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EFFECT OF RED BELL PEPPER AND SHRIMP HEAD WASTE ON PIGMENTATION, GROWTH AND SURVIVAL IN KOI CARP (*Cyprinus carpio* L.)

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ABSTRACT

Sixty days feeding trial was conducted to evaluate the comparative efficacy of plant and animal derived natural pigment sources on growth performance, feed utilisation, survival and colour enhancement of koi carp (*Cyprinus carpio* L.) The experiment was conducted in the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. Juvenile koi carp (initial mean body weight of 2.10 ± 0.013 g) were randomly distributed into seven dietary treatments in a completely randomised design. The control group (T0) was fed a basal diet without addition of the pigment. Experimental diets were supplemented with Red bell pepper at 3% (T1), 6% (T2) and 9% (T3) as well as Shrimp head waste at 3% (T4), 6% (T5) and 9% (T6). All diets were formulated to be isonitrogenous with 30% crude protein. Results showed that dietary supplementation with both red bell pepper and shrimp head waste significantly ($p < 0.05$) improved growth performance, feed conversion ratio, protein efficiency ratio, and skin colour pigmentation compared with the control. Among the red bell pepper treatments, T1 (3% red bell pepper) showed the most favourable growth, feed utilisation, and pigmentation, while higher inclusion levels showed comparatively reduced performance. Shrimp head waste, however, showed a clear dose-dependent improvement, with T6 (9% shrimp head waste) recording the highest final body weight, weight gain, specific growth rate, feed efficiency, and the most intense colour pigmentation among all treatments. Survival was consistently high across all dietary groups and did not differ significantly. Red bell pepper and shrimp head waste were effective, economical and eco-friendly sources of natural pigments for the culture of koi carp. The 9% shrimp head waste treatment showed the best growth, feed efficiency and colour enhancement among all treatments and thus is a promising sustainable alternative to synthetic pigments in ornamental fish aquaculture.

Keywords: Red Bell Pepper, Shrimp Head Waste, Pigmentation, Growth and Survival, Koi Carp (*Cyprinus carpio* L.)

Introduction

The ornamental fish farming industry is one of the most rapidly growing areas in the fishing industry due to heightened demand for such fish in the global market as well as strong profit margins. In India, ornamental aquaculture has been encouraged due to the favourable climatic conditions, diversity of water habitats, and the resultant advancement in the domestic as well as global market (Ghosh *et al.*, 2003; Anusha *et al.*, 2014). Among the ornamental fish, koi carp (*Cyprinus carpio* L.) are in high demand globally for their distinctive colours, ability to thrive in different

farming conditions, and strong market demand (Kusrini *et al.*, 2015). In ornamental fish culture, body coloration and growth performance are key determinants of consumer preference and profitability.

Colour presentation in koi fish is mainly driven by their diet of carotenoids because these fish cannot produce these pigments in their bodies (Armstrong & Hearst, 1996). Apart from being used for enhancing fish colours, these pigments possess antioxidant properties, improve the immune response of fish, and are precursors of vitamin A hence they improve growth rates in fish (Shahidi & Brown, 1998; Chen *et al.*,

2017). Some natural sources of these compounds include Red Bell Pepper (*Capsicum annuum*), which has high levels of capsanthin and antioxidants, as well as Shrimp Head Waste, which is a product of shrimp processing that has high levels of Astaxanthin and nutrients important for growth (Sachindra *et al.*, 2005; Venugopal, 2008). However, comparative information on the effectiveness of plant-based and animal-based carotenoid sources in koi carp is limited. Therefore, the present study aimed to evaluate and compare the effects of dietary supplementation with red bell pepper and shrimp head waste on growth performance, feed utilization, survival, and colour pigmentation of koi carp (*Cyprinus carpio* L.), to identify cost-effective and environmentally sustainable natural alternatives to synthetic pigments in ornamental aquaculture.

Materials and Method

The feeding experiment was conducted for 60 days (October–December 2024) at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. Juvenile koi carp (*Cyprinus carpio* L.) with an average initial weight of 2.10 ± 0.013 g were acclimatized for seven days and randomly stocked into 21 plastic aquaria (50 L capacity) following a Completely Randomized Design with seven treatments and three replicates each. Seven isonitrogenous diets (30% crude protein) were formulated by incorporating two natural pigment sources, red bell pepper (*Capsicum annuum*) powder and shrimp head waste powder, at different inclusion levels. Red bell pepper was washed, sun-dried, and powdered, while shrimp head waste was collected, cleaned, dried, and powdered before feed preparation.

