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GROWTH RESPONSE OF JUVENILE ROHU (*Labeo rohita*) TO WHEATGRASS POWDER SUPPLEMENTED DIET

Md. Abdus Salam^{1*}, K. M. Shakil Rana¹, Md. Rakib Ahmmed¹ and Al Minan Noor²

¹Department of Aquaculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; ²Department of Fisheries, 13, Shohid Captain Moonsur Ali Sharani, Matshya Bhaban, Ramna, Dhaka, Bangladesh.

*Corresponding author: Dr. Md. Abdus Salam; E-mail: masalambau@gmail.com

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ABSTRACT

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Wheatgrass was evaluated as a potential non-conventional feedstuff to supplement fish meal in juvenile rohu (*Labeo rohita*) diet to reduce feed cost. Green leafy sprouted wheatgrass (*Triticum aestivum*), inexpensive quality nutrient source, was processed into powder to formulate sinking pellet feed. Four isonitrogenous test diets were applied in four treatments (T) with three replications (R) each. The basal inclusion rate of fish meal was 30% in the control (T₀), of which 10, 20 and 30% was replaced with wheatgrass powder in T₁₀, T₂₀ and T₃₀ respectively to feed the experimental fish. Rohu fingerlings (7.63±0.41 cm; 4.66±0.15 g) were stocked in twelve glass aquaria (60×40×45 cm³) at 10 fish in 75 L water per aquarium, fed experimental diets at 5% of body weight twice daily. After 60 days of feeding trial, significantly better growth was observed in T₂₀ with the highest production (3.23±0.44 tons/ha/60days), SGR (1.01±0.08 %/day) and the lowest FCR (2.68±1.40). Importantly, fish survival rate was improved with the progressive addition of wheatgrass in T₁₀ (90%), T₂₀ (93.33%) and T₃₀ (100%) compared to the lowest survival in T₀ (86.67%). Correspondingly fish in T₃₀ were most resilient to low pH stress test (LT₅₀ = 17 minutes) followed by T₂₀ and T₁₀ than T₀ (LT₅₀ = 9 minutes). Supplementation also resulted in better fish carcass quality with lowest carcass lipid (3.96±0.15%) and highest protein (15.72±0.53%) in T₂₀ feed cost was reduced by 2.73, 5.13 and 8.06% in T₁₀, T₂₀ and T₃₀ respectively than T₀. Therefore, wheatgrass has prospect in juvenile rohu diet.

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www.agroaid-bd.org/ralf, E-mail: editor.ralf@gmail.com

INTRODUCTION

Fish has always been a good source of quality protein for public health. Global fish consumption has been dramatically increased with the increase of community awareness. It has been reported that more than 50% of globally consumed fish come from aquaculture (Gasco et al., 2018). Therefore, world aquaculture industry has undergone exponential growth over the last few decades to feed the ever-increasing population. Sustainability of this intensification is largely dependent on the continuous supply of quality aqua-feed at reasonable price. Traditionally, fish meal is being used as the prime protein source in aqua-feed because of its balanced nutrients, palatability and growth potential (Al-Thobaiti et al., 2018). However, this world wide reliance on fish meal render an enormous loss to the wild fisheries resources and has challenged its availability (Tacon and Metian, 2008; FAO, 2012). Therefore, the resulting price hike, associated adulteration and greater propensity of fish meal to pollute the environment have forced the researchers thinking about balanced and cost effective alternate feed source to sustain the industry (Martinez-Llorens et al., 2009). However, different efforts have been made with a number of non-conventional animal and plant sources at varied success (Booth and Sheppard, 1984; Goda et al., 2007; Audu et al., 2010, Rana et al., 2015). But in most cases, low cost and balanced amino acid contents have put the plant based ingredients one step ahead of animal sources (Mahboob, 2014; Azeredo et al., 2017).

