

# Potential effects of fermented moringa (*Moringa oleifera*) leaf extract to increase the color brightness of the Oranda goldfish (*Carassius auratus*)

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**Abstract.** Umayah S, Putra AN, Herjayanto M, Syamsunarno MB. 2023. Potential effects of fermented moringa (*Moringa oleifera*) leaf extract to increase the color brightness of the Oranda goldfish (*Carassius auratus*). *Nusantara Bioscience* 15: 289-297. Moringa (*Moringa oleifera* Lam.) leaf extract serves as a natural reservoir of carotenoids, prominently  $\beta$ -carotene, effectively employed to augment the color quality of ornamental fish. This study aimed to evaluate the potential effects of adding fermented moringa leaf extract to feed to enhance the color vibrancy of Oranda goldfish (*Carassius auratus* (Linnaeus, 1758)). Moringa leaves were also fermented using *Aspergillus niger* Tiegh. at a dosage of 0.5 g/100 g for 48 hours, followed by extraction using 70% ethanol. Four doses of fermented moringa leaf extract (0, 40, 50, 60 mL/kg) with three replications were added to a commercial feed using the coating method. The results showed that the carotene in fermented moringa leaf extract is 94.22 ppm/50 mL. Adding moringa leaf extract to the feed enhanced the Oranda goldfish's brightness and color diversity. The addition of fermented moringa leaf extract at a dose of 60 mL/kg led to the highest color diversity values on the body (57.81%), caudal (71.71%), and head (71.13%) compared to other treatments. Moringa leaf extract did not affect growth rate, survival, blood profile, and water quality parameters for maintaining Oranda goldfish. The extract from *A. niger* had the potential to be used in the cultivation of Oranda goldfish, and the addition of 60 mL/kg achieved the best color brightness and diversity.

**Keywords:** *Aspergillus niger*, feed, fermented, ornamental fish

## INTRODUCTION

The Oranda goldfish (*Carassius auratus* (Linnaeus, 1758)) is one of the ornamental fish species with high economic value, resulting in significant demand and cultivation potential (Hedayati et al. 2017). The fish has various colors, such as black, white, red, orange, brown, and yellow (Gurung et al. 2018) and belongs to the Cyprinidae family (Hedayati et al. 2017). Oranda is a goldfish widely cultivated in Indonesia due to its beautiful colors and unique body shape (Yan et al. 2022). Oranda goldfish has long fins, including the dorsal fin and long double caudal fin, the goldfish most widely cultivated in Indonesia (Prakoso et al. 2023). Improving fish color quality is an effort that can lead to higher selling value of ornamental fish (Sawant et al. 2020). The better and unique the color and the more perfect the physical form of the ornamental fish, the more expensive the selling price will be on the market (Hoseinifar et al. 2023). The color quality and appearance of the Oranda goldfish are directly proportional to the market price (Kautsar et al. 2022). However, the cultivation faces many challenges, such as the degradation of color quality, both in brightness and diversity (Andriani et al. 2021). Enhancing fish color can be achieved by providing feed containing color pigments or carotenoids (Dananjaya et al. 2017), and carotenoids in the diet naturally enhance the color brightness (Sahin et al. 2022).

Carotenoids are natural pigments in animals, plants, and microorganisms (Astari et al. 2016; Wongphonprateep and

Pichitkul 2021). Carotenoids are the primary source of coloration on fish skin, and based on their chemical structure, carotenoids are divided into 2 groups: carotenes and xanthophyll (Sathyaruban et al. 2021). Fish cannot synthesize carotenoids, which must be added to their diet (Hekimoglu et al. 2017; Koncara et al. 2019). The improvement in fish color begins with the intake of carotenoid-containing feed, which enters the fish body and is synthesized into pigments. Furthermore, these pigments were carried through the bloodstream and deposited in chromatophores in the dermis/skin (Das and Biswas 2016). Fish pigmentation depends on the additional feed containing carotenoids. Fish only modify pigments from their dietary sources (Meilisza et al. 2019). However, the high cost of synthetic carotenoids limits their use in feed formulations due to increased expenses (Besen et al. 2019).

Moringa leaves (*Moringa oleifera* Lam.) can serve as an alternative source of carotenoids due to their beta-carotene content, one of the provitamin A compounds (Angelica et al. 2020). Jebaraja et al. (2013) found that Moringa leaf contains a significant amount of  $\beta$ -carotene, specifically lutein, at 520 mg/kg. Beta-carotene belongs to the carotenoid group and is crucial in skin color regulation by providing orange to red colors (Chapman and Miles 2018). The findings of Takhir et al. (2022) explained that applying Moringa leaf extract in feed enhances growth and color vibrancy in betta fish. However, the leaves also contain anti-nutritional compounds such as saponins, tannins, phytates, and polyphenols (Putra et al. 2020a). These anti-nutritional substances limit the use of Moringa

