

STUDY ON EFFECTS OF DIETARY WHEAT GRASS ON BEHAVIOURAL RESPONSES OF FRESH WATER FISH *Channa striata* (BLOCH, 1793)

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ABSTRACT

The present investigation aimed to evaluate the dietary wheatgrass induced behavioural responses of freshwater fish *Channa striata* (Bloch, 1793). The experiment was performed during September to December 2021 under laboratory condition at Karanja (Lad), Maharashtra, India. The behavioural responses by the fish fed on control and wheatgrass based formulated fish feed shows the improved frequency and duration of feeding, swimming, aggression and surface visit while decreased in rest and arousal. The behavioural responses were observed to be improved with increased wheatgrass content in diet. In considering the overall performance, wheatgrass supplementation in diet is recommended for successful aquaculture of this important species. As the aquaculture and agriculture are joint ventures; so the successful aquaculture production will surely help the farmers to enrich their economy.

(Key words : Agriculture, aquaculture, behaviour, *Channa striata*, fresh water fish, supplementation, wheatgrass)

INTRODUCTION

Fish feeding is one of the most important factors in commercial fish farming because feeding regime may have consequences on both, growth performance and feed wastage (Azzaydi *et al.*, 2000). Hence nutrient composition of feed, such as protein, carbohydrate, lipid, vitamins, and minerals are the most important factors affecting the health and growth of fish; hence, properly balanced supplemental feeds with a reliable feeding rate can be helpful to enhance survival and growth (Ammar, 2008; Alina *et al.*, 2013; Lee *et al.*, 2016; Shabana *et al.*, 2019). In recent years, plant products (leaf, root, stem, bark, etc.) have been used as a natural growth promoters and immunostimulant instead of antibiotics in aquaculture feed formulations. It is due to their eco-friendly and cost-effective properties compared to synthetic drugs.

The wheatgrass (*Triticum aestivum*) is potential herb having the effective utility in production of fish feed pellets (Butle *et al.*, 2019; Hesham *et al.*, 2020; Pradhan *et al.*, 2021). It refers to young grass of the common wheat plant, which belongs to Poaceae family. Wheatgrass is a source of potassium, dietary fiber, vitamin A, vitamin C, vitamin E, vitamin K, thiamin, riboflavin, niacin, vitamin B6, pantothenic acid, iron, zinc, copper, manganese and selenium. Wheatgrass is also a source of protein (Nath *et al.*, 2014; Islam *et al.*, 2017; Abdus *et al.*, 2020; Rana *et al.*, 2020). Plant has been shown to have anti-inflammatory, antioxidant, anticarcinogenic, immunomodulatory, laxative, astringent, diuretic, antibacterial, antihemolytic and anti-aging properties as well improve reproductive health. Its

use in acidity, colitis, kidney malfunctions, athero-sclerosis and swelling has been shown to be beneficial (Sharma *et al.*, 2016; Johri and Khan, 2017, Joshi 2021).

In this concern, present study aims to evaluate the dietary wheatgrass induced behavioural responses of freshwater fish *Channa striata* (Bloch, 1793).

MATERIALS AND METHODS

The protocol of Joshi (2021) was adopted during the evaluate the dietary wheatgrass induced behavioural responses of freshwater fish *Channa striata* (Bloch, 1793).

Experimental containers

The experimental containers were rectangular plastic aquaria of 10 liter capacity (100 X 100 X 100 cm). The container had flat bottom. They were easy to clean and no material collected at corners and cracks. They were rinsed with tap water, cleaned with detergent and rinsed once with 10% of HCl and then rinsed twice with tap water before use in each experiment. Each container was covered with a mosquito net to prevent the fish from escaping. The containers were divided into following 6 groups with 20 fishes each and observed for next 60 days.

