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Enhancing Growth Performance and Coloration of Koi Fish (*Cyprinus rubrofuscus*) through Feed Supplementation with Rumen-Fermented Carrots and *Bacillus* sp.

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Abstract

Koi fish (*Cyprinus rubrofuscus*) is an ornamental fish with a relatively high percentage of domestic and international market demand, and its price is highly dependent on the body shape and color quality. Adding carotenoids to feed is one technique to improve the quality and brightness of koi fish colors. This research aimed to analyze the effectiveness of carrot meal fermented using rumen fluid and *Bacillus* sp. in feed to improve the color and growth performance of koi. This experiment was designed using a completely randomized design method, consisting of four types of treatments, each of which was repeated three times, resulting in a total of twelve test units. The treatments tested included the use of carrot meal fermented by rumen fluid and *Bacillus* sp. at levels of 0% (control), 10%, 15%, and 20%. The parameters observed were carrot meal carotenoids after fermentation, fish color performance, feed utilization efficiency, feed conversion ratio, muscle glycogen content, specific growth rate, absolute growth, and survival of koi fish. Data were analyzed using analysis of variance and further tested using Duncan. The results showed the best in treatment C, 15% rumen microbe fermented carrot meal and *Bacillus* sp. in feed, and produced the best color performance, TFC 89.91%, FUE 87.54%, FCR 1.99%, MGC 16.79%, SGR 22.62%, AG 34.33g, and SR 100%. This information can help koi breeders improve the color and growth performance of koi fish by using fermented carrot feed, rumen fluid, and *Bacillus* sp.

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INTRODUCTION

Koi fish (*Cyprinus rubrofuscus*) is one among ornamental fish that is economically significant due to substantial demand from both domestic and international markets (Aries *et al*., 2022), especially the magnificence of its body color (Amuneke *et al*., 2025). Bright and contrasting colors are one of the quality indicators of koi fish (Domasevich *et al*., 2022), in addition to growth and body shape (Serban *et al*., 2024). Based on this, in aquaculture activities, the aspect of improving color performance and growth is the main focus that koi fish farmers pay attention to (Dwiastuti *et al*., 2024). However, improving the color quality of koi fish is a challenge for farmers since the color of the fish fades and is not striking if it is cultivated overlong in a pond or aquarium (Firdaus *et al*., 2022), compared to rearing them in an environment that simulates their natural habitat (Liu *et al*., 2024).

Feed manipulation is currently one of the parts of cultivation science and technology that has been widely developed to improve the color performance and growth of koi fish (Fahruddin *et al*., 2025; Syamsinar *et al*., 2023). Some studies report that feeding raw materials containing natural pigments such as carotenoids, especially β-carotene, has been shown to increase the brightness of fish colors (Dwiastuti *et al*., 2024; Khairunnisa *et al*., 2020). Carotenoids are natural pigments that give yellow, orange, and red colors to fruits and vegetables (Gumilarsah *et al*., 2019). Carrot (*Daucus carota*) is a source of β-carotene that is abundant and easy to obtain, but its bioavailability in raw form is still relatively low (Anjani *et al*., 2022; Wagde *et al*., 2018). Therefore, one of the biotechnologies to increase the availability of nutrients and the effectiveness of feed absorption by fish through the fermentation process (Anwar *et al*., 2023; Murni *et al*., 2021).

Fermentation using rumen fluid (Murni *et al.*, 2021) and *Bacillus* sp. can increase the nutritional value of feed ingredients through the process of breaking down complex compounds (Anwar *et al*., 2024a, 2023), increasing the availability of amino acids, and producing enzymes and bioactive metabolites (Jannathulla *et al*., 2017). Rumen fluid contains various microorganisms, especially cellulolytic, amylolytic, proteolytic, and methanogenic bacteria, which are able to produce hydrolytic enzymes such as amylase, lichenase, endoglucanase, and xylanase (Raffrenato *et al*., 2021). Rumen fluids have high lignocellulolytic abilities, namely being able to break down plant cell walls (Vargas-Ortiz *et al*., 2022), so that active compounds such as β-carotene become more easily absorbed. Meanwhile, *Bacillus* sp., known as a probiotic that not only helps the digestive process (Salim *et al*., 2017; Vargas-Ortiz *et al*., 2022) but can also improve the health of the fish digestive tract and the efficiency of feed utilization (Anwar *et al*., 2024a).

The use of carrot meal fermented by a combination of rumen fluid and *Bacillus* sp. is expected to increase the content and availability of β-carotene in feed, as well as have a positive impact on improving color performance and growth of koi fish. Research on the application of fermented carrot meal using rumen fluid and *Bacillus* sp. in feed is important to increase feed availability to support the production of high-quality koi fish.

