STUDY ON EFFECTS OF DIETARY HERBS ON BEHAVIOURAL RESPONSES OF FRESH WATER FISH *LABEO ROHITA* (HAMILTON 1822)

M. R. Nirban and P. S. Patil
Post Graduate and Research Department of Zoology, Rajasthan Aryan Arts, Shri Mithulal Kacholia Commerce and Shri Satyanarayanji Ramkrishnaji Rathi Science College, Washim

ABSTRACT
The present work is "Preparation of herbal feed and its impact on growth performance of fish Labeo rohita (Hamilton 1822)". The behavioural responses by the fish fed on control and experimental feed containing Hibiscus rosa-sinensis followed by Beta vulgaris, Phyllanthus emblica, Tinospora cordifolia and Moringa oleifera. showed 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 in fish fed on herb-based diets. In considering the overall performance, herb supplementation in diet is recommended for successful aquaculture of this important species.
Keywords: *Aquaculture, behaviour, herbs, freshwater fish, Labeo rohita supplementation.*

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, herb parts (leaf, root, stem, bark, flower 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 *Hibiscus rosa-sinensis* followed by *Beta vulgaris, Phyllanthus emblica, Tinospora cordifolia* and *Moringa oleifera* are potential herb known for their phytochemical and pharmacological potential. These variety make them useful in production of fish feed pellets. In this concern, present study aims to evaluate the effects of herb-based feeds on behavioural responses of fish *Labeo rohita* (Hamilton 1822)".

Materials And Methods

The protocol of Joshi (2021) was adopted during the evaluate the herbs induced behavioural responses of freshwater fis*h Channa striata* (Bloch, 1793).

Experimental containers

The experimental containers were rectangular plastic aquaria of 10 Lt. 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% $KMNO_4$ 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 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, *Hibiscus rosa-sinensis* (flower) followed by *Beta vulgaris* (root), *Phyllanthus emblica* (fruit), *Tinospora cordifolia* (stem) and *Moringa oleifera* (leaves). The harvested herbs were cleaned, sliced and were laid on a clean baking sheet separately and placed into food dehydrator. The temperature was set to 150^{O} 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

proximate compositions of feed were estimated by using the Association of Analytical Chemists (Anonymous, 1995) methods with some modifications (Mohammad *et al.*, 2019). The composition of experimental diet is given in Table 1.

Table 3.1.1: Ingredient composition of the experimental feeds (on % basis)

Ingredients (%)	H0	H1	H2	H3	H4	H5
Wheat flour	45	40	40	40	40	40
Soybean flour	25	25	25	25	25	25
Corn flour	10	10	10	10	10	10
Meat powder	15	15	15	15	15	15
Soybean oil	05	05	05	05	05	05
Herb Powder	-	-	-	-	-	-
Phyllanthus emblica (Fruit)	-	05	-	-	-	-
Beta vulgaris (Root)	-	-	05	-	-	-
Moringa oleifera (Leaves)	-	-	-	05	-	-
Tinospora cordifolia (Stem)	-	-	-	-	05	-
Hibiscus rosa-sinensis (Flower)	-	-	-	-	-	05
Proximate Composition (%)						
Moisture	05.25 ± 0.30					
Crude protein	36.26 ± 1.76					
Crude lipid	6.62 ± 0.06					
Crude fibre	20.87 ± 2.62					
Crude Ash	13.75 ± 2.98					
NFE	17.17 ± 1.04					
Gross energy (kJ/g)	17.73 ± 0.66					

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 20 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.

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 that the behavioural responses by the fish fed on control and herb based formulated fish feed (Table 2).

The behavioural responses by the fish fed on control and experimental feed containing *Hibiscus rosa-sinensis* followed by *Beta vulgaris*, *Phyllanthus emblica*, *Tinospora cordifolia* and *Moringa oleifera.* showed 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 in fish fed on herb-based diets.

The behavioural patterns of species were observed to be influenced by herb supplementation. As in case of feeding frequency and duration in which there was a significant increase in group in which fish fed with experimental diet. It may be attributed to that increase the nutrient rich diet increases 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 agreed 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 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 (Hassan and Soltan, 2016; Sharma *et al.*, 2016; Butle *et al.*, 2018; Joshi, 2021; Pradhan *et al.*, 2021). The aquaculture and agriculture are very common joint ventures. The preformed aquaculture experiment evaluated the dietary wheatgrass induced behavioural responses of fish *Labeo rohita* (Hamilton 1822). The results indicated that the supplementation of herbs *Hibiscus rosa-sinensis* (flower), *Beta vulgaris* (root), *Phyllanthus emblica* (fruit), *Tinospora cordifolia* (stem) and *Moringa oleifera* (leaves) in diet improved the behavioural responses of the fish. In considering the overall responses, herb 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 4.1.0: Behavioural responses by *Labeo rohita* fed on herb added feed

