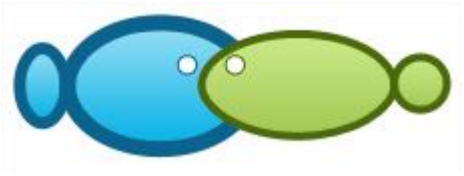

The level of parasitic infection and growth of red tilapia (*Oreochromis* sp.) fed with vegetable fern (*Diplazium esculentum*) flour

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Abstract. This research aims to analyze the level of parasitic infections and the growth of red tilapia (*Oreochromis* sp.) fed with vegetable fern (*Diplazium esculentum*) flour. This study was conducted with an experimental method using a Completely Randomized Design (CRD) research, with four feed dosages treatments: 6%, 8%, 10%, and 12%. Data obtained were analyzed descriptively and quantitatively. The effect of treatments on variables was determined using Analysis of Variance (ANOVA). The results show that there are three types of ectoparasites, namely: *Trichodina* sp., *Cichlidogyrus* sp., and *Chilodonella* sp. The fish organs that were attacked by the ectoparasites were gills, mucous membranes, scales, and caudal fins. Vegetable fern flour as artificial feed ingredients given to red tilapia can reduce the intensity and prevalence of ectoparasites. The intensity of ectoparasites ranged from 15 to 104 individuals fish^{-1}. Prevalence rates range from 17.67 to 66.67%. The highest intensity and prevalence were found in red tilapia without the treatments and low in the treated fish. The addition of vegetable fern flour to artificial feed influences the growth of red tilapia. An 8% dose of artificial feed made from vegetable fern flour resulted in the best weight gain of 0.3913 grams.
Key Words: artificial feed, *Diplazium*, edible fern, ectoparasites.

Introduction. Red tilapia fish (*Oreochromis* sp.) is a freshwater fisheries commodity with significant economic value and potential to be developed. The FAO (Food and Agriculture Organization) Department of Fisheries and Aquaculture consider tilapia as the third best success example of in the world of aquaculture fisheries (after shrimp and salmon) (Irwandi et al 2017). Red tilapia has several advantages such as being easy to cultivate, relatively fast growth, easy to breed, and it is euryhaline and able to resist adverse environmental conditions.

The intensive development of red tilapia aquaculture in Indonesia has been carried out for a long time. However, in practice, it is prone to disease due to the intensive cultivation that maintained high-density fish stocking with intensive artificial feeding. This practice can reduce water quality, potentially triggering diseases, inhibiting growth, and can even result in death (Larasati et al 2020; Putra et al 2017).

According to the Director-General for Aquaculture, Ministry of Marine Affairs and Fisheries of Indonesia, tilapia fish production in Indonesia in 2018 amounted to 1,085 thousand tons. Tilapia production has decreased compared to production in 2017, which reached 1,265 thousand tons. It is due to infection by ectoparasites (Jansen & Mohan 2017).

The three largest tilapia producers in 2015 were Republic of China (producing 1.78 MMT), Indonesia (1.12 MMT), and Egypt (0.88 MMT) (FAO 2017). According to the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries of Indonesia, tilapia fish production in Indonesia has continued to increase since 2011. It increased from 567,078 tons to 999,695 tons in 2014, while in 2015 the production increased to 1,084,281 tons with Indonesia's export value of 14,681 tons. However, in 2016 export value decreased to 11,879 tons due to the infection of ectoparasites.

Ectoparasites are parasites that live on the outer surface of the host body and often infect the skin, fins, and gills of fish. Damage to the gills due to ectoparasitic infection will affect the respiratory system, disrupt the physiological process, inhibit growth, cause mass death, and consumer rejection of fish due to the quality degradation (Purbomartono et al 2010; Purwanti et al 2012). Information regarding the types and levels of parasitic infections that attack fish is required to fight ectoparasite infections appropriately and effectively.

