the protein consumed by the Wader pari fish, the higher the growth. The three treatments that affected the growth of Wader pari sequentially are mixed feed, maggot feed, and commercial feed. The histological structure of the wader pari intestine is composed of tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. Maggot and mixed feed can affect the histological structure of the Wader pari intestine compared to commercial feed. This can be seen in the results of the length of the villi and the distribution of the number of goblet cells.

Keywords: Fish digestive system; intestinal histological structure; maggot (Black Soldier Fly larvae); silkworm (*Tubifex*); wader pari fish

INTRODUCTION

Indonesia has huge fresh water with the potential for freshwater aquaculture. Compared with the existing waters, the yield of freshwater aquaculture in Indonesia has yet to reach its maximum, with various methods, this resource still has the potential to be developed (Cahyono, 2000). One type of fish often consumed by the public is "wader" fish. Wader pari fish is one of the fish that is often found in Indonesian waters. The distribution of this fish is very wide, including Sumatra, Java, Kalimantan, Bali, Nusa Tenggara, and Sulawesi (Budiharjo, 2002). This wader fish has a high economic value as a popular culinary fish because it has a unique and delicious taste. The demand for the availability and supply of fish is increasing dramatically (Retnoaji *et al.*, 2016).

Wader pari (*Rasbora lateristriata* Bleeker, 1854) usually called as yellow rasbora in Indonesia. This fish is an endemic fish to Southeast Asia. Has a slender body with a maximum length of 170 mm (17 cm), a brownish yellow colour on the back and silver on the lateral-ventral side (Retnoaji *et al.*, 2016). Wader pari fish live in tropical areas where the temperature range is between 22-24 °C, and the water pH is between 6.0-6.5. Wader pari fish live in groups at the bottom or within flowing or stagnant water columns. Wader pari fish also need space to swim freely, although they generally are relatively calm fish in their motion (Sterba, 1989). The following is a picture of the morphology of the Wader pari fish.

Figure 1. Morphology of wader pari fish (*R. lateristriata*) (Sentosa & Djumanto, 2017).

One of the problems regarding fish farming is the problem of feed. Feed is one of the essential parts of production in fish farming activities. The nutritional content in feed required by the fish is generally formulated from plant-based and animal-based raw materials combined to achieve a balanced nutritional content (Yanti *et al.*, 2013). Some of the local raw materials that can be used as a source of animal protein are maggots and silkworms. Maggot is an alternative feed that can be used as a source of protein. Silkworms are natural feed that is generally used by fish hatcheries for the advantage of good nutrition for the growth of fish larvae (Kurniawan *et al.*, 2018; Sutra *et al.*, 2019).

The absorption process takes place in the intestine with a very diverse structure related to eating habits and functional needs. Wader fish have a simpler intestine compared to animals of higher ranks. There are four layers of histological structure of wader fish that consist of tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa (Kierszenbaum, 2002; Ereschenko, 2008). Research on the effects of feeding maggots and silkworms, especially on the growth and histological structure of the intestine, has not been carried out. Hence, this study aims to compare the effect of several feeds on the growth of weight and length of Wader pari within each treatment and to determine the effect of each treatment on the histological structure of the intestine, the length of the villi and the number of goblet cells.

MATERIALS AND METHODS

Location and time of research

This research was conducted at the Laboratory of Animal Structure and Development and at the maintenance and cultivation facility of wader pari, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, from January to May 2022.

Materials

The materials used in this study were wader pari fish (*R. lateristriata*), maggot and silkworms as the fish feed, water and wheat flour, cornstarch, and cooking oil. commercial fish feed compositions are fish meal, soybeans, wheat, and vitamins. The nutritional content of maggot and silk worm feeds is 11.15% water content, 7.61% ash content, 38.42% fat, 31.49% protein and 11.33% carbohydrates. The materials used to construct histological preparations were wader pari, aquades, absolute alcohol, and graded alcohol which are 30%, 40%, 50%, 60%, 70%, 80%, 90% and 96%, paraffin, toluol, HE (Hematoxylin and eosin) stain, PAS-AB (*alcian blue*, *periodic acid*, *schiff reagent*) stain Canada balsam (entellan) NBF, xylol, and nitric acid (decalcification).

