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Kinjalkumari J Patel
 Department of Aquaculture,
 College of Fisheries Science,
 Veraval, Kamdhenu
 University, Gujarat, India

Ketan V Tank
 Department of Aquaculture,
 College of Fisheries Science,
 Veraval, Kamdhenu
 University, Gujarat, India

Prakashkumar V Parmar
 Department of Aquaculture,
 College of Fisheries Science,
 Veraval, Kamdhenu
 University, Gujarat, India

Viral C Bajaniya
 Department of Aquaculture,
 College of Fisheries Science,
 Veraval, Kamdhenu
 University, Gujarat, India

Corresponding Author:
Kinjalkumari J Patel
 Department of Aquaculture,
 College of Fisheries Science,
 Veraval, Kamdhenu
 University, Gujarat, India

Supplementation of pumpkin *Cucurbita Maxima* seed powder as a feed additive on growth and survival of rohu, *Labeo rohita* (Hamilton, 1822)

Kinjalkumari J Patel, Ketan V Tank, Prakashkumar V Parmar and Viral C Bajaniya

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Abstract

This study evaluates the effects of the dietary inclusion of Pumpkin (*Cucurbita maxima*) seed powder (PSP) on the growth and survival of *Labeo rohita* fingerlings over 60 days. Pumpkin seeds are rich in antioxidants and vitamin E. It has antinutritional and toxic factors, such as polyphenols, flavonoids, tannins, and cyanogenic glycosides. PSP was incorporated at varying levels (T₀ (0%), T₁ (1.5%), T₂ (3%), T₃ (4.5%), and T₄ (6%) Pumpkin seed powder) into fish diets. Growth parameters and survival were assessed, and it showed that 4.5% PSP diet (T₃) significantly improved growth rates and feed conversion ratio. However, survival rates were unaffected across treatments. Thus, a 4.5% PSP diet optimally enhances fish growth and Survival.

Keywords: *Labeo rohita*, pumpkin seed powder (PSP), growth, survival

Introduction

The aquaculture sector is vital to maintaining fish supply, especially with the decline in capture fish industry. Total production of aquatic animals has increased over the decades, going from 19 million tonnes (Live weight equivalent) in 1950 to a record of over 185 million tonnes in 2022, with an average annual growth rate of 3.2% (FAO, 2024). In 2022, global aquaculture production increased to 130.9 million tonnes, up 8.1 million tonnes from the 122.8 million tonnes produced in 2020. The most significant of the three Indian major carp species employed in carp polyculture systems is Rohu (*Labeo rohita*). It is a well-known component of aquaculture because of its quick growth rate, capacity to adapt to a wide range of environmental circumstances, and strong market demand. Fish growth at various stages is primarily influenced by the type of feed, feeding frequency, feed intake, and the fish's ability to absorb nutrients (Abid & Ahmed, 2009) ^[1]. Farmed rohu is omnivorous and has a diet containing 30-35% protein (Rasal & Sundaray, 2020) ^[11]. Medicinal plants have been proven as growth promoters. They Introduction have the properties of growth-promoting ability, a tonic to improve the antimicrobial capability, and stimulating appetite and anti-stress characteristics (Citarasu *et al.*, 2010) ^[2]. Pumpkin is a well-known edible plant with substantial medicinal properties due to its unique natural edible substances (Yadav *et al.*, 2010) ^[15]. Pumpkin seed has considerable economic importance, generating huge amounts of seeds. Pumpkin seeds have been used traditionally for herbal, medicinal, and clinical purposes. Pumpkin seeds are rich in essential fatty acids such as palmitic, oleic, linoleic, and steric acids as well as arginine and glutamic acid. However, it has antinutritional and toxic factors, such as polyphenols, flavonoids, tannins and cyanogenic glycosides (Del Vechio *et al.*, 2005) ^[4]. Pumpkin seeds contain approximately 30% crude protein (Sharma *et al.* 1986; Esuoso *et al.* 1998) ^[5, 12]. The amount and quality of protein in fish feed significantly impact their growth and survival rates (Hua *et al.*, 2019) ^[7]. Formulating fish feed utilizing relatively cheap, easily available, and extremely high-nutrient terrestrial resources, including *Cucurbita maxima* seeds is necessary.

Materials and Methods

The experiment was conducted at the College of Fisheries Science, KU, Veraval for 60 days.

The experiment was conducted in 20 rectangular plastic aquarium tanks (2 x 1 x 1 feet) with adequate aeration. The *Labeo rohita* seed was sourced from a commercial IMC and acclimatized in a 500-liter FRP tank for 7 days. Fish with an average weight of 1.310 ± 0.009 g was selected for the experiment.

Experimental Diet Preparation

Five experimental diets (T_0 to T_4) were prepared. Treatment groups include T_0 (control, no Pumpkin seed powder (PSP)),

T_1 with (1.5% PSP), T_2 (3% PSP), T_3 (4.5% PSP) and T_4 (6% PSP). The feed mixture was then subjected to heat treatment at 121 °C under 15 lbs of pressure for 15 minutes. After the steam cooking process, vitamins and minerals were added and carefully blended into the mixture.

Proximate Analysis of Ingredients

The proximate composition of the ingredients was analysed using the standard method of AOAC (2019).

Table 1: Proximate Analysis of Ingredients

Ingredients (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Moisture (%)
Fishmeal	55.03	4.20	15.20	7.91
Pumpkin seed powder (PSP)	24.12	22.17	3.31	6.184
Wheat flour	11.94	1.3	5.32	9.81
GNOC	35.56	4.92	8.07	7.19
Tapioca	4.81	0.61	0.14	8.13

The composition of the experimental diet was formulated using Pearson's square method at a 30% protein level. The formula for the experimental diets, including their

percentage composition, is detailed in Table 2 based on the treatment specifications.

Table 2: Composition of Experimental Diet (in gms)

Ingredients	Diet (30% Protein)				
	T_0 (Control)	T_1 (1.5% PSP)	T_2 (3.0% PSP)	T_3 (4.5% PSP)	T_4 (6% PSP)
Pumpkin seed powder	0	15	30	45	60
Sterilized fish meal	25	24	24	24	24
Wheat flour	15	14	14	13	13
Tapioca powder	13	13	12	12	12
Gnoc	41	41.5	41	40.5	39
Plant oil	2	2	2	2	2
Fish oil	2	2	2	2	2
Vitamin and minerals	2	2	2	2	2
Total	100	100	100	100	100

During the experimental period, *Labeo rohita* was fed twice daily at 5% of its body weight. The feed used contained 30% protein. Key parameters measured include total weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), Average weight gain (AWG) and survival rate across all five treatment groups. Completely Randomised Design (CRD) experimental design will be used in the experiment and estimate the arithmetic mean, standard deviation, and coefficient of variation using standard statistical Methods (Snedecor & Cochran, 2014) [13].

The collected data was analyzed using a one-way analysis of variance (ANOVA) to identify significant differences between the means. The statistical analysis was performed using IBM SPSS version 22 software.

Results

The study examines Body weight gain, feed conversion ratio, specific growth rate, Protein efficiency ratio, survival rate, Average weight gain and Water quality parameters in *L. rohita*-fed diets with varying levels of Pumpkin seed powder.

Table 3: Growth performance and survival of *L. rohita* fed a diet with 0% (Control), 1.5%, 3%, 4.5%, and 6% supplementation of pumpkin (*Cucurbita maxima*) seed powder

Parameters	Treatments				
	T_0 (Control)	T_1 (PSP 1.5%)	T_2 (PSP 3%)	T_3 (PSP 4.5%)	T_4 (PSP 6%)
IW (g)	1.310 ± 0.009^a	1.317 ± 0.006^a	1.325 ± 0.006^a	1.315 ± 0.006^a	1.310 ± 0.010^a
FW (g)	3.407 ± 0.042^e	3.535 ± 0.104^d	3.745 ± 0.011^c	4.297 ± 0.012^a	4.097 ± 0.008^b
MWG (g)	2.097 ± 0.033^e	2.217 ± 0.007^d	2.420 ± 0.012^c	2.982 ± 0.007^a	2.787 ± 0.016^b
SGR (%)	0.775 ± 0.003^e	0.803 ± 0.006^d	0.851 ± 0.008^c	1.001 ± 0.005^a	0.960 ± 0.014^b
Survival (%)	91.75 ± 2.39^a	92.50 ± 3.22^a	93.00 ± 3.53^a	95.25 ± 2.05^a	95.75 ± 2.05^a
AWG (%)	160.08 ± 1.581^e	168.32 ± 0.920^d	182.66 ± 1.569^c	226.81 ± 0.895^a	212.85 ± 2.908^b

*Mean $\pm$ standard deviation (SD). Different lowercase letters on the values within the same sample indicate significant differences ($p < 0.05$)

** IW- Initial weight, FW- Final Weight, MWG-Mean weight gain, SGR- Specific growth rate, AWG - Average weight gain

Discussion

The growth of *Labeo rohita* was assessed by sampling 15 fish per tank at 15-day intervals. After 60 days, final weights

were highest in T_3 (4.297 ± 0.012 g), indicating pumpkin seed powder significantly enhanced growth.

Over 60 days, *Labeo rohita* fry showed significant differences ($p < 0.05$) in mean weight gain across treatments,

with T₃ achieving the highest (2.982±0.007 g). Similar findings were reported by Teves *et al.* (2014) [14] for Nile tilapia (*Oreochromis niloticus*) fed squash (*Cucurbita maxima*) seed meal, where a 5% inclusion level resulted in the highest weight gain and specific growth rate. Musthafa *et al.* (2017) [8] also demonstrated that pumpkin (*C. mixta*) seed meal at 4 g kg⁻¹ and 6 g kg⁻¹ significantly ($p < 0.05$) improved weight gain and survival rates in fish. Its high protein and vitamin E content as key factors for improved growth and survival.