Considering this phenomenon, wheatgrass (freshly sprouted first leaves of the common wheat plant, *Triticum aestivum*) has been evaluated in this experiment as an alternate (plant origin) to fish meal in the diet of rohu (*Labeo rohita*) fingerlings with a view to minimizing feed cost at optimal growth and wellbeing of fish. Besides the basic nutrients (viz., lipid, protein and carbohydrates), young sprouted wheatgrass has a myriad nutrient profile containing good proportion of fiber, antioxidants, essential minerals and vitamins (Meyerowitz, 1992; Murphy, 2002; Shirude, 2011; Devi et al., 2015). Because of its health benefits, wheatgrass is often termed as “power house of nutrients” (Mujoriya and Bodla, 2011). Correspondingly, Devi et al. (2015); reported that fifteen pounds of green wheatgrass is nutritionally equal to 350 pounds of ordinary garden vegetables. Neglecting this inexpensively available nutritious feedstuff, the use of wheatgrass as fish feed ingredients is still under recognized. Although some feeding experiments has brought this alternate source as a strong candidate to be focused in aqua-feed industry (Nath et al., 2014; Islam et al., 2017). The authors in their previous study used wheatgrass in the diet of grass carp (*Ctenopharyngodon idella*) and in this current venture rohu was considered to brief on overall response of Indian major carp fed wheatgrass incorporated diets (Rana et al., 2020b).

MATERIALS AND METHODS

Experimental overview

A sixty days feeding trial (from 7th September to 6th November, 2018) with rohu (*L. rohita*) fed wheatgrass supplemented test diets was conducted in the “BAU Aquaponics Oasis” laboratory of the Department of Aquaculture, Bangladesh Agricultural University. There were four treatments each with three replications and applied in twelve glass aquaria of 100 liters (size: 60×40×45 cm³), each containing 75 L of underground deep tube well water. Two air pumps (RESUN, Model ACO-003 and 35 watts) with twelve outlets (with one air stone) were used for continuous aeration in the aquaria. In addition, the aquaria were numbered randomly as T₀R₁, T₁R₂, T₀R₃, T₁₀R₁, T₁₀R₂, T₁₀R₃, T₂₀R₁, T₂₀R₂, T₂₀R₃, T₃₀R₁, T₃₀R₂ and T₃₀R₃ according to the respective test diets. Complete randomized block design was followed during the arrangement of aquarium for the congeniality. The aquariums were covered with fine meshed net frame to prevent the fish escaping or predatory animals or birds attack.

Sprouting and blanching of wheatgrass to obtain powder

At first, nine perforated trays were washed properly with tap water and dried under sunlight to use them as wheatgrass sprouting bed. Subsequently, three clean plastic buckets were taken with 1 kg wheat seed in each that was already collected from local market. Then wheat was washed and soaked into water for overnight. In the following morning the soaked wheat was sieved, wrapped with cotton cloth and kept in a perforated bucket covered with cloths for 24 hours. After that the germinated wheat seed was spread over each sprouting bed

(tray). Then water was sprayed over the trays and covered it for 2 days. After 2 days, the sprouted young wheatgrass of yellowish color was uncovered and brought to passive sunlight. Until the harvest, the water was sprayed over the sprouting bed twice daily (morning and evening). When the seedlings (dark green colors) become 6-7-inch-long within 8 days it is termed as sprouted and then the stems were cut down as well as weighed for further use. These sprouted wheatgrass were then subjected to blanching, a process of scalding vegetables in boiling or steaming water (75 to 105 °C) for a short period (1 to 10minutes). The objective of the process is to inactivate enzymes, remove air and gases, set the color, improve the texture, retard the changing of flavor and leaching of water soluble sugars. Simultaneously, blanched vegetables need to cool down promptly to minimize the degradation of heat liable nutrients. Here, the green wheatgrass stems were boiled for 7 minutes. Then the boiled stems were cooled down in a big bowl by adding ice and one pinch of sea salt (NaCl). After blanching, the wheatgrass was kept in a well-ventilated room under fan to facilitate air cooling. Then the wheatgrass was dried into dryer and resulting crunchy stems were cut into small pieces with scissors and blended into powder (Figure 1) to use as fish feed ingredients later.



