RESEARCH ARTICLE
eISSN 2234-1757
Fish Aquat Sci. 2023;26(10):583-592
https://doi.org/10.47853/FAS.2023.e50
FAS *Fisheries and Aquatic Sciences*

Evaluation of feed types based on growth performance, survival, hematology, and resistance in celebes rainbow (*Marosantherina ladigesi*)

Amrullah[1,*], Wahidah[1], Khusnul Khatimah[1], Ardiansyah[1], Eka Rosyida[2], Imam Taufik[3]

[1] *Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, Indonesia*
[2] *Aquaculture Study Program, Fisheries and Marine Department, Tadulako University, Kampus Bumi Tadulako, Palu 94148, Indonesia*
[3] *National Research and Innovation Agency, Research Center for Fishery, Cibinong Science Center, Bogor 16911, Indonesia*

Abstract

Celebes rainbow (*Marosantherina ladigesi*) is one of Indonesia's exported ornamental fish commodities, but the exploitation of this fish only relies on wild catches. The rise of unlimited fishing, especially those using poison, has changed the aquatic environment, threatening sustainability and causing fish extinction. This study aimed to evaluate the effectiveness of several types of feed in improving the absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), feed efficiency (FE), hematology, and immune response of Celebes rainbow. The fish used in this study were male ornamental Celebes rainbow (*M. ladigesi*) weighing 1.32 ± 0.21 g/ind, reared in 54 L-aquariums at a stocking density of 30 individuals/aquarium for six weeks. The fish were fed according to the test diet consisting of live *Tubifex* sp worms, dry *Tubifex* sp worms, *Spirulina platensis*, and crumble pellets. The parameters observed were AGR, SGR, SR, FCR, FE, hematology, intestinal histology, liver histology, and a challenge test with the pathogenic bacteria *Aeromonas hydrophila*. The results showed that fish-fed live *Tubifex* sp worms had better AGR, SGR, SR, FCR, FE, hematology, and disease resistance compared to all other treatments. These results indicate that live *Tubifex* sp worms are the best feed for rearing Celebes rainbow.

Keywords: Aeromonasis, Fresh water fish, Non-specific immune, Ornamental fish, Tubifex

Introduction

Ornamental fish exhibit a variety of attractive features, such as body color, body shape, and suitability in aquariums (Khomdram, 2018). Various types of ornamental fish are traded on a global scale with approximately 5,000–6,500 freshwater species and 1,600 marine species (Moorhead & Zeng, 2010). According to Moorhead & Zeng (2010), the ornamental fishing industry has reached 15 billion US dollars (USD).

Growing global demand for ornamental fish at rising prices has not been followed by sustainable conservation and farming efforts because of growing pressures (Nunez et al., 2019), especially for wild-caught fishes, due to over-fishing, environmental degradation (Lotze et al., 2006), or climate

Received: May 17, 2023 **Revised:** Jun 21, 2023 **Accepted:** Jul 27, 2023
***Corresponding author:** Amrullah
Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, Indonesia
Tel: +62-82312747489, **E-mail:** ulla_285@yahoo.com

change (Beaugrand & Kirby, 2018). Among the aforementioned problems, freshwater ecosystems face more severe problems than seawater ecosystems (IPBES, 2019). However, conservation efforts have been relatively less extensive than in seawater ecosystems (Strayer & Dudgeon, 2010).

The Celebes rainbow (*Marosantherina ladigesi*) is a type of Indonesian ornamental fish that has been exported since 1976. One of the major importing countries is Germany (Hadiaty, 2007). Exports of rainbow fish depend solely on wild catches, and while these fish may be found in significant quantities in rivers some time ago, unlimited fishing even involves poison (Hadiaty, 2007) and changes in the aquatic environment due to settlements and agricultural activities around the rivers (Gebrekiros, 2016) make the fish increasingly difficult to find and indicate that they have almost dissapear.

Research on Celebes rainbow is limited, especially regarding its cultivation. There have been no specific research reports studying the use of feed at the domestication stage; therefore, this study focused on rainbow fish culture by trying several types of feed by adopting the feeding habits of fish in nature. According to Bilio (2007), feeding habits are important information in the context of limited aquaculture activities. Feeding habits for each species of fish vary, but feeds containing fishmeal and live food are usually more readily accepted by domesticated fish (Li et al., 2019). On the other hand, differences in type, nutrition, and many other differences, will affect feed utilization and ultimately fish growth (Hien et al., 2016). The feed must be available in sufficient quantities, ready to eat and digest effectively and contain nutrients that support fish growth and health. Therefore, it is important to gradually introduce new types of feed to allow the fish to adjust to the type of feed during the rearing period which should be cheap, readily available, and sustainable (Dietrich et al., 2021). However, in Celebes rainbow, no information on suitable foods at the domestication stage, which is why research on four types of feed with different characteristics was tested; live *Tubifex* sp, dry *Tubifex* sp, artificial crumble pellets, and natural feed *Spirulina platensis*. These four experimental feeds have almost the same nutritional content, in particular protein, but have different physical and biological characteristics

The main objective of this pilot study was to investigate the potential of several types of optimum feed to improve the absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), feed efficiency (FE), hematology, immunity and resistance to pathogen of Celebes rainbow.