leaves due to their potential to inhibit growth and physiological processes within the fish body (Abdel-Latif et al. 2022). Fermentation process using *Aspergillus niger* Tiegh. can reduce the crude fiber and anti-nutritional content in fish feed ingredients (Putra et al. 2022a). Several studies have reported natural potential sources of carotenoid to improve the color quality of goldfish such as astaxanthin (Weeratunge and Perera 2016), red paprika (Kumar et al. 2017), bixin (Dananjaya et al. 2017), Lutein (Besen et al. 2019), annatto seeds extract (Dananjaya et al. 2020), red yam flour (Putra and Romdhonah 2019), carrot flour (Tiewsoh et al. 2019), lobster meal (Bell et al. 2019), *Spirulina platensis* (Gomont) Geitler (Kargun and Dikbas 2020), sweet potato and yellow pumpkin flour (Kautsar et al. 2022). However, no data reports the utilization of fermented moringa leaf extract for enhancing pigmentation of goldfish. Therefore, the study is needed to explore the potential of the Moringa leaves as a source of carotenoids in fish feed. This study aimed to evaluate the potential effects of adding the fermented extract of Moringa leaf to feed to enhance the color brightness of Oranda goldfish.

## MATERIALS AND METHODS

### Fermented moringa leaf extract preparation

The fermentation of moringa leaf powder started with steaming for 30 min at a temperature of 100°C. *A. niger* inoculum with a dosage of 0.5 g/100 g was mixed with moringa leaf powder and incubated for 48 hours, following the study findings of Ikhwanuddin et al. (2018). Moringa leaf powder was dried in an oven at 60°C for 10 min. The powder was extracted using 70% ethanol in a 1:1 (w/v) ratio and stirred using a magnetic stirrer for 15 hours at room temperature, following the method described by Putra et al. (2015). The powder was filtered using filter paper, and the obtained filtrate was evaporated with a vacuum evaporator at 60 °C for 45 min to remove ethanol residue. Subsequently, the remaining solution was diluted with distilled water until the total dissolved solids reached 5%.

### Preparation of test feed and experimental design

Fermented Moringa leaf extract was weighed according to the treatment doses and homogenized at 250 mL/kg of water and 2% egg yolk, following Putra et al. (2020b). The extract was then coated by evenly spraying onto the commercial feed and air-drying at room temperature for 40 minutes. The feed coating process followed the method described by Putra and Romdhonah (2019). The commercial feed used as the test diet was ornamental (PF500, Indonesia), with a particle size of 0.5-0.7 mm, minimum protein content of 39.5%, fat 5.3%, crude fiber 4.0%, ash content 11.6%, moisture content 10.4%, nitrogen-free extracts 39.6%, dry matter 89.6%, organic matter 78.0%, and energy 433.78 kcal/g (protein: 5.6 kcal/g, fat: 9.4 kcal/g, BETN: 4.11 kcal/g). Feeding was conducted three times a day ad libitum at 08:00, 12:00, and 16:00 local time. This study employed an experimental method with a Completely Randomized Design (CRD)

consisting of four groups of fermented Moringa leaf extract treatments. The treatments included A (without the addition of fermented Moringa leaf extract, 0 mL/kg), B (fermented Moringa leaf extract 40 mL/kg), C (fermented Moringa leaf extract 50 mL/kg), and D (fermented Moringa leaf extract 60 mL/kg).








The test fish used were Oranda goldfish sourced from an ornamental fish breeder in Pandeglang, Banten, Indonesia, with an average length of 4.5±0.20 cm and weight of 3.2±0.27 g. The fish were acclimated and fasting for 24 hours to eliminate the influence of residue in their bodies. A total of 120 were randomly distributed into 30x30x30 cm aquariums with a water volume of 20 L/aquarium and a density of 10 fish per aquarium. The aquariums used are colorless (colored glass), and all treatments are given the same light, namely bright during the day and dark at night, to minimize the effect of light and tank color on Oranda goldfish. Fish maintenance was carried out for 40 days using a recirculation system, with one aerator as an oxygen source and one water heater (Amara HT-50) in each aquarium. Water quality management is carried out by daily siphoning of waste and feed residue was performed, and 30% of the total aquarium water volume was replaced daily. Water quality measurements consist of temperature with a digital thermometer (TPM 10), pH with a pH meter (Backlight PH-2011), and oxygen content with a DO meter (Lutron DO-551).

### Test parameters

#### Fish color quality

Color quality measurements were taken every 10 days (10, 20, 30, and 40 days) using the measurement method proposed by Kautsar et al. (2022). The measurements were conducted visually using the Toca Color Finder (TCF) standard values by 8 healthy and non-colorblind panelists. The assessment ranged from a score of 1 (lowest) to 7 (highest), with color gradations from yellow to orange and red. Three test fish were assessed per replication at each measurement interval, and the scores and colors are presented in Table 1.