The specific growth rate (SGR) data (Table 3) showed that T₃ and T₄ had the highest SGR, significantly exceeding T₀, T₁, and T₂, while T₀ and T₁ had the lowest SGR with no significant difference ($p > 0.05$) between them. These findings align with Dada *et al.* (2015) [3] for African catfish (*Clarias gariepinus*) fed fluted pumpkin leaf meal, where 5 g/kg feed led to improved growth, feed conversion ratio, and SGR. Similarly, Yanti *et al.* (2022) [16] observed that Barramundi (*Lates calcarifer*) fingerlings fed a diet with 5% pumpkin flour showed significant improvements in weight gain, daily growth, and SGR.

At the end of the 60-day experimental period, the final mean percentage weight gain for Treatment T₃, with 4.5% pumpkin seed powder, showed the highest weight gain, followed by T₄ with 6% inclusion of PSP. Similarly, Teves *et al.* (2014) [14] found that *Oreochromis niloticus* fed squash (*Cucurbita maxima*) seed meal at a 5% inclusion level achieved the highest weight gain and SGR. Naiel *et al.* (2024) [9] reported that *Nile tilapia*-fed ochratoxin A-contaminated diets supplemented with pumpkin seed extract exhibited significantly improved final biomass, daily weight gain, SGR, and percentage weight gain.

The survival rates of *L. rohita* are represented in Table 3. Statistical analysis revealed no significant difference ($p > 0.05$) among treatments, indicating consistent survival rates across all groups. Similarly, Yanti *et al.* (2022) [16] reported that a 5% pumpkin flour diet significantly improved survival, in Barramundi (*Lates calcarifer*). Musthafa *et al.* (2017) [8] demonstrated that diets with 4 g kg⁻¹ and 6 g kg⁻¹ pumpkin (*C. mixta*) seed meal significantly ($p < 0.05$) enhanced survival rates.

Feed utilization by *Labeo rohita*

Table 4: Evolution of Feed Conversion ratio (FCR) and Protein efficiency ratio (PER) of *L. Rohita* recorded in different treatments at the end of 60 days

Parameters	Treatments				
	T ₀ (Control)	T ₁ (PSP 1.5%)	T ₂ (PSP 3%)	T ₃ (PSP 4.5%)	T ₄ (PSP 6%)
FCR	2.895±0.033 ^a	2.802±0.010 ^b	2.631±0.018 ^c	2.248±0.011 ^e	2.376±0.015 ^d
PER	1.151±0.013 ^c	1.189±0.004 ^d	1.266±0.009 ^c	1.482±0.007 ^a	1.402±0.009 ^b

FCR - Feed Conversion ratio, PER- Protein efficiency ratio, PSP- pumpkin seed powder

Discussion

After 60 days, the feed conversion ratio (FCR) for T₃ was 2.248±0.011 (Table 4). Statistical analysis revealed significant differences ($p > 0.05$) among treatments, with T₃ showing the lowest FCR, indicating superior feed efficiency. Mounes *et al.* (2024) demonstrated that replacing soybean meal with pumpkin seed cake in *Nile tilapia* diets significantly improved FCR. Similarly, Omosowone and Ogunrinde (2018) [10] found that a diet with a 25% inclusion level of pumpkin seed meal resulted in the best FCR for *Heteroclinus*. These findings highlight the effectiveness of pumpkin seed as a dietary supplement for improving FCR, enhancing growth performance, and potentially reducing feed costs in aquaculture.

Analysis showed significant variation in protein efficiency ratio (PER) among treatments, with T₃ achieving the highest PER (1.482±0.007), followed by T₄ (1.402±0.009), and T₀ the lowest. High PER reflects efficient feed protein conversion, consistent with studies by Musthafa *et al.* (2017) [8] and Teves *et al.* (2014) [14], highlighting the benefits of pumpkin seed meal in enhancing growth efficiency.

Physico-chemical water parameters

Water quality was monitored weekly for temperature, pH, dissolved oxygen, and total hardness throughout the 60-day study. It was in the ideal range throughout the experiment.

Conclusion

The study concluded that supplementing the *Labeo rohita* diet with pumpkin seed powder (PSP) positively affected growth, feed conversion, and immune response. The best growth was observed in fish fed 4.5% PSP (T₃), showing the

highest mean weight gain, specific growth rate (SGR), and favorable feed conversion ratio (FCR). Survival rates remained high, and no significant difference in mortality was noted. The study concluded that 4.5% PSP supplementation improved both growth and Survival in *Labeo rohita*, yielding the best outcomes.

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