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EFFECT OF ADDING *Moringa oliverfera* LEAF EXTRACT TO FEED ON INCREASING IMMUNITY PERFORMANCE AND GROWTH OF TAWES FISH FRY (*Barbonimus gonionotus*)

Pengaruh Penambahan Ekstrak Daun Kelor (*Moringa oliefera*) pada Pakan terhadap Peningkatan Performa Imunitas dan Pertumbuhan Benih Ikan Tawes (*Barbonimus gonionotus*)

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ABSTRACT

Tawes fish (*Barbonimus gonionotus*) is a freshwater fish commodity with considerable economic value, but its growth and immunity often experience constraints due to feed quality and disease attacks. Efforts to improve fish performance can be carried out through supplementation of natural ingredients, one of which is moringa leaf extract (*Moringa oleifera*), which contains vitamins, proteins, bioactive compounds, and minerals. This study aimed to evaluate the effect of adding moringa leaf extract to feed on the immunity and growth of tawes fish juveniles. The research was conducted for 40 days using a Completely Randomized Design (CRD) with four treatments and four replications. Treatments consisted of commercial feed without moringa extract (A), and commercial feed supplemented with moringa extract at doses of 0.125 g/kg (B), 0.15 g/kg (C), and 0.175 g/kg (D). The results showed that the addition of moringa leaf extract had a significant effect on fish immunity and growth. The best treatment was obtained at a dose of 0.175 g/kg with erythrocyte count of 2.09 × 10^6 cells/mm^3, leukocyte count of 91.1 × 10^3 cells/mm^3, length growth of 1.97 cm, weight of 23.20 g, SGR of 2.98%/day, FCR of 1.59, feed efficiency of 62.99%, and SR of 95%. Therefore, supplementation of 0.175 g/kg moringa leaf extract in feed can enhance the immune system and growth performance of tawes fish juveniles.

Keywords: Tawes Fish, *Moringa oleifera*, Immunity, Growth

ABSTRAK

Ikan tawes (*Barbonimus gonionotus*) merupakan komoditas ikan air tawar dengan nilai ekonomis cukup tinggi yang sering mengalami kendala pertumbuhan dan imunitas akibat kualitas pakan dan serangan penyakit. Upaya peningkatan performa ikan dapat dilakukan melalui suplementasi bahan alami, salah satunya dengan ekstrak daun kelor (*Moringa oleifera*) yang memiliki kandungan vitamin, protein, senyawa bioaktif, dan mineral. Penelitian ini

bertujuan untuk mengevaluasi efek penambahan ekstrak daun kelor yang ditambahkan pada pakan terhadap imunitas dan pertumbuhan benih ikan tawes. Waktu penelitian selama 40 hari dengan metode RAL empat perlakuan dan empat ulangan. Perlakuan terdiri atas pakan komersial tanpa penambahan ekstrak daun kelor (A), serta penambahan ekstrak daun kelor dengan dosis 0,125 g/kg (B), 0,15 g/kg (C), dan 0,175 g/kg (D). Hasil penelitian menunjukkan dengan ditambahkannya ekstrak daun kelor berpengaruh nyata terhadap imunitas dan pertumbuhan ikan. Perlakuan terbaik diperoleh pada dosis 0,175 g/kg dengan jumlah eritrosit 2,09 × 10^6 sel/mm^3, leukosit 91,1 × 10^3 sel/mm^3, pertumbuhan panjang 1,97 cm, bobot 23,20 g, SGR 2,98%/hari, FCR 1,59, efisiensi pakan 62,99%, dan SR 95%. Dengan demikian, penambahan ekstrak daun kelor pada dosis 0,175 g/kg pakan dapat meningkatkan kekebalan tubuh dan kinerja pertumbuhan benih ikan tawes.

Kata kunci: Ikan Tawes, *Moringa oleifera*, Imunitas, Pertumbuhan

INTRODUCTION

Tawes fish (*Barbonimus gonionotus*) is a freshwater fish species that is currently being developed as a fisheries commodity due to its high economic value and its ease of cultivation by Indonesian communities. As fish farmers, the main objective in aquaculture is to increase the growth and health of tawes fish so that they can reach optimal harvest size. In fish farming, feed is a key factor in achieving good growth performance. High-quality feed contains balanced nutritional components.

The cultivation of tawes fish is often confronted with various problems that hinder growth and increase the risk of disease. One of these issues is the frequent occurrence of pathogenic infections that can lead to reduced growth and mass mortality within fish populations. Environmental factors such as poor water quality can weaken the fish's resistance to disease. Tawes fish seedlings, as the initial stage of fish production, have specific nutritional requirements to support growth rate and strengthen the fish's defense system. Studies have shown that the addition of natural extracts to feed can provide significant positive effects.

One natural ingredient that has been widely studied for improving fish health and growth is moringa leaf extract (*Moringa oleifera*). The essential nutrients in *Moringa oleifera* include proteins, vitamins, minerals, and bioactive compounds that provide benefits to fish. Numerous previous studies reported that the addition of moringa leaf extract to fish feed has the potential to enhance immunity while supporting fish growth. These findings align with the research of Mbokane and Moyo (2019), which revealed that moringa leaves contain alkaloids, flavonoids, saponins, tannins, and terpenoids that have been proven to play a role in increasing immunity in fish.

With the addition of moringa leaf extract into the feed, it is expected that disturbances from various types of diseases can be minimized so that fish are protected from stress and the energy from the feed can be optimally utilized for growth. Research on the addition of moringa leaf extract through feed is expected to enhance immunity as well as influence the growth of tawes fish seedlings. This study aims to determine the effective dosage of moringa leaf extract in improving the immune system and supporting the growth of tawes fish.

METHODS

Place and Time of Research

The research took 40 days, from May to June 2024, at the Aquaculture Laboratory of the Faculty of Fisheries and Marine Sciences, Padjadjaran University. *Moringa oloifera* leaf extract was produced at the Central Laboratory of Padjadjaran University.

Tools and materials

The tools used are scales, sieves, blenders, measuring cups, filter paper, rotary vacuum evaporators, jerry cans, air pumps, aeration hoses, aeration stones, aquariums, fiber tanks, do meters, pH meters, mercury thermometers, heaters, cell phones, hoses, trays, sprays, ziplocks, counting chambers, cover glasses, dessecting kits, hand counters, microscopes, dropper pipettes, hemocytometers, millimeter blocks, rulers, and scoops. The research materials used are pf-800 tawes fish, moringa leaf extract, distilled water, hayem solution, turk solution, and progol.

Research Design

This study used a completely randomized design (CRD) experimental method, consisting of four treatments with four replications. The treatments included the addition of *Moringa oleifera* leaf extract to the feed at different doses:

Treatment A : 100% commercial feed (without added moringa leaf extract)
Treatment B : Commercial feed + 0.125 g/kg moringa leaf extract
Treatment C : Commercial feed + 0.15 g/kg moringa leaf extract
Treatment D : Commercial feed + 0.175 g/kg moringa leaf extract

The determination of treatment concentrations was based on research by Rosidah *et al*., (2018) who evaluated the administration of moringa leaf extract to Sangkuriang catfish fry feed with a dose range of 0–0.2 g/kg feed. In that study, the 0.15 g/kg treatment showed the best results with the lowest mortality compared to other concentrations, thus being used as a reference for the optimum dose. Meanwhile, concentrations of 0.125 g/kg and 0.175 g/kg were selected as dose variations below and above the optimum value, respectively, and 0 g/kg was used as a control. The results of preliminary tests at concentrations of 0.15 g/kg and 10 g/kg also showed survival rates of 90% and 60%, respectively, thus supporting a more relevant concentration range for further research. Therefore, in this study the treatments applied were 0 g/kg, 0.125 g/kg, 0.15 g/kg, and 0.175 g/kg as a dose range that is expected to be able to observe the growth and immunity responses of tawes fish optimally.

RESEARCH PROCEDURE

Moringa Leaf Extract

Ten kg of moringa leaves were washed with water, then dried in the sun for 3 days. After the moringa leaves were dry, the simplex was made by blending them, then placed in a macerator to be macerated with the addition of 70% ethanol as a solvent for 3x24 hours until a clear supernatant was produced. The macerated extract was then evaporated using a vacuum rotary evaporator at 60°C for 60 minutes at a speed of 65 rpm until the solvent evaporated and a concentrated extract was obtained. The moringa leaf extract was placed in a water bath dish to complete the thickening process until a paste-like extract was produced.