METHODOLOGY

Ethical Approval

This research has obtained ethical approval from the Research Ethics Committee to maintain and respect the dignity of experimental animals, with registration number: UMI022504266.

Place and Time

The research was carried out from January to March 2025. The feed fermentation process and maintenance are carried out in the laboratory of the University of Muhammadiyah Makassar.

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Proximate and muscle glycogen analysis was carried out at the Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University.

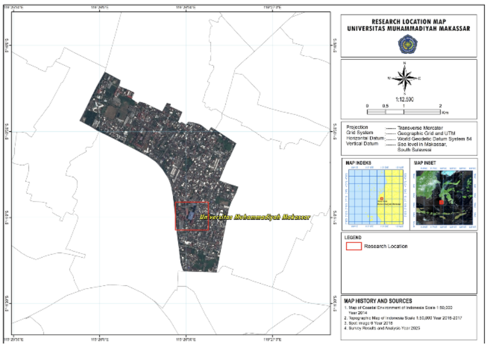

Figure 1. Research Location Map.

Research Materials

Equipment used in this study: glass aquarium size 50 x 30 x 35cm, blower (Resun LP200, BWS, China), shaker orbital digital SK0330-Pro LCD (DLAB Scientific Co., Ltd, China), suction hose, aerator, digital scales, ruler, jigs, thermometer, pH meter, Dissolved Oxygen meter (Do9100)(HEDAO, China), M-TCF (Toca Color Finder) paper and buckets. Research materials: koi fish (*C. rubrofuscus*) size ±3 cm, rumen fluid and *Bacillus* sp, flour, bran meal, soybean meal, cornstarch, carrot meal, fish oil, and vitamin mixed.

Research Design

The research design used a Completely Randomized Design (CRD), four treatments with three replications, as follows: Treatment A: Without the addition of fermented carrot meal (control); Treatment B: Fermented carrot meal 10%/100g feed; Treatment C: Fermented carrot meal 15%/100g feed; and Treatment D: Fermented carrot meal 20%/100g feed.

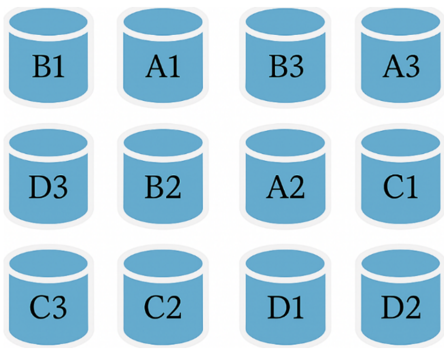

Figure 2. Research layout.

Work Procedure

Rearing Media Preparation

The tested fish-rearing medium uses a glass aquarium measuring 50 x 30 x 35cm. It was previously washed thoroughly with soap, then filled with fresh water and equipped with aeration.

Preparation of Rumen Fluid and *Bacillus* sp.

Cattle rumen fluid was taken from the Sungguminasa Slaughterhouse, Gowa Regency. Cattle rumen fluid was taken from the contents of the cattle rumen by filtration (filtering with cotton cloth) at a temperature of 4°C. The rumen fluid was then centrifuged at a speed of 2000 rpm for 15 minutes to separate the sediment and supernatant.

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Bacillus sp. from Micro, Small, and Medium Enterprises Biopocall Makassar - Indonesia NIB 0220000862736, density 4.82×10^6 CFU/mL, added 100mL of water and 10g of sugar, then refreshed by stirring for approximately one hour.

Carrot Fermentation Process

Carrots were obtained from the Sungguminasa Central Market, Somba Opu District, Gowa Regency, South Sulawesi Province, Indonesia. Carrots are washed and thinly sliced, then dried in the sun until they reach a moisture level of 90%. It is then blended and sifted through a 60-mesh screen. Then, 100g carrot meal plus 2.25 mL rumen fluid and *Bacillus* sp. with a ratio of 1:1, were put in an airtight plastic clip (anaerobic) at a temperature of about ±37°C and fermented for 120 hours (Murni *et al.*, 2018).

Test Feed Preparation

The preparation of test feed was started with the preparation of feed raw materials, mixed evenly, then molded using a Mahkota brand pellet machine Mpm-400b. Then dried using sunlight until it reached a dryness level of 90%, after which it was then packaged in plastic. Feed formulation by adding rumen fluid, fermented carrot meal, and *Bacillus* sp. as treatment, referring to Anwar *et al.* (2024a), modified, presented in Table 1 as follows:

Table 1. Feed formulation with fermented carrots.