Table 1: Composition of Experimental Diets

Ingredients (%)	Diets						
	T0 Control	T1 (3%)	T2 (6%)	T3 (9%)	T4 (3%)	T5 (6%)	T6 (9%)
Red bell pepper	0.00	3.00	6.00	9.00	0.00	0.00	0.00
Shrimp head waste	0.00	0.00	0.00	0.00	3.00	6.00	9.00
Fish meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Groundnut oil cake	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Wheat flour	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Tapioca powder	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Plant oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Fish oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamins and minerals	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100	100	100

Fish were fed twice daily at 2–3% of body weight, with feeding rates adjusted fortnightly. Continuous aeration and daily partial water exchange were maintained, and water quality parameters were monitored regularly. Growth performance parameters including weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate were recorded at 15-day intervals. Colour pigmentation was assessed by estimating total carotenoid content in skin and muscle tissues using a spectrophotometric method. Tissue samples were extracted using chloroform and ethanol, and carotenoid concentrations were quantified spectrophotometrically at wavelengths between 375 and 500 nm. The

combined assessment of growth indices and carotenoid deposition enabled evaluation of the efficacy of dietary red bell pepper supplementation in koi carp. Completely Randomised Design (CRD) with three replications was used for each treatment.

Result and Discussion

The present study confirmed that both plant-based (Red bell pepper) and animal-based (Shrimp head waste) carotenoid sources significantly influenced growth performance, feed utilization, survival and colour pigmentation in koi carp, with responses strongly dependent on dietary source and inclusion level.

Table 2: Mean weight (g) of Koi carp fed with diets containing Red bell pepper (RBP) and Shrimp Head Waste (SHW) during the experimental period (Mean±SD)

Treatment	Experimental Period				
	0 Days	15 Days	30 Days	45 Days	60 Days
T0	2.11±0.06 ^a	2.56±0.03 ^b	3.03±0.06 ^b	3.53±0.07 ^b	4.08±0.03 ^b
T1 (3% RBP)	2.10±0.07 ^a	2.59±0.05 ^a	3.17±0.06 ^a	3.72±0.07 ^a	4.27±0.05 ^a

T2(6% RBP)	2.11±0.08 ^a	2.57±0.07 ^{ab}	3.16±0.03 ^a	3.69±0.02 ^{ab}	4.13±0.03 ^{ab}
T3(9% SHW)	2.11±0.07 ^a	2.63±0.02 ^a	3.08±0.06 ^{ab}	3.50±0.06 ^b	3.88±0.04 ^c
T4(3% SHW)	2.11±0.05 ^a	2.63±0.03 ^{ab}	3.24±0.04 ^a	3.84±0.03 ^b	4.50±0.02 ^b
T5(6% SHW)	2.10±0.05 ^a	2.65±0.02 ^a	3.31±0.07 ^a	3.90±0.04 ^b	4.61±0.07 ^b
T6(9% SHW)	2.11±0.08 ^a	2.66±0.06 ^a	3.34±0.07 ^a	4.12±0.03 ^a	5.06±0.2 ^a

Maximum growth was obtained with supplementation of red bell pepper at 3%, indicating that this level of inclusion offers an optimal balance of carotenoids, vitamins and antioxidants, as well as good palatability and nutrient digestibility. These bioactive compounds probably helped improve feed intake, digestive efficiency and nutrient use, leading to better growth. On the other hand, the inclusion levels above 6.6% (especially 9%) hurt growth possibly due to the increase in cellulose and crude fibre which fish poorly digest due to less cellulolytic activity. Increased inclusion can also result in decreased feed palatability and increased antinutritional factors that can impair nutrient absorption and utilisation. Similar findings were reported by Buyukcapar *et al.*, who observed that moderate inclusion of red pepper enhanced growth, whereas excessive levels adversely affected performance. These results indicate that 3% red bell pepper is the optimum dietary level for maximizing growth in koi carp.

Inclusion of shrimp head waste (%) in the diet produced a dose-dependent (3, 6 and 9%) significant

increase of growth performance in koi carp as illustrated by linear values for final weight, daily gain that peaked at 7.5% (21 g), along with improving feed efficiency with feeding levels up to 9% shrimp head waste. At higher inclusion levels, the enhanced performance is likely due to availability of the protein needed for muscle and exoskeletal growth, essential amino acids, phospholipids, minerals as well as bioavailable carotenoid–protein complexes that enhance nutrient assimilation metabolic efficiency and somatic tissue deposition. Growth stimulation against the control was lower when inclusion levels were reduced to 3% and 6% as these inclusion rates may not provide sufficient quantities of growth-promoting nutrients. Comparable dose-dependent growth responses following dietary incorporation of shrimp by-products have been documented by Boonyaratpalin and Unprasert, Fall *et al.*, and Pattanaik *et al.* Collectively, these findings establish 9% shrimp head waste as the optimal dietary inclusion level for maximizing growth in koi carp.