**Table 1.** Toca Color Finder (TCF) score in ornamental fish

Color Score	Color	TCF Code
1		TCF code 0305
2		TCF code 0405
3		TCF code 0505
4		TCF code 0604
5		TCF code 0625
6		TCF code 0605
7		TCF code 0616

### *Fish color diversity*

Observations were made at the end of the rearing period, covering three sample points: head, body, and caudal peduncle. Color diversity observations followed the method proposed by Andriani et al. (2021). Test fish were photographed using a 13-megapixel camera, and the obtained photos were analyzed using color gradient conversion methods on a scale and percentage basis using Adobe Photoshop CS4. Color diversity observations were conducted on 3 fish from each treatment.

### *Chromatophore cell count*

Four randomly selected fish were used to prepare histological slides at the end of the rearing period. The preparation was conducted at the Laboratory of Histopathology, Fish and Environmental Disease Examination Station, Serang, Banten. Following the method described by Putra et al. (2021a). Histological preparation started with the fixation step, where the sampled fish organ was immersed in 10% phosphate-buffered formalin and 5% ethylenediaminetetraacetic acid solution for 24 hours. Dehydration followed, involving immersion of the fish organ samples in alcohol with gradually increasing concentrations of 70%, 80%, 96%, and absolute alcohol for 3 hours; the next step involved immersing the samples in xylene solution for 1 hour before embedding them in paraffin. The staining phase was then conducted using hematoxylin and eosin (H and E), and the prepared slides were observed under a microscope (Leica DM500) at 10x magnification. Meanwhile, chromatophore cell counts were performed at three randomly selected points on each slide. The number of cells in each treatment group of Oranda goldfish was counted and statistically analyzed based on the obtained data.

### *Fish blood profile*

The measurement of fish blood profiles, including leukocyte, erythrocyte, hematocrit, and hemoglobin counts, was conducted at the end of the rearing period. Blood samples were taken from four fish in each treatment using a 1 mL syringe containing 0.1 mL of anticoagulant to prevent blood coagulation. Blood samples from each treatment were mixed with Hayem (for erythrocyte measurement) and Turk solutions (for leukocyte measurement) until homogenous. Subsequently, 1-2 drops of blood were placed into a hemocytometer and counted under a microscope at 400x magnification. Erythrocyte and leukocyte measurements followed the method described by Kumar et al. (2015), and hemoglobin levels were measured using the Sahli method (Pawar and Bhilave 2019). Blood was drawn into a Sahli pipette up to the 2 mL mark and was transferred to a Hb-meter tube containing 0.1 N HCl. The solution was allowed to stand for 3-5 minutes for hemoglobin to react with HCl, forming acid hematin, and distilled water was added until the color matched the standard in the Hb-meter tube. Hemoglobin values were also determined based on the height scale of the formed surface of the solution, indicating the amount in grams per 100 mL of blood. The hematocrit level was calculated according to the method proposed by Yaji et al. (2018).

Hematocrit tubes containing fish blood samples were centrifuged for 5 minutes at 5,000 rpm, and the levels were expressed as a percentage of the volume of packed blood cells.

### *Growth parameters*

Growth parameters, including feed consumption, specific growth rate, and survival rate, were measured following the formulas proposed by Rifai et al. (2022). The feed conversion ratio was calculated using the formula proposed by Hermawan et al. (2021). These parameters included (i) Feed consumption (g) = initial feed amount (g) - final feed amount (g), (ii) Absolute weight (g) = mean final fish weight (g) - mean initial fish weight (g), (iii) Absolute length (cm) = mean final fish length (cm) - mean initial fish length (cm), (iv) Weight/length growth rate = ((Ln final weight/length) - (Ln initial weight/length)) / 40 days, (v) Feed conversion ratio = feed consumption / (final weight - initial weight), (vi) Survival rate (%) = (number of fish at the end/ beginning of the study) x 100.

### **Chemical analysis**

Proximate analysis was conducted to determine the protein, fat, crude fiber, ash, and moisture content of the test feed at the Fish Nutrition Laboratory, Institut Pertanian Bogor, Bogor, Indonesia, following the procedures outlined by AOAC (2005). Carotenoid content analysis in the fermented Moringa leaf extract was conducted at the Postharvest Agricultural Research and Development Center, Bogor, using the method proposed by Meiliszka et al. (2019) with a spectrophotometer at 480 nm.

### **Statistical analysis**

Color variation data, the number of chromatophore cells, and growth were analyzed using Analysis of Variance (ANOVA) through SPSS software version 17.0. Significant differences in the results were tested using Duncan's Multiple Range Test with a confidence interval of 95%. The obtained data were presented in tables and graphs, and the color brightness and fish blood profile data were analyzed descriptively.

## **RESULTS AND DISCUSSION**

### **Fish color brightness**

The values for Oranda goldfish with the addition of fermented Moringa leaf extract are presented in Figure 1. The results indicated that adding fermented Moringa leaf extract to the feed enhances the intensity of color brightness in Oranda goldfish. The color brightness scores of Oranda goldfish on the 10<sup>th</sup> day and 20<sup>th</sup> day ranged from 3.72 to 3.75 and 3.73 to 3.80, respectively. The improvement in color enhancement was noticed on the 20<sup>th</sup> day, with the highest score recorded in treatment D at 4.75, followed by C, B, and A at 4.53, 4.34, and 3.75, respectively. The same trend was observed on the 40<sup>th</sup> day, where the highest score was found in treatment D at 5.04, followed by C, B, and A at 4.79, 4.44, and 3.70.