Materials and Methods

Preparation of experimental fish and rearing conditions

The experimental animals were male Celebes rainbow obtained from the Bantimurung river, South Sulawesi, Indonesia. The fish were kept in the laboratory for 14 days for acclimatization to study conditions, size selection, and determination of fish health status. Acclimatization is also necessary for changing the habits of fish that are lotic in the wild. A total of 360 male fish with an average weight of 1.32 ± 0.21 g/individual and standard length of 4.0 ± 0.55 mm were randomly stocked into 12 aquariums with a volume of 54 liters (30 fish/aquarium). During feed treatment, water parameters were maintained within the same range as in the wild: water temperature 27℃–29℃, dissolved oxygen 6.0–6.4 ppm, pH 7.0–7.3, alkalinity 115–141 ppm, and hardness 109–125 ppm. A change of water was done at 20% daily in addition to siphoning which was done at any time to remove accumulated residual feed or fish feces.

Preparation of the experimental diets

The four types of feed tested consisted of live *Tubifex* sp worms, dry *Tubifex* sp worms, flour *S. platensis* meal, and artificial crumb feed. Live *Tubifex* sp worm was prepared under optimal culture condition provided by the aquaculturist surround, while *S. platensis*, dry *Tubifex* sp, and crumble feed are commercial products. Each test feed was given randomly to an aquarium filled with fish with three repetitions, and the fish were hand-fed to satiation twice daily for six weeks. The nutritional content of the test feeds is presented in Table 1.

Study design

The design used in this study was a completely randomized design with four types of feed treatments, consisting of live *Tubifex* sp worms, dry *Tubifex* sp worms, *S. platensis* meal, and artificial crumble pellets, each triplicate.

Analytical procedures of the diets and fish

The research parameters consisted of the AGR, SGR, SR, FCR, FE, total leukocytes, phagocytic activity, hematocrit (Ht), hemoglobin, total erythrocytes, intestinal and liver histology, and fish immune system assay. Growth performance was observed by weighing 15 fish/aquarium at the beginning and the end of the study. The fish's AGR, SR and FE were calculated based on Busti et al. (2020) and Farhad et al. (2023). Fish hematology was observed by randomly harvesting three fish per study unit at the

Table 1. The nutritional content of the feeds used on the Celebes rainbow (*Marosantherina ladigesi*)

No.	Feed nutrients	Experimental feed			
		Live Tubifex sp	*Dry Tubifex* sp	*S. platensis* meal	Crumble pellet
1	Protein (%)	52	50	55	49
2	Fat (%)	10	8	6	6
3	Carbohydrate (%)	2.1	9	17	10
	Ash	2.1	2	3	4
	Water	10	5	5	12

end of the study (on the 42nd day) and anesthetizing them using MS-222 (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 50 mg/L (Priborsky & Velisek, 2018). The hematological parameters total leukocytes, total erythrocytes, and the differential leukocyte were carried out based on Blaxhall & Daisley (1973), while the phagocytic activity was based on Anderson & Siwicki (1993) and Ht based on Alexpandi et al. (2020).

To observe the development of the fish intestines and the storage of energy reserves in the fish liver, at the end of the study, histological preparations were made from three fish from each type of feed treatment. To make histological preparations, the fish were anesthetized with MS 222 (50.0 mg/L), then the fish was carefully dissected through the ventral cavity. The fish livers and intestines were removed and fixed in 10% formaldehyde (Ogueji et al., 2020) and processed using histological methods. Tissues embedded in paraffin were sliced 5 μm thick and stained with hematoxylin and eosin. The slides were observed using a light microscope to see changes in the intestinal and liver tissues after being treated with several types of feed. Intestine and liver histology results were observed descriptively. Intestine histology of fish is described and scored based on the growth of the fish intestinal villi, presence of goblet cells and lamina propria, as well as liver histology based on differences in the cohesiveness of the uterine cells containing glycogen as energy reserves.

Challenge test

The challenge test was conducted at the end of the study by rearing the fish for seven days in a 5-liter container, each duplicate. The infection was done by spreading *Aeromonas hydrophila* at a density of 10^7 CFU/mL into a receptacle containing 2 liters of aerated water. After the bacteria are homogeneous, five fish were put into the container. After 24 hours, the fish were transferred to a 5-liter container of water and reared for seven days. The accumulation SR was observed daily until the 7th-day post-challenge test.

Data analysis

The data for AGR, SGR, SR, FCR, FE, hematology, and SR post-challenge test were statistically analyzed using SPSS version 22 (IBM, Armonk, NY, USA) with one-way analysis of variance test followed by with Tukey's multiple range test to determine the effect of treatments. The histology of the fish intestines and liver were analyzed descriptively.

Results

Survival rate

The survival of the Celebes rainbow fish during the study period (Table 2) ranged from 64.45%–83.33%. The highest survival percentage of the tested fish was found in the fish-fed live tubifex, followed by fish-fed artificial feed, *S. platensis* meal, and the lowest in dry Tubifex. In general, it demonstrated that the test feed could be utilized by the fish. This is closely related to the omnivorous eating habits of rainbow fish.

Fish growth

Fish growth measured by AGR and SGR (Table 2) showed an increase during the study. The different feeds had a significant effect on fish growth ($p < 0.05$). Tukey's follow-up test showed that the highest AGR, SGR, and final weight of fish were found in the fish-fed live *Tubifex* sp worms, then followed by the artificial feed treatment, *S. platensis* meal, and lastly dry *Tubifex* sp worms ($p < 0.05$).