The most widely used method for tackling cultured fish diseases is antibiotic treatment. Antibiotics are divided into two categories, synthetic antibiotics (which come from chemicals) and natural antibiotics. The use of synthetic antibiotics is now banned due to it can cause resistant effects on pathogenic bacteria and cause pollution in the environment. The use of antibiotics in fish consumption can leave residues on the host body. Therefore, it is harmfully consumed by humans as it can cause resistance effects on infectious parasites. Therefore we need other treatment alternatives that are more environmentally friendly, inexpensive, and do not cause resistance to parasites and not prejudicial to the farmers and consumers of fish (Rosenova 2014).

To avoid the negative impact of antibiotic usage, the prevention of fish diseases is pursued through the use of natural ingredients with antimicrobial, antibacterial, and antioxidant properties. Vegetable fern (*Diplazium esculentum*) is such a material. Koniyo (2020) revealed that vegetable fern leaves contain seven secondary metabolites, namely: flavonoids, tannins, phenolics, triterpenoids, steroids, saponins, and glycosides. While, the active compounds that generally act as antioxidants are tannins, flavonoids, phenolics, saponins, and terpenoids. Ajizah (2004) described that flavonoids, saponins, terpenoids, phenolics, and tannins as active compounds that also function as antimicrobial. The content of steroids in vegetable fern can also increase the growth and survival of aquaculture commodities (Koniyo 2020). The purpose of this research is to analyze the level of parasitic infections and growth of red tilapia (*Oreochromis* sp.) that are fed with vegetable fern flour (*Diplazium esculentum*).

Material and Method

Location. The experiment was conducted at the Bube Baru Bone Bolango Aquaculture Laboratory of Gorontalo Province, and at the Center for Fish Quarantine, Quality Control and Safety of Fisheries Products of Gorontalo Province.

Research method. Test animals used in this research were 150 red tilapia fish with a length of ±4 cm and a density of 10 fish/5 liters of water. The research was done in an aquarium with a volume of 10 liters of water equipped with aeration. The fresh water that has been filtered and aerated for one night was used as the culture medium.

The vegetable fern material used to make the flour was old vegetable fern leaves that were not eaten and considered as waste. The dried leaves of the vegetable fern are then made into flour. Artificial feed composition for each raw ingredient within 1000 grams of feed can be seen in Table 1.

Table 1

Composition of artificial feed ingredients within 1.000 gram of feed

No.	*Material*	*Quantity (grams)*
1	Vegetable fern flour	400
2	Soybean flour	170
3	Fine bran	144
4	Fish flour	241
5	Vitamin	25
6	Mineral	20
	Total	1000 grams

This study was done with an experimental method using a Completely Randomized Design (CRD) research approach with four treatments and three replications. The doses given for each treatment are as follows:

- treatment A, feed dose 6% per day;
- treatment B, feed dose 8% per day;
- treatment C, feed dose 10% per day;
- treatment D, feed dose 12% per day.

The experimental procedure was carried out by examining and identifying parasites in fish samples before the experiment. The fish are then kept for 30 days with feeding frequency twice a day.

Weight (gram) measurement for each fish was conducted every week. Ectoparasite examination was taken by scraping the skin of fish, scales, head to tail using a scalpel to collect mucus. The collected mucus is placed on an object-glass, flattened, and dripped with distilled water (aquades), covered with a glass cover, observed under a microscope. Ectoparasite examination on the gills performed by cutting the gills using scissors and then placing them on a petri dish that has been given distilled water, and then observed under a microscope. The parasites were observed using a microscope, and identification of the parasites was made using the guide book Kabata (1985).

Additionally, several supporting parameters on water quality, such as temperature, pH, and dissolved oxygen, were also measured every week.

Data analysis. The data obtained were analyzed descriptively and quantitatively. Analysis of variance (ANOVA) was performed to check the effect of treatment on the observation. Furthermore, the Tukey posthoc test was applied when the results of ANOVA show significantly different among the treatments. Data analysis was done using SPSS software version 22.