Figure 1. Wheat grass powder

Formulation of test diets from the selected ingredients

Locally available conventional feed stuffs, besides prepared wheatgrass powder, were selected based on their nutritional profiles (Table 1) to formulate four isonitrogenous (crude protein content around 30 %) test diets following Pearson square method. Experimental diets were formulated by giving emphasize on progressive replacement of fishmeal with wheatgrass powder. Correspondingly, four different feeds were formulated by substituting 0, 10, 20, and 30% fish meal with wheatgrass powder. The control feed (T_0) contain 30% fishmeal as basal inclusion but no wheatgrass powder. By contrast, in T_{10} ten percent (10%) of basal fishmeal was replaced with the wheatgrass powder; hence it contained 27% fishmeal and 3% wheatgrass powder. Twenty percent (20%) fishmeal was replaced with wheatgrass powder in T_{20} containing 24% fishmeal and 6% wheatgrass powder. In T_{30} where the replacement was 30% and resulting diet contained 21% fishmeal and 9% of wheatgrass powder (Table 2).

In order to prepare the experimental feeds all the dietary ingredients (calculated amount) were ground finely and sieved through a particle size of 0.5 mm to obtain a homogenous mixture. Before grinding, measured mustard oil cake was soaked overnight and soybean meal was pre-boiled to minimize their glucocyanate effects. However, after sieving, all the ingredients along with the vitamins-minerals premix were mixed thoroughly and water was added to make the mixture moisten. The resultant dough was then processed through an extruded pellet machine (0.8 mm diameter) to prepare sinking dry pellet feed for rohu fingerlings. The prepared feeds were initially sun dried and then stored in air tight polythene bags at 4°C in refrigerator before feeding the fish. The analyzed proximate composition (AOAC, 1990) of different test diets has been shown in Table 3.

Table 1. Major energy sources and market price of the selected feed ingredients for juvenile rohu feed formulation

Ingredients	% Crude protein	% Crude lipid	% Carbohydrates	Price (BDT/kg)
Fish meal	56	8.5	2.7	80
Wheatgrass seed	16.5	2	60	23
Mustard oil cake	30	11	35	35
Rice bran	12	12	55	35
Soya bean meal	40	15	30	42
Wheat bran	12	7.5	60	20
Wheat flower	12	2.5	70	25
Soya oil	0	100	0	80
Minerals and vitamin premix	0	0	0	100

Table 2. Incorporation rates (g) of different feed stuffs used in formulating 100 g of the test diets for rohu fingerlings

Feed ingredients	T ₀	T ₁₀	T ₂₀	T ₃₀
Fish meal	30	27	24	21
Wheatgrass	0	3	6	9
Mustard oil cake	20	18	18	17
Rice bran	20	17	17	12
Soya bean meal	10	15	18	23
Wheat bran	10	10	10	10
Wheat flower	5	5	2	3
Soya oil	3	3	3	3
Minerals and vitamin premix	2	2	2	2
Grand Total	100	100	100	100

Table 3. Nutritional composition (%) of different test diets (wet weight basis)

Treatments	Moisture	Crude lipid	Crude protein	Ash	Crude fiber	Nitrogen Free Extract (NFE)
T ₀	11.88	7.12	30.97	12.05	4.63	33.02
T ₁₀	11.31	6.78	30.57	12.13	5.17	33.16
T ₂₀	11.42	6.87	30.95	11.67	5.34	33.74
T ₃₀	11.73	6.93	30.41	11.75	6.11	32.58

Fish stocking in the aquaria and feeding trial

Fingerlings of rohu (initial size: 7.63±0.41 cm; 4.66±0.15 g) were collected from a local fish hatchery and transported to the experimental station by using oxygenated plastic bag to avoid stress and injury. The fishes were acclimatized to the laboratory condition into glass aquaria at room temperature ranging (25-30°C) for a period of 8-10 days at the beginning of the experiment. During the acclimation period, fish were fed control diet twice daily (morning and evening) at approximately 3% of live body weight per day.

Feeding trial began with stocking of fish in the aquaria (75 L of water in each aquarium) at a rate of 10 fish per aquarium. Fish were fed with experimental diets twice daily at the rate of 5% of their body weight throughout the experimental period. The uneaten feed and feces were removed by discharging 25% of aquarium water daily and subsequently balanced by adding same amount of new water. However, the aquaria were completely drained fortnightly over the study period to assure convenient water for the fish to live.