Raw Material	Percentages of feed (%)			
	A	B	C	D
Fish meal	33	33	33	33
Soybean meal	25	25	25	25
Bran meal	25	14	9	4
Cornstarch	7	7	7	7
Fermented carrot meal	0	10	15	20
Flour	9	9	9	9
Fish oil	1	1	1	1
Vitamin mixed	1	1	1	1
Total	100	100	100	100

Test Feed Proximate

Proximate analysis of feed that has been formulated using fermented carrot meal of rumen fluid and *Bacillus* sp. according to the treatment dose is presented in Table 2:

Table 2. Treatment feed proximate analysis.

Sample	Composition (%)						µg/100g
	Moisture	Crude protein	Crude fat	Crude fiber	NFE	Ash	β-carotene
Feed A	20.21	30.21	16.60	9,86	25.30	17.98	0
Feed B	20.24	31.23	16.61	9.77	24.72	17.66	928.9
Feed C	20.22	32.32	16.65	9.75	23.41	17.87	1.392
Feed D	20.23	32.35	16.64	9.76	23.48	17.77	1.855

Test Fish Rearing and Sampling Process

Koi fish from the Freshwater Fish Seed Center Gowa, with an initial weight of ± 3 grams, a solid spread of 1 head/2 liters of water, in 12 glass aquariums measuring 50x30x35cm^3 with a capacity of 50 liters of water, equipped with aeration. The rearing of test animals, as many as 120 individuals, was acclimatized: pH, temperature, and dissolved oxygen, in addition to being acclimatized and adjusted to the control feed for 5 days, then weighed to get the initial weight. The rearing or cultivation process of koi fish was carried out for 60 days (until harvest), fed with a protein content of 30%, as much as 5% of the biomass, 3 times a day,

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at 08:00, 12:00, and 18:00 WITA. Test fish sampling was carried out every 10 days to determine the increase in test fish weight and adjustment of the amount of feed. Water change per sampling of 30% (Zainuddin *et al.*, 2014) modified.

Water Quality Management

Water quality management during the study and water replacement were carried out as much as 10% of the total volume every day. Water quality measurements are carried out daily, namely pH, dissolved oxygen (DO), and temperature *in situ*.

Research Parameters

The parameters measured during the study were proximate content of fermented carrot meal of rumen fluid and *Bacillus* sp., testing of fermented carrot meal β-carotene, total feed consumption, Feed Utilization Efficiency (FUE), Feed Conversion Ratio (FCR), muscle glycogen content (MGC), specific growth rate (SGR), absolute growth (AG), and survival rate (SR).

Proximate Analysis

The proximate analysis of fermented carrots of rumen fluid and *Bacillus* sp., as well as feed treatment, namely moisture, crude protein, crude fat, crude fiber, nitrogen-free extract (NFE), and ash (AOAC, 2010).

Fermented Carotene Beta-carotene Testing

The extraction of β-carotene was dissolved from carrot meal using an ethanol solvent. Then, the separation was continued by centrifugation to separate the β-carotene from the dregs. The measurement of β-carotene content was analyzed using HPLC. The method of calculation is the absorbance result compared to the standard for determining β-carotene levels, expressed in mg/100g of meal.

Koi Fish Color Observation

Observation of koi fish color was carried out by comparing the color increase in each treatment using paper M-TCF (Toca Color Finder) (Figure 3) (Fahruddin *et al.*, 2025).

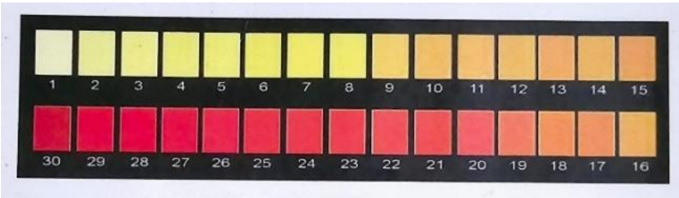

Figure 3. M-TCF (Toca Color Finder).

Total Feed Consumption (TFC)

$$\text{TFC (g)} = 1^{st}\text{ day of feed (g)} + 2^{nd}\text{ day of feed (g)} + .. + n^{th}\text{ day of feed (g)}$$

(Watanabe, 2002).

Feed utilization efficiency is calculated using the following formula (Effendie, 2002).

$$\text{FUE} = \frac{W_t - W_0}{F}\ \text{x}100\%$$

Information:
FUE = feed utilization efficiency (%)
Wo = initial biomass of koi fish (g)
Wt = final biomass of koi fish (g)
F = amount of feed given (g)

The feed conversion ratio is calculated using the following formula (Effendie, 2002).

$$\text{FCR} = \frac{F}{(W_t + D) - W_0}$$

Information:
FCR = feed conversion ratio
F = amount of feed given (g)
Wt = final average weight (g)
D = weight of dead fish (g)
Wo = initial average weight (g)

Koi fish muscle glycogen is calculated using the formula (Carroll *et al.*, 1956):

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$$\text{glycogen}\left(\frac{mg}{g}\text{sample}\right) = \frac{\text{abs.}\frac{spl}{abs}\text{.std x kons.std x Df x 1/1000}}{\text{Sample weight(g)}}$$

Information:
Abs. spl = sample absorbance at λ 670 nm
Abs.stda = absorbance standard
Kons. Std = Standard concentration (500µg /mL)
Df = dilution factor (5X)
1/1000 = change from micrograms to milligrams

The specific growth rate is the percent of the difference between the final and initial weight, divided by the length of maintenance time. According to Tacon (1987), the formula for calculating specific growth rate is:

$$SGR = \frac{\ln Wt - \ln Wo}{T} x100\%$$

Information:
SGR = specific growth rate (%)
Wo = average initial weight (g)
Wt = final initial weight (g)
T = time (days)

Absolute body weight growth is calculated using the (Effendie, 2002).
Wm = Wt – Wo
Information:
Wm = absolute body weight (gram)
Wt = final weight (gram)
Wo = initial weight (gram)

Survival rate (SR) is calculated using the formula (Tacon, 1987):

$$SR = \frac{Nt}{No} x100\%$$

Information:
SR = survival rate (%)
Nt = final number of koi fish
No = initial number of koi fish

Data Analysis

The color performance of fish was observed descriptively, and feed utilization efficiency, feed conversion ratio, muscle glycogen levels, specific growth rate, absolute growth, and survival of koi fish were evaluated using analysis of variance (ANOVA) to determine the differences among treatments using the Duncan test with a 95% confidence interval. Statistical analysis was carried out using SPSS software version 26. Water quality data, evaluated descriptively.

RESULTS AND DISCUSSION

Proximate Composition and β-Carotene Content of Carrot Meal Fermented with Rumen Fluid and *Bacillus* sp.

The results of the study on proximate and β-carotene levels of carrot meal fermented using rumen fluid and *Bacillus* sp, are presented in Table 3.

Table 3. Proximate analysis and β-carotene content of fermented carrots of rumen fluid and *Bacillus* sp.

Sample	Composition (%)						(µg/100g)
(Carrot meal)	Moisture	Crude protein	Crude fat	Crude fiber	NFE	Ash	β-carotene
After Fermentation	07.88	3.50	0.59	1.02	77.77	17.12	9289
Before Fermentation	89.80	1.20	0.50	1.65	81.55	15.10	8285

Carrots are abundant, cheap, and easy to reach as a local raw material. They contain a lot of β-carotene, which increases the brightness of the color of ornamental fish (Wang *et al*., 2024). Carrots are fermented using rumen fluid, and *Bacillus* sp. can potentially be a raw material for koi fish feed with β-carotene content. Analysis of the nutritional content of fermented carrots, rumen fluid, and *Bacillus* sp. provides information about the nutritional composition and β-carotene in carrots as raw materials for producing fish feed. Its nutritional composition comprises moisture, crude protein, fiber, fat, NFE, ash, and β-carotene. Based on the results of the study, the proximate content of fermented carrots of rumen fluid and *Bacillus* sp. showed a total of moisture of 87.88, crude protein of 3.50%, crude fiber of 1.12%, NFE 77.77%,

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ash of 17.12%, crude fat 0.59%, and β-carotene of 9289 µg (Table 3).

The results of the study showed that the moisture content of carrots after fermentation was relatively high but still within normal limits, which was 87.88%. High moisture content, in general, can support the growth of fermentative microbes but also risks increasing the development of decaying microbes if not under optimal conditions (Roslan *et al*., 2024). Generally, fermented products produce water as one of the by-products, but not as the main product. Water is formed by microorganisms' metabolic process during fermentation (Jiang *et al*., 2023).

The results of the study on the increase in the crude protein content of carrots after fermentation were 3.50%. It is suspected that fermentation using rumen fluid and *Bacillus* increases nitrogen content with the contribution of microbes that grow during fermentation. Reports that 70% of the weight of microbial dry cells consists of proteins (Koukoumaki *et al*., 2024).

The crude fat content of fermented carrot meal is 0.59%, similar to the characteristics of carrots, which are not a source of lipids. Although fat contributes to metabolic energy. In nutrition, low-fat content tends to be beneficial because it does not speed up the fattening process and prolongs the shelf life of feed (Motegaonkar *et al*., 2024).