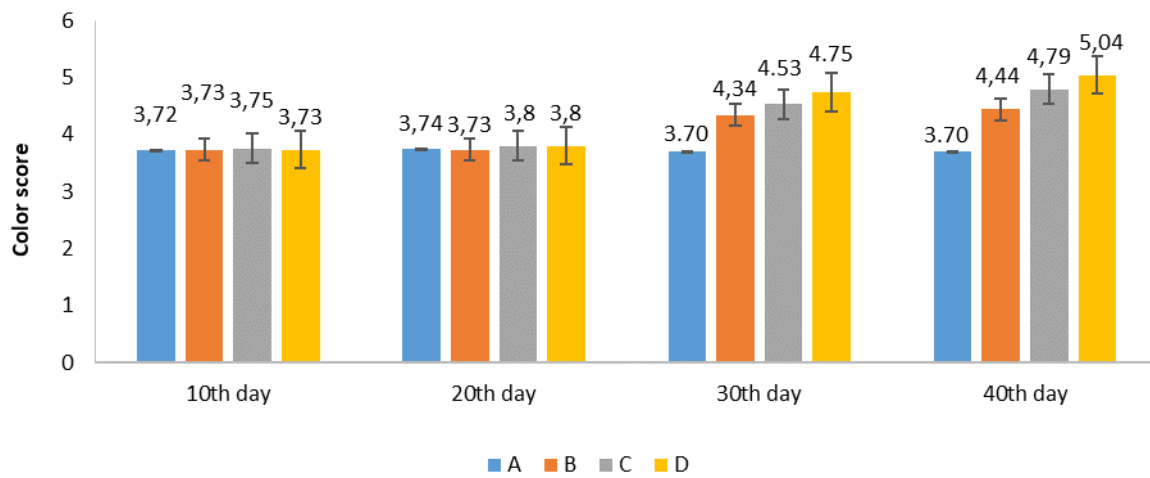
**Color variation in Oranda goldfish**

Color variation calculations were observed at the head, body, and caudal peduncle. The color variation analysis in Oranda goldfish with different doses of fermented Moringa leaf extract is presented in Table 2, while the images of Oranda goldfish at the end of the rearing period are shown in Figure 2. The color range values on the body, caudal, and head ranged from 47.28% to 57.81%, 48.37% to 71.71%, and 54.59% to 71.13%, respectively.

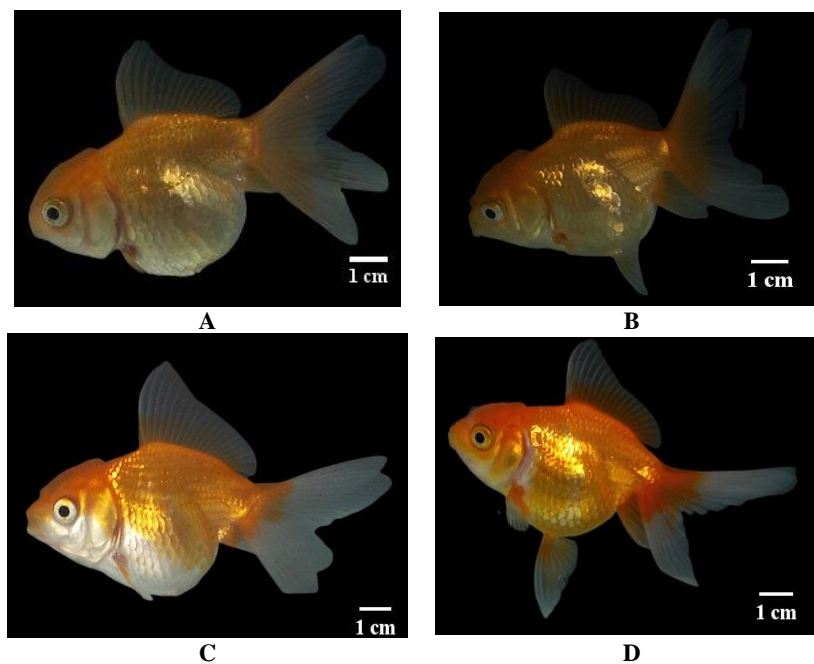
**Table 2.** Color variation analysis in *C. auratus* Oranda goldfish using Adobe Photoshop CS4\*

Feed Test**	Color Variation (%)		
	Body	Caudal	Head
A	47.28 ± 5.58 <sup>a</sup>	48.37 ± 6.93 <sup>a</sup>	54.59 ± 3.71 <sup>a</sup>
B	50.65 ± 2.40 <sup>ab</sup>	54.14 ± 8.88 <sup>a</sup>	62.96 ± 0.45 <sup>b</sup>
C	52.22 ± 4.64 <sup>ab</sup>	55.86 ± 1.98 <sup>a</sup>	65.93 ± 0.87 <sup>bc</sup>
D	57.81 ± 6.31 <sup>b</sup>	71.71 ± 2.70 <sup>b</sup>	71.13 ± 4.45 <sup>c</sup>

Note: \*Different superscript letters behind the standard deviation (±) values indicate significant differences (P < 0.05). \*\* A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)



**Figure 1.** Scores of color brightness in Oranda goldfish with different fermented Moringa leaf extract additions. A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)



**Figure 2.** Visual appearance of color quality in Oranda goldfish at the end of the rearing period. A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)

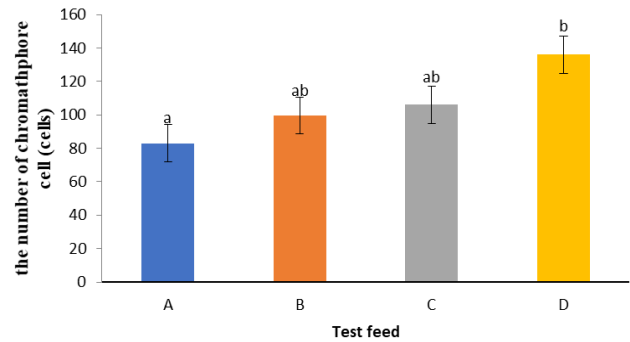
**Number of chromatophore cells**

The number of cells in Oranda goldfish with different doses of the extract in the feed is presented in Figure 3, while the histological results are shown in Figure 4. The result showed that the highest ( $P < 0.05$ ) number of chromatophore cells was found in treatment D of 136 cells, followed by treatment C of 106 cells, treatment B of 100 cells and treatment A of 83 cells. Chromatophore cells appear as dark purple spherical cells under a 10x microscope magnification.

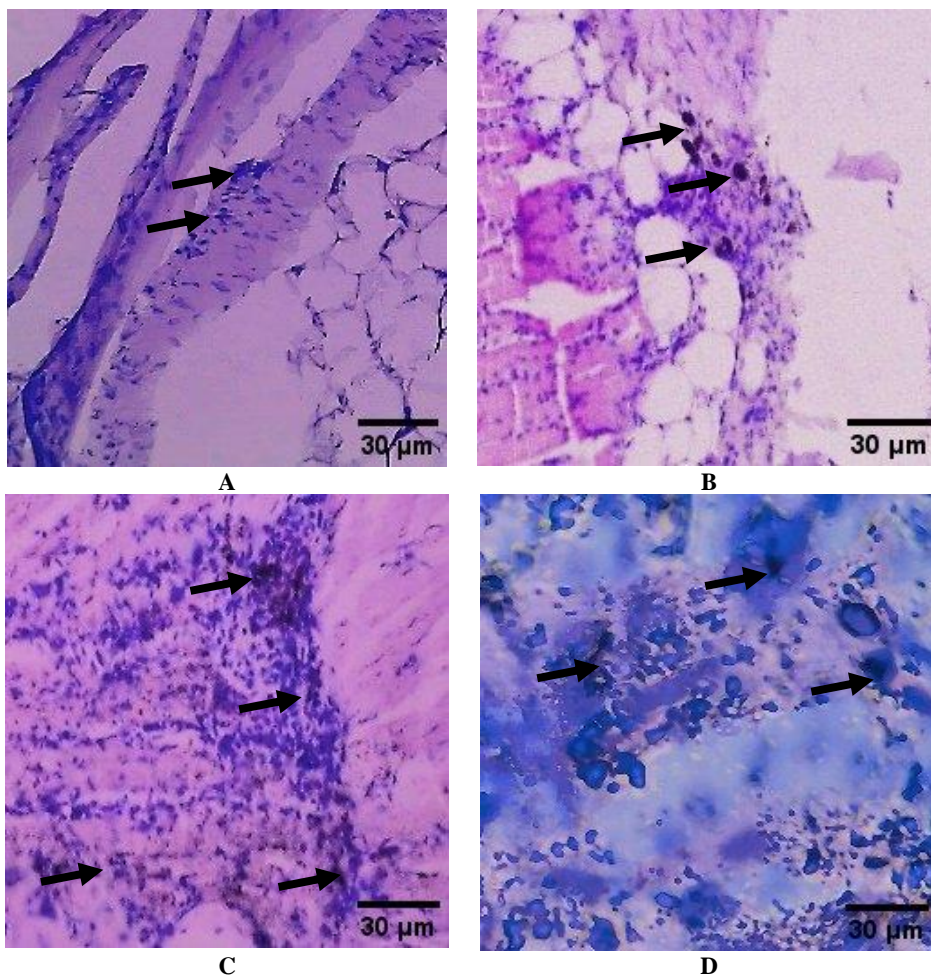
**Growth and survival rate of Oranda goldfish**

The growth and survival rate of Oranda goldfish with different doses of fermented Moringa leaf extract in the feed are presented in Table 3. The average final, absolute, and specific growth rate length ranged from 5.90 to 6.24 cm, 1.57 to 1.64 cm, and 0.74 to 0.78 % cm/day. The final weight ranged from 7.14 to 7.25 g, while the absolute weight and specific growth rate in weight ranged from 3.41 to 3.52 g and 1.62 to 1.66 % g/day, respectively. Feed consumption values, feed conversion ratio, and survival rate did not differ significantly ( $P > 0.05$ ) among treatments. The feed consumption and conversion ratio ranged from

12.17 to 12.88 g and 3.54 to 3.76, and the treatments showed the same survival rate of 100%.



**Figure 3.** Number of chromatophore cells in Oranda goldfish with fermented Moringa leaf extract in the feed. Different superscript letters (a, ab, b) above the bar graph indicate significant differences ( $P < 0.05$ ). A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)



**Figure 4.** Histological observation results of chromatophore cells (yellow circle) in the skin tissue of *C. auratus* Oranda goldfish. A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)

### Blood profile of Oranda goldfish

The blood profile values of Oranda goldfish, including hemoglobin, hematocrit, erythrocytes, and leukocytes, are presented in Table 4. The study indicated that hemoglobin and hematocrit values ranged from 5.0 to 5.8 g/dL and 10.81 to 22.58%. The erythrocyte values ranged from 0.55 to  $2.11 \times 10^6$  cells/mm<sup>3</sup>. Leukocyte values also ranged from  $4.75$  to  $7.17 \times 10^4$  cells/mm<sup>3</sup>.

### Water quality

Water quality is crucial as the primary medium for fish survival, and the range of values in the environment for Oranda goldfish with the addition of fermented Moringa leaf extract is presented in Table 5.

### Discussion

Color brightness is a primary factor in determining the quality of ornamental fish and is influenced by genetics, nutrition, color pigment content in the feed, and environment (Koncara et al. 2019). The result showed that adding fermented Moringa leaf extract produced higher scores of color brightness in Oranda goldfish on the 30<sup>th</sup>

and 40<sup>th</sup> day. The higher scores in the treatments with the addition of fermented Moringa leaf extract were attributed to the presence of carotenoid pigments efficiently absorbed and utilized by the fish to enhance and maintain their color quality. Sathyaruban et al. (2021) stated that goldfish cannot improve and maintain their body color without carotenoid content in their diet. One of the functions of carotenoids is to maintain and enhance color brightness (Chapman and Miles 2018). These compounds are absorbed into pigment cells through various processes, and the distribution of pigment cells affects the increase and decrease of fish color. Similar findings have been reported by Takdir et al. (2022) that Moringa leaf extract supplementation significantly influenced the coloring of betta fish. This was supported by Angelica et al. (2020), who said that one of the antioxidant compounds in Moringa leaves was beta-carotene, which is responsible for orange, red-orange, and yellow pigments. According to Dananjaya et al. (2020), increasing pigmentation on the skin and fins of goldfish occurs at different times depending on the environment and carotenoid concentration in the diet.

**Table 3.** Average survival rate, absolute weight, absolute length, and feed consumption of Oranda goldfish (*C. auratus*)\*

Growth Parameters	Feed Test**			
	A	B	C	D
Initial length (cm)	4.65±0.10 <sup>a</sup>	4.51±0.28 <sup>a</sup>	4.33±0.15 <sup>a</sup>	4.56±0.10 <sup>a</sup>
Final length (cm)	6.24±0.03 <sup>a</sup>	6.15±0.37 <sup>a</sup>	5.90±0.08 <sup>a</sup>	6.15±0.13 <sup>a</sup>
Absolute length (cm)	1.59±0.13 <sup>a</sup>	1.64±0.32 <sup>a</sup>	1.57±0.21 <sup>a</sup>	1.59±0.28 <sup>a</sup>
Specific growth rate of length (%.cm/hari)	0.74±0.07 <sup>a</sup>	0.78±0.14 <sup>a</sup>	0.77±0.11 <sup>a</sup>	0.75±0.13 <sup>a</sup>
Initial weight (g)	3.73±0.06 <sup>a</sup>	3.68±0.09 <sup>a</sup>	3.73±0.06 <sup>a</sup>	3.73±0.06 <sup>a</sup>
Final weight (g)	7.25±0.69 <sup>a</sup>	7.16±0.66 <sup>a</sup>	7.14±0.40 <sup>a</sup>	7.24±0.34 <sup>a</sup>
Absolute weight (g)	3.52±0.66 <sup>a</sup>	3.49±0.60 <sup>a</sup>	3.41±0.43 <sup>a</sup>	3.50±0.30 <sup>a</sup>
Specific growth rate of weight (%.g/hari)	1.65±0.23 <sup>a</sup>	1.66±0.20 <sup>a</sup>	1.62±0.16 <sup>a</sup>	1.65±0.09 <sup>a</sup>
Feed consumption (g)	12.88±0.14 <sup>a</sup>	12.17±0.30 <sup>a</sup>	12.60±0.41 <sup>a</sup>	12.31±0.48 <sup>a</sup>
Feed conversion ratio	3.76±0.81 <sup>a</sup>	3.56±0.63 <sup>a</sup>	3.74±0.56 <sup>a</sup>	3.54±0.43 <sup>a</sup>
Survival rate (%)	100.00±0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>

Note: \*Different superscript letters behind the standard deviation (±) values indicate significant differences ( $P < 0.05$ ). \*\* A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)

**Table 4.** Blood profile of Oranda goldfish with the addition of fermented Moringa leaf extract in the feed

Feed Test*	Blood Profiles			
	Hemoglobin (g.%)	Hematocrit (%)	Erythrocyte ( $10^6$ cells/mm <sup>3</sup> )	Leukocyte ( $10^4$ cells/mm <sup>3</sup> )
A	5.7±1.6	13.33±1.2	1.38±15.6	4.75±28.6
B	5.4±2.1	10.81±1.7	0.82±16.4	5.70±31.6
C	5.0±1.8	20.68±1.4	0.55±18.2	6.36±15.7
D	5.8±1.1	22.58±1.6	2.11±21.4	7.17±14.0

Note: \*A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)

**Table 5.** Water quality parameters in the environment for Oranda goldfish rearing

Parameters	Feed Test*			
	A	B	C	D
Temperature (°C)	26-29	26-29	26-29	26-29
pH	7.89-8.63	7.77-8.70	7.53-8.50	7.81-8.55
DO (mg/L)	5.80-6.7	5.31-7.3	5.85-7.50	5.21-7.7
Ammonia (mg/L)	0-0.025	0-0.25	0-0.25	0-0.25

Note: \*A (control), B (40 mL/kg fermented Moringa leaf extract), C (50 mL/kg fermented Moringa leaf extract), and D (60 mL/kg fermented Moringa leaf extract)

In the present study, adding the highest dose of fermented Moringa leaf extract (60 mL/kg) resulted in the highest color brightness score compared to other treatments. This suggests that a higher dose has a higher carotene content, resulting in better color quality of the Oranda goldfish. The same results have been reported by Andriani et al. (2021). The addition of butterfly pea leaf meal as a carotenoid source in the feed with the highest dose of 12% produced the best quality score (87.33) compared to treatment with additional doses of 6% (73.83), 1% (32.17) and 0% (24.67). Nutritional strategy is an effort widely used to improve the color quality of ornamental fish. Using fish feed rich in carotenoids has been proven to increase the brightness of ornamental fish (Sawant et al. 2020). Visual color variation in the present study was also observed at the end of the rearing period at the head, body, and caudal. The result demonstrated that the percentage of visual color variation increased with the fermented Moringa leaf extract dose. Treatment D significantly ( $P < 0.05$ ) resulted in the highest color variation level in the body (57.81%), caudal (71.71%), and head (71.13%) compared to the others. This finding suggested that higher doses of the extract lead to higher carotenoid content in the feed, resulting in better color variation than other treatments. Moringa leaves are a source of carotenoids in the feed, enhancing the color quality of ornamental fish (Angelica et al. 2020). The results showed that the leaf extract's carotene content is 94.22 ppm/50 mL. Similar findings have been reported by other studies using natural ingredients as a source of carotenoids.

Dananjaya et al. (2017) reported that increasing the dietary carotenoid level enhances pigmentation on the skin and fin of goldfish. Kurnia et al. (2019) stated that the addition of red dragon fruit peel meal extract as a potential carotenoid source with the highest dose (15%) resulted in the best color quality in koi carp compared to other treatments (0%, 5% and 10%). Chromatophores are cells responsible for increasing or decreasing the brightness of fish scales (Astari et al. 2016). When the number of chromatophore cells increases, the color of the fish body becomes brighter and more attractive when the number of cells increases. In the present study, we found the highest number of chromatophore cells in treatment D, while the smallest number was found in treatment A. These results align with the brightness level and color variation of goldfish in this study. The number and combination of chromatophore cells in each fish species vary depending on the feed and environment (Sabrina et al. 2023).

Carotenoids have a positive effect on increasing color pigmentation and goldfish growth (Besen et al. 2019). However, the present study showed no significant differences in the growth parameters of Oranda goldfish among treatments. The addition of fermented Moringa leaf extract to the feed is thought not to affect macro and micronutrient content in the feed so that the growth performance of the Oranda goldfish produced in each treatment is the same. The same results have been reported by Hekimoglu et al. (2017), who found that supplementation of algae carotenoids in the diet did not affect the growth and feed conversion ratio of tomato

clownfish. Gurung et al. (2018) also reported that adding natural carotenoid sources (cassava leaves, potato leaves, and colocasia leaves) in the feed does not affect the Oranda goldfish's specific growth rate and feed conversion ratio. In a similar study, Bell et al. (2019) revealed that including lobster meal (0-15%) in the diet did not affect the growth performance of goldfish. Similarly, there is no significant effect on the diet's absolute weight, absolute length specific growth rate and survival rate of goldfish with different concentrations of red yam flour (0-12%) as carotenoid sources (Putra and Romdhonah 2019). These results are also in line with the report by Kautsar et al. (2022) that the addition of natural ingredients (sweet potato, carrot, and pumpkin meal) as a source of  $\beta$ -carotene has no impact on absolute weight growth and absolute length growth of Oranda goldfish. There were no significant differences ( $P > 0.05$ ) in the amount of feed consumption, where the addition of fermented extract to the feed did not affect the palatability of Oranda goldfish. Feed palatability is determined by taste, smell, and color, which affect feed consumption, digestibility, and fish growth levels. Putra et al. (2020a) stated that using Moringa leaves as feed ingredients did not affect the feed consumption of catfish. This study found no difference in the survival rate of Oranda goldfish; the same results were found by Dananjaya et al. (2017). Using natural bixin as a potential carotenoid with different levels in feed did not affect the survival rate of goldfish. A similar result was found by Besen et al. (2019), who found that dietary lutein as a carotenoid in the diet (50 mg/kg) did not influence goldfish juvenile survival rate, growth, and feed efficiency.

The blood profile values of Oranda goldfish in each treatment fall within the normal range. Hemoglobin in the blood functions as an oxygen transport tool found within erythrocytes, while hematocrit represents the percentage of the volume serving as an indicator of fish stress (Madyowati and Muhajir 2018). The normal hemoglobin value of fish is 4.70-16.6 g/dL (Fazio et al. 2019), while the hematocrit range for teleosts fish is 9.4-33.53% (Witeska et al. 2022). According to Esmaeili et al. (2021), the normal range of red and white blood cells in fish was  $0.4$  to  $5.2 \times 10^6$  cells/mm<sup>3</sup> and  $2.17$  to  $116.5 \times 10^4$  cells/mm<sup>3</sup>. Based on these results, adding fermented Moringa leaf extract to the feed is assumed not to affect the physiological processes within the body of Oranda goldfish. The analysis can evaluate the physiological responses within the fish's body, indicating stress. Sources of stress, such as environmental factors, including temperature, pH, fish handling, and light, as well as biotic factors, including pathogenic infections, can negatively impact the physiological state (Royan et al. 2014). The anti-nutritional substances present in Moringa leaves can interfere with the physiological processes within the body of fish (Putra et al. 2022b). The fermentation process of *A. niger* is believed to have reduced the crude fiber value and anti-nutritional content in the leaves. This aligns with the statement by Putra et al. (2022a) that the fermentation of feed ingredients using *A. niger* can decrease the crude fiber value and anti-nutritional substances. However, in the present study, the authors did

not measure the anti-nutritional substances in Moringa leaf extract before and after fermentation.

Water quality plays an important role for ornamental fish because good water quality produces bright colors and fish growth (Indriani et al. 2023). Changes in rearing water temperature will affect the metabolism, growth, and feed consumption of the fish's body (Charlot et al. 2017). In this study, the obtained temperature values from 26 to 29°C indicated the normal range of fish rearing temperature. According to Chen et al. (2019), the optimal temperature value for goldfish is 28°C. Catalano et al. (2019) added that goldfish still grow to a temperature of 18°C. Roslan et al. (2021) stated that the normal range for Dissolved Oxygen (DO) and pH values in freshwater fish are >4 mg/L and 6.5-8.5. In this study, DO and pH values ranged from 5.21 to 7.50 mg/L and 7.53 to 8.70; these results indicate that the DO and pH values in this study are in the normal range. Ammonia is toxic and the most abundant product of nitrogen metabolism produced by intensive aquaculture systems (Putra et al. (2021b)). The result showed that the value of ammonia produced was 0 to 0.25 mg/L; the results are within the normal range of freshwater fish rearing. Mustaqim et al. (2022) stated that the ammonia value for fish rearing should not exceed 1.5 mg/L. The addition of fermented Moringa leaf extract in the feed did not affect the water quality values in the environment for Oranda goldfish.

In conclusion, the carotene in fermented Moringa leaf extract showed a content of 94.22 ppm/50 mL. Adding Moringa leaf extract to the feed enhanced and maintained the color of the Oranda goldfish. The addition of fermented Moringa leaf extract at a dosage of 60 mL/kg resulted in the highest color diversity values on the body (57.81%), caudal peduncle (71.71%), and head (71.13%) compared to others. The addition of fermented Moringa leaf extract did not affect growth, survival rate, blood profile, and water quality. Fermented Moringa leaf extract using *A. niger* at a dosage of 60 mL/kg could be used in Oranda goldfish cultivation. Further study should be conducted to explore the addition of carotenoid content in Oranda goldfish after being fed with fermented Moringa leaf extract to optimize the dosage.

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