Making Test Feed

Mix moringa leaf extract dissolved in distilled water (10% of the feed weight) into a spray bottle containing progol (2% of the feed weight). Shake the solution until homogeneous and spray it evenly over the entire surface of the feed. After spraying the solution evenly over the feed, let it air-dry for approximately one day at room temperature and store it in a container.

Maintenance

The fish to be tested were placed in 16 aquariums at a rate of 1 fish per liter of water (Putri, 2023). The fish were then fed artificial feed mixed with moringa leaf extract according to the treatment, three times daily (Rakhfid *et al*., 2020).

Observation Parameters

Erythrocyte Count

The number of erythrocytes is calculated using the formula of Nabib and Pasaribu (1989):

$$Erythrocytes = \left(\frac{A}{N}\right) X \left(\frac{1}{V}\right) X\ Fp$$

Information:
A = Number of cells counted
V = Volume of the hemocytometer box
N = Number of hemocytometer boxes during observation
Fp = Dilution factor

Leukocyte Count

The number of leukocytes is calculated according to the Simmons formula (1980) :

$$\sum \text{Leukocytes} = \frac{\text{Ni x Multiplier Factor}}{0.4}$$

Information:
Ni = Number of leukocytes
Multiplier = Dilution number (10 times)
0.4 = Total blood volume in the box

Absolute Length Growth

Absolute length growth formula (Hidayat *et al*., 2013):

$$\Delta L = Lt - Lo$$

Information:
ΔL = Absolute increase in fish length (cm)
Lt = Fish length at the end of the cultivation period (cm)
Lo = Fish length at the beginning of the cultivation period (cm)

Absolute Weight Gain

Absolute weight growth formula (Hidayat *et al*., 2013):

$$\Delta W = Wt - Wo$$

Information:
ΔW = Absolute weight gain (g)
Wt = Fish weight at the end of cultivation (g)
Wo = Fish weight at the beginning of cultivation (g)
t = Research duration (days)

Specific Growth Rate (SGR)

Specific growth rate formula (Effendie 1997):

$$SGR = \frac{lnWt - lnWo}{t} x\ 100\%$$

Information:
SGR = Daily growth rate of individual (%/day)
Wt = Average weight of test fish at the end of cultivation (g)
Wo = Average weight of test fish at the beginning of cultivation (g)
t = Duration of study (days)

Feed Conversion Ratio (FCR)

Feed Conversion Ratio (FCR) can be calculated based on the Effendie (1997) formula below :

$$FCR = \frac{F}{(Wt + D) - Wo} \times 100$$

Information:
FCR = Feed Conversion Ratio
Wo = Initial weight of test fish (g)
Wt = Final weight of test fish (g)
D = Weight of dead fish (g)
F = Amount of feed given (g)

Feed Efficiency

According to (Watanabe 1988) the formula for calculating feed efficiency is :

$$EP = \frac{(Bt + Bd) - Bo}{F} \; x \; 100\%$$

Information:
EP = Feed Efficiency (%)
Bt = Weight of fish biomass at the end of cultivation (g)
Bo = Weight of fish biomass at the beginning of cultivation (g)
Bd = Weight of fish biomass that died during cultivation (g)
F = Feed consumed by fish during cultivation (g)

Survival Rate (SR)

Survival rate data is calculated using the formula according to Effendie (2002):

$$SR = \frac{Nt}{No} x \; 100\%$$

Information:
SR = Survival Rate (%)
Nt = Final number of fish (tails)
No = Initial number of fish (tails)

Water Quality

In this study, the water quality tested was dissolved oxygen, temperature, and pH, with measurements taken every ten days.

Data analysis

The collected data were analyzed using an F-test (ANOVA) with a 95% confidence interval. If significant differences were found, Duncan's Multiple Range test was used. Meanwhile, water quality was analyzed descriptively.

RESULTS

Red Blood Cell Count

The erythrocyte counts of tawes fish before and after treatment, over a 40-day rearing period, showed an increase of 12-63%. This increase occurred in the control treatment and the addition of moringa leaf extract. The data are presented in Figure 1.