Feed conversion ratio (FCR) and feed efficiency (FE)

The FCR and FE of rainbow fish given each of the four types of feed treatments are presented in Table 2. Tests with the four different types of feed showed a significant effect on both the FCR and FE ($p < 0.05$). Among the types of feed tested, the application of live *Tubifex* sp demonstrated the lowest FCR in rainbow fish compared to fish in other treatments, whereas the

Table 2. Growth performance and feed utilization of Celebes rainbow (*Marosantherina ladigesi*) fed four different feeds for six weeks

Parameters	Treatment				*p*-value
	Live *Tubifex* sp	Dry *Tubifex* sp	*Spirulina platensis*	Crumble pellet	
IBW (g)	1.22 ± 0.015	1.22 ± 0.010	1.26 ± 0.030	1.25 ± 0.031	0.514
FBW (g)	1.92 ± 0.0^{a}	1.59 ± 0.03^{c}	1.75 ± 0.03^{b}	1.86 ± 0.06^{ab}	0.001
SGR (%BW/day)	0.021 ± 0.0008^{a}	0.010 ± 0.0005^{c}	0.012 ± 0.0012^{c}	0.015 ± 0.0004^{b}	0.000
PBW (g)	0.82 ± 0.02^{a}	0.42 ± 0.02^{c}	0.49 ± 0.03^{c}	0.61 ± 0.02^{b}	0.000
SR (%)	83.33 ± 3.33^{a}	64.45 ± 2.22^{b}	68.89 ± 1.11^{b}	73.33 ± 3.33^{ab}	0.006
FCR (g feed/g gain)	1.20 ± 0.02^{a}	1.34 ± 0.05^{c}	1.30 ± 0.02^{bc}	1.24 ± 0.01^{ab}	0.030
PE (%)	83.54 ± 0.34^{a}	74.52 ± 0.16^{d}	76.80 ± 0.1^{c}	80.71 ± 0.26^{b}	0.000

Each value represents mean ± SE (n = 3). Differences between means were tested with Duncan's multiple range test.
[a–c] Different letters indicate significantly different ($p<0.05$) by Tukey's test; Different superscripts in the same row indicate a significant difference ($p < 0.05$).
IBW, initial body weight; FBW, final body weight; SGR, specific growth rate; PBW, body weight gain; SR, survival rate; FCR, food conversion rate; PE, feed efficiency.

highest FCR was observed in dry *Tubifex* sp feed. Likewise, the FE in fish treated with live *Tubifex* sp feed was more efficient than the other treatments, and the lowest FE was in the dry Tubifex feed ($p < 0.05$).

Fish hematology

The hematology of the test fish in this study all exhibited the effects of the four feed treatments ($p < 0.05$; Table 3). The four types of different feed showed different increases in the fish hematology, but the live *Tubifex* sp feed had the highest and most significant increase in hematology for all the hematological parameters compared to the dry *Tubifex* sp and *S. platensis* feeds.

Fish intestinal and liver histology

Descriptively, the fish's intestinal and liver histology exhibited differences in transverse intestinal slices, intestinal villi slices, and liver slices between the test fish based on feed treatment. In the fish fed live Tubifex, the intestinal villi were well-developed and were denser, allowing them to break down the feed mechanically, the number of lamina propria, goblet cells, and mononuclear immune cell infiltration were also higher, especially in fish that were fed live *Tubifex* sp and commercial feed, whereas the fish fed dry *Tubifex* sp and *S. platensis* had fewer lamina propria and fewer goblet cells (Table 4 and Fig. 1).

The histology of the fish's liver also exhibited differences in terms of cell cohesiveness, glycogen content, which is an energy reserve, and fat content. The livers of the fish-fed live *Tubifex* sp were better than those of the other 3 treatments (Fig. 2).

Challenge test

A challenge test was conducted to determine the fish's resistance to Aeromonas after six weeks of rearing using different types of feed. The SR accumulation post-challenge test until the 7th-day post-challenge test can be seen in Fig. 3. The fish's SR began to decrease on the 3rd day after the challenge test indicated by fish mortality in the treatment using dry *Tubifex* sp, *S. platensis,* and

Table 3. Hematology of the Celebes rainbow (*Marosantherina ladigesi*) fed four different feeds for six weeks: live *Tubifex* sp (A), dry *Tubifex* sp (B), *Spirulina platensis* (C), and crumble pellet (D)

Hematology	Treatment				*p*-value
	Live *Tubifex* sp	Dry *Tubifex* sp	*S. platensis*	Crumble pellet	
Total leukocyte (Cell/mL)	$2.38 \times 10^{4} \pm 0.04^{a}$	$2.01 \times 10^{4} \pm 0.03^{c}$	$2.03 \times 10^{4} \pm 0.04^{c}$	$2.18 \times 10^{4} \pm 0.04^{b}$	0.00
Total erythrocyte (Cell/mL)	$1.02 \times 10^{5} \pm 0.14^{a}$	$8.76 \times 10^{4} \pm 0.13^{b}$	$8.78 \times 10^{4} \pm 0.17^{b}$	$9.6 \times 10^{4} \pm 0.20^{ab}$	0.00
Hemoglobin (%)	6.6233 ± 0.19^{a}	5.4033 ± 0.13^{b}	5.3500 ± 0.18^{b}	6.0867 ± 0.16^{ab}	0.02
Hematocrit (%)	12.2333 ± 0.33^{a}	9.3000 ± 0.33^{c}	8.8667 ± 0.33^{c}	10.5667 ± 0.58^{b}	0.00
Phagocytic activity (%)	7.3333 ± 0.24^{a}	4.3333 ± 0.31^{b}	5.3333 ± 0.15^{b}	6.0000 ± 0.19^{ab}	0.05