Prevalence. Prevalence is the percentage of fish infested by parasites out of the overall number of fish examined. The prevalence formula is as follows (Kabata 1985):

$$\text{Prevalence} = \frac{\sum \text{Number of parasitic fish}}{\sum \text{Examined fish}} \text{ X } 100$$

The prevalence rate criteria are shown in Table 2.

Table 2

Prevalence rate criteria of parasitic infections in fish

No.	*Prevalence*	*Category*	*Information*
1	100-99%	Always	Very severe infection
2	98-90%	Almost always	Severe infection
3	89-70%	Usual	Moderate infection
4	69-50%	Very often	Persistent infections
5	49-30%	Often	Regular infections
6	29-10%	Irregulaar	Frequent infections
7	9-1%	Somewhile	Irregular infection
8	< 1-0.1%	Rare	Infrequent infections
9	< 0.1-0.1%	Very rare	Very rare Infection
10	< 0.01	Almost never	Never infected

Intensity. Intensity is the number of ectoparasites that infect for each sample observed. The calculation of fish parasite intensity value is as follows (Kabata 1985):

$$\text{Intensity (ind/fish)} = \frac{\sum \text{The parasite found}}{\sum \text{Examined fish}} \text{ X } 100$$

The criteria for the intensity of parasitic infections were provided in Table 3.

Table 3

Criteria for the intensity of parasitic infections in fish

No.	*Intensity (individual/fish)*	*Category*
1	< 1	Very low
2	1-5	Low
3	6-55	Moderate
4	51-100	Severe
5	> 100	Very severe
6	> 1000	Super infection

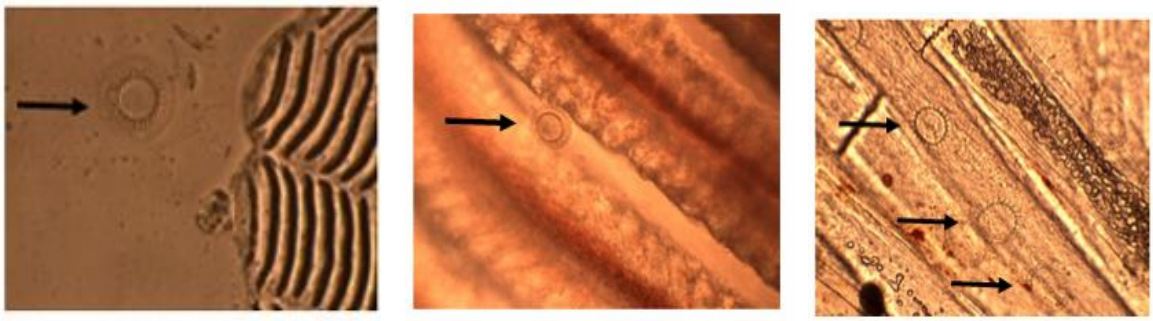
Fish growth. Calculation of growth in absolute weight of red tilapia (W) according to Cholik et al (2005) is as follows:

$$W = W_t - W_0$$

where: W = absolute weight of red tilapia (gram);
W_t = final weight of the research stock on week-t (gram);
W_0 = initial weight of red tilapia stock (gram).

Results

Identification of parasite types. Based on the experiment conducted on 150 samples of red tilapia, there are three types of ectoparasites identified based on their morphological shape, namely *Trichodina* sp. (Figure 1), *Cichlidogyrus* sp. (Figure 2) and *Chilodonella* sp. (Figure 3).



(a) (b) (c)

Figure 1. (a) *Trichodina* sp. (b) in the mucus/scales (c) on the gills of red tilapia.

(a) (b) (c)

Figure 2. (a) *Cichlidogyrus* sp. on the fish gills; (b) morphology of *Cichlidogyrus* sp. in V-shaped ventral transverse bar; (c) morphology of *Cichlidogyrus* sp. in dorsal transverse bar.