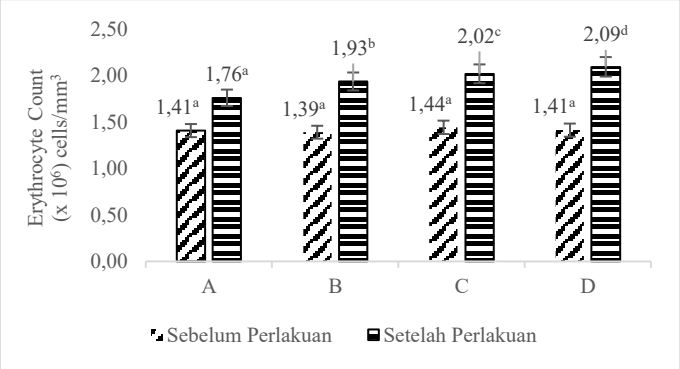

Figure 1. Number of Red Blood Cells (Erythrocytes) in Tawes Fish Seed During Research (A: Control; B: Feed + 0.125 g/kg moringa leaf extract; C: Feed + 0.15 g/kg moringa leaf extract; D: Feed + 0.175 g/kg moringa leaf extract)

White Blood Cell Count

Based on Figure 2, tilapia fry fed with moringa leaf extract for 40 days showed an increase in white blood cell count. Increasing the concentration of moringa leaf extract was directly proportional to the increase in white blood cell count in tilapia fry.

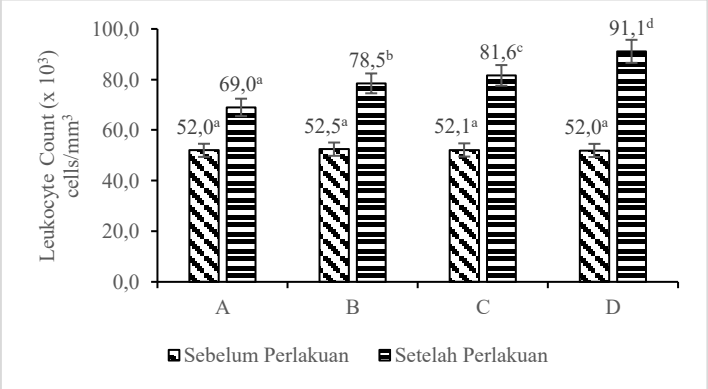

Figure 2. White Blood Cell (Leukocyte) Count in Tawes Seedlings During the Research (A: Control; B: Feed + 0.125 g/kg Moringa leaf extract; C: Feed + 0.15 g/kg Moringa leaf extract; D: Feed + 0.175 g/kg Moringa leaf extract)

Growth of Tawes Fish Seed (*Barbonimus gonionotus*)

The following are the results of data collection on the growth parameters of tawes fish seeds (Table 1) observed during the research.

Table 1. Data on Growth Parameters of Tawes Fish Seeds

Observation Parameters	Treatment			
	A	B	C	D
Absolute Length Growth (cm)	1,65 ± 0.04[a]	1,78 ± 0,07[b]	1,84 ± 0,09[c]	1,97 ± 0,05[d]
Absolute Weight Gain (g)	19,80 ± 0,43[a]	20,70 ± 0,45[b]	21,28 ± 0,8[c]	23,20 ± 0,54[d]
SGR (% ind/ day)	2,72 ± 0,01[a]	2,78 ± 0,04[a]	2,83 ± 0,02[b]	2,98 ± 0,03[c]
FCR	1,66 ± 0,07	1,64 ± 0,06	1,65 ± 0,04	1,59 ± 0,03
FE (%)	60,3 ± 0,02	61,2 ± 0,03	61,4 ± 0,01	63,0 ± 0,03
SR (%)	85 ± 0,06 [a]	90 ± 0,8 [b]	93 ± 0,05[c]	95 ± 0,10 [c]

Information:
SGR = Daily growth rate or Specific Growth Rate (%)
FCR = Feed conversion ratio or Food Conversion Ratio
FE = Feed Efficiency (%)
SR = Survival Rate (%)

Absolute Length Growth

Observation results after 40 days of raising tawes fish seeds (Figure 3) show that the absolute length growth of fish in each treatment has varying values. Treatment A (100% commercial feed) has the lowest average absolute length growth value, which is around 1.65 ± 0.04 cm and treatment D (addition of moringa leaf extract (0.175 g/kg) is the treatment that produces the highest average absolute length growth, which is around 1.97 ± 0.05 cm.

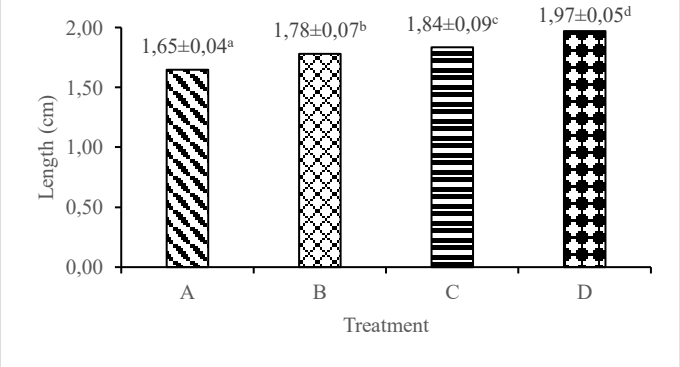

Figure 3. Absolute Length Growth (ΔL)
(A: Control; B: Feed + 0.125 g/kg Moringa leaf extract; C: Feed + 0.15 g/kg Moringa leaf extract; D: Feed + 0.175 g/kg Moringa leaf extract)

Absolute Weight Gain

Based on the observation results after maintaining tawes fish seeds with feed treatment for 40 days, Figure 4 shows that the absolute weight gain value of fish in all treatments has varying weight values. Treatment A (100% commercial feed) has the lowest average absolute

weight growth value, which is around 19.80 ± 0.43 grams and treatment D (addition of moringa leaf extract (150 g/kg) is the treatment that has the highest average absolute weight growth value, which is around 23.20 ± 0.54 grams.

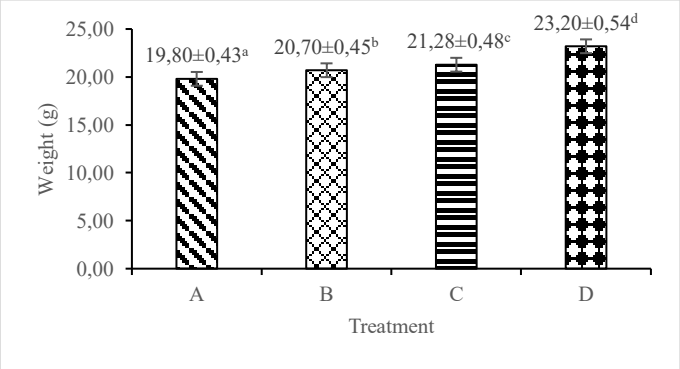

Figure 4. Absolute Weight Growth of Tawes Fish Fry (ΔW)
(A: Control; B: Feed + 0.125 g/kg Moringa leaf extract; C: Feed + 0.15 g/kg Moringa leaf extract; D: Feed + 0.175 g/kg Moringa leaf extract)

Specific Growth Rate (SGR)

Moringa leaf extract in feed has a significant effect on the daily growth rate of tawes fish seeds (Figure 5).

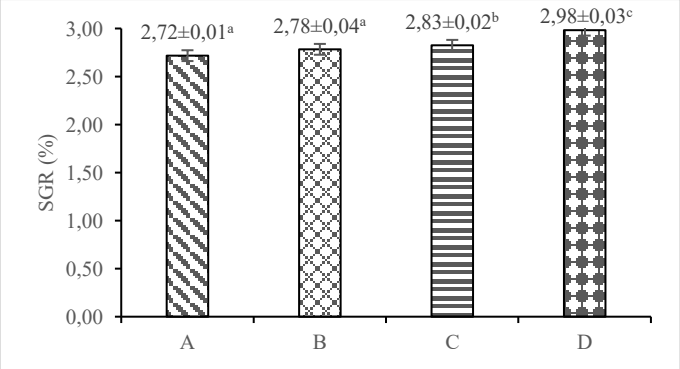

Figure 5. Specific Growth Rate (SGR%)
(A: Control; B: Feed + 0.125 g/kg Moringa leaf extract; C: Feed + 0.15 g/kg Moringa leaf extract; D: Feed + 0.175 g/kg Moringa leaf extract)

***Feed Convertion Ratio* (FCR)**

The feed conversion ratio varied between 1.59 and 1.66. The graph below (Figure 6) shows an increase in the *feed conversion ratio* for each treatment.

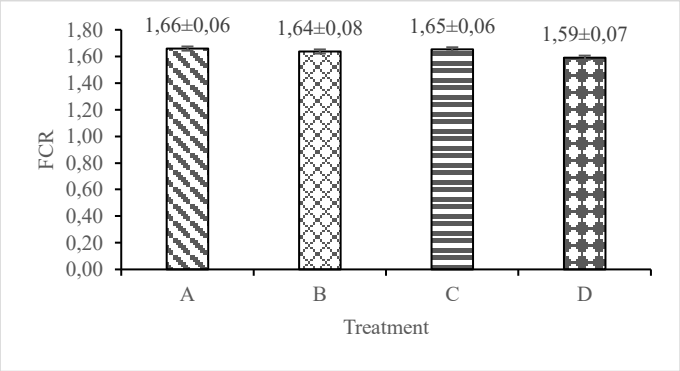

Figure 6. Feed Conversion Ratio (FCR)
(A: Control; B: Feed + 0.125 g/kg moringa leaf extract; C: Feed + 0.15 g/kg moringa leaf extract; D: Feed + 0.175 g/kg moringa leaf extract)

Feed Efficiency

Feed Efficiency (FE) is a parameter used to determine the level of effectiveness of feed in increasing the percentage of fish growth.

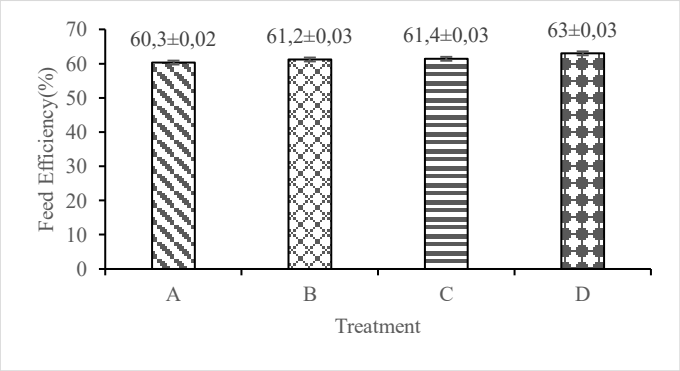

Figure 7. Feed Efficiency
(A: Control; B: Feed + 0.125 g/kg Moringa leaf extract; C: Feed + 0.15 g/kg Moringa leaf extract; D: Feed + 0.175 g/kg Moringa leaf extract)

***Survival Rate* (SR)**

The survival rate of tawes fish fry cultivated for 40 days showed that the lowest treatment was treatment A with an SR value of 85±0.06% and the highest SR value was treatment D at 95±0.10%. The results of statistical analysis showed that the survival rate of tawes fish fry was significantly different in each treatment, as can be seen in (Table 2).

Table 2. Survival Rate (SR)

Treatment	Survival Rate
A (100% Commercial Feed)	85±0.06%
B (0.125 g/kg moringa leaf extract)	90±0.08%
C (0.15 g/kg moringa leaf extract)	93±0.05%
D (0.175 g/kg moringa leaf extract)	95±0.10%

Water Quality

The results of water quality parameter measurements during the study are shown in Table 3.

Table 3. Water Quality

Parameter	Treatment				Optimum Range (SNI 01-6133-1999)
	A	B	C	D	
Temperature (°C)	27,5-28,2	27,3-28	28-29,2	27,4-28	25 – 30
pH	7-7,8	7,2-8	7-7,9	7-8,1	6.5-8.5
DO (mg/L)	6,3-7,6	5,8-7,1	6,5-7,4	6,2-7,8	> 5

DISCUSSION

Based on the research results, the addition of moringa leaf extract was proven to have a significant effect on the immunity and growth of *tawes* fish seeds. The highest increase in erythrocytes was obtained in treatment D (0.175 g/kg), amounting to 2.09 × 10^6 cells/mm^3, higher than the findings of Eko *et al.,* (2022) in common carp (1.79 × 10^6 cells/mm^3) and still within the normal range as reported by Syawal *et al.* (2011). This increase is presumably due to the flavonoids contained in moringa leaves functioning to stimulate hematopoietic organs, causing the number of erythrocytes to increase (Herawati, 2011). These findings indicate that supplementation of moringa leaf extract is capable of improving the physiological capacity of fish in binding oxygen and adapting to their environment (Shabrina *et al.*, 2018).

The number of leukocytes also increased in line with the dose of extract added, with the highest value found in treatment D at 91.1 × 10^3 cells/mm^3. This value is within the normal range of teleost fish (Legler, 1977) and indicates activation of the non-specific immune system in responding to foreign agents in the fish body (Nainggolan *et al.*, 2021). The increase in leukocytes is influenced by flavonoids, saponins, and alkaloids in moringa leaves which serve as immunostimulants (Rosidah *et al.*, 2018), as also demonstrated in the study of Rousdy and Wijayanti (2015) using humic acid in common carp.

In terms of growth, the administration of moringa leaf extract resulted in greater values compared to the control treatment. Absolute length and weight growth increased significantly in treatment D, which is consistent with the research of Aliyas *et al.* (2023) on common carp with the addition of moringa leaf powder. Bioactive compounds such as flavonoids, carotenoids, and terpenoids are known to play a role in increasing metabolism and feed consumption, thereby promoting growth (Ahmadifar *et al.*, 2020; Safitri *et al.*, 2020). The SGR values in this study ranged from 2.72–2.98%, with the highest value in treatment D, higher than the findings of Naria *et al.* (2022) in common carp at 1.53%. This reinforces the assumption that bioflavonoids in moringa leaves can stimulate fish growth through metabolic and hormonal mechanisms (Shakya, 2017; Puspitasari, 2017).

Feed performance also showed better results with the addition of moringa leaf extract. The lowest FCR in treatment D was 1.59, indicating better feed efficiency. Feed efficiency (FE) was also highest in treatment D (62.99%). This condition is presumably associated with the role of flavonoids as prebiotics that enhance digestive enzyme activity so that nutrients are more easily absorbed by the body (Yuriana *et al.*, 2019). Sumarjan *et al.* (2022) stated that moringa leaves are capable of increasing feed digestibility through their protein content.

In addition to improving immunity and growth, the addition of moringa leaf extract also had a positive effect on the survival rate (SR) of fish. The highest SR value was obtained in treatment D at 95%, far above the survival standard for *tawes* fish (KEPMEN-KP, 2017) and higher than the study of Basir and Nursyahran (2018) on tilapia (91%). This increase in survival is influenced by better immune status and optimal stocking density, thereby reducing mortality

(Syawal *et al.*, 2019). During the study, water quality parameters were within the optimal range for *tawes* fish culture, namely temperature 27.3–29.2 °C, dissolved oxygen 5.8–7.8 mg/L, and pH 7–8.1. These values comply with freshwater fish culture standards according to Zonneveld (1991) and Moniruzzaman *et al.* (2015). Good water quality supports fish growth and health, while polluted conditions can increase the risk of disease (Rojo *et al.*, 2018).

CONCLUSION

The addition of moringa leaf extract in the feed of tawes fish fry can improve immunity and growth performance. This is proven by an increase in total erythrocytes of 2.09 x 106 cells/mm3, total leukocytes of 91.1 × 103 cells/mm3, absolute length growth of 1.97 cm, absolute weight of 23.30 g, daily growth rate (SGR) of 2.98%, feed conversion ratio (FCR) of 1.59, feed utilization efficiency (EPP) of 63.0% and fish survival rate (SR) of 95%. Where the addition of 0.175 g/kg of moringa leaf extract in the feed is the most optimal treatment and has an effect on increasing immunity and growth performance of tawes fish fry (*Barbonimus gonionotus*).

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