Feed production

The production of maggot feed was done by washing the maggot thoroughly. A total of 200 g of maggot was mashed until it became a paste. The maggot paste was mixed with 250 g of wheat flour, 25 g of cornstarch, and 50 g of oil. The dough was then kneaded until smooth, and then the fish feed was dried in the oven and coarsely crushed according to the size of the fish's mouth.

Fish adaptation and maintenance

The larvae obtained from spawning eggs were then placed in the pond that had been provided. Wader pari larvae were raised and cared for in each pond under different treatments. Each pond contained 50 fish. Feeding was done twice daily in the morning and evening with high protein feed until the fish was 2 months old.

Water quality measurement and fish growth measurement

In this study, water quality measurements included physical parameters such as pH, DO, and temperature. After measuring the water quality, the weight and length of the fish were measured by taking 5 fish and then measured with a caliper for the length and a digital scale for the fish's weight. Each parameter and fish growth were measured once a week. Absolute growth in length and weight can be calculated through the formula by Effendie (2002) as follows:

$$L = Lt - Lo$$

Note :
L = absolute growth in length of the fish seed that are tended (cm);
Lt = length of fish seed at the end of maintenance (cm);
Lo = length of fish seed at the beginning of maintenance (cm).

The absolute weight growth can be calculated by formula the Effendie (2002) as follows:

$$W = Wt - Wo$$

Note :
W = absolute growth in weight of fish seeds that are tended (g);
Wt = weight of fish seed at the end of maintenance (g);
Wo = weight of fish at the beginning of maintenance (g).

Survival rate observation (survival rate)

Observation of the survival of wader pari fish can be done by comparing the number of live fish at the end of maintenance with the number of fish at the beginning. The formula used to calculate the fish's survival, according to Effendie (2002), is as follows:

$$SR = \frac{\text{Final number of live fish}}{\text{Initial number of live fish}} \times 100\%$$

Construction of fish intestinal histological preparations

The fish that were tended for 2 months were taken and fixed using an NBF solution that was soaked for 24 hours. Then the decalcification was carried out using nitric acid for 12 hours. After that, the fixative solution was washed using graded alcohol, namely 70%, 80%, 90%, 96% and absolute alcohol. Then the fish was soaked in toluene for 5 hours to clean the tissue. Next, the histological preparations were constructed using the paraffin method at a thickness of 5-6 m and were stained with HE and PAS-AB staining following the protocol described by Bancroft and Cook (Bancroft & Cook, 1998) with modifications.

Data analysis

The data analysis was tested using the DMRT test on various ANOVA with a significance level of P>0.05. The histological structure of the intestine was analyzed descriptively and quantitatively. The descriptive-comparative data compare each treatment's intestine histological structure and treatment based on the morphometry observations. The quantitative data in this study measured the villi's length and the number of goblet cells using the Image J application.

RESULTS AND DISCUSSION

Water quality is an essential parameter in the process of fish farming. Each type of fish has different characteristics to water conditions such as pH, temperature, and dissolved oxygen (DO) levels (Pramana, 2018). The observations of the water quality on the fish maintenance for 2 months is as table 1 .

According to Boyd (1990), the ideal temperature and pH in fish farming range from 20-30°C, and the pH ranges from 6,5-9. The dissolved oxygen (DO) in fish farming ranges above 5 mg/L (Effendi, 2003). Based on the results of the data obtained from the three treatments (P1, P2, and P3), the temperature and pH were still in the normal range as a medium for the maintenance of wader pari (*R. lateristriata*), while dissolved oxygen (DO) in the three treatments (P1, P2, and P3) obtained was lower than the literature, thus allowing the death of the wader pari fish (*R. lateristriata*).

Table 1. Water quality parameters for 2 months of wader pari fish (*R. lateristriata*) maintenance.

Treatment	Average Water Quality Parameters		
	Temperature (°C)	pH	Dissolved oxygen (mg/L)
P1	25.5-29.9	7.5-8.9	3.3-4.1
P2	26.1-29.3	7.5-9.0	3.6-4.5
P3	25.5-29.3	7.5-9.0	3.5-4.6

Note: P1: Treatment of fish maintenance with commercial feed (control); P2: Treatment of fish maintenance with maggot feed; P3: Treatment of fish maintenance with maggot feed and silkworms.