Each value represents mean ± SE (n = 3).
[a–c] Different letters indicate statistically significant differences ($p < 0.05$) by the Tukey test.

https://doi.org/10.47853/FAS.2023.e50

Table 4. Histological description of the intestines and liver of Celebes rainbow (*Marosantherina ladigesi*) fed four different feeds: treatment using live *Tubifex* sp feed (A), dry *Tubifex* sp worms (B), *Spirulina platensis* (C), and artificial feed (D)

Organ	Organ description	Treatment			
		Live *Tubifex* sp	Dry *Tubifex* sp	*S. platensis*	Crumble pellet
Intestines	Intestinal villi length, number of lamina propria, goblet cells	++++	++	++	+++
Liver	Compact cell structure, solid, red colour	++++	+	++	+++

'+' indicates qualitative descriptive from lowest to the highest score.

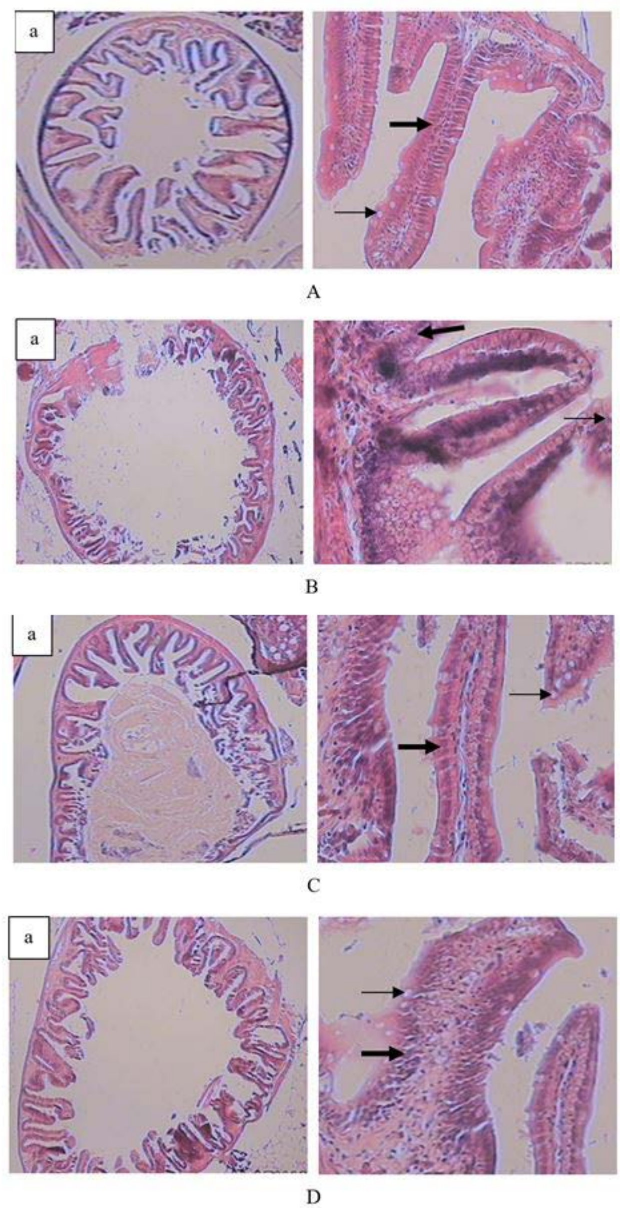

Fig. 1. Histology of the intestines of Celebes rainbow (*Marosantherina ladigesi*) fed four different feeds for six weeks: live *Tubifex* sp (H&E staining; A), dry *Tubifex* sp (H&E staining; B), *Spirulina platensis* (H&E staining; C), and crumble pellet (H&E staining; D). Villi in cross section of fish intestine (a), Goblet cells (→), lamina propria (➡). H&E, hematoxylin and eosin.