Table 4. Growth parameters of juvenile rohu fed test diets

Parameters	T ₀	T ₁₀	T ₂₀	T ₃₀	F value	p-value	Level of Sig.
Mean initial length(cm)	7.66(±0.79) ^a	7.59(±0.15) ^a	7.72(±0.57) ^a	7.52(±0.12) ^a	1.99	0.19	NS
Mean final length(cm)	8.57(±0.21) ^a	8.62(±0.28) ^a	8.64(±0.06) ^a	8.39(±0.70) ^b	4.06	0.05	*
Mean length gain	0.91(±0.33) ^a	1.01(±0.25) ^a	0.92(±0.18) ^a	0.87(±0.02) ^a	1.22	0.37	NS
Mean initial weight(g)	4.65(±0.28) ^a	4.56(±0.12) ^a	4.85(±0.11) ^a	4.59(±0.09) ^a	1.44	0.30	NS
Mean final weight	8.44(±0.32) ^a	8.13(±0.63) ^a	8.21(±0.38) ^a	7.27(±0.14) ^b	4.67	0.04	*
Mean weight gain	3.79(±0.46) ^a	3.28(±0.23) ^a	3.65(±0.66) ^a	2.68(±0.16) ^b	4.73	0.038	*
Percent weight gain	81.51(±12.92) ^a	67.63(±1.87) ^b	80.04(±8.95) ^a	58.35(±4.88) ^b	4.86	0.035	*
FCR	2.81(±0.29) ^b	3.20(±2.02) ^a	2.68(±1.40) ^b	3.43(±1.51) ^a	3.76	0.048	*
SGR (%/day)	0.98(±0.22) ^a	0.99(±0.31) ^a	1.01(±0.08) ^a	0.94(±0.19) ^a	0.06	0.98	NS
Survival rate (%)	86.67 ^b	90.00 ^{ab}	93.33 ^{ab}	100 ^a	3.83	0.045	*
Production (tons/ha/60days)	3.05(±0.28) ^{ab}	2.82(±0.20) ^b	3.23(±0.44) ^a	2.73(±0.19) ^b	4.36	0.04	*

Note: Values are mean ±Standard deviation from triplicate groups. Values in a row having similar letters (s) or without letters do not differ significantly whereas values bearing the dissimilar letter (s) differ significantly as per DMRT (Duncan's New Multiple Range Test). *significant at P≤ 0.05; ** significant at P≤ 0.01; NS non-significant at P> 0.05; Sig. : significance

Sampling of fish and water

The experimental fish and aquarium water were sampled biweekly. During sampling, three fish from each aquarium (9 fish per treatment) were randomly sampled to observe their average length and weight ascertaining their response to the test diets by calculating the growth parameters viz., length gain (cm), weight gain (g), percent weight gain, specific growth rate (SGR, %/day), food conversion ratio (FCR), survival rate (%) and fish production (kg/ha). Fish carcass composition was also determined to assess dietary wheatgrass effect following the standard procedure of AOAC (1990). Along with the test fish, aquarium water was also investigated for assuring its compatibility by measuring water quality parameters such as dissolved oxygen (mg/L), water temperature (°C), pH, ammonia and nitrite contents with portable DO meter, thermometer, pH meter and ammonia testing kits, respectively.

Extreme pH stress test

After the final harvest, the experimental fish from different treatments were challenged with acute pH stressor (pH 3) to conclude whether dietary wheatgrass could have effect on their resilience against adverse condition. This trial was accomplished by stocking 6 fish (randomly selected) from each treatment in a 20 L bucket containing water of pH 3. Water from Deep tube-well was robustly aerated for 24 h and gradually mixed with nitric acid (HNO₃) to avail this low pH water (pH 3). The containers for stress test had continuous aeration and kept under ambient temperature. Time required for the fish of individual treatment to reach 50% mortality was calculated as median lethal time (LT₅₀).