The crude fiber in fish feed helps the digestive process, accelerates the excretion of feed residues, and improves the performance of the digestive tract; in addition, crude fiber is also used as a stomach filler, helps intestinal peristaltic movement, prevents feed clumping, and spurs the development of digestive organs. However, high crude fiber in fish feed can negatively impact fish digestibility and growth. Excessive coarse fiber reduces digestion efficiency and absorption of essential nutrients, thereby inhibiting the growth and development of fish (Cahya *et al*., 2022). The results showed a crude fiber content of 1.02%, which was suitable for fish feed. Crude fiber that is not recommended for fish feed is greater than 10% (Anwar *et al*., 2024a). This showed that fermentation could reduce some of the carrots' crude fiber content. Rumen fluid and *Bacillus* sp. produce the enzymes cellulase and hemicellulase, which can degrade carrots' cell walls, especially cellulose and hemicellulose.

NFE describes the content of non-fiber carbohydrates (starches and soluble sugars). The fermentation yield was 77.77% (Table 3), indicating that fermented carrots contain high energy, which comes from the natural sugars contained in carrots. Despite fermentation by microbes, most non-structural carbohydrates remain. This condition suggests that fermentation does not completely transform the substrate into microbial products. In addition, NFE contained in carrots is rich in reducing and non-reducing sugars in fructose, sucrose, dextrose, lactose, and maltose (Hosen *et al*., 2022). Sugar is part of carbohydrates and is readily fermented by rumen fluid and *Bacillus* sp. Another possibility is that carrots are rich in antioxidants that protect cells from damage caused by free radicals (Tian *et al*., 2024), so that they can help increase the viability of rumen microbial cells and *Bacillus* sp., which impacts the fermentation process, especially the conversion of carbohydrates into fatty acids.

Ash indicates the total mineral content. The ash content that increased after the carrots were fermented during the study was 17.12%, suspected to be contaminated with inorganic materials during the fermentation process. The increase in ash content in the feed after fermentation occurred because the fermentation process overhauls the components of organic matter, so that the undecomposed residues would be a higher percentage. The fermentation process, especially by microorganisms, decomposes the substrate (feed material) and degrades the organic matter (Fernandes *et al*., 2021).

The bioavailability of β-carotene in carrots is challenging due to the attachment of carotenoid compounds in the cellulose matrix and lignin of carrot cell walls. The

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carotenoid compounds are essential as a precursor to vitamin A (Britton *et al.*, 2012). The results of the study after fermentation using rumen fluid and *Bacillus* sp. anaerobically produced β-carotene of 9289 µg/100g (Table 3). This is supported by the performance of rumen fluid and *Bacillus* sp., which are combined to synergize and produce more diverse enzymes. Rumen fluid produces the enzymes cellulose breakdown cellulose; hemicellulose breaks down hemicellulose, pectinase, and xylanase (Hao *et al.*, 2021; Xie *et al.*, 2022), which helps destroy the cell wall of carrots, whereas *Bacillus* sp. can produce the enzymes amylase and protease (Danilova and Sharipova, 2020; Sulistiyani *et al.*, 2021) as well as some antioxidants (Etesami *et al.*, 2023), which can improve the stability of bioactive compounds β-carotene in carrots so that they are easy to use by koi fish.

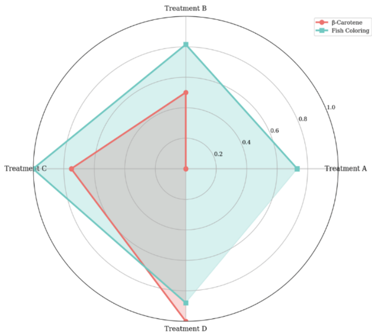

Figure 4. The relationship between β-carotene content and the level of fish coloration in all treatments.

Based on the polar plot in Figure 4, it can be seen that there is an interesting relationship between β-carotene content and fish coloration levels in various treatments. Treatment A (control) showed the lowest value, while treatment C showed an optimal balance with high β-carotene content (1392.35) and the best fish coloration (24.5). Although treatment D had the highest β-carotene content (1855.8), fish coloration actually decreased to 21.53, indicating a saturation point or a negative effect of too high β-carotene concentration. Treatment B, with a value of (928.9), showed a moderate increase in both parameters. This pattern showed that the relationship between β-carotene and fish coloration is not linear, but followed a dose-response curve with an optimal point at treatment C, where further increases could actually reduce the effectiveness of coloration.

Koi Fish Color Performance

The color performance of koi fish reared for 60 days with the treatment of fermented carrots of rumen fluid, and *Bacillus* sp. in feed is shown in Figure 5.

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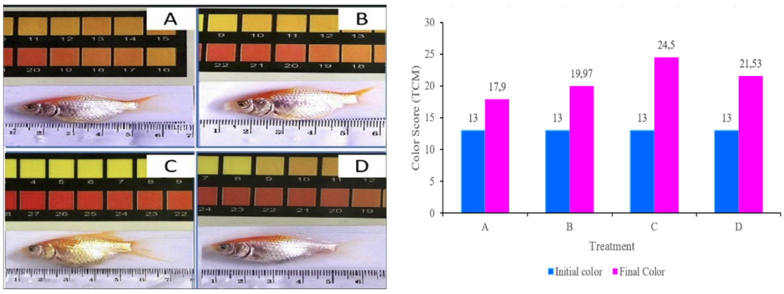

(a) (b)

Figure 5. (5a)The color performance, (5b) Coloring score of koi fish fed fermented carrots with rumen fluid and *Bacillus* sp.