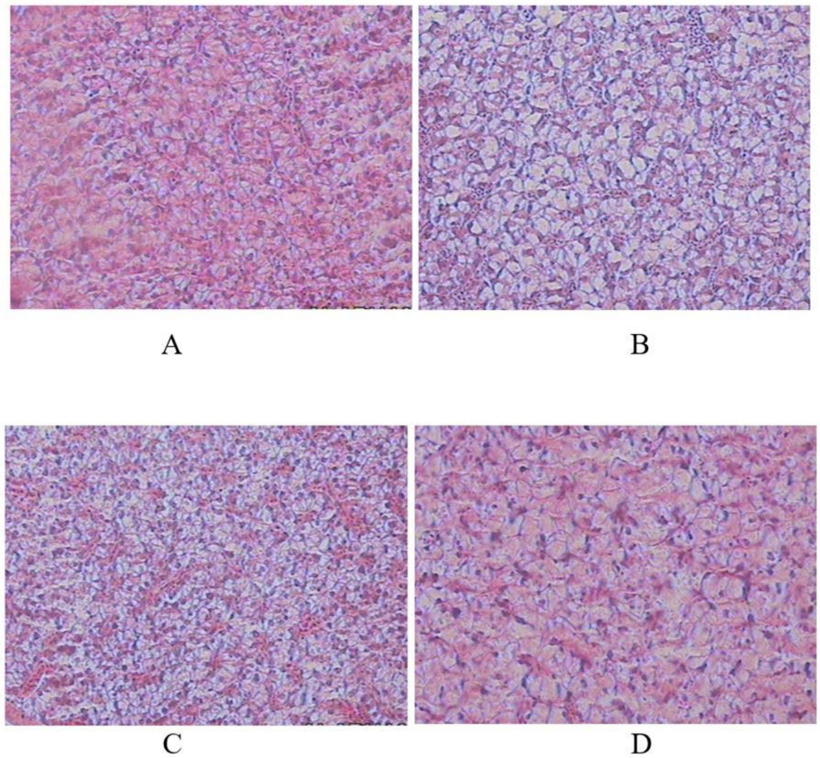

Fig. 2. Histology of the liver of Celebes rainbow (*Marosantherina ladigesi*) fed four different feeds for six weeks: live *Tubifex* sp (H&E staining; A), dry *Tubifex* sp (H&E staining; B), *Spirulina platensis* (H&E staining; C), and crumble pellet (H&E staining; D). H&E, hematoxylin and eosin.

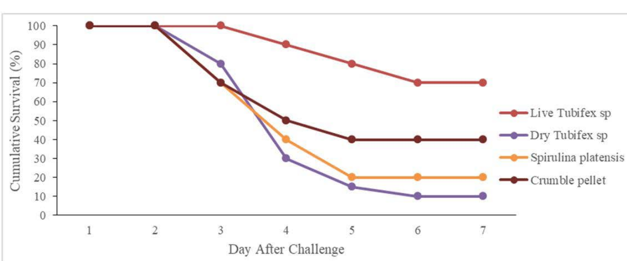

Fig. 3. The cumulative survival rate after the challenge test using *Aeromonas hydrophila* for seven days in Celebes rainbow (*Marosantherina ladigesi*) fed the four experimental diets for six weeks. Each value represents mean ± SE.

crumble pellets test feeds. SR accumulation was lower after high fish mortality in all treatments from day 4 to 7 post-challenge test. The statistical analysis of the fish's SR on the 7th-day post-challenge test showed different fish resistance to infection based on the type of test feed ($p < 0.05$). Tukey's follow-up tests showed that fish-fed live *Tubifex* sp had the highest resistance to disease ($p < 0.05$) while the lowest was found in fish-fed dry *Tubifex* sp and *S. platensis*.

Discussion

The study of maintaining Celebes rainbow with the input of various types of feed has been conducted. This study showed that Celebes rainbow fish could utilize all types of feed tested, although based on statistical tests live *Tubifex* sp was better than the other types of feed tested ($p < 0.05$). The fish fed live *Tubifex* sp worm performed better AGR, SGR, SR, FE, hematology, immunity, and lower FCR compared to pelleted feed, *S. platensis*, and dry *Tubifex* sp (Tables 2 and 3). These results are in line with the use of *Tubifex* sp worms in guppy fish (*Poecilia reticulata*; Perera & Bhujel, 2022) and Clown loach fish, *Cromobotia macracantus* (Putra et al., 2019), demonstrating the best growth and SR. A similar study about several types of feed on *Notopterus chitala* seeds for 60 days showed that *Tubifex* sp increased growth and digestive enzyme activity, and improved the SR (Sontakke

https://doi.org/10.47853/FAS.2023.e50

et al., 2019). Likewise, the use of *Tubifex* sp worm feed for eight weeks (Arslan et al., 2009) resulted in the highest growth rate among live feeds, commercial feeds, and formulated feeds on juvenile South American catfish, surubim (*Pseudoplatystoma fasciatum*). The use of *Tubifex* sp worms also increased the SGR and resulted in the highest SR of *Chitala chitala* after rearing for 28 days (Sarkar et al., 2006).

Hematological observations in this study showed results in the optimal range for the total leukocytes, phagocytic activity, total erythrocytes, Ht, and hemoglobin (Table 3); all these play an important role in the fish's immunity and nutritional metabolism. Hematological parameters are important determinants for assessing fish feed quality and fish health status (Tabassum et al., 2021). This study is also similar to one about the domestication of the Mandarin fish (*Siniperca* sp) which affects growth performance by influencing appetite regulation, digestive enzyme activity, and immune response (Li et al., 2023), gut microbiota, and metabolic analysis (Li et al., 2023).

Although fish fed with Tubifex had higher hematology than other treatments, in general, fish in all treatments showed optimal hematology. This indicates that the physiological condition of fish with feed treatment during the study was normal. Hematology parameters; total leukocytes and phagocytosis activity play a role in fish immunity. Hemoglobin plays a role in metabolism, red blood cells (RBCs) play a role in oxygen transportation to tissues, and Ht determines the volume of RBCs (Taherpour et al., 2023).