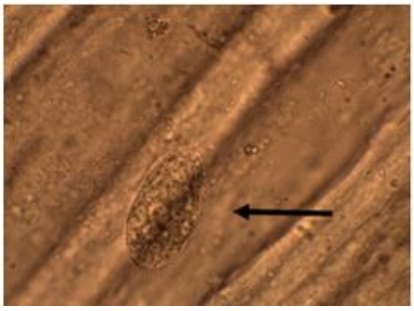


Figure 3. *Chilodonella* sp. on the caudal fin.

Parasitic infection. The detailed level of parasitic infection seen from the prevalence and intensity of attacks on tilapia with variations in treatment doses are presented in Table 4 and Table 5.

Table 4

Intensity of each ectoparasite type found in red tilapia (*Oreochromis* sp.) for each research treatment type

Treatment	*Type of ectoparasites*	*∑Attacked fish*	*∑Parasites*	*Intensity (ind fish^{-1})*	*Category*
Pre-treatment	*Trichodina* sp.	25	2600	104	Very severe
	Cichlidogyrus sp.	18	665	36.94	Moderate
	Chilodonella sp.	17	516	30.35	Moderate
A (6%)	*Trichodina* sp.	19	197	10.36	Moderate
	Cichlidogyrus sp.	10	188	18.8	Moderate
	Chilodonella sp.	10	292	29.2	Moderate
B (8%)	*Trichodina* sp.	10	159	15.9	Moderate
	Cichlidogyrus sp.	11	105	9.54	Moderate
	Chilodonella sp.	7	52	7.42	Moderate
C (10%)	*Trichodina* sp.	14	144	10.28	Moderate
	Cichlidogyrus sp.	8	59	7.375	Moderate
	Chilodonella sp.	7	29	4.14	Low
D (12%)	*Trichodina* sp.	18	239	13.28	Moderate
	Cichlidogyrus sp.	5	23	4.6	Low
	Chilodonella sp.	2	30	15	Moderate

Table 5

Prevalence based on ectoparasites found in red tilapia (*Oreochromis* sp.)

Treatment	*Prevalence*			*Average prevalence*	*Category*
	Trichodina sp.	*Cichlidogyrus sp.*	*Chilodonella sp.*		
Pre-treatment	83.33	60	56.67	66.67	Very often
A (6%)	63.33	33.33	33.33	43.33	General
B (8%)	33.33	36.67	23.33	17.67	Often
C (10%)	46.67	26.67	23.33	32.22	General
D (12%)	60	16	7	27.67	Often

Vegetable fern can be used as medicine because it produces secondary metabolites. Quantitative test results of secondary metabolite compounds of the fern can be seen in Table 6.

Table 6

Quantitative test results of chemical compounds from vegetable fern extract

No	*Compound name*	*Type of compound*	*Area(%)*
1	Tetrahydroxycyclo-hexane carboxylic acid	Tannin	8.6566
2	gamma-Sitosterol	Steroids	7.8705
3	Neophytadiene	Steroids	6.7438
4	5,5a,6,7,8,9,10,10a,11,12 Decahydrobenzo[B]Heptalene	Saponin	4.3542
5	Hexadecanoic acid	Alkaloids	4.2542
6	1,4-Benzenediol	Flavonoids	3,5192
7	5-Hydroxymethylfurfural	Glycosides	3.4544
8	n-Hexadecanoic acid	Alkaloids	2.9183
9	Phytol	Tripernoid	2.9183
10	1,2-Benzenediol	Flavonoids	1.9802
11	Phenol	Phenolic	1.9071
12	3,7,11,15- Tetramethyl-2-Hexadecen-1-ol	Alkaloids	1.7814
13	Benzeneethanol, 4-hydroxy-	Tannin	1.7387
14	2-Pentadecanone, 6,10,14-trimethyl	Tannin	1.6919

Source: Koniyo (2020).