Maintenance and acclimatization

The collected fishes were disinfected with 0.1% KMNO₄ solution to avoid fungal infection. These fishes were acclimatized for a week and maintained glass aquarium. Aquariums were aerated by air pump for supply of oxygen to individuals. The drain settled in tanks is collected by filtration net. Water quality was maintained during the

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feeding trial with light: dark cycle of 12:12 h during study. The specimens were fed on control diet during the course of experiment.

Water quality standards

The water analysis is performed according to American Public Health Association (Anonymous, 2000). The aquarium water was aerated continuously during experimental tenure. The water composition and characteristics were maintained within the effective range (Bhatnagar and Devi, 2013).

Formulation fish feed

For the experiment, wheatgrass from conventional organic farm was used. A crop of mature wheatgrass (8 inches) trimmed to 1/2-inch above the soil. It took 6 to 9 days for wheatgrass to be mature. Then the fresh meat was

thin sliced. The harvested grass blades and meat slices were laid on a clean baking sheet separately and placed into food dehydrator. The temperature was set to 150° F for eight hour or until dry; then grinded in food processor. The ingredients were weighed, mixed and pelleted. After pelleting, the feeds were air dried and put in an air-tight container. The composition of experimental diet is given in Table 1 and 2.

Feeding regime

During the acclimation, fish were fed the control diet to satiation twice a day at 09:00 and 15:00 hours. After acclimation, fish were fasted for one day; batch weighted and randomly distributed among density of 10 fish tank⁻¹. During the experiment, fish were fed on experimental diet to satiation third a day at 08:00, 12:00 and 16:00 hours.

Table 1. Ingredient composition of the experimental feeds (on % basis)

Ingredients (%)	W0	W1	W2	W3	W4
Wheat flour	45.0	42.5	40.0	37.5	35.0
Soybean flour	24.0	24.0	24.0	24.0	24.0
Corn flour	10.0	10.0	10.0	10.0	10.0
Meat powder	15.0	15.0	15.0	15.0	15.0
Soybean oil	05.0	05.0	05.0	05.0	05.0
Watrmin® Forte*	01.0	01.0	01.0	01.0	01.0
Wheatgrass Powder	00.0	2.5.0	5.0	7.5	10.0

* Vitamins, Minerals and Amino Acids supplements for Aquatic feed. Manufactured by Virbac Animal Health India Pvt. Ltd.

Table 2. Proximate composition of the experimental feeds (on % basis)

Proximate Composition (%)	W0	W1	W2	W3	W4
Moisture	7.79	6.35	5.83	5.29	4.83
Crude protein	39.81	38.37	39.41	40.95	44.16
Crude lipid	6.45	7.26	7.27	7.31	7.38
Crude fibre	20.46	27.91	30.77	30.75	31.35
Crude Ash	25.94	20.10	16.72	15.71	12.27
Gross energy (kjg ⁻¹)	12.64	13.03	13.44	14.95	16.39

Behavioural responses

Behavioural changes are physiological responses shown by the animal, which are often used as the sensitive measure of stress syndrome in the organism experiencing it, consequently the behavioural changes were observed in control and exposed fish. The behaviour of experimental fishes were recorded in the period between 09:00 till 15:00 h for initial 60 days by using focal sample technique for 15 sec. with intervals during one hour daily. The observations were recorded according to Altmann (1974). It was made visually by using a note book for recording behaviour, a digital stop watch, and video camera. The following patterns were recorded according to Stephan (2008).

a. **Feeding:** Frequency and duration (Sec.) spent in feeding.

b. **Swimming:** Frequency and duration (Sec.) spent in swimming.

c. **Aggression:** Frequency and duration (Sec.) spent in attacking each other.

d. **Rest:** Frequency and duration (Sec.) in which fish completely immobile and rest on the bottom of their tank.

e. **Arousal:** Frequency and duration (Sec.) in which fish has a locomotors activity.

f. **Surface visit:** Freq. and duration (Sec.) in which fish hanging around top of tank

Statistical analysis

Results were recorded as Mean ± Standard Deviation of triplicate (Joshi *et al.*, 2015).