Table 3: Growth parameters after a diet with Red bell pepper (RBP) and Shrimp Head Waste (SHW) and during the experimental period (Mean±SD)

Parameter	T ₀ Control	T ₁ (3 % RBP)	T ₂ (6 % RBP)	T ₃ (9 % RBP)	T ₄ (3% SHW)	T ₅ (6% SHW)	T ₆ (9 % SHW)
MWG (%)	0.45±0.08	0.49±0.02	0.46±0.01	0.52±0.07	0.52±0.01	0.55±0.02	0.55±0.03
SGR (%)	1.099±0.06	1.183±0.04	1.159±0.05	1.015±0.06	1.262±0.03	1.31±0.01	1.458±0.05
Survival (%)	76.66±5.77	83.33±5.77	80.00±10.0	73.33±5.77	83.33±5.77	86.66±5.77	90±10.00

In the present study, specific growth rate (SGR) differed among treatments, with the highest value recorded in T6 (9% shrimp head waste), followed by T5, T4, T1, T2 and the control (T0) and T3, which showed the lowest growth performance. This pattern indicates that shrimp head waste supplementation, particularly at higher inclusion levels, is more effective in enhancing growth than red bell pepper. Similar findings have been reported in Koi carp, where the highest SGR was observed in T6 and was significantly higher ($P < 0.05$) than other treatments, supporting the superior nutritional contribution of shrimp-based ingredients (Pattanaik *et al.*, 2021; Pai *et al.*, 2016). In terms of survival, T6 also exhibited the highest rate, followed by T5, T4, T1, T2 and T0 however, differences were not statistically significant ($P > 0.05$),

suggesting a general improvement in survival with dietary supplementation. These results are consistent with previous studies (Vijayagopal *et al.*, 2008; Anka *et al.*, 2016), confirming that shrimp-derived feed additives enhance both growth and survival performance in fish.

Feed Utilization

Dietary supplementation showed a marked enhancement in feed utilisation. Among the treatments with red bell pepper, the best results were obtained in T1 (lowest FCR and highest PER) and the least efficient performance was obtained in T3. The results showed that moderate inclusion levels were optimal for feed efficiency, whereas high levels of inclusion negatively impacted FCR and PER, indicating less efficient nutrient utilisation at higher doses. These

findings are in agreement with the previous reports of Yigit *et al.* (2021) and Jain and Kour (2016) who showed improved FCR and growth performance in fish fed with carotenoid enriched diets. However, Kop *et al.* (2010) observed no significant effect of red pepper supplementation.

The result was accompanied by a gradual improvement in performance in the diets containing shrimp head waste, with T6 presenting the best FCR and PER, while the control group (T0) showed the lowest efficiency. The changing enhancement in T4–T6 suggests a positive influence of shrimp by-products, supporting the findings of Vasanthakumaran *et al.*

(2020) and Fall *et al.* (2011), who also observed enhanced FCR and PER with the incorporation of shrimp waste. Additional evidence from Boonyaratpalin and Unprasert (1989), Kalinowski *et al.* (2005) and Mohanta *et al.* (2013) demonstrated that carotenoid-rich shrimp by-products significantly improved feed utilisation and protein efficiency in fish. Taken together, these findings suggest that dietary sources of carotenoids from both plant and animal sources can be utilised to improve feed efficiency but the key to optimising the benefits is the determination of optimal inclusion levels.

Table 4: FCR (%) and PER (%) of Koi carp fed with diets containing Red bell pepper (RBP) and Shrimp Head Waste (SHW) during the experimental period (Mean±SD)

Parameter	T ₀ Control	T ₁ (3 % RBP)	T ₂ (6 % RBP)	T ₃ (9 % RBP)	T ₄ (3% SHW)	T ₅ (6% SHW)	T ₆ (9 % SHW)
FCR (%)	2.63±0.20	2.44±0.09	2.49±0.1	3.02±0.1	2.29±0.02	2.25±0.02	1.99±0.09
PER (%)	1.27±0.11	1.36±0.05	1.33±0.06	1.10±0.04	1.45±0.01	1.47±0.01	1.67±0.07

Pigmentation

Pigment responses followed a similar trend, with red pepper enhancing colour via capsanthin and capsorubin at optimal levels, while shrimp head waste led to higher carotenoid deposition related to the higher absorption efficiency of astaxanthin (Sachindra *et al.*, 2005; Breithaupt & Schwack, 2000). Survival was high and statistically similar in all treatments indicating physiological safety of both supplements and

antioxidant and immunomodulatory roles of dietary carotenoids (Vijayagopal *et al.*, 2008; Kumar *et al.*, 2017). Red pepper was effective in controlling pigmentation and enhancing growth at moderate inclusion levels. Shrimp head waste was a superior additive that enhanced both growth and colouration at higher dietary levels, and provided economic and environmental benefits through waste processing.