The high hematological parameters in this study, especially in fish-fed *Tubifex* sp, are in line with the study by Abarike et al. (2022) who used several natural ingredients in tilapia. Total leukocyte and phagocytic activity play an important role directly in the fish's non-specific immune response (Abarike et al., 2022; Chen et al., 2021). This study also showed that the application of live *Tubifex* sp increased the Celebes rainbow's resistance to pathogenic diseases, especially post-challenge test with *A. hydrophila* (Fig. 3). The challenge test conducted after fish rearing showed that up to day 7, *Tubifex* sp-fed fish had the highest resistance to *A. hydrophila* infection compared to the other treatments. The high resistance of fish treated with *Tubifex* sp is in line with the high hematology of fish, namely total leukocytes and phagocytosis activity (Kim et al., 2021a, 2021b; Table 3) and also many mononuclear immune cells and goblet cells in the intestine (Table 4) which play a role in increasing the immune response. This is especially true in fish-fed *S. platensis* and dry *Tubifex* sp, where the activity of pagosity, total leukocytes, mononuclear immune cells, and goblet cells in the intestine is lower, making them more susceptible to attack by pathogenic bacteria.

Although the four types of feed tested had relatively the same nutritional content, there were differences in the fish's AGR, SGR, SR, FCR, FE, immunity, hematology, and disease resistance. Some of the advantages of *Tubifex* sp compared to other feeds include the size of the *Tubifex* sp feed which is ideal for the size of the fish's mouth and that live *Tubifex* sp will float in the water and will be chased by fish (Busti et al., 2020). *Tubifex* sp worms also do not have a skeleton so they are easily digested by the fish. In addition, the smell and color of live *Tubifex* sp worms stimulate fish to eat them (Budiardi et al., 2005). The nutritional components of *Tubifex* sp are rich in n-3 (C18: 3n-3 and C20: 5n-3), n-6 fatty acids (C18: 2n-6 and C20: 4n-6). *Tubifex* sp worms contain ten essential amino acids; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan.

The ability of fish to digest a feed depends on the feed's physical and chemical characteristics, the type of feed, the nutritional content of the feed, the size and age of the fish, the type of digestive enzymes, the feeding frequency, the water temperature and chemical properties (Jannathulla et al., 2019). In addition, live *Tubifex* sp worms are more palatable to rainbow fish (Jannathulla et al., 2019; Yadav et al., 2020), directly affecting fish growth (Jingting et al., 2020). Live feed such as *Tubifex* sp contains all the nutrients required by fish such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids (New, 1999), therefore the type of feed applied will play a significant role in feed consumption and growth, as well as its effect on the reproductive aspects of fish. Several studies using live *Tubifex* sp feed have shown an increase in fish growth performance, including the ornamental fish *C. chitala* (Sarkar et al., 2006), *Betta splendens* (Mandal et al., 2010) and Guppy fish (Busti et al., 2020). This study also showed that the application of live *Tubifex* sp increased the Celebes rainbow's resistance to pathogenic diseases, especially *A. hydrophila*.

Fish fed with dried *Tubifex* sp, *S. platensis*, and artificial feed showed lower performance in all test parameters compared to fish fed with live *Tubifex* sp. Physically, Spirulina meal is very small in size so it has a large percentage of not being eaten by the fish. Similarly, the artificial feed will be destroyed, thus both types of feed will tend to spread and ultimately not be eaten by fish and can even cause damage to the maintenance water. While the dry *Tubifex* sp test feed had low palatability compared to the live *Tubifex* sp. The physical and biological deficiencies of the test feed caused low feed consumption. It is different for fish fed with live *Tubifex* sp. Although in this study the application of the crumble feed formula improved growth performance, immunity, and resistance not as good as with live *Tubifex* sp but better than other test feeds, thus the application of crumble feed can be used to replace live *Tubifex* sp. According to Mandal et al. (2010), the disadvantages of live food such as *Tubifex* sp are not available at all times which makes it impractical and may contain diseases that can be transmitted to the fish that eat it.

Moreover, the lower growth performance of fish fed with dry *Tubifex* sp, *S. platensis*, and crumble feed was due to the high carbohydrate content. According to Rahman et al. (2023), feed containing high carbohydrates and fiber interfere with enzymes from accessing their substrates or directly interacting with enzymes decelerating digestive processes, thereby impairing the digestibility of feed ingredients. Information on the nutrient digestibility of feed ingredients is indispensable for improving the accuracy of diets for fish species in terms of formulating cost-effective feeds.

The intestinal histology (Fig. 1) of fish fed a diet of live blood worms showed denser, well-developed intestinal villi, allowing them to break down the feed mechanically. The development of these intestinal villi indicates the quality of the feeds tested during rearing (Goda et al., 2020). The villi cell development was almost the same as the intestinal villi in fish-fed artificial feed.

Intestinal villi increase the absorptive surface area while enterocytes are absorptive cells, acting as the main structural component of the villous epithelium, and regulating the passage of nutrient molecules in aquatic animals (Tabassum et al., 2021). Goblet cells are a type of intestinal epithelial cell that secrete mucin, intestinal trefoil factor, and resistin-like molecules which coat the surface of the intestinal tract (Chen et al., 2021). Under certain conditions, mucus secretion will increase, including those related to the immune response that will play a role in protecting fish from disease (Tabassum et al., 2021). Therefore, histological analysis of the intestinal tract of fish is important to evaluate changes at the tissue level of fish during feed testing (Yadav et al., 2020).

The histology of the liver (Fig. 2) showed differences in terms of cohesiveness, where the livers of fish-fed live *Tubifex* sp had a dense and compact liver structure. It can also be seen that the dominantly red color indicates the amount of glycogen which indicates that energy reserves are higher and the fat content is low; on the other hand, in the treatment of dry *Tubifex* sp worms, the livers are dominated by fat and are low in glycogen. This supports the previous statement that live *Tubifex* sp feed has better digestibility.