RESULTS AND DISCUSSION

Obtained results shows the behavioural responses by the fish fed on control and wheatgrass based formulated fish feed. It shows that the feeding frequency, swimming frequency, aggression and surface visit frequency increased while rest frequency and arousal frequency decreased with the increase in wheatgrass content in feed (Table 3). The feeding duration, swimming duration, aggression duration and surface visit duration increased, while rest duration and arousal duration decreased with the increase in wheatgrass content in feed (Table 4).

The behavioural patterns of species were observed to be influenced by dietary wheatgrass. As in case of feeding frequency and duration in which there was a significant increase in group in which fish fed high wheatgrass level in diet. It may be attributed to that increase the nutrient rich diet increase the feed intake fish and growth rate which correlated with increase oxidative metabolism and protein synthesis. This result could be supported by Borge *et al.* (2006), who reported that high numbers of factors including feeding affect fish welfare for maintaining homeostasis and normal development and protected against physical damages.

The swimming frequency and duration showed significant increase in experimental group. This result was in harmony with that mentioned by Brannas *et al.* (2003), who illustrated that, food deprivation and deficiency in the diet leads to changes in metabolic activity and changes in territorial behaviour strategies and activity pattern especially swimming. The aggressive frequency and duration showed significant increase in moderate group. It may be due to decrease dietary protein levels which enhance the aggressive behaviour. This result was in agreement with

that of Höglund *et al.* (2005), who illustrated that increase nutrient levels has been shown to suppress aggressive activity.

The rest and arousal behaviour and duration have a significant increase in control group. These results agreed with Khalil *et al.* (2016), who suggested that the availability of nutrient rich food increase the activity while decrease the rest and arousal duration of fish. The fish coming to the surface of aquaria behaviour showed a significant increase in moderate group. This might be due to that the nutrients deficiency in diet acts as a stress factor in which fish become aggressive and try to visit the surface to get more food in order to be more growth rate and development as suggested by Bhalerao (2017).

The behavioural changes are physiological responses shown by the animal, which are often used as the sensitive measure of stress syndrome in the organism experiencing it. The observation clears the suitability of dietary wheatgrass for selected fish. These findings are in well agreement with the observations of Hassan and Soltan (2016), Butle *et al.* (2018) and Joshi (2021), who suggested that the herbal feed additive improves the behavioural responses of fresh water fish.

The aquaculture and agriculture are very common joint ventures. The preformed aquaculture experiment evaluated the dietary wheatgrass induced behavioural responses of freshwater fish *Channa striata* (Bloch, 1793). The results indicated that the increased level of wheatgrass in diet improved the behavioural responses by the fish. In considering the overall responses, wheatgrass supplementation in diet is recommended for successful culture of this important fish species. The successful aquaculture production will surely help the farmers to enrich their economy.

Table 3. Frequency of behavioural responses by fish fed on wheatgrass based fish feed

Behavioural Patterns		W0	W1	W2	W3	W4
Feeding Frequency (n)	Mean	1.24	2.61	3.53	3.75	4.77
	±SD	0.07	0.10	0.17	0.19	0.20
Swimming Frequency (n)	Mean	0.89	2.25	2.83	3.38	4.27
	±SD	0.12	0.18	0.38	0.55	0.18
Aggression Frequency (n)	Mean	0.54	1.00	2.43	3.74	7.62
	±SD	0.21	0.17	0.31	0.40	1.44
Surface visit Frequency (n)	Mean	0.56	0.99	2.02	2.78	3.58
	±SD	0.12	0.12	0.15	0.30	0.26
Rest Frequency (n)	Mean	5.21	4.87	4.43	2.70	1.49
	±SD	1.31	1.07	0.81	0.40	0.30
Arousal Frequency (n)	Mean	1.48	1.29	1.15	1.12	0.74
	±SD	0.07	0.04	0.04	0.02	0.02