Behavioural Patterns		**HF**	**HF1**	**HF2**	**HF3**	**HF4**	**HF5**
		Control	*Hibiscus rosa-sinensis*	*Beta vulgaris*	*Phyllanthus emblica*	*Tinospora cordifolia*	*Moringa oleifera*
Feeding Frequency (n)	Mean	1.49	3.15	4.28	4.53	5.77	6.81
	±SD	0.09	0.12	0.20	0.22	0.25	0.33
Feeding Duration(Sec.)	Mean	11.51	26.80	38.16	71.95	109.35	118.42
	±SD	1.21	2.68	4.01	4.42	5.48	6.97
Swimming Frequency (n)	Mean	1.08	2.72	3.42	4.09	5.16	7.84
	±SD	0.15	0.21	0.46	0.66	0.21	0.87
Swimming Duration(Sec.)	Mean	39.26	40.77	41.76	46.27	55.28	63.24
	±SD	2.68	3.05	4.83	7.00	5.78	7.04
Aggression Frequency (n)	Mean	0.65	1.21	2.93	4.51	9.21	12.54
	±SD	0.26	0.20	0.37	0.49	1.74	1.97
Aggression Duration(Sec.)	Mean	6.09	7.31	10.28	14.69	23.61	31.06
	±SD	1.35	2.86	2.38	3.63	3.68	4.33
Surface visit Frequency (n)	Mean	0.67	1.19	2.44	3.37	4.33	4.90
	±SD	0.15	0.15	0.18	0.36	0.32	0.25
Surface visit Duration(Sec.)	Mean	141.09	147.48	148.34	155.97	160.00	169.07
	±SD	6.03	5.57	6.93	3.14	7.44	10.08
Rest Frequency (n)	Mean	6.30	5.89	5.35	3.26	1.80	0.70
	±SD	1.58	1.29	0.98	0.49	0.36	0.22
Rest Duration(Sec.)	Mean	43.06	30.87	23.48	20.67	17.08	15.99
	±SD	5.85	5.21	4.41	3.64	2.89	2.82
Arousal Frequency (n)	Mean	1.78	1.56	1.40	1.35	0.90	0.51
	±SD	0.09	0.04	0.04	0.03	0.03	0.03
Arousal Duration(Sec.)	Mean	156.66	155.56	151.97	138.59	102.27	62.45
	±SD	8.42	5.08	5.78	5.12	5.16	4.18

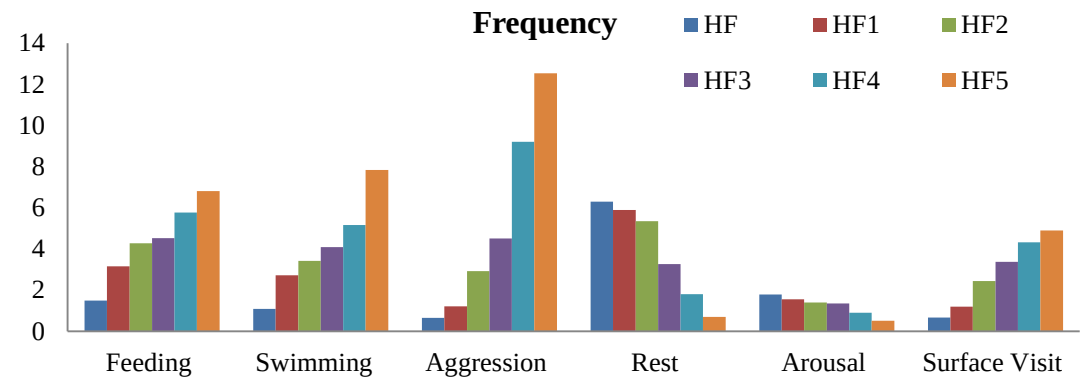

Figure 1. Frequency of behavioural responses by fish fed on herb-based fish feed

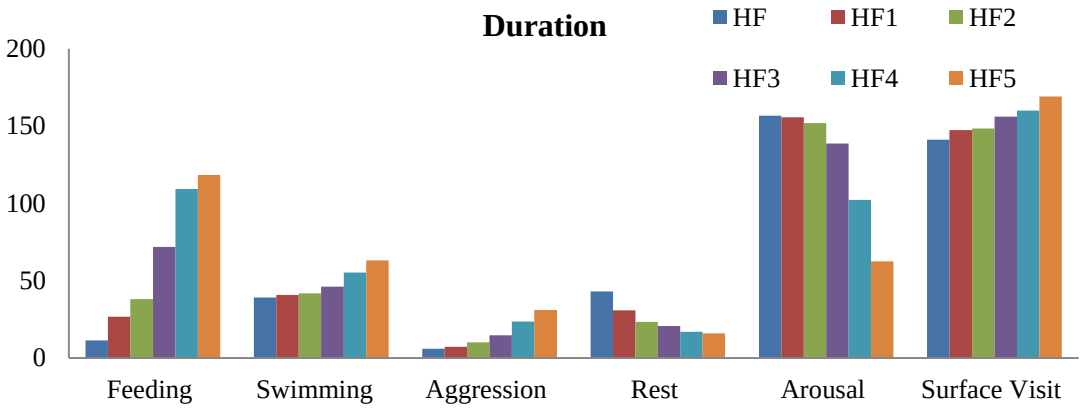

Figure 2: Duration of behavioural responses by fish fed on herb-based fish feed

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