The results of the study descriptively showed that the addition of fermented carrots of rumen fluid and *Bacillus* sp.15% had the highest effect on increasing the color brightness of koi carp, compared to the control treatments 0%, 10%, and 20% (Fig.5a and 5b). This increase in color brightness was supported by total feed consumption and high feed efficiency (Table 4). It was previously reported that the difference in the brightness level of koi fish color was influenced by the ability of fish to absorb carotenoids in feed (Fahruddin *et al.*, 2025). In addition, it is thought to be related to fermentation, increasing the bioavailability of the natural pigment carotenoids β-carotene contained in carrot material (Xu *et al.*, 2020). Rumen fluid and *Bacillus* sp. together produce the enzymes hemicellulase, xylanase, and cellulase (Anwar *et al.*, 2024b; Murni *et al.*, 2018), which break down complex bonds in the cell walls of carrots so that nutrients and β-carotene to improve skin coloration and scales become more easily absorbed by the koi fish's body without disturbing the nutritional balance. In addition, the rumen microbe *Bacillus* sp. enzyme activity as an exogenous enzyme is thought to improve the feed absorption system and nutrient metabolism so that pigment absorption becomes more efficient. Meanwhile, the addition of 10% fermented carrot meal did show an increase in brightness compared to the control, but it was not as optimal as at 15%. This showed that the fermented content of the fermented product at a dose of 10% was not enough to provide the maximum effect on the synthesis and deposition of pigments in the body of koi fish. On the other hand, adding 20% did not provide a better color enhancement and even tended to decrease compared to 15%.

This, allegedly influenced by overly high doses of fermented carrots, impacts the nutritional stability of feed or even causes micronutrient imbalances, which actually harm pigment metabolism. Thus, adding fermented carrots at a level of 15% is the right dose to increase the brightness of the color of koi fish optimally. This research is in line with (Firdaus *et al.*, 2022), the 15% carrot dose in the feed can provide an ideal balance between improving the nutritional quality of the feed and the effectiveness of pigment absorption by the koi fish's body.

Total Feed Consumption, Feed Utilization Efficiency, Feed Conversion Ratio, and Koi Fish Muscle Glycogen Content

The results of the variety-based analysis (ANOVA) showed that fermented carrots with rumen fluid and *Bacillus* sp in feed had a real effect on total feed consumption, feed utilization efficiency, feed conversion ratio, and muscle glycogen content. Follow-up tests using Duncan showed that treatment with a dose of 15% fermented carrots in feed, significantly different ($P<0.05$), was significantly highest and lowest for feed conversion ratio values, followed by 20%, 10%, and 0% treatment(control). This showed that an increase of up to 15% results in TFC, FUE,

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FCR, and MGC at optimal conditions, presented in Table 4.

Table 4. Total feed consumption (TFC), feed utilization efficiency (FUE), feed conversion ratio (FCR), and muscle glycogen content (MGC) in feed supplemented with fermented carrots of rumen fluid and *Bacillus* sp. in all treatments.

Treatments	Parameters ± std			
	TFC (g)	FUE(%)	FCR	MGC(%)
A (0%)	85.32±0.28[a]	72.21±0.36[a]	2.64±0.30[d]	13.09±0,07[a]
B (10%)	88.67±0.27[b]	74.61±0.09[b]	2.48±0.57[c]	14.45±0.03[b]
C (15%)	89.91±0.06[d]	87.54±0.40[d]	1.99±0.04[a]	16.79±0.14[d]
D (20%)	89.12±0.50[c]	86.39±0.09[c]	2.17±0.11[b]	15.73±0.05[c]

Note: The same letter superscript in a different column indicates insignificance (P>0.05) at the 95% confidence level.