Table 2. The effect of using maggot feed on the survival rate of wader pari fish (*R. lateristriata*) for 2 months of maintenance.

Treatment	Reiteration		Survival rate (%)
	Beginning of maintenance (number of fish)	End of maintenance (number of fish)	
P1	50	47	94±2.1
P2	50	49	98±0.7
P3	50	48	96±1.4

Note: P1: Treatment of fish maintenance with commercial feed (control); P2: Treatment of fish maintenance with maggot feed; P3: Treatment of fish maintenance with maggot feed and silkworms.

The survival rate of fish is a percentage of the final number of live fish during the harvesting process compared to the number of live fish at the beginning of maintenance (Arzad *et al*., 2019). The research results regarding the survival rate of Wader pari (*R. lateristriata*) for 2 months of maintenance is as table 2.

In the three results, P2 (maggot and silkworms) had the highest value of 98%, while P1 (control) had the lowest survival rate for Wader pari fish; the water quality could influence this. According to Fahrizal & Nasir (2018), the survival rate or survival of the fish is strongly influenced by water quality factors and feed factors in the maintenance media which can cause death in fish. Considering the water quality data results obtained from the three treatments (P1, P2, and P3), P1 had the lowest dissolved oxygen at 4.8 mg/L, so more fish died than in the other two treatments. Low dissolved oxygen (DO) can result in hypoxic conditions.

The results of the study on the average absolute length growth of Wader pari fish (*R. lateristriata*) in the 1st and 2nd months during the treatment are as follows:

Table 3. Average absolute growth in length of Wader pari (*R. lateristriata*) during 1 month of maintenance.

Treatment	Reiteration (cm)					Average (cm)
	1	2	3	4	5	
P1	1.5	1.6	1.3	1.4	1.6	1.4±0.1^a
P2	1.8	1.9	1.6	1.3	1.5	1.6±0.2^a
P3	1.8	1.9	1.6	1.3	1.5	1.6±0.2^a

Note: Values with the same superscript show results that are not significantly different from the level of significance (P>0.05).

Based on the observation of the absolute length growth of Wader pari fish (*R. lateristriata*) for one month in each treatment, values did not differ significantly between the treatment results. In this observation, the largest to the smallest growth was shown in fish with P2 and P3 treatments, then P1 treatment. Based on the results of statistical tests on each treatment, it was shown that P1, P2, and P3 did not have a significantly different effect on each other's treatment.

Table 4. Average absolute growth in length of wader pari fish (*R. lateristriata*) for 2 months of maintenance.

Treatment	Reiteration (cm)					Average (cm)
	1	2	3	4	5	
P1	2.9	2.8	2.6	2.7	3	2.8±0.2^a
P2	3.2	2.9	3.3	3.1	34	3.2±0.2^b
P3	3.6	3.2	3.6	3.1	3.3	3.4±0.2^b

Note: Values with the same superscript showed results that were not significantly different from the level of significance (P>0.05).

Based on these observations, the largest to the smallest growth was shown in the wader pari fish with treatments P3 and P2 and the smallest in treatment P1. Based on the results of statistical tests for each treatment, it was shown that P1 had a significantly different effect on P2 and P3. The P2 and P3 treatments had different values, but the statistical test showed that the two treatments did not have significantly different treatment effects. The average absolute growth in length increases in the first 1 month to the last 2 months in each treatment was 1.3 cm in P1 treatment, 1.5 cm in P2 treatment, and 1.7 cm in P3 treatment.

Based on the observations of the average absolute weight gain in wader pari fish (*R. lateristriata*) from the largest to the smallest, namely the P3 treatment had the enormous fish weight, while the most negligible fish

Table 5. Average absolute growth in weight of wader pari fish (*R. lateristriata*) during 1 month of maintenance.

Treatment	Reiteration (g)					Average (g)
	1	2	3	4	5	
P1	0.051	0.058	0.029	0.035	0.058	0.046±0.013[a]
P2	0.043	0.09	0.041	0.055	0.03	0.052±0.023[a]
P3	0.068	0.072	0.065	0.033	0.07	0.062±0.016[a]

Note: Values with the same superscript show results that are not significantly different from the level of significance (P> 0.05).

Table 6. Average absolute growth in weight of Wader pari fish (*R. lateristriata*) for 2 months of maintenance.