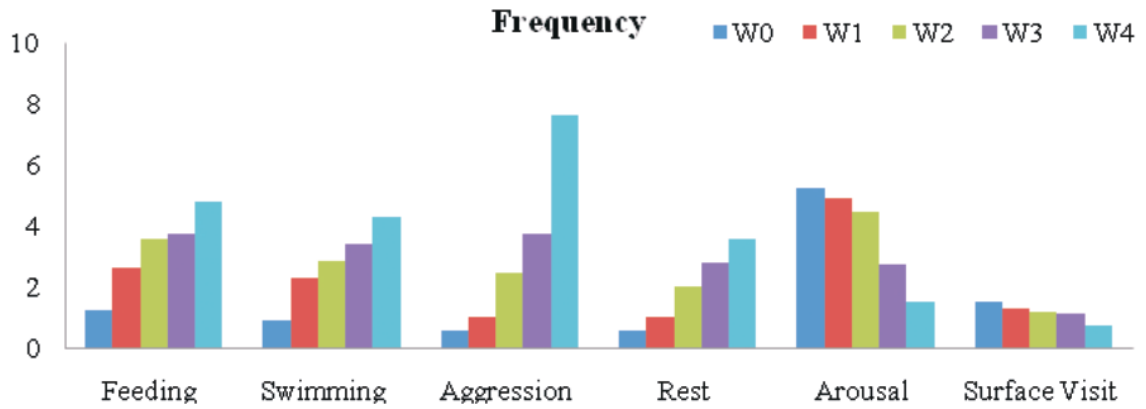


Figure 1. Frequency of behavioural responses by fish fed on wheatgrass based fish feed

Table 4. Duration of behavioural responses by fish fed on wheatgrass based fish feed

Behavioural Patterns		W0	W1	W2	W3	W4
Feeding Duration (Sec.)	Mean	9.52	22.16	31.56	59.51	90.44
	±SD	1.00	2.21	3.32	3.65	4.53
Swimming Duration (Sec.)	Mean	32.46	33.72	34.55	38.27	45.72
	±SD	2.21	2.52	4.00	5.78	4.78
Aggression Duration (Sec.)	Mean	5.03	6.05	8.51	12.15	19.53
	±SD	1.12	2.37	1.96	3.00	3.05
Surface visit Duration (Sec.)	Mean	116.69	121.98	122.69	129.00	132.33
	±SD	4.99	4.61	5.74	2.59	6.15
Rest Duration (Sec.)	Mean	35.62	25.54	19.42	17.10	14.13
	±SD	4.83	4.31	3.64	3.01	2.39
Arousal Duration (Sec.)	Mean	129.57	128.66	125.69	114.62	84.59
	±SD	6.96	4.20	4.78	4.24	4.27