Statistical analysis

Collected data were loaded on computer for statistical analysis. One-way ANOVA was performed with the collected data (Snedecor and Cochran, 1994). Comparison between treatments' mean was done by Duncan's test to analyze the significance of variation in between (Duncan, 1955). All statistical analyses were carried out by MS EXCEL 2010 version and SPSS 16.0 software. The outcomes have been presented in tabular and graphical forms.

RESULTS AND DISCUSSION

Sprouted wheatgrass production rate

The collected wheat seeds were sprouted in nine perforated trays (sprouting bed) to obtain young greenish sprouted wheatgrass. Each tray (60x30x8 cm³) was initially sprayed with 250 g of raw wheat seeds. After processing, total weight of wheatgrass powder was 620 g from the 9 trays, which was around 23% of total production (2695 g) of sprouted wheatgrass (live weight). Rana et al. (2020b), in a similar research got around 500 g of sprouted wheatgrass (live weight) from the same bedding area. Seasonal variation could be attributed to this higher production rate of sprouted wheatgrass in the current study.

Palatability of test diets

In natural habitat, fry of rohu (*L. rohita*) is planktivorous surface feeder which is transformed into water column and bottom feeding nature during fingerling stage fed predominantly on filamentous algae, decomposed vegetation, mud and sand (Chondar, 1999). However, rohu fingerlings have also been reported to accept formulated diet in culture condition (Rahman et al., 2006). Therefore, considering their natural food components, it was presumed that wheatgrass (plant origin) powder supplemented formulated diets would be acceptable to the test fish. Throughout the feeding trial, the experimental diets were well accepted by the rohu fingerlings as there was almost no left over after twenty minutes of feed delivery. Thus in conformity of previous trials with wheatgrass powder, dietary inclusion of wheatgrass did not hamper the palatability of the test diets rather it has increase length and weight gain, production and survival means it acted as wellbeing of rohu juveniles in the experiment (Nath et al., 2014; Islam et al., 2017; Rana et al., 2020b).

Growth response of rohu to test diets

Respond of juvenile rohu (growth parameters) to the test diets has been depicted in Table 4. Initially, there was no significant difference ($P>0.05$) in terms of initial length and weight of fish among the treatments. However, after 60 days of rearing, the highest mean length gains (cm) was observed in T₁₀ (1.01±0.25 cm), followed by T₂₀ (0.92±0.18 cm), T₀ (0.91±0.33 cm) and T₃₀ (0.87±0.02 cm), while the differences were statistically insignificant ($P>0.05$). In comparison, significantly lowest ($P<0.05$) mean weight gain (g) was associated with the fish in T₃₀ (2.68±0.16 g), whereas the other treatments T₀ (3.79±0.46 g), T₁₀ (3.28±0.23 g), and T₂₀ (3.65±0.66 g) gave statistically similar results. In a similar pattern, values for specific growth rate (SGR, %/day) did not varied a lot among the treatments ($P>0.05$). The highest SGR was recorder in T₂₀ (1.01±0.08 %/day) followed by T₁₀ (0.99±0.31 %/day), T₀ (0.98±0.22 %/day) and T₃₀ (0.94±0.19%/day). Comparatively short rearing period and some inevitable error occurred during sampling might have contributed to this statistical non-significance. Similar response has been found in the authors previous wheatgrass based feeding trial with grass carp (*Ctenopharyngodon idella*) where the SGR ranged from 0.95 to 1.13 %/day (Rana et al. 2020b). Although the observed SGR values are quite higher than that of Islam et al. (2017), who documented SGR values between 0.46 and 0.77 %/day for stinging catfish (*Heteropneustes fossilis*) and findings of Nath et al. (2014), who reported SGR value 0.29 %/day for Asian catfish (*Clarias batrachus*) fed sprouted wheatgrass supplemented diets. Concerned species and seasonal variation might be the reason behind these differences.