Treatment	Reiteration (g)					Average (g)
	1	2	3	4	5	
P1	0.227	0.198	0.129	0.159	0.316	0.206±0.072[a]
P2	0.23	0.147	0.28	0.21	0.358	0.245±0.079[ab]
P3	0.315	0.259	0.424	0.279	0.258	0.307±0.069[b]

Note: Values with the same superscript show results that are not significantly different from the level of significance (P> 0.05).

weight was in the P1 treatment. Based on the results of statistical tests regarding the average absolute growth in weight of wader pari (*R. lateristriata*) in each treatment, it was shown that P1, P2, and P3 did not have a significantly different effect on each other's treatment.

Based on the observations of the average absolute growth in weight in wader pari fish (*R. lateristriata*) from the largest to the smallest, namely the P3 treatment had the enormous fish weight, while the most negligible fish weight was in the P1 treatment. Based on the results of statistical tests regarding the average absolute weight growth of Wader pari fish (*Rasbora lateristriata*) in each treatment, it was shown that treatment P1 had no significant effect on treatment P2 as treatment P2 had no significant effect on treatment P3. However, statistical tests showed that treatment P1 had a significantly different effect on treatment P3. The average absolute growth in weight in the first 1 month to the last 2 months in each treatment is 0.206 g in P1 treatment, 0.245 g in P2 treatment, and 0.307 g in P3 treatment.

From these results, it can be seen that the Wader pari fish (*R. lateristriata*) experienced a tremendous increase in length and weight growth rate, namely in the supply of P3 treatment with a mixture of maggot and silkworm feed (*Tubifex* sp.), where the growth rate of fish was closely related to the availability of protein in the feed, as protein is a source of energy for fish and protein is a nutrient that fish need for growth. Energy balance and protein content are essential for fish growth since if the need for energy sources is lacking, protein will be broken down and used as an energy source (Iskandar, 2011). Based on the results of the proximate test, the protein content in the mixture of maggot and silkworm feed obtained relatively high results, which are 31.49%, 38.42% fat, and 11.33% carbohydrate content.

The histological structure of Wader pari fish is known to have an intestinal wall composed of four layers: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa (Retnoaji *et al*., 2016).

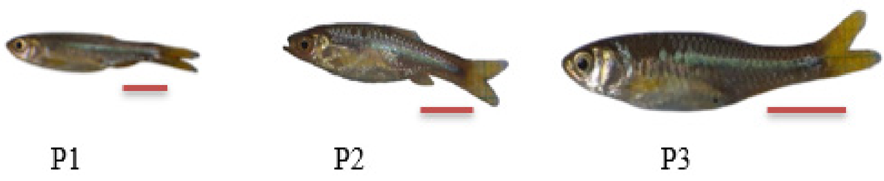

Figure 2. Comparison of morphological sizes of wader pari fish after being treated for 2 months, P1: 2-month-old fish treated with commercial feed, P2: 2-month-old fish treated with maggot feed, P3: 2-month-old fish treated with mixed maggot feed with silkworms. Stem = 0.5 cm.

Based on the figure above, it can be seen that the morphology of P3 had the most significant fish body compared to P1 and P2. In these three treatments, P3 in the form of treatment with maggot feed and silkworms showed the best results, where the nutrient content of the feed mixture was very supportive of the growth of Wader pari (*R. lateristriata*). From this observation, it can be seen that there is absolute growth in length of Wader pari fish (*R. lateristriata*) along with increasing age and maintenance time.

Figures 3 and 4 show the intestine's histological structure, consisting of four tunics: mucosa, submucosa, muscularis, and serosa. The tunica mucosa is most profound in the lumen, which forms folds to form the protrusion of the villi and crypts in the form of pits (Genten *et al*., 2009). Tunica mucosa consists of several parts, namely the lamina propria and the lamina muscularis mucosa. Lamina epithelium is composed of simple cylindrical epithelium consisting of goblet cells. Goblet cells produce mucus to protect the intestinal mucosa from mechanical and chemical damage, transport molecules through membranes and aid absorption (Prakasa, 2015; Fizikri *et al*., 2018).

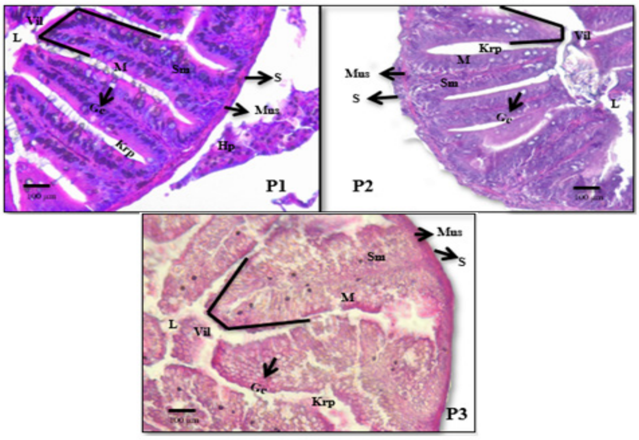

Figure 3. Histological structure of the distal intestine of the wader pari fish in each treatment P1 (retention of fish with commercial feeding), P2 (retention of fish by feeding maggots), P3 (retention of fish with mixed feed and silkworms). Hematoxylin Eosin (HE) staining. 40x magnification. Description: Lumen (L), Villi (Vil), Krypte (Krp), Tunica Mucosa (M), Goblet Cells (GC), Liver (Hp), Tunica Submucosa (Sm), Tunica Muscularis (Mus), Tunica Serosa (S).

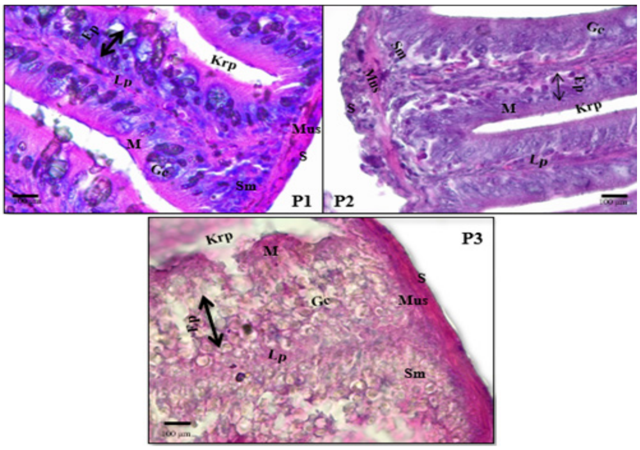

Figure 4. Histological structure of the distal intestine of the wader pari fish in each treatment P1 (retention of fish with commercial feeding), P2 (retention of fish by feeding maggots), P3 (keeping fish with mixed feed and silk- worms). Hematoxylin Eosin (HE) staining. 100x magnifi-cation Description: Lamina propria (Lp), epithelial cell nucleus (Ep), Krypte (Krp), Tunica Mucosa (M), Goblet cells (GC), Tunica Submucosa (Sm), Tunica Muscularis (Mus), Tunica Serosa (S).

Tunica submucosa comprises connective tissue with vessels, lymph, and nerve vessels. Tunica serosa is the outermost part of the intestine that contains connective tissue and collagen fibres. Tunica serosa consists of a thin layer of connective tissue lined by a simple squamous epithelium with blood vessels and tissues (Prakasa, 2015; Emha, 2018). The results of observations of the intestine structure of Wader pari fish with Hematoxylin-Eosin (HE) staining at 40x and 100x magnifications in each treatment showed differences in the lengths of the villi, yet the structure which was observed in each treatment was not different.

In this study, we also observed the number of goblet cells in the histological structure of the Wader pari fish's intestine found on the protrusions of the villi. The distribution of goblet cells dramatically affects the activity of the intestine and the mucus content within it.