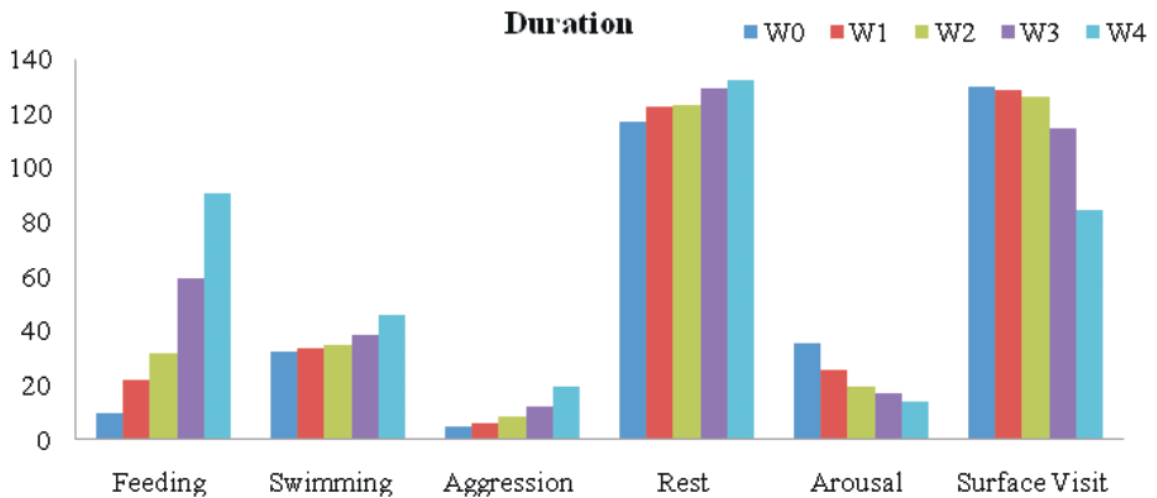


Figure 2. Duration of behavioural responses by fish fed on wheatgrass based fish feed