Regarding the fish production (tons/ha), significantly highest ($P<0.05$) production has been ascertained in T₂₀ (3.23±0.44 tons/ha/60 days) and the lowest in T₃₀ (2.73±0.19 tons/ha/60days) which was statistically similar to T₁₀ 2.82(±0.20 tons/ha/60days) and T₀ (3.05±0.28 tons/ha/60days). Therefore, dietary supplementation of wheatgrass powder positively impacted the production performance of juvenile rohu to a certain level (20% substitution with fish meal) after which it started to decline in conformity with the previous wheatgrass based fish feeding experiments (Nath et al., 2014; Islam et al., 2017; Rana et al. 2020b). Furthermore, the findings enriched the literature with the established phenomenon that improved growth could be experienced from substitution of fish meal with plant based feedstuffs up to a certain level in fish diets but higher dietary substitution may result in a reduction of growth and immune responses (Lin and Luo 2011, Mokrani et al., 2020).

Survival rate

Effect of wheatgrass powder inclusion in the test diets has been more prominent while counting the survival rate of the test fish (Table 4). Progressive addition of wheatgrass in the test diets has resulted in significantly better survival compared to the fish in control (T_0). As a result, the highest survival was enjoyed by the fish in T_{30} (100%) followed by T_{20} (93.33%) and T_{10} (90%) which were statistically different ($P < 0.05$) to the lowest survival in T_0 (86.67%). The antioxidant property and the supply of basic nutrients with quality minerals (K, Ca, Fe, Mg, Na and S) and vitamins (A, B, C and E) from the green sprouted wheatgrass is believed to play the key role in escalating the survival of rohu fingerlings (Mujoriya and Bodla, 2011; Shirude, 2011; Anwar et al., 2015; Devi et al., 2015; Rana et al., 2020b).

Evaluation of test diets

The test diets have been evaluated by calculating associated food conversion ratio (FCR) and feed formulation cost. Considering the FCR value, the significantly lowest ($P < 0.05$) FCR was found with the diet applied in T_{20} (2.68 ± 1.40) that was statistically similar to that of T_0 (2.81 ± 0.29). However, there was no significant difference ($P > 0.05$) among the higher FCR values of T_{10} (3.20 ± 2.02) and T_{30} (3.43 ± 1.51). These variations in FCR could be attributed to the promising impact of dietary wheatgrass as well as the survival and production differences among the treatments. Moreover, the observed FCR values were quite similar to the findings of Rana et al, 2020b; where the lowest FCR (2.13) was gained in 20% fish meal replacement with wheatgrass powder for grass carp (*C. idella*).

Notably, feed formulation cost was diminished with the amplified inclusion of wheatgrass in the test diets (Figure 2). Hence, feed formulation cost was the highest in T_0 (49.85 BDT/Kg). The progressive addition of wheatgrass powder has subsequently lowered the feed cost in T_{10} (48.49 BDT/Kg), T_{20} (47.29 BDT/Kg) and T_{30} (45.83 BDT/Kg) by 2.73, 5.13 and 8.06% respectively. The availability of the wheatgrass seed (non-conventional plant based feed stuff) at relatively lower price than fish meal (animal source) is the reason behind this cost reduction. In the previous studies, it was also possible to cut off the feed manufacturing costs by utilizing non-conventional plant based feed stuffs (Rana et al., 2020a; 2020b). The issue of feed cost, comprising more than 60% of aquaculture expenses (Gadzama and Ndudim, 2019), has therefore crystallized young sprouted wheatgrass as a potential substitute for fish meal in carp's diet.