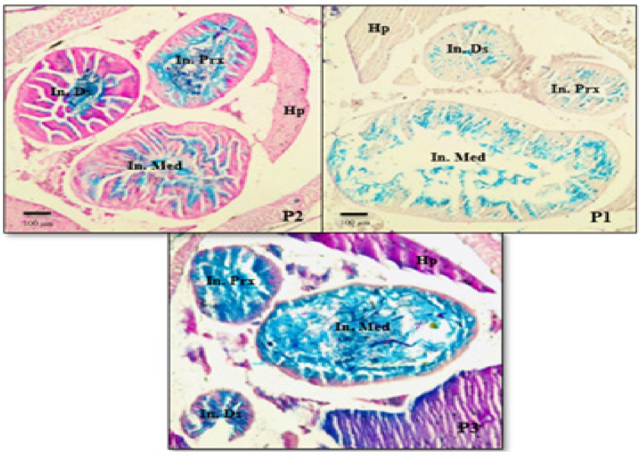

Figure 5. Histological structure of the distal intestine of the wader pari fish in each treatment P1 (retention of fish with commercial feeding), P2 (retention of fish by feeding maggots), P3 (retention of fish with mixed feed and silkworms). Periodic Acid Schif-Alcian Blue (PAS-AB) staining. 4x magnification. information: Proximal Intestinum (In.Prx), Medial Intestinum (In.Med), Distal Intestinum (In.Ds), Liver (Hp).

Figure 5 shows that the intestine is located in the medial part of the digestive cavity, surrounded by the liver that looks circular in the digestive tract. The histological structure of the intestine consists of three parts: the proximal intestine, medial intestine, and distal intestine. In the figure above, PAS-AB staining was used to view the distribution of goblet cells and mucus on the histological structure of the intestine. Goblet cells and mucus were stained positively in blue.

According to the results above on the wader pari fish, goblet cells were distributed more in the distal intestine than in the proximal and medial intestines. Senarat *et al*. (2013) stated that the large number of goblet cells distributed in the distal intestine could facilitate the expulsion of faeces in fish. It can be seen that the proximal and medial intestines produce more mucus than the distal intestines. The existence of mucus that secretes mucus plays a role in facilitating the passage of food through the organs, regulating pH, and protecting from acids (da Silva, 2012).

The results of the length measurement of the villi and the number of goblet cells in the histological structure of the intestine of the wader pari fish can be seen as follows.

Table 7. The average length of villi and the number of goblet cells in the intestines of wader pari fish (*R. lateristriata*).

Treatment	Length of Vili Micrometer (µm)	Number of goblet cells
P1	211.05±14.43[a]	18.2±1.1[a]
P2	248.52±16.90[b]	22.4±1.5[b]
P3	280.73±38.45[c]	31.7±3.7[c]

Note: Values with the same superscript show results that are not significantly different from the level of significance (P>0.05).

Based on these results, it was found that the length of the villi from each treatment was directly proportional to the number of goblet cells. Meanwhile, the histological tests on the intestines of Wader Pari fish with the villi length and number of villi showed that the treatments P1, P2, and P3 were significantly different.

According to da Silva *et al*. (2012), the length of the villi in the fish's intestine can increase the number of enterocyte cells that can function for digestion and absorption of nutrients. According to Kuperman & Kuz'mina (1994), the villi's size can affect food absorption. Longer microvilli absorb food faster than shorter microvilli. The process of villi absorption is closely related to the presence of goblet cells in the intestines of Wader Pari fish.

Goblet cells contain mucin, which lubricates food, protects the walls and surfaces of the intestine and is a defence against parasitic infections. Mucin will become mucus if it is secreted and reacts with water (Zainuddin *et al*., 2017). Mucus can neutralize HCl because it contains bicarbonate that possesses alkaline properties (Sharp, 1982). Fish's digestive activity requires many enzyme secretions. The secretion of these enzymes can stimulate goblet cells to produce more mucus to protect the intestines from mechanical damage and irritation (Dellman & Brown, 1992).

The calculation results of the number of goblet cells in Wader Pari fish in treatment P3 obtained the highest distribution of goblet cells compared to treatments P1 and P2. The number of distributions of goblet cells in the P3 treatment is increasing, which is thought to be an adaptation of the intestinal epithelium in assisting the digestive process in that part of the intestine (Zainuddin *et al*., 2017). Purbomartono *et al*. (2004) stated that the increasing number of goblet cells could help the digestive process of the types of food consumed by fish.

CONCLUSION

In this study, it can be concluded that the higher the protein consumed by Wader Pari fish, the greater the growth. The three treatments affected the growth of Wader Pari sequentially: mixed feed, maggot, and commercial feed. The histological structure of the Wader Pari fish's intestine is composed of tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa. Maggot and mixed feed can affect the histological structure of the Wader Pari's intestine compared to commercial feed. This can be seen in the results of the villi's lengths and the distribution of the number of goblet cells.

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