REFERENCES

- Abdus, S., R. Shakil, A. Rakib and M. Noor, 2020. Growth response of juvenile rohu (*Labeo rohita*) to wheatgrass powder supplemented diet. Res. Agri. Live. Fish. **7**(3): 533-543
- Alina, A., C. Victor, R. G. Julia, I. Sndina and M. Mirela, 2013. The Influence of Rosemary, Sea Buckthorn and Ginger on Oxidative Stress at *Oreochromis niloticus* Reared in a Recirculating Aquaculture System. Bul. Anim. Sci. Biotech. **70**(1): 110-116.
- Altmann, J. 1974. Observational study of behaviour: sampling methods. Behaviour, **49**: 227-267.
- Ammar, A. A. 2008. Effect of extruded and trash fish diets on growth performance and pond productivity of sea bream, *Sparus aurata*, the sea bass, *Dicentrarchus labrax* and the flat head grey mullet, *Mugil cephalus* reared in polyculture system in earthen ponds. Egypt. J. Aqua. Biol. Fish. **12**:43-58.
- Anonymous, 1995. Method of Analysis, Association of Analytical Chemistry, 16th Edn., AOAC International, Washington, USA, pp.1141.
- Anonymous, 2000. Standard methods for examination of water and waste water. 21st edition. American Public Health Association, Washington DC, USA. pp. 1268.
- Azzaydi, M., F. J. Martinez, S. Zamora, F. J. Sanchez-Vazquez and J. A. Madrid, 2000. The influence of nocturnal vs. diurnal feeding condition under winter condition on growth and feed conversion of European sea bass, *Dicentrarchus labrax* (L.) Aquacult. **18**(2):329-38.
- Bhalerao, S. N. 2017. Mercury toxicity; behavioral changes, and recovery in a fresh water teleost *Heteropneustus fossilis* (Bloch). Biosci. Discover. **8**(1):61-63.
- Bhatnagar, A. and P. Devi, 2013. Water quality guidelines for the management of pond fish culture. Int. J. Env. Sci. **3**(6): 1980-1996.
- Borge, D., J. Erik Juell and B. Brastad, 2006. Welfare in farmed fish. Published by Norwegian Institute of fisheries and aquaculture research, pp. 10.
- Brannas, E., S. Jonsson and H. Lundquist, 2003. Influence of food abundance on individual behavior strategy and growth rate in juvenile brown trout (*Salmo trutta*). Can. J. Zool. **8**(1): 684-691.
- Butle, S. S., R. A. Gulhane and P. S. Joshi, 2019. Evaluation of Phytochemical and Antioxidant Potential of Aqueous Wheatgrass Extract for fish feed formulation. Vidya. Int. Intd. Res. **1**(2): 65-70.
- Butle, S. S., V. G. Thakare and P. S. Joshi, 2018. Study of dietary garlic induced effects on behaviour responses, growth performance and feed utilization in *Clarias batrachus* (Linnaeus, 1758). Biosci. Discover., **9**(3):403-408
- Hassan, M. S. and M. A. Soltan, 2016. Evaluation of Essential Oil of Fennel and Garlic Separately or Combined with *Bacillus licheniformis* on the Growth, Feeding Behaviour, Hemato-biochemical Indices of *Oreochromis niloticus* fry. J. Aquac. Res. Develop. **7**(4): 1-8.
- Hesham, A. E., S. M. Sherif and A. M. Hussein, 2020. Nutritional value and impact of wheatgrass juice (Green Blood Therapy) on increasing fertility in male albino rats. Bul. Nat. Res. Cen. **44**(30): 1-11.
- Höglund, E., F. A. Weltzien, J. Schjolden, S. Winberg and H. Ursin, 2005. Avoidance behavior and brain monoamines in fish. Brain Res. **10** (32): 104-110.
- Islam, T., K. M. Rana and M. A. Salam, 2017. Potential of wheatgrass based feed for stinging catfish fry nursing in laboratory condition. Int. J. Fish. Aqua. Stud. **5**(6): 179-184.
- Johri, S. and N. Khan, 2017. *In vitro* Antioxidant and Antihemolytic Potential of *Triticum aestivum* Grass. Int. J. Compl. And Alt. Med. **9**(5): 1-5.
- Joshi, P. A., H. H. Pilley, V. P. Wadegaonkar and P. A. Wadegaonkar, 2015. Comparative Assessment of Antioxidant Potential of *Cassia Auriculata* (Linn.) Flower, Leaf and Seed Methanolic Extracts. Int. J. Pharm. Pharmaceut. Sci. **7**(9): 381-385.
- Joshi, P. S. 2021. Evaluation of dietary wheatgrass induced developmental performance of freshwater fish *Channa punctatus* (bloch, 1793) reared in closed recirculating aquaculture system. D.Sc. Thesis Submitted to Srinivas University, Mangaluru.
- Khalil, A., W. EL-Husseiny, F. Azhar and W. Ghonimi, 2016. Effect of Feeding with Different Dietary Protein Levels and Starvation on the Health, Nonspecific Immune Parameters, Behavior and Histoarchitectures of Fantail Goldfish *Carassius auratus* (L.). J. Vet. Sci. Technol. **7**(1): 01-12.
- Lee, S., M. Mohammad, B. Jinho, S. Minji, S. Yu-jin, D. Bakshish and C. B. Sungchul, 2016. Effects of extruded pellet and moist pellet on growth performance, body composition, and hematology of juvenile olive flounder, *Paralichthys olivaceus*. Fish. Aqua. Sci. **19**(32):2-6.
- Nath, T., S. Hashem and M. Salam, 2014. Asian catfish fry (*Clarias batrachus*) rearing with wheatgrass powder mixed formulated feed in plastic half drum. Int. J. Fish. Aqua. Stud. **1**(5): 162-168.
- Pradhan, C., S. S. Giri, S. N. Mohanty and K. C. Nayak, 2021. Influence of fishmeal-replaced diet on nutrient digestibility, digestive enzyme activity, and whole-body fatty acid profile of Indian major carp, *Cirrhinus mrigala*. J. Basic Appl. Zool. **82**(1):1-10.
- Rana, K. M., M. A. Salam, M. R. Ahmed and A. M. Noor, 2020. Dietary supplementation of wheatgrass powder to assess somatic response of juvenile grass carp (*Ctenopharyngodon idella*). Asian J. Med. Biol. Res. **6**(3): 482-490.
- Shabana, M. S., M. Karthika and V. Ramasubramanian, 2019. Effect of dietary *Citrus sinensis* peel extract on growth performance, digestive enzyme activity, muscle biochemical composition, and metabolic enzyme status of the freshwater fish, *Catla catla*. J. Bas. Appl. Zool. **80**(51):1-9.
- Sharma, R., A. Nair, S. Harak, T. Patil and S. Shelke, 2016. Wheat Grass Juice: Nature's Powerful Medicine. World J. Pharma. Pharmac. Sci. **5**(7): 384-391.
- Stephan, G. R., 2008. Sleep in fishes. Universite de Moncton, Canada, pp. 13.