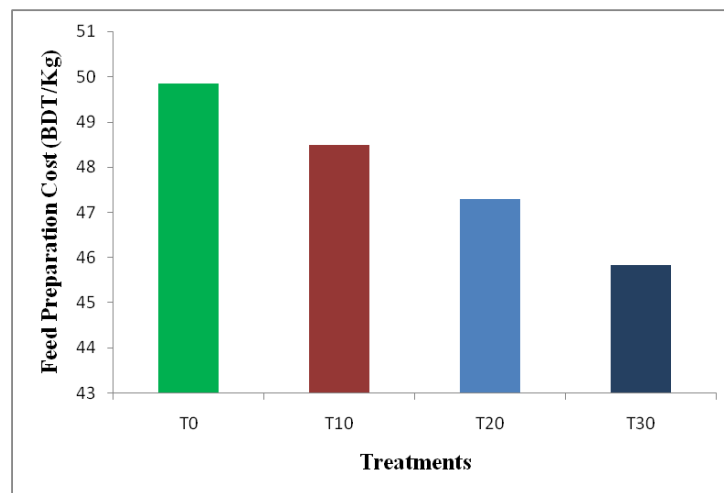


Figure 2. Feed formulation cost in different treatments

Fish nutrient profile

Harvested fish fed test diets had no obnoxious odor during tissue collection for proximate composition analysis. The major carcass compositions determined were moisture, crude lipid and crude protein. Ash (minerals), fiber and Carbohydrates collectively comprised around 4% of the fish whole carcass (Table 5). However, fish moisture content significantly varied among the treatments with the highest in T_{10} ($76.17 \pm 0.21\%$) and lowest in T_{30} ($74.74 \pm 0.08\%$). Importantly, fish in T_0 gained the significantly highest carcass

lipid ($5.61 \pm 0.54\%$) and lowest protein ($14.57 \pm 0.42\%$), which were just conversed in T₂₀ having the lowest carcass lipid ($3.96 \pm 0.15\%$) and highest protein ($15.72 \pm 0.53\%$) contents. Therefore, supplementation with wheatgrass powder has considerably increased carcass protein and lowered fat content of the experimental fish. However, the nutrient composition of the experimental rohu were within the reported range for rohu except lipid content (slightly higher) which might be due to the variation in feed ingredients, formulation process or unintentional experimental error (Paul et al., 2016). Moreover, the carcass ash (minerals) and fiber contents were statistically similar among the treatments, though increased addition of wheatgrass in the treatments has raised their proportion considerably (Table 5). Consequently, dietary substitution of fish meal with sprouted wheatgrass resulted in better carcass quality of rohu and signifying the contribution of plant based diets to boost up nutrient profile in Indian major carps at improved consumer digestibility (Nandeesh et al., 1995, Rana et al., 2020a; 2020b).

Table 5. Carcass composition of experimental rohu (% wet weight basis) after feeding trial

Parameters	T ₀	T ₁₀	T ₂₀	T ₃₀	F value	p-value	Level of Significance
Moisture	75.62(±0.17) ^b	76.17(±0.21) ^a	75.76(±0.09) ^b	74.74(±0.08) ^c	34.83	0.001	**
Crude lipid	5.61(±0.54) ^b	4.44(±0.21) ^a	3.96(±0.15) ^a	4.16(±0.06) ^a	18.06	0.001	**
Crude protein	14.57(±0.42) ^b	15.17(±0.09) ^{ab}	15.72(±0.53) ^a	14.74(±0.06) ^{ab}	5.70	0.02	*
Ash	3.07(±0.06) ^a	2.99(±0.22) ^a	3.32(±0.09) ^b	3.08(±0.04) ^a	3.80	0.06	NS
Fiber	0.66(±0.22) ^a	0.68(±0.13) ^a	0.69(±0.25) ^a	0.71(±0.16) ^a	0.40	0.99	NS
Carbohydrate	0.41(±0.19) ^a	0.53(±0.13) ^a	0.57(±0.43) ^a	0.49(±0.14) ^a	1.37	0.32	NS

Note: Values are mean ±Standard deviation from triplicate groups. Values in a row having similar letters (s) or without letters do not differ significantly whereas values bearing the dissimilar letter (s) differ significantly as per DMRT (Duncan's New Multiple Range Test). *significant at P ≤ 0.05; **significant at P ≤ 0.01; NS non-significant at P > 0.05

Water quality parameters

The water quality parameters such as temperature, pH, dissolved oxygen (DO), ammonia, nitrite content etc of the experimental tank could produce biased result if not within the recommended range. Over the feeding trial, water quality indicators did not vary much among the treatments viz., temperature (26.5 to 27.77 °C), pH (7.6 to 8.62), DO (5.96 to 6.80 ppm), ammonia (0.07 to 0.28 mg/L) and nitrite (0.08 to 0.68 mg/L). The water quality parameters depicted in Table 6, therefore imply that the values were within the suitability range for rohu culture (Swingle, 1967; DAS et al., 2015). Thus, it could be assumed that the rearing environment was secure for fish wellbeing and had no influential effect on the response of rohu to the experimental diets.