The results of a study related to the total feed consumption of 89.91g showed that adding fermented carrots of rumen fluid and *Bacillus* sp. with a dose of 15% in the feed had an optimal effect. This showed a balance among nutrients, aromas, and feed textures compared to 0, 10, and 20% controls. This condition is thought to be caused by fermentation to increase the palatability and digestibility of feed. Researchers have previously reported that fermentation by rumen fluid and *Bacillus* sp. can break down anti-nutrient compounds, increasing nutrient content and bioactive compounds (Jiang *et al*., 2023), so that it is easier to digest and attractive to fish. This causes koi fish to be more active in consuming feed. This means the dose of 15% fermented carrots in the feed can balance increased nutritional value and feed palatability. Previous researchers reported the same thing: that the fermentation method significantly affects the deliciousness of feed (Yao *et al*., 2020). Although the 10% treatment showed an improvement compared to the control, it did not provide the optimal effect as with the 15% dose increase. This indicated that at a dose of 10%, the fermentation content was not optimal enough to affect feed quality significantly. Meanwhile, the total feed consumption in the 20% treatment showed a decrease compared to 15%; it was suspected that the fermented carrot content was too high in the feed, resulting in a decrease in palatability, changes in the aroma, or texture of the feed that koi fish do not like. Another possibility is the emergence of secondary metabolites resulting from over-fermentation that reduce appetite in eating fish (Jannathulla *et al*., 2017).

The highest feed efficiency was achieved by adding fermented carrot using rumen fluid and *Bacillus* sp. 15%. This is related to total feed consumption (Table 4). In addition, it is suspected that the content of carotenoids, exogenous enzymes from fermentation, and optimal nutritional values support fish metabolism. Fermented carrots, at a dose of 15%, provided enough bioavailable nutrients to support growth (Table 5). Meanwhile, in the control treatment, the feed efficiency value was lowest due to not being enriched with fermented carrots, which did not add exogenous enzymes for digestion. Furthermore, feed efficiency increased in the 10% treatment, but it was still limited because the amount of nutrients was not optimal. On the other hand, a dose of 20% decreased the feed efficiency value as the total feed consumption decreased (Aboelyzed *et al*., 2024).

The FCR at the time of the study was obtained at a dose of 15% fermented carrots in the feed. This showed that koi fish could efficiently convert feed into body biomass at a dose of 15%. This fact showed that koi fish grew faster (Table 5). Another condition, the control treatment produced the highest FCR value, illustrating inefficient feed being converted into body biomass (Table 4). At an

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additional dose of 10%, there was a decrease in FCR, but it was not significant. Meanwhile, 20% FCR increased again, likely due to excess or unbalanced nutrients, so not all were utilized efficiently. A low FCR value indicates that the fish efficiently convert the feed into body mass; conversely, if the FCR value is high, it indicates that the fish converts the inefficient feed into body mass (Iskandar *et al*., 2024; Safir *et al*., 2025).

Muscle glycogen is a reserve of carbohydrates in the muscles (Guo *et al*., 2024). The highest muscle glycogen content was recorded in the addition of a 15% dose of fermented carrots; this fact explained that the fish obtained enough energy with a high total feed consumption and stored it efficiently (Table 4). It also showed that the physiological condition of koi fish is excellent. This meant that a dose of 15% was optimal for energy metabolism and was thought to be showing no physiological symptoms of stress in koi fish, which was evidenced by its high growth rate (Table 5). Meanwhile, in the control treatment, the lowest glycogen content was due to the absence of additional fermented feed (Table 4), so it was not able to support energy metabolism. At a dose of 10%, glycogen levels increased as simple fermented carbohydrates were available. However, in addition to 20%, there was a slight decrease in glycogen levels, possibly due to metabolic stress due to excess nutrition or feed imbalances. The same thing was reported (Shimul *et al.,* 2024; Siddik *et al.,* 2018) that the optimal fermented raw materials in feed could increase energy stability, which affected the health of energy metabolism and energy storage in the form of muscle and liver glycogen.

Absolute Growth, Specific Growth Rate, and Survival Rate

The results showed that the addition of 15% of fermented carrots of rumen fluid and *Bacillus* sp. in the feed showed the highest and significantly different values (P<0.05) to the specific growth rate (SGR) and absolute growth (AG), but had no significant effect on the survival rate of koi fish. Treatment C showed the best growth, as presented in Table 5.

Table 5. Effect of rumen microbial fermented carrots and *Bacillus* sp. in feed on the Specific growth rate (SGR), Absolute growth (AG), and Survival rate (SR) of koi fish (*C. rubrofuscus*).

Treatments	Parameters ± std		
	SGR (%)	(AG) (g)	SR (%)
A (0%)	18.20±0.07[a]	30.47±0.31[a]	100±0
B (10%)	19.32±0.12[b]	31.56±0.40[b]	100±0
C (15%)	22.62±0.26[d]	34.33±0.32[d]	100±0
D (20%)	20.66±0.05[c]	32.74±0.08[c]	100±0

Note: The same letter superscript in a different column indicates insignificance (P>0.05) at the 95% confidence level.