Growth of red tilapia (Oreochromis* sp.*). The results of research on the growth of tilapia fed with different doses of vegetable fern can be seen in Table 7 and Figure 4.

Table 7

Absolute weight growth rate of red tilapia (*Oreochromis* sp.)

Treatment	*N*	*Mean*	*Std. deviation*	*Std. error*	*95% Confidence interval for mean*	
					Lower bound	*Upper bound*
A (6 %)	30	0.1717	0.10901	0.01990	0.1310	0.2124
B (8 %)	30	0.3913	0.33224	0.06066	0.2673	0.5154
C (10 %)	30	0.2143	0.07994	0.01460	0.1845	0.2442
D (12 %)	30	0.2957	0.16237	0.02964	0.2350	0.3563
Total	120	0.2682	0.21183	0.01934	0.2300	0.3065

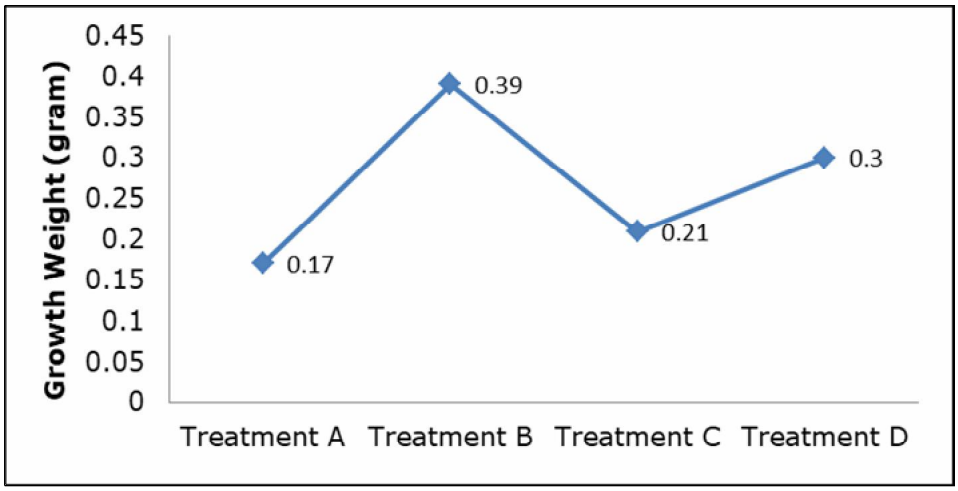


Figure 4. Average of absolute weight growth for red tilapia (*Oreochromis* sp.).

Table 7 and Figure 4 show the average weight growth results from the study:
- weight gain for treatment A (feed dose 6%) was 0.1717 gram;
- weight gain for treatment B (feed dose 8%) was 0.3913 gram;
- weight gain for treatment C (feed dose 10%) was 0.2143 gram;
- weight gain for treatment D (feed dose 12%) was 0.2957 gram.

The highest weight gain was observed in treatment B (feed dose 8%). Analysis of variance (Table 8) and post hoc Tukey tests (Table 9) show that there is no difference in the growth rate of fish weight given feed by adding vegetable fern flour at different doses in treatment A, C, and D. However, they are different when compared to treatment B. Furthermore, treatment B with 8% feeding dose is the optimum dose to trigger the weight growth of red tilapia (0.3913 gram).

Table 8

ANOVA for weight growth of red tilapia (*Oreochromis* sp.)

Source of variation	*Sum of squares*	*Degree of freedom*	*Mean square*	*F*	*Significance F*
Treatment	0.844	3	0.281	7.260	0.000024
Error	4.496	116	0.039		
Total	5.340	119			

Table 9

Tukey test for weight growth of red tilapia (*Oreochromis* sp.)

Treatment	*N*	*Subset for alpha = 0.05*	
		1	*2*
A	30	0.1717	
C	30	0.2143	
D	30	0.2957	0.2957
B	30		0.3913
Significance		0.7500	0.2410

Water quality. The results of water quality measurements during the study period (Table 10) show that water temperatures ranged from 28.23 to 29.36^{o}C, pH between 7.27 and 8.47, and DO rangeg from 4.15 to 5.97 ppm.

Table 10

Results of Water Quality Measurement

Parameters	*Treatment*	*Week*				
		0	*1*	*2*	*3*	*4*
Temperature (^{o}C)		28.23	29.26	29.07	29. 01	28.72
pH	A	7.27	7.83	8.24	8.25	8.20
DO (ppm)		5.69	5.35	5.39	5.22	5.71
Temperature (^{o}C)		2 8.9 5	29.01	29.36	29.17	2 8.91
pH	B	7.37	7.79	8.15	8.01	8.22
DO (ppm)		5.78	5.77	5.65	5.52	5.50
Temperature (^{o}C)		2 8.77	2 9.21	2 9.15	28.44	28.71
pH	C	7.35	7.79	8.23	8.31	8.23
DO (ppm)		5.97	5.2 3	5.30	4.15	4.16
Temperature (^{o}C)		28.65	28.37	28. 33	28. 57	28.63
pH	D	7.31	8.36	7.59	7.66	8.47
DO (ppm)		5.25	5.37	5.48	5.66	5.7 9

Discussion

Ectoparasites found in the fish. *Trichodina* sp. is a pathogenic ectoparasite from the group of Protozoa that usually attacks freshwater fish. *Trichodina* sp. is a member of the protozoan group Ciliata. Their body is flat or round discs in shape with a bowl of teeth in the middle, shaped like a flying saucer, and surrounded by cilia, which they use to swim. The body sides of *Trichodina* sp. have a convex shape. On the disk, there are ring

denture organelles. The structure of *Trichodina* sp. consists of membrane borders, blades, thorns, adhesive disks, and radial pins (Kabata 1985; Anshary et al 2014). *Trichodina* sp. prefers to infect body surfaces, fins, and gills of fishes. *Trichodina* sp. causes a disease in fish called Trichodiniasis. Fish that are infected by *Trichodina* sp. are characterized by grayish-white spots and an increase in mucus production (Gusrina 2008).

Cichlidogyrus sp. is a monogenic class of parasites with an elongated shape and a body that is slightly tapered towards the posterior at both ends, often developing four anterior lobes. They generally have a pair of eyes, though sometimes can be found eyes without a lens. They have an opisthaptor with two pairs of anchors and 14 marginal hooks. *Chichlidogyrus* sp. usually infects cichlid fish such as tilapia, causing severe damage to the gills (Kabata 1985). According to Anshary et al (2014), *Chichlidogyrus* sp. has three pairs of cephalic glands, two pairs of anchors (one ventral and one dorsal), two transverse bars, dorsal bars with auricle and V-shaped ventral transverse bars.

Chilodonella sp. is a monogenic class of parasites with characteristics of 80 µm in size, oval and flat dorsoventral, dorsal convex, having cilia, living as a unicellular individual or as a colony eukaryote. The side of their convex-shaped body is not covered with cilia except on the ventral surface. They also have cytoskeletal in the mouth for helping to eat debris cells (Kabata 1985; Anshary et al 2014).

The intensity of ectoparasites in red tilapia was found to be within the very severe, moderate, and low categories. Ectoparasite intensity ranged from 15 to 104 individuals fish^{-1}. Prevalence is in the category of very often, general, and often with a prevalence range of 17.67 to 66.67%. The highest intensity and prevalence was found in red tilapia, which had not been given the additional treatment of vegetable fern. In contrast, while in fish that were given feed treated with vegetable fern, the intensity, and prevalence of parasites were performed to be lower. The fish organs that were attacked were gills, mucous membranes, scales, and caudal fins. The high value of the intensity and prevalence of ectoparasites that infect red tilapia, especially in the initial fish samples, was due to the fish used as samples are small-sized seedlings (±4 cm) and have not been given feed treatment based on vegetable fern. The endurance level of a larger-sized fish seedling is still weak and very susceptible to changes in the environment, making them more susceptible to parasites. Ectoparasite attack on fish will continue to decrease as the age and size of fish increases. The larger the fish size leads the better immune system of the fish (Rustikawati et al 2004).

Based on observations in Tables 4 and 5, it appears that there are differences in the intensity and prevalence of ectoparasites. *Trichodina* sp. had the highest intensity and prevalence (104 individuals fish^{-1} and 66.67% respectively). The high intensity and prevalence of *Trichodina* sp. is due to this ectoparasite's ability to multiply rapidly through self-division or binary reproduction. According to Kabata (1985), *Trichodina* sp. has an active movement, fast-breeding, tends to attack seedling-sized fish, and generally attacks freshwater fish.

On average, the intensity and prevalence of ectoparasites in red tilapia samples that have been fed with vegetable fern are lower than the average intensity and prevalence of ectoparasites in those that have not been treated. This happens because the addition of vegetable fern to the feed given to red tilapia has an effect of reducing ectoparasite attack.

According to Maulianawati et al (2018), chemical compounds contained in the ferns, namely alkaloids, polyphenols, tannins, steroids, saponins, triterpenoids, show potential as an antibacterial agent, antioxidant, hormone sources, increasing endurance and boosting growth. Vegetable fern contains many active compounds, including steroids, triterpenoids, quinones, and flavonoids, which have many health benefits (Syafitri et al 2017).

Fish growth. In general, the addition of vegetable fern into the feed with different doses increased the growth of the absolute weight of tilapia. Table 7 and Figure 4 show that weight gain is different for each treatment. The growth response of the tilapia is

influenced by differences in the feed doses due to vegetable ferns contain complete secondary metabolites as a trigger for fish growth hormone (Table 6). Based on the proximate analysis of vegetable fern, it has a fat content of 2.47±0.97%, protein 21.52±2.70%, water 16.16±0.69%, and ash 2.67±0.79% (Koniyo et al 2019).

An 8% feed dose can stimulate tilapia to grow optimally as the phyto ecdysterone hormone and nutrient content in vegetable fern can work optimally. In comparison, a low dose of 6% is unable to stimulate growth properly. Similarly, a high dose of 10% and 12% feed results in suboptimal growth. Fujaya et al (2012) stated that tissue or organ growth is strongly influenced by feed quality, hormones, growth stimulants, and the environment. High concentrations of secondary metabolites inhibit the production of receptor hormones and decrease the ability of cell receptors to bind to hormones. If the receptor performance decreases, the formation of new products such as proteins will be inhibited so that growth will be inhibited. High growth in treatment B is caused by the positive response of tilapia to various phytochemical compounds found in vegetable fern extract. It is in line with the results of research conducted by Koniyo (2020) that the use of vegetable fern can increase the percentage of molting, growth, and survival in mangrove crabs.

Overall, water quality measurements during the rearing of red tilapia seedlings show that parameters are still in a good range for the life of tilapia seedlings. Kordi (2004) stated that the range of optimum water quality for tilapia growth is a temperature range of 23-30^{o}C, pH range from 6 to 8.5, and DO > 5 ppm.

Conclusions. Three types of ectoparasites were found in red tilapia, namely *Trichodina* sp., *Cichlidogyrus* sp., and *Chilodonella* sp. Treatment by adding vegetable fern flour as artificial feed ingredients reduced the intensity and prevalence of ectoparasites and positively influenced the growth of red tilapia. Moreover, the best growth of tilapia fish was shown when they were given an 8% dose of artificial feed made from vegetable fern flour.

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