Table 6. Water quality parameters over the feeding trial

Parameters	T ₀	T ₁₀	T ₂₀	T ₃₀
Temperature (°C)	27.57±0.40	27.6±0.17	27.00±0.5	27.4±0.40
pH	8.2±0.32	7.8±0.2	8.3±0.32	7.93±0.14
DO (ppm)	6.76±0.04	6.34±0.05	6.13±0.17	6.64±0.18
Ammonia(mg/L)	0.22±0.06	0.18±0.11	0.19±0.05	0.21±0.08
Nitrite(mg/L)	0.38±0.30	0.31±0.18	0.25±0.02	0.21±0.13

Response of juvenile rohu to low pH stressor

pH of rearing water is an important factor in determining the resilience of fish in water. The recommended pH range for fish culture is 6.8-9.0, and below 4 is lethal to fish irrespective to species (Swingle, 1967). Rohu, showed negative growth in acidic (pH 4.5-6.5) environment because of poor blood sodium concentration, somatic protein and oxygen depletion (Mukhopadhyay et al., 2003). Therefore, in-order-to justify the robustness of the test fish, they were confronting to low pH stress test (pH 3.0) following the feeding trial. The

thumb role was 'the hardy the fish is the more time it will take before die'. Results revealed that 50% of the fish in T₀ died after 9 minutes (LT₅₀ = 9 minutes) of exposure to low pH stress (Figure 3). This tolerance time was subsequently increased in T₁₀ (LT₅₀ = 11 minutes), T₂₀ (LT₅₀ = 15 minutes) and T₃₀ (LT₅₀ = 17 minutes). Therefore, the findings showed conformity with the previous study where dietary addition of wheatgrass (high mineral content) had improved resilience of the test fish (Rana et al., 2020a; 2020b).

To sum up, through the experiment, non-conventional feedstuffs (animal or plant origin) have been proven again to be promising in fish meal substitution to a definite level in fish diet at improved survival and growth (Ayoola, 2010; Rana et al., 2015; Sing et al., 2016; Islam et al., 2017; Daniel, 2018; Osho et al., 2019; Rana et al., 2020a; 2020b).

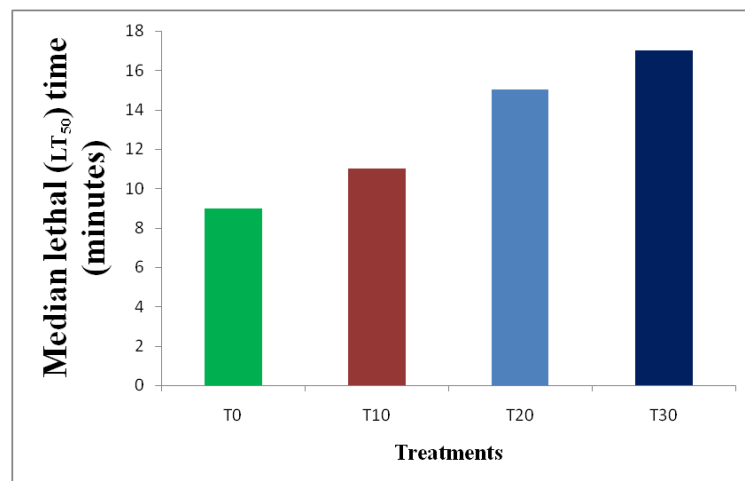


Figure 3. Response of fish to low pH stress test

CONCLUSION

Cost effective substitution of fish meal has become the core of research interest now-a-days. Addressing the issue, the present experiment has validated wheatgrass powder as a promising and cheap alternate to fish meal in the diet of juvenile rohu. However, further research is needed to ensure concise bio-molecular response of fish to wheat grass for wider acceptability in fish feed industry.

CONFLICT OF INTEREST

The authors have declared that no conflicts of interest exist.

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