The addition of 15% fermented carrots (fermented using rumen fluid and *Bacillus* sp.) had the most significant effect on the specific growth rate of koi fish, which was 22.62%, indicating high growth due to increased nutrient availability, efficiency, and digestibility of feed (Table 5). Adding fermented carrots at this level could produce a nutritionally balanced feed, increase nutrient absorption, and promote faster growth. The addition of 20% lowered the specific growth rate to 20.66%, which might be due to an excess of fermentation components that reduced the metabolic efficiency of koi fish. Meanwhile, the 10% dose only resulted in a growth of 19.32%, although better than the control, it was still not optimal. These results showed that the 15% dose was the most effective dose of fermented carrots in feed to increase the specific growth rate of koi fish significantly (Herawati *et al*., 2020).

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The addition of fermented carrots using rumen fluid and *Bacillus* sp. showed a noticeable effect on the absolute growth increase of koi fish, where a 15% dose resulted in the highest growth of 34.33g, and this result was in line with the specific growth rate (Table 5). This suggested that the balance between nutrient availability, digestibility, and feed palatability is optimal at this dose. This was strongly related to total feed consumption and high efficiency (Table 4). Meanwhile, the addition of 20% lowered the absolute growth to 32.74g, allegedly due to excess fermented carrots in the feed, thereby reducing the quality of the feed. The addition of 10% resulted in a growth of 31.56g, showing an improvement over controls but not as efficient as the 15% dose. In the control treatment, which resulted in the lowest growth, this fact confirmed that fermenting carrots with microbes. This is the same as that reported by Amaral *et al.* (2023), that fermented fish feed has an optimal limit to support the growth performance of European Seabass (*Dicentrarchus labrax*).

The results showed that adding fermented carrots to the feed, either in various doses or without, did not significantly affect the survival of koi fish (Table 5). This showed that the presence or absence of fermented carrots in the feed does not significantly contribute to the survival rate of koi fish. All treatments showed a high and uniform survival rate, which could be explained by the fact that the conditions of the cultivation environment were optimal and did not cause stress to the fish.

Water Quality

Water quality greatly determines the success of koi fish cultivation. Measurements of water quality parameters during the study included pH, temperature, and dissolved oxygen content. The data from the water quality measurement is presented in Table 6.

Table 6. Water quality analysis at all treatments.

Parameter	Treatment A	Treatment B	Treatment C	Treatment D	Standard	Description
pH	7.3	7.2	7.3	7.3	6.5-8.5	(Amalia *et al*., 2023; Fahruddin *et al*., 2025)
Temperature (°C)	28	28	28	28	25-35°C	(Fahruddin *et al*., 2025; SNI, 2017)
Dissolved Oxygen (mg/l)	5.4	5.4	5.4	5.4	> 5	(SNI, 2017)

The degree of acidity (pH) is one of the chemical parameters used to review the stability of the water. The pH value can affect the growth of biota; if it is excessive or insufficient, it can cause death. The pH value during the study ranged from 7.2 to 7.3. Amalia *et al*. (2023) reported that koi fish farming was in the range of 6.5 to 8. A pH value of less than 4 and more than 11 is the critical point for the fish, while a pH of less than 6.5 and more than 9.5 harms the growth of fish (Elsaidy *et al*., 2015). Table 6 shows the water quality for 60 maintenance days according to koi farming standards, with temperatures ranging from 27–30°C (SNI, 2017).

Temperature significantly affects the appetite, metabolism, and endurance of the fish. Low temperatures can inhibit growth, while high temperatures lower feed consumption and energy efficiency (Fahruddin *et al*., 2025). Meanwhile, the dissolved oxygen level during the study was 5.4 mg/l, which was in an optimal condition. Optimal dissolved oxygen can increase koi fish growth, feed efficiency, immunity, and survival rate in aquaculture waters (Zhou *et al.*, 2024).

CONCLUSION

Based on the results of proximate analysis of rumen microbial fermented carrots and *Bacillus* sp., produce crude protein content of 3.50%, crude fat 0.59%, crude fiber 1.02%, NFE 77.77%, ash 17.12%, and β-carotene 9289 µg. The best

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results obtained were TFC values of 89.91%, FUE of 87.54%, FCR of 1.99%, MGC of 16.79%, SGR of 22.62%, and AG of 34.33g at the addition of a dose of 15% fermented carrots in feed. These results are the optimal values of applying fermented carrots in feed without showing physiological disorders in koi fish.

CONFLICT OF INTEREST

All authors stated that they agreed with the manuscript being published and did not submit it to other journals. In addition, there is also no conflict of interest in the writing and publication of this manuscript.

AUTHOR CONTRIBUTION

The respective authors were: Asni Anwar, Murni, and Hamsah (Experimental conceptualization, design, and project acquisition). Syawaluddin Soadiq, Imam Taukhid, Nurwahyudi, and Burhanuddin (Data collection and formal analysis). Andi Khaeriyah, Harnita Agusanty, and Ching Fui Fui (Manuscript preparation and revision).

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