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Potential of wheatgrass powder based feed for stinging catfish fry nursing in laboratory condition

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Abstract
Effectiveness of young sprouted wheatgrass powder over fishmeal for stinging catfish fry as an alternative animal protein source was assessed by this experiment. Wheat grain was sorted, washed and soaked overnight, kept in cotton bag for 24 hours darkness, sprouted for eight days on sand, cut and blanched before drying and made powder. Test diets (T_1, T_2, T_3, T_4 and T_5) were formulated replacing fish meal with wheatgrass powder at 100, 75, 50, 25 and 0%, respectively. A sixty days trial was conducted with three replications each at a density of 10 fish/20 liter water in plastic tanks. Survival rate was 100% in all the treatments except T_2 (95%). Whole fish protein was significantly higher in T_2 (17.04±0.98%) than T_5 (15.17±0.9%). Significantly higher fish production (1183.67 kg/ha), SGR (0.77 %/day) and lower FCR (7.18), feed cost (37.82 TK/kg) of T_1 signifying wheatgrass powder as a potential substitute of fish meal.

Keywords: wheat grass, fish meal, proximate composition, additives, protein

1. Introduction
Fisheries and aquaculture is the important source of food, nutrition, income and livelihoods for hundreds of millions of people around the world. At present, aquaculture is one of the promising sectors in Bangladesh. During the last seven years, the aquaculture production increased more than double 10.06 lakh MT in 2007-08 to 20 lakh MT (DoF, 2016)[1] and the current fish production of the country is 38.78 million tons (DoF, 2017) [2]. Indian major carps, perches and catfishes are the main commercial aquaculture species in our country among which catfishes especially stinging catfish (*Heteropneustes fossilis* Bloch, 1794*)* is vital due to its fast growth, tolerance to high stocking densities, high market value, ability to survive in low water quality, low fat, high protein, iron, antioxidant and medicinal value (Khan *et al.*, 2003 and Dehadrai *et al.*, 1985) [3, 4]. Continuous supply of good quality feed with competitive price is needed for sustaining the species. The culture fishes are generally offered with wild caught fish meal and fish oil for their nourishment as the major source of dietary protein. This trend has threatened the wild fisheries drastically. Hence, it is urgent to find out alternative feed ingredients that are easily available, cheap and good quality. In this case wheatgrass powder can be an alternative source of fish feed formulation which are already using as animal feed for cattle, chicken, good, sheep as well as nutrient supplement for human (Mujoriya and Bodla, 2011) [5]. Wheat grass is one of the green foods that contain 70% chlorophyll and also a good source amino acids, minerals, vitamins, and enzymes and proteins (Devi *et al.*, 2015) [6]. Mujoriya and Bodla (2011) [5] have mentioned wheatgrass as a powerhouse of nutrients and vitamins for the human body. In a similar study by Nath *et al.* (2014) [7] mentioned wheatgrass powder, as a feed ingredient, had significant impact on growth and survival of Asian catfish fry. So, the present experiment was apprehended to see the potentiality of wheatgrass powder when replace with fishmeal on stinging catfish fry feed formulation as an alternative of animal protein sources.

2. Materials and methods
2.1 Site and period of experiment
The experiment was carried out in the Aquaponics laboratory at the Department of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh. The duration of the study was 60 days from 7th September to 6th November 2015.

2.2 Experimental design

Fifteen plastic tanks of 30L capacity containing 20L water were used as fish rearing tanks. All the tanks were placed in two rows to facilitate better management such as feeding, cleaning and setting aerators for aeration. In this experiment four feeds were formulated replacing fish meal with wheatgrass powder of 100, 75, 50 and 25% respectively, which indicated as T_1, T_2, $T_3$and $T_4$and $T_5$was control where no replacement of fish meal was done. All the experiments were carried out in triplicates. The experimental tanks were numbered as T_1R_1, T_1R_2, T_1R_3,T_2R_1, T_2R_2, T_2R_3, T_3R_1, T_3R_2, T_3R_3, T_4R_1, T_4R_2, T_4R_3,T_5R_1, T_5R_2, and T_5R_1, and the tanks were arranged in complete randomized block design. Plastic pipes of one foot long were placed at the bottom of the tanks for hiding and resting the fry. The tanks were covered with nets to prevent the fish jumping or predatory animals attack. Ten fry of stinging catfish was released per tank and acclimatized for a week with commercial feed before start the experiment.

2.3 Fry collection and acclimatization

Stinging catfish fry was collected using oxygenated plastic bag from the Satota Matshya Hatchery, Digarkandha, Mymensingh. The fry was acclimatized to the laboratory condition and adapted with the small water tank at room temperature and kept for a period of 8 days prior to start the experiment. During this time aeration was provided using aerator and air stones, fry fed commercial powder feed containing 35% protein twice daily at 10 am and 5 pm.

2.4 Wheatgrass sprouting procedure

The wheat was sprouted in galvanized trays ($73\times37\times3cm^3$). At the beginning the trays washed with tap water and Sun dried and a thin layer of sand was spread on the trays. Then ½ kg wheat seed was taken in 4 separate plastic buckets each and cleaned, washed and soaked overnight in water. In the following morning the soaked wheat was washed again and sieved, wrapped with cotton bag and kept in cool and dark place for 24 hours. After that soaked and slightly germinated wheat was spread over each trays on sand and sprayed water on the seeds and covered with another tray for 3 days and watered twice daily uncovering the trays. After 3 days, the trays were uncovered when the wheat was sprouted fully and young wheatgrass looked yellowish and the trays kept in passive light but not direct sunlight and watered 3 times daily for another 4 days before harvest. In 7-8 days the wheat sprout became 6-7 inch long and dark green colors then the stems were cut down and weighed.

2.5 Blanching and powder preparation

Blanching process was applied on wheatgrass to keep the color intact and all other contents as it is in wheatgrass. The water was boiled in a big tub at 75 to 105ºC temperature and harvested wheatgrass soaked in boiled water for 7minutes. After boiling wheat stems were put into an ice cold water where a pinch of sea salt was added to keep the water cooler. The process facilitated to stop the enzymatic activities, removed air and gases, kept the color green, improved texture, stopped changing flavor and leaching water soluble sugars from the wheatgrass.

Following blanching the wheatgrass was kept spreading on a table in room temperature over night under fan to remove the excess water then dried in a homemade drier for 24 hours at 48^0Ctemperature. When the wheatgrass became crispy it was cut into small pieces with scissors and made powder by blender and kept in fridge at 4^0C before use in fish feed preparation.

2.6 Feed ingredients collection and analyze proximate composition

The feed ingredients were collected from local market and analyzed proximate composition at the Fish Nutrition Laboratory at the Dept. of Aquaculture, BAU that is shown in Table 1.

Table 1: The ingredients that were used in feed formulation of stinging catfish fry

Name of item	Moisture (%)	Crude lipid (%)	Crude protein (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)
Wheatgrass	9.055	2.25	22.935	6.875	4.65	54.235
Maize	13.11	5.2	17.69	1.31	6.2	56.49
Soybean meal	12.66	7.8	38.66	4.9	5.8	30.18
Rice bran	12.6	12.2	13.1	13.3	6.2	42.6
Wheat bran	11.8	6.6	17.03	10.4	5.6	48.57
Mustard oil cake	13.1	13.2	33.42	7.4	3.6	29.28
Treatment (T_1)	12.50	7.31	32.55	6.51	4.80	36.33
Treatment (T_2)	12.21	8.13	35.35	8.08	4.10	32.13
Treatment (T_3)	11.81	7.92	32.90	9.06	4.70	33.61
Treatment (T_4)	12.05	8.20	33.60	9.80	5.10	31.25
Treatment (T_5)	10.83	8.87	36.75	11.19	4.20	28.16

T_1=Feed with100% wheatgrass powder; T_2=Feed with75%wheatgrass powder; T_3=Feed with50% wheatgrass powder; T_4=Feed with25%wheatgrass powder; T_5= Control (Feed with 0% wheatgrass powder).

2.7 Feed formulation and feeding

Prior to feed formulation, all the ingredients were ground and sieved through a sieve of 0.5 mm mesh size to have homogenous mixtures. Feed was formulated by giving emphasize on progressive replacement of fishmeal with wheatgrass powder. After sieving the ingredients they were weighted as per experimental design (Table 2) along with the vitamins and mineral premixes in an electronic balance (Electronic Precision Balance, EK 600i) and mixed well and add water to moisten the mixtures. The resultant dough sieved again with 1.0 mm mesh size sieve and kept in fridge in air tight plastic bags after Sun dried for feeding later. Some precautionary measures were taken such as Mustard oil cake soaked overnight and Soybean boiled before add in feed to abrogate glucocyanates growth inhibiting effect. The formulated feed Fed to the fry in all the treatments twice daily at the rate of 10% of their body weight at 10 am and 5 pm. The uneaten feed and faces were siphoned and 25%water was exchanged daily and all the water changed fortnightly to maintain good water quality in the containers.

Table 2: Feed ingredients used in formulating the test diets

Feed ingredients	Treatment T_1 (g)	Treatment T_2 (g)	Treatment T_3 (g)	Treatment T_4 (g)	Treatment T_5 (g)
Wheatgrass powder	19.00	14.25	9.50	4.75	0.00
Fish meal	0.00	4.75	9.50	14.25	19.00
Mustard oilcake	20.00	27.00	29.00	28.50	24.00
Rice bran	5.00	6.00	8.00	11.00	15.00
Soya bean	50.00	41.00	34.00	28.50	26.00
Wheat bran	4.00	5.00	8.00	11.00	14.00
Molasses	0.50	0.50	0.50	0.50	0.50
Vitamin	0.50	0.50	0.50	0.50	0.50
Binder	1.00	1.00	1.00	1.00	1.00
Grand Total	100	100	100	100	100

2.8 Sampling of fish
Sampling was done fortnightly. Six fries from each tank were randomly collected by scope net and length and weight were measured using millimeter ruler and electronic balance.

2.9 Growth performance of fish
Several parameters were measured to evaluate the growth of stinging catfish fry.

Length gain (cm) = mean final length (cm) – mean initial length (cm).

Weight gain (g) = mean final weight (g) – mean initial weight (g).

$$\text{Percent weight gain (\%)} = \frac{\text{Mean final weightin (\%) - mean initia}}{\text{Mean initial weight}} \times 100$$

$$\text{Specific Growth Rate(\%per day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

$$\text{Food Conversion Ratio} = \frac{\text{Amount of dry feed used}}{\text{Weight gain}}$$

$$\text{Survival rate (\%)} = \frac{\text{No.of fish harvested}}{\text{No.of fish stocked}} \times 100$$

Fish production = No. of fish harvested × mean weight gain

3. Results
3.1 Water quality parameters in the fish tank
In the present experiment, the physico-chemical parameters of fish tank water like temperature (ºC), dissolved oxygen (ppm), pH and ammonia (mg/l), nitrate (mg/l) and phosphate (mg/l) test results were within the suitable range for stinging catfish fry rearing. In all the five experiments the DO and temperature ranges were 5.08(±0.68) to 5.56(±1.11) ppm and 27.49(±1.54) to 27.67(±1.37) ºC and not significantly differed among the treatments. The water quality parameters measured in different experimental tanks are presented in Table 3.

Table 3: The water quality parameters of different treatments during the experimental period

Parameters	T_1(with 100% wheatgrass)	T_2(with 75% wheatgrass)	T_3(with 50% wheatgrass)	T_4(with 25% wheatgrass)	T_5(0% wheatgrassas Control)
pH	7.91±0.30	8.01±0.34	8.15±0.40	8.02±0.33	8.11±0.37
Dissolved Oxygen(ppm)	5.08±0.68	5.29±0.83	5.56±1.11	5.19±0.74	5.39±0.84
Temperature (˚C)	27.58±1.41	27.49±1.54	27.54±1.41	27.63±1.39	27.67±1.37
Ammonia(mg/L)	0.14±0.05	0.17±0.09	0.22±0.11	0.14±0.06	1.90±2.27
Nitrate(mg/L)	0.48±0.10	0.12±0.04	0.06±0.00	0.31±0.18	0.33±0.30
Phosphate(mg/L)	3.59±0.81	0.08±0.01	3.65±1.29	1.57±2.14	4±0.71
Sulphate(mg/L)	13±1.41	15±1.41	15±0.00	12±1.41	12.5±0.71

3.2 Wheatgrass Production
The average sprouted wheatgrass production was 341.98(±45.07) g where the range of production was300.38 to 384.38 g. On the other hand, total weight of wheatgrass powder was 208.85g and around 15.27% powder was retained from the live wheatgrass.

3.3 Growth performance of stinging catfish
Growth performance of stinging catfish (Table 4) indicated the length and weight gain, specific growth rate, daily growth rate, survival rate, feed conversion ratio and fish production. Significantly higher fish production was achieved with T_1 followed by T_5, T_3, T_4 and T_2 ($p<0.05$) treatments respectively. Fish production range was 732.87 to 1183.67 tons/ha/60 days, where highest production was with $T_1$and lowest with T_2. Likewise, FCR and feed cost was lowest and SGR was highest in T_1 among all the treatments. The highest FCR was with T_2 where 75%fish meal was replaced with wheatgrass powder. The overall growth performance of experimental fish is given in

Table 4: Overall growth performance of stinging catfish fry fed experimental feed

Parameters	T_1(with 100% wheatgrass)	T_2(with 75% wheatgrass)	T_3(with 50% wheatgrass)	T_4 (with 25% wheatgrass)	T_5(0% wheatgrass as Control)
Mean initial length (cm)	$(7.12\pm0.12)^{bc}$	$(7.48\pm0.12)^{a}$	$(7.30\pm0.14)^{ab}$	$(7.03\pm0.08)^{c}$	$(7.45\pm0.15)^{a}$
Mean final length (cm)	$(9.22\pm0.11)^{ab}$	$(9.01\pm0.06)^{b}$	$(9.05\pm0.05)^{ab}$	$(8.81\pm0.11)^{b}$	$(9.45\pm0.46)^{a}$
Mean length gain (cm)	2.10	1.53	1.78	1.78	2.00
% length gain	29.51	20.38	24.40	25.24	26.85
Mean initial weight (g)	$(2.03\pm0.04)^{b}$	$(2.3\pm0.15)^{a}$	$(2.13\pm0.14)^{ab}$	$(2.12\pm0.12)^{ab}$	$(2.34\pm0.10)^{a}$
Mean final weight (g)	$(4.93\pm0.43)^{ab}$	$(4.19\pm0.19)^{c}$	$(4.41\pm0.42)^{bc}$	$(4.08\pm0.10)^{c}$	$(5.09\pm0.11)^{a}$

Mean weight gain	2.90	1.89	2.28	1.96	2.75
% weight gain	142.86	82.17	107.04	92.45	117.52
Daily growth rate (%)	4.83	3.15	3.8	3.27	4.58
Correlation coefficient (r)	0.92	0.89	0.96	0.92	0.92
Fish production(kg/ha)	1183.67^{a}	732.87^{e}	930.61^{c}	800^{d}	1122.45^{b}
FCR	7.18^{c}	11.27^{a}	8.77^{b}	8.77^{b}	7.21^{c}
Specific growth rate (%/day)	0.77^{a}	0.46^{c}	0.60^{bc}	0.49^{c}	0.73^{ab}
Survival (%)	100	95	100	100	100

Mean (±SD); Significant level indicates in a rightward and mean with same superscript values are insignificantly different (P>0.05).

3.4 Length weight relationship

Positive linear relationships were observed between dependent and independent variables when length-weight relationships were computed from the sampled data of present experiment. The linear relationship of regression equations showed in Figure 2, 3, 4, 5 and 6.The positive correlation were observed where r values were highly correlated (r=0.92, 0.89, 0.96, 0.92 and 0.92)between length and weight of stinging catfish fry in T_1, T_2, T_3, T_4 and T_5 respectively.

3.5 Condition factor

The condition factor was also calculated from collected sampled data. The condition factor for five different treatments showed the length and weight relationship in figure 2, 3, 4, 5 and 6. The average condition factor in T_1, T_2, T_3, T_4 and T_5 treatments were 0.58, 0.55, 0.56, 0.58 and 0.56 respectively.

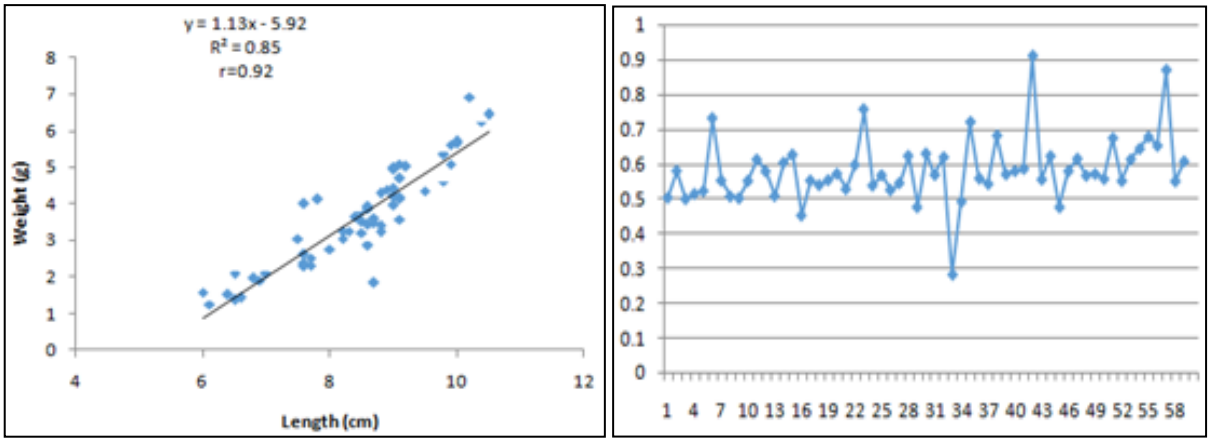

Fig 2: Length weight relationship and condition factor for T_1 treatment.

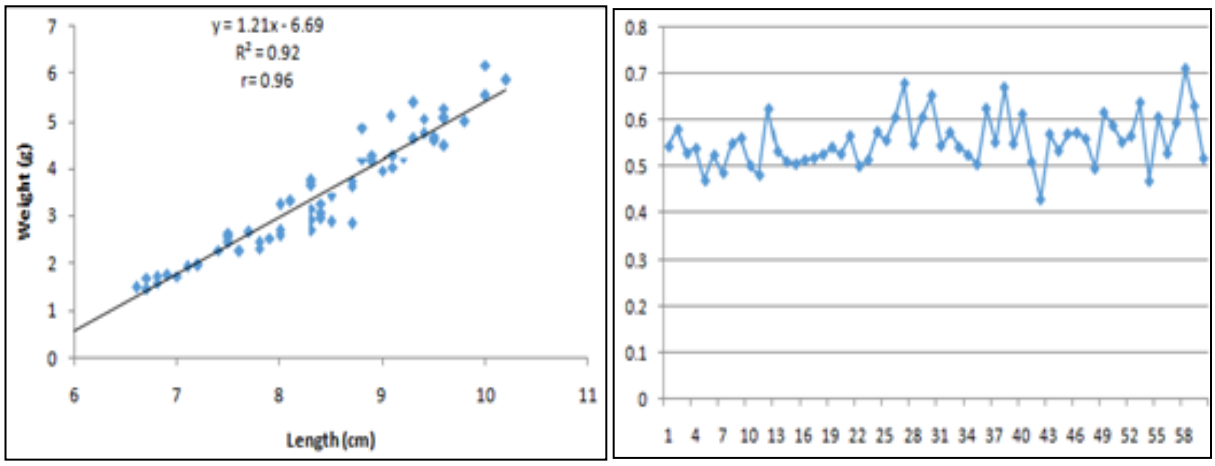

Fig 3: Length weight relationship and condition factor for T_2 treatment.

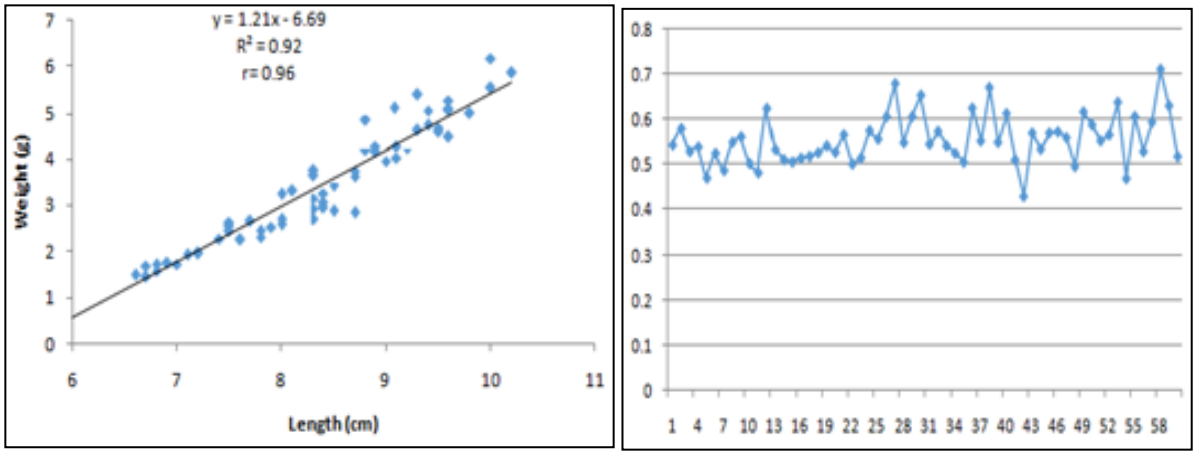

Fig 4: Length weight relationship and condition factor for T_3 treatment

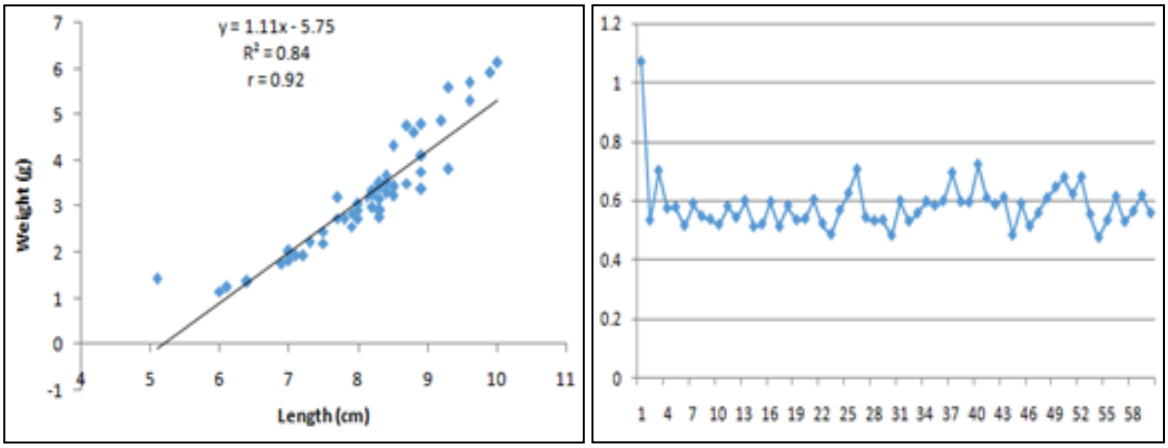

Fig 5: Length weight relationship and condition factor for T_4 treatment.

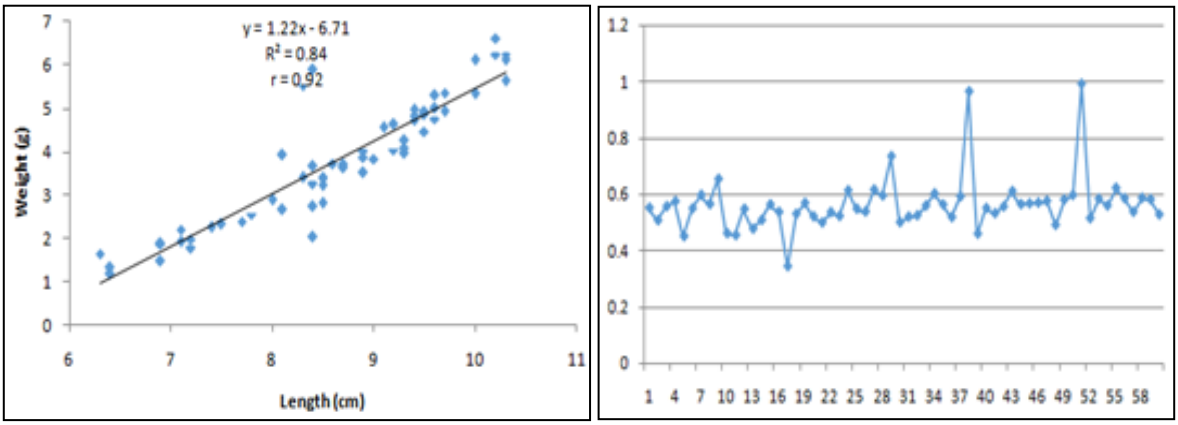

Fig 6: Length weