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Penggunaan tepung daun *Indigofera zollingeriana* dalam formulasi pakan untuk kecerahan warna dan pertumbuhan ikan koi (*Cyprinus carpio*)

Utilization of *Indigofera zollingeriana* leaves meal in feed formulation to color brightness and growth in koi fish (*Cyprinus carpio*)

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Abstract

This study aims to determine the effect of the addition of *Indigofera zollingeriana* in feed formulations with different doses on the color brightness of koi fish. The research design was Completely Randomized Design (CRD) with five treatments and three replications. Treatment P0 was control (without the use of Indigofera leaf meal), P1 was the addition of 5% indigofera meal, P2 was the addition of 10% indigofera meal, P3 was the addition of 15% indigofera meal, and P4 was the addition of 20% indigofera meal. Fingerlings measuring 6 ± 1 cm with a stocking density of 1 fish for 2 L were reared for 30 days in this study. The results showed that P2 with a percentage of 10% *Indigofera zollingeriana* meal was the best treatment for the brightness of koi fish color (27.23). Still, they had no significant effect on the growth of absolute length (1.04 cm) and absolute weight (1.05 g), feed efficiency (23.08%), and survival rate (100%). The water quality obtained during maintenance was temperature 26-27°C, pH 6.1-7.2, dissolved oxygen 5.2-6.3 mg/L, and ammonia 0.02-0.036 mg/L.

Keywords: *Fish Color Brightness; Indigofera zollingeriana; Koi Fish*

Abstrak

Penelitian ini bertujuan untuk mengetahui pengaruh penambahan tepung daun *Indigofera zollingeriana* dalam formulasi pakan dengan dosis yang berbeda untuk kecerahan warna ikan koi. Rancangan penelitian yang digunakan yaitu Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan tiga ulangan. Perlakuan P0 yaitu kontrol (tanpa penggunaan tepung daun indigofera), P1 yaitu penambahan tepung daun indigofera 5 %, P2 yaitu penambahan tepung daun indigofera 10 %, P3 yaitu penambahan tepung indigofera 15 % dan P4 yaitu penambahan tepung daun indigofera 20 %. Ukuran ikan yang digunakan adalah 6±1 cm dengan padat tebar 1 ekor per 2 L. Pemeliharaan penelitian selama 30 hari. Hasil penelitian menunjukkan bahwa P2 dengan persentase 10% tepung daun *Indigofera zolllingeriana* adalah hasil terbaik dengan kecerahan warna ikan koi (27,23), tetapi tidak berpengaruh nyata untuk pertumbuhan panjang mutlak (1,04 cm) dan bobot mutlak (1,05 g), efisiensi pakan (23,08 %), dan kelangsungan hidup (100%). Kualitas air yang didapatkan selama pemeliharaan yaitu suhu 26-27°C, pH 6,1-7,2, oksigen terlarut 5,2-6,3 mg/L dan amonia 0,02-0,036 mg/L.

Kata kunci: Ikan Koi; Indigofera zollingeriana; Kecerahan Warna ikan

1. Introduction

Ornamental fish is a fishery commodity that has high economic value. The Marine and Fisheries Ministry of the Republic of Indonesia (2022) stated that demand for national ornamental fish production has increased from 2017-2021, with an average increase in ornamental fish production of 4.35% per year. One ornamental fish that has long been a prospect for farming is the koi fish.

Koi fish (*Cyprinus carpio*) is a fish that has an attractive color and body shape, so it is in great demand by consumers. Koi fish are an ornamental fish in great demand because of their beautiful body shape and attractive colors (Kusrini *et al.,* 2015).

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The brightness of color in fish can be increased by adding carotenoids to the feed. This is because fish
cannot synthesize carotenoids themselves, so it is necessary to provide feed containing carotenoids.

Carotenoids are natural dyes that can produce orange, red, yellow, blue, green, and purple colors from vegetable and animal sources (Haser, 2015). One type of carotenoid that produces yellow, orange, and red pigments is β-carotene (Syukri, 2021).

Currently, the price of commercial feed for ornamental fish, especially koi fish, is relatively high compared to commercial feed for consumption fish, IDR 110,000/kg (Wachin, 2022). This is because ornamental fish feed contains high levels of β-carotene. Therefore, there is a need for alternative ingredients that contain β-carotene. Several natural ingredients that contain β-carotene include carrot meal with a β-carotene content of 33.74 mg/kg (Rochimiwati *et al.,* 2011), shrimp head meal has a β-carotene content of 13.6 µg ml-1 (Solihah *et al.*, 2015), and yellow pumpkin contains β-carotene of 0.82 mg/g (Yulianawati and Isworo, 2012). One other natural ingredient that contains β-carotene is Indigofera zollingeriana leaves, namely 507.6 mg/kg (Palupi *et al.,* 2014).

Research on the use of natural ingredients containing carotenoids to increase the brightness of the color of koi fish has been carried out, including the use of carrot meal at the best dose of 5-10% (Budi and Mardiana, 2021), shrimp head meal at the best dose of 10% (Riansah *et al.,* 2020), and pumpkin meal with the best dose of 15% (Nazhira *et al.,* 2017).

In fish farming, feed is an important factor. The primary growth energy for fish is protein, and this is because the composition of the body's largest constituent besides water is protein (Mawalgi *et al.,* 2017). According to Juliana et al. (2018), protein is an important nutrient that fish need for growth. Indigofera leaves contain high protein, 29.16% (Abdullah, 2010). Therefore, indigofera leaves can be used to grow koi fish.
Research on the use of indigofera leaf meal for the growth of consumption fish has been carried out, including on tilapia (Tampubolon, 2017), gourami fish (Mawalgi, 2018), catfish (Mukti *et al.,* 2019), catfish (Yurtiana, 2021), and fish. addition (Fransiska, 2022). Meanwhile, research on the use of indigofera leaves to increase the brightness of fish color has been carried out, including on Sumatran fish with the best dose of 15% (Pratama *et al.,* 2019), rainbow fish with the best dose of 10% (Maryanto, 2019) and goldfish with the best dose of 5 % (Firdaus, 2022). Therefore, it is necessary to research the use of indigofera leaf meal in feed formulations to increase the brightness of the color of koi fish.

2. Materials and Methods

2.1. Research design

This research used a Completely Randomized Design (CRD) with five treatments and three replications. The treatment used is the addition of indigofera leaf meal at different doses to the feed formulation as follows:
P0 = Control (without use of indigofera leaf meal).
P1 = Addition of 5% indigofera leaf meal.
P2 = Addition of 10% indigofera leaf meal.
P3 = Addition of 15% indigofera leaf meal.
P4 = Addition of 20% indigofera leaf meal.

2.2. Feed formulation

The feed formulation used for this research is presented in Table 1.

Table 1
Feed formulation.

Ingredient	Treatment (%)				
	P0	P1	P2	P3	P4
Indigofera leaf meal	0	5	10	15	20
Soybean meal	35	30	25	20	15
Fish meal	30	30	30	30	30
Rice bran meal	18	18	18	18	18
Tapioca	13	13	13	13	13
Fish oil	2	2	2	2	2
Vitamin mix	2	2	2	2	2
Total (%)	100	100	100	100	100
Protein* (%)	30.54	30.74	30.82	32.24	32.49
Lipid* (%)	8.08	8.30	8.05	8.68	8.75
Nitrogen Free Extract (NFE) * (%)	30.82	32.18	31.87	31.20	30.01
Fiber* (%)	5.44	6.61	4.82	6.45	7.96
Ash* (%)	17.74	14.78	16.14	13.57	12.75
Water content* (%)	7.37	7.39	8.30	7.86	8.04
Carotenoid**	15.73	26.64	43.16	55.73	76.67
GE*** (Kcal/100 g)	395.642	409.203	398.691	416.501	419.871
GE/P (Kcal/g protein)	12.95	13.31	12.94	12.92	12.92

Note: *Gross Energy (GE) value is calculated based on the amount of protein energy 5.6 kcal/g, lipid 9.4 kcal/g, and carbohydrates 4.1 kcal/g (NRC, 1993).

2.3. Preparation of Indigofera zollingeriana leaf meal

Preparation of *Indigofera zollingeriana* leaf meal starts with taking the young leaves, namely the shoots (Fransiska, 2022), then washing them clean to avoid dirt. Then, the leaves are dried in the sun. After drying, the leaves are ground using a blender until they become a meal and then sifted.

2.4. Production of treatment feed

The ingredients used are first weighed according to the formulation of each treatment and then mixed into a container from the smallest amount to the largest. The ingredients that have been mixed are homogenized, and 30% of the weight of the mixture, or 300 mL of warm water, is added. Then, stir until

smooth, then mold using a feed maker. The molded feed is then dried by drying the feed in the sun until dry. After drying, the feed is stored in a closed container and protected from direct sun.

2.5. Preparation of rearing containers

The rearing container uses 15 aquariums measuring 25x25x25 cm^3. The container was cleaned first using a potassium permanganate solution of 2.5 mg/L (Agustini *et al.,* 2020). Then, the aquarium is rinsed until clean, filled with 13 cm or 8 L of water, given aeration, and left for 24 hours (Mutiarasari, 2017). The aquariums were then labelled according to randomization based on each treatment.

2.6. Koi fish rearing

The fish in this study used Kohaku koi fish with a size of 6 ± 1 cm and a fish stocking density of 1 fish per 2 L of water (Ervina, 2016). The fish used has an almost uniform color, namely orange. The fish are acclimatized and adapted first for five days to reduce stress levels. After that, the fish's weight and length were measured as initial sampling data. Fish are given food according to their respective treatments. The feed given to fish is 5% of the fish's body weight, with a feeding frequency of once every three days at 8.00 a.m., 12.00 a.m., and 4.00 p.m. (Mutiarasari, 2017). Cleaning is done every two days. Water is added after syphoning as much as the water released during syphoning. Fish rearing is carried out for 30 days. Fish color brightness measurements were carried out at the beginning and end of rearing. Fish sampling is carried out every 15 days. Fish that died during rearing were measured for weight and length.

2.6. Parameters

The parameters measured in this study included color brightness, absolute weight growth, absolute length growth, feed efficiency, survival and water quality.

2.6.5. Water quality

The water quality parameters measured in the research were temperature, pH, ammonia, and dissolved oxygen (DO). Temperature and pH measurements were measured daily during rearing, while ammonia and dissolved oxygen were measured at the rearing period's beginning, middle, and end.

2.7. Data analysis

The data obtained on color brightness, growth in absolute length and weight, feed efficiency, and survival were analyzed using analysis of variance at a 95% confidence interval. If it has a significant effect, then proceed with the least significant difference test (LSD). Water quality data was analyzed descriptively.

3. Results and Discussion

3.1. Results

Data on the color brightness of koi fish kept for 30 days are presented in Table 2.

Table 2
Average color brightness values for koi fish.

Treatment	Color Brightness (LSD= 8.11)
P0	16.93 ± 2.85^{ab}
P1	22.13 ± 2.67^{bc}
P2	27.23 ± 7.86^{c}
P3	11.56 ± 4.03^{a}
P4	9.95 ± 2.49^{a}

Note: Numbers in the same column followed by different superscript letters show significantly different effects on the 95% LSD test.

Data on the absolute growth and feed efficiency of koi fish obtained during 30 days of rearing are presented in Table 3.

Table 3
Average growth and feed efficiency value of koi fish.

Treatment	Absolute Growth		Feed efficiency (%)
	Growth (g)	Length (cm)	
P0	0.94 ± 0.18	0.95 ± 0.13	16.72 ± 5.39
P1	1.01 ± 0.13	0.99 ± 0.10	18.49 ± 1.17
P2	1.05 ± 0.23	1.04 ± 0.21	23.08 ± 8.51
P3	1.17 ± 0.17	1.13 ± 0.13	22.32 ± 2.42
P4	1.18 ± 0.09	1.16 ± 0.19	25.56 ± 2.71

Note: Numbers in the same column followed by different superscript letters show significantly different effects on the 95% LSD test.

Data on the survival of koi fish that were given the addition of indigofera leaf meal in their feed during 30 days of rearing can be seen in Table 4.

Table 4
Average survival rate value of koi fish.

Treatment	Survival rate (%)
P0	100
P1	100
P2	100
P3	100
P4	100

The water quality values obtained during 30 days of koi fish rearing can be seen in Table 5.

Table 5
Water quality for koi fish rearing.

Treatment	Temperature (°C)	pH	DO (mg/L)	Ammonia (mg/L)
P0	26-27.3	6-7.1	5.3-6.5	0.028-0.041
P1	26-27.2	6-7.2	5-6.6	0.021-0.038
P2	26-27	6.1-7.2	5.2-6.3	0.020-0.036
P3	26-27.3	6.1-7.2	5.1-6.7	0.024-0.038
P4	26-27.1	6-7.2	5-6.1	0.021-0.033

3.2. Discussion

Based on Table 2, the color brightness of koi fish provides results that have a real effect. Koi fish in P2 with the addition of 10% indigofera leaf meal were significantly different from P0, P3, and P4 but not significantly different from P1.

The best increase in color brightness of koi fish in P2 is because the carotenoids contained in P2 can be utilized optimally, and koi fish directly absorb carotenoids to become pigment cells or chromatophores. Color brightness increases due to changes in the chromatophore cells (Sari *et al.,* 2012). The process of color formation in the fish's body occurs due to the translocation of pigments in chromatophores, namely carotenoids, together with proteins and help from lipids to spread and gather in the cytoskeleton to increase the brightness of the fish's color (Ahi *et al.,* 2020). In addition, the carotenoid content in P2 feed is 43.16 mg/g (Table 2). This follows what was reported by Ninwichian *et al.* (2020) that the brightness of the color of koi fish increased when fed food with a carotenoid content of 47.40 mg/kg.

The P0 treatment did not produce the best color brightness, presumably because the carotenoid content in the feed was low, namely 15.73 mg/g (Table 2). According to Amin *et al.* (2012), to get the best color appearance in fish, the dose of colored carotenoids given must be correct. Meanwhile, treatments P3 and P4 had higher carotenoid content than P2, namely 55.73 mg/g (Table 2.) and 76.67 mg/g (Table 2.) but

produced low color brightness. This is thought to be due to a buildup of carotenoids in the fish's body. According to Kusuma (2012), feed containing carotenoids has a maximum limit that the fish's body can absorb, so excessive doses of carotenoids affect the brightness of the fish's color and even decrease the color quality. Seed-sized koi fish require relatively fewer carotenoids because their body color changes are not fixed (Utomo, 2006). According to Chavarría and Flores (2013), fish's carotenoid metabolism occurs in the fish's intestines and liver. Continuous administration of excess carotenoids is thought to cause a buildup in the liver.

The results of growth in absolute length, absolute weight, and feed efficiency in koi fish in all treatments showed no significant effect. Feed is the main factor that influences fish growth. Mawalgi *et al.* (2017) stated that feed contains protein, the main energy for fish growth. According to Juliana et al. (2018), protein is an important nutrient that fish need for growth.

Ornamental fish have attractive colors. Color brightness in ornamental fish can be increased by providing carotenoid feed (Nur *et al.,* 2020). The carotenoid content in the feed can increase the brightness of the color of the koi fish compared to its growth. Prayogo *et al. (*2012) stated that ornamental fish fed with carotenoids are thought to utilize these dyes to increase their body color compared to the growth of ornamental fish. Research on the use of carotenoid ingredients such as pumpkin (Nur et al., 2020), shrimp head meal (Subamia *et al.,* 2013), and carrot meal (Firdaus *et al.,* 2022) in feed for koi fish can increase color brightness, however, produces slow growth. According to Jha and Barat (2005), koi fish produce a weight of 4 g within three months from an initial weight of 0.14 g.

The feed efficiency produced in this study had no significant effect. According to Setiawati et al. (2013), the feed efficiency value is related to the growth rate of the fish. The greater the weight of the fish, the greater the feed efficiency value. According to Saade *et al*. (2014), seed-sized koi fish produced the best feed efficiency of 9.17% when rearing for 30 days.

The results of the analysis of variance with a 95% confidence interval had no significant effect on the survival of koi fish. The survival rate obtained in the study was 100% for all treatments. The high survival rate of koi fish is likely due to adequate food requirements and supportive water quality during the research. According to Fran and Akbar (2013), the survival rate is influenced by several factors: environment (water quality), age, food, and pests and diseases.

The analysis of variance with a 95% confidence interval showed that adding indigofera leaf flour to the feed formulation had no significant effect on koi fish hematocrit. Even though it produces values that have no real effect, the total hematocrit obtained from H0 to H30 has increased by 11 - 28.33%. The increase in hematocrit is still within the normal range for koi fish. According to Shabirah et al. (2019), normal hematocrit levels for goldfish range from 21-44%.

The results of the lsd follow-up test at the 5% level showed that at H0 to H30, the total hemoglobin value in treatment P0 was significantly higher than in treatments P2, P3, and P4 but not significantly different from P1. The total hemoglobin value in all treatments increased from the total hemoglobin value from H0 to H30, namely 4-7.37 g %. This increase in hemoglobin is still within the normal range for koi fish. According to Kusrini et al. (2019), normal hemoglobin levels in goldfish range from 4.9-9.65 g %. Erythrocyte and hemoglobin values are interrelated. According to *Lagler et al.* (1977) and Nuradi and Jangga (2020), fish blood hemoglobin levels are related to the number of erythrocytes. If the erythrocyte value increases, hemoglobin will also increase, and vice versa.

The results in Table 5 obtained during the research show the water quality values that can be tolerated for keeping koi fish. Good water quality for the growth of koi fish is a temperature ranging from 25.1-27.3 oC, pH 5-8 and ammonia of 0.05-0.24 mg/L (Andayani *et al*., 2022), and dissolved oxygen of 5-8 mg/L (Twigg, 2013).

4. Conclusion

The use of Indigofera zollingeriana leaf flour in feed formulation significantly affected the brightness of the color of koi fish but had no significant effect on absolute growth, feed efficiency, and survival. The best color brightness value was found by adding *Indigofera zollingeriana* leaf flour to the feed at 10% (P2).

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