Accumulation of fat in the liver is the most determining parameter for larval growth and liver metabolic performance of a type of feed. A normal liver with rich hepatocyte glycogen, sinusoids, and central veins suggests that the diet does not cause gastric upset. The opposite can result in poor feed absorption and digestion in fish. However, there were no signs of liver damage in any of the fish, indicating that the feeds tested had a positive effect as a source of energy for the fish (Chen et al., 2021).

Conclusion

Feeding tests demonstrated that Celebes rainbow fish fed live *Tubifex* sp had higher survival, growth, and FE, better hematology and disease resistance, and lower FCR compared to fish fed crumble pellets, *S. platensis* meal, and dry *Tubifex* sp.

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

Not applicable.

Acknowledgements

We would like to thank the Director of Pangkep State Polytechnic of Agriculture and P3M for their advice and assistance in conducting this research.

Availability of data and materials

Upon a reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

The use of animals in this study has fulfilled the principles of animal ethics through research contract number 57.007/K24.6.1/PG.

ORCID

Amrullah	https://orcid.org/0000-0002-1219-9788
Wahidah	https://orcid.org/0000-0001-9177-4957
Khusnul Khatimah	https://orcid.org/0000-0002-6898-1154
Ardiansyah	https://orcid.org/0000-0002-3734-2747
Eka Rosyida	https://orcid.org/0009-0006-7273-0346

Imam Taufik https://orcid.org/0000-0001-8118-5294

References

Abarike ED, Dandi SO, Ampofo-Yeboah A. A blend of Guava, Bitter, and Neem Leaf extracts improves haematology and resistance to co-infection of *Streptococcus agalactiae* and *Aeromonas jandaie* but not liver health in Nile tilapia. Fish Shellfish Immunol. 2022;3:100066.

Alexpandi R, Gopi CVM, Durgadevi R, Kim HJ, Pandian SK, Ravi AV. Metal sensing-carbon dots loaded TiO_2-nanocomposite for photocatalytic bacterial deactivation and application in aquaculture. Sci Rep. 2020;10:1-12883.

Anderson DP, Siwicki AK. Basic hematology and serology for fish health programs. In: Proceedings of the Second Symposium on Diseases in Asian Aquaculture; 1993; Phuket, Thailand.

Arslan M, Dabrowski K, Portella MC. Growth, fat content and fatty acid profile of South American catfish, surubim (*Pseudoplatystoma fasciatum*) juveniles fed live, commercial and formulated diets. J Appl Ichthyol. 2009;25:73-8.

Beaugrand G, Kirby RR. How do marine pelagic species respond to climate change? Theories and observations. Ann Rev Mar Sci. 2018;10:169-97.

Bilio M. Controlled reproduction and domestication in aquaculture: the current state of the art. Part I. Aquac Eur. 2007;32:5-14.

Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. J Fish Biol. 1973;5:771-81.

Budiardi T, Nursyams T, Sudrajat AO. Survival rate and growth of fighting fish larvae (*Betta splendens* Regan) fed on various live foods. J Akuak Indones. 2005;4:13-6.

Busti S, Bonaldo A, Dondi F, Cavallini D, Yùfera M, Gilannejad N, et al. Effects of different feeding frequencies on growth, feed utilisation, digestive enzyme activities and plasma biochemistry of gilthead sea bream (*Sparus aurata*) fed with different fishmeal and fish oil dietary levels. Aquaculture. 2020;529:735616.

Chen Z, Dong S, Dai L, Xie M, Fu W, Yuan X, et al. Effect of food domestication on the growth of *Elopichthys bambusa*. Reprod Breed. 2021;1:157-66.

Dietrich MA, Judycka S, Żarski D, Malinowska A, Świderska B, Palińska-Żarska K, et al. Proteomic analysis of pikeperch seminal plasma provides novel insight into the testicular development of domesticated fish stocks. Animal. 2021;15:100279.

Farhad FB, Hashem S, Rana KMS, Salam MA. Growth performance and hematological responses of silver barb (*Barbonymus gonionotus* bleeker, 1850) fingerlings to dietary blanched moringa (*Moringa oleifera* lam.) leaf meal as a substitute of soybean meal. Heliyon. 2023;9:E13552.

Gebrekiros ST. Factors affecting stream fish community composition and habitat suitability. J Aquac Mar Biol. 2016;4:00076.

Goda AMAS, Ahmed SR, Nazmi HM, Aboseif AM, Taha MKS, Fadda SH, et al. Assessment of a high protein distillers dried grain (HP-DDG) augmented with phytase in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, haematological status, immune response and related gut and liver histology. Aquaculture. 2020;529:735617.

Hadiaty RK. Scientific review of a rainbow fish, *Marosatherina ladigesi* (Ahl 1936) an endemic fauna of Sulawesi. Berita Biol. 2007;8:473-9.

Hien TTT, Trung NHD, Tâm BM, Chau VMQ, Huy NH, Lee CM, et al. Replacement of freshwater small-size fish by formulated feed in snakehead (*Channa striata*) aquaculture: experimental and commercial-scale pond trials, with economic analysis. Aquac Rep. 2016;4:42-7.