Table 5: Comparison of Carotenoid Content in Koi Carp Across Different Treatments Over the Experimental Period

Treatment	0 Days	15 Days	30 Days	45 Days	60 Days
T0	3.45±0.04 ^a	3.31±0.06 ^e	3.18±0.05 ^e	3.09±0.07 ^f	3±0.18 ^e
T1	3.32±0.1 ^{abc}	3.85±0.02 ^{cd}	5.05±0.3 ^b	5.58±0.06 ^b	6.1±0.14 ^b
T2	3.41±0.05 ^{ab}	3.75±0.2 ^{cd}	4.25±0.1 ^d	4.51±0.08 ^d	5.6±0.14 ^{cd}
T3	3.36±0.1 ^{abc}	3.68±0.06 ^d	3.60±0.1 ^c	3.17±0.11 ^e	2.90±0.09 ^d
T4	3.27±0.02 ^{bc}	3.91±0.03 ^c	4.33±0.04 ^c	5.02±0.11 ^c	5.8±0.07 ^c
T5	3.24±0.07 ^c	4.31±0.12 ^b	5.16±0.1 ^b	5.54±0.1 ^b	6.2±0.1 ^b
T6	3.23±0.01 ^c	4.82±0.1 ^a	5.95±0.04 ^a	6.66±0.07 ^a	7.4±0.05 ^a

Conclusion

The present study indicates that dietary supplementation of natural pigment sources has a significant effect on growth performance, feed utilisation, survival and colour pigmentation in koi carp (*Cyprinus carpio* L.) and the responses are strongly dependent on the source and inclusion level. Red bell pepper (*Capsicum annuum*) was effective at moderate inclusion (3%) to improve growth, feed efficiency and pigmentation but higher levels negatively impacted performance due to reduced palatability and antinutritional factors. In contrast,

prawn head waste enhanced growth and colouration in a dose dependent manner, which is attributed to its high-quality protein and better bioavailability of astaxanthin, without affecting survival. Overall, red bell pepper is suitable for controlled pigmentation enhancement, whereas shrimp head waste functions as a superior dual-purpose and sustainable feed ingredient, improving both growth and aesthetic quality. Strategic use of these natural additives can enhance the commercial value of koi carp while promoting environmentally sustainable ornamental aquaculture.

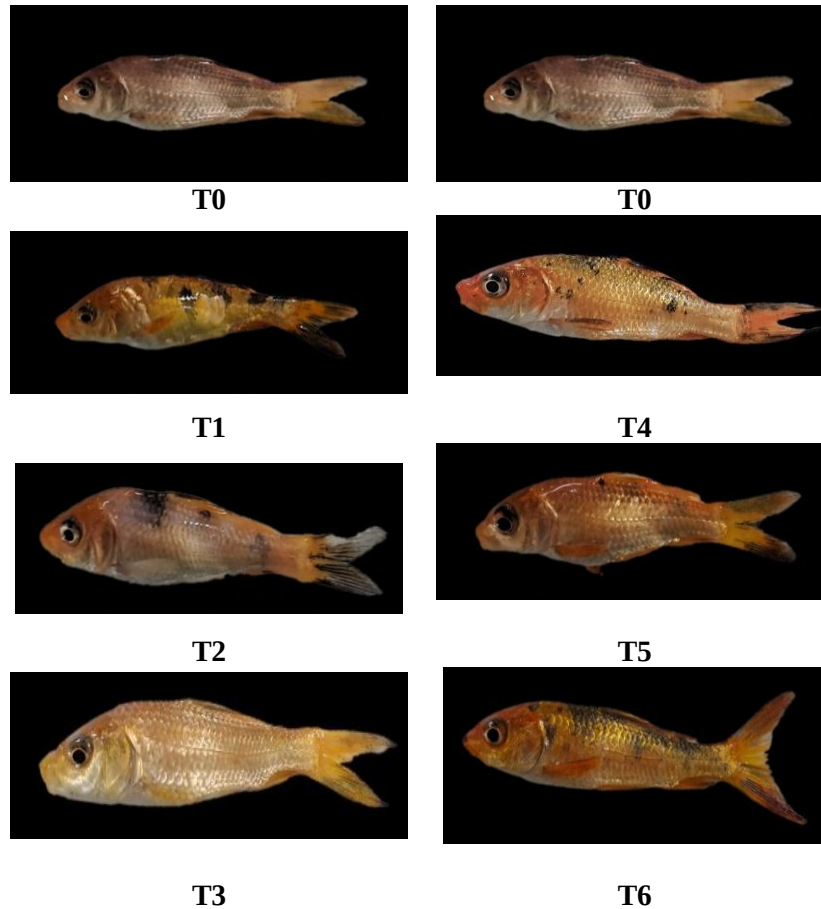


PLATE 1 : Pigmentation in Different treatments

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