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [IPBES]. Global assessment summary for policymakers. Bonn: IPBES; 2019.

Jannathulla R, Rajaram V, Kalanjiam R, Ambasankar K, Muralidhar M, Dayal JS. Fishmeal availability in the scenarios of climate change: inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquac Res. 2019;50:3493-506.

Jingting Y, Danting G, Chun K, Min J, Xueming H. Effect of soybean antigenic protein on feed palatability of fishmeal replaced diets for obscure puffer (*Takifugu fasciatus*) and the alternation of diet preference by domestication. Aquac Rep. 2020;17:100332.

Khomdram BD. A small leap toward DNA barcode library creation of ornamental fishes: development of 17 DNA barcodes from Manipur, India. J Asia Pac Biodivers. 2018;11:452-8.

Kim SM, Lee DW, Kim YJ, Jun LJ, Park HK, Kim YJ, et al. Field experiment on effect of butaphosphan and cyanocobalamin complex on the immunity and stress of olive flounder at low temperature. Fish Aquat Sci. 2021a;24:153-62.

Kim YO, Oh SY, Lee WS. Feeding ratio affects growth, body composition, and blood chemistry of mandarin fish (*Siniperca scherzeri*) in recirculating aquaculture system. Fish Aquat Sci. 2021b;24:219-27.

Li H, Niu S, Pan H, Wang G, Xie J, Tian J, et al. Integrated miRNA-mRNA analysis reveals the molecular mechanism in mandarin fish (*Siniperca chuatsi*) in response to fresh baits and artificial diets feeding. Aquac Rep. 2023;30:101554.

Li H, Xu W, Jin J, Zhu X, Yang Y, Han D, et al. Effects of dietary carbohydrate and lipid concentrations on growth performance, feed utilization, glucose, and lipid metabolism in two strains of gibel carp. Front Vet Sci. 2019;6:165.

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, et al. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science. 2006;312:1806-9.

Mandal SC, Sahu NP, Kohli MPS, Das P, Gupta SK, Munilkumar S. Replacement of live feed by formulated feed: effect on the growth and spawning performance of Siamese fighting fish (*Betta splendens*, Regan, 1910). Aquac Res. 2010;41:1707-16.

Moorhead JA, Zeng C. Development of captive breeding techniques for marine ornamental fish: a review. Rev Fish Sci. 2010;18:315-43.

New MB. Global aquaculture: current trends and challenges for the 21st century. World Aquac. 1999;30:8-13.

Nunez S, Arets E, Alkemade R, Verwer C, Leemans R. Assessing the impacts of climate change on biodiversity: is below 2℃ enough? Clim Change. 2019;154:351-65.

Ogueji EO, Iheanacho SC, Mbah CE, Yaji AJ, Ezemagu U. Effect of partial and complete replacement of soybean with discarded cashew nut (*Anacardium occidentale* L) on liver and stomach histology of *Clarias gariepinus* (Burchell, 1822). Aquac Fish. 2020;5:86-91.

Perera GSC, Bhujel RC. Replacement of fishmeal by house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*) meals: effect for growth, pigmentation, and breeding performances of guppy (*Poecilia reticulata*). Aquac Rep. 2022;25:101260.

Priborsky J, Velisek J. A review of three commonly used fish anesthetics. Rev Fish Sci Aquac. 2018;26:417-42.

Putra DF, Nur F, Rahimi SAE, Othman N. Effects of different live feed on growth and survival rate of clown loach, *Cromobotia macracantus*. IOP Conf Ser Earth Environ Sci. 2019;348:012099.

Rahman MM, Gunathilaka BE, You SG, Kim KW, Lee SM. Apparent digestibility coefficients of plant feed ingredients for olive flounder (*Paralichthys olivaceus*). Fish Aquat Sci. 2023;26:87-96.

Sarkar UK, Lakra WS, Deepak PK, Negi RS, Paul SK, Srivastava A. Performance of different types of diets on experimental larval rearing of endangered *Chitala chitala* (Hamilton) in recirculatory system. Aquaculture. 2006;261:141-50.

Sontakke R, Chaturvedi CS, Saharan N, Tiwari VK, Haridas H, Rani BAM. Growth response, digestive enzyme activity and stress enzyme status in early stages of an endangered fish, *Notopterus chitala* (Hamilton, 1822) fed with live feed and formulated diet. Aquaculture. 2019;510:182-90.

Strayer DL, Dudgeon D. Freshwater biodiversity conservation: recent progress and future challenges. J North Am Benthol Soc. 2010;29:344-58.

Tabassum T, Mahamud AGMSU, Acharjee TK, Hassan R, Snigdha TA, Islam T, et al. Probiotic supplementations improve growth, water quality, hematology, gut microbiota and intestinal morphology of Nile tilapia. Aquac Rep. 2021;21:100972.

Taherpour M, Roomiani L, Islami HR, Mehrgan MS. Effect of dietary butyric acid, *Bacillus licheniformis* (probiotic), and their combination on hemato-biochemical indices, antioxidant enzymes, immunological parameters, and growth performance of Rainbow trout (*Oncorhynchus mykiss*). Aquac Rep. 2023;30:101534.

Yadav G, Meena DK, Sahoo AK, Das BK, Sen R. Effective valorization of microalgal biomass for the production of nutritional fish-feed supplements. J Clean Prod. 2020;243:118697.

https://doi.org/10.47853/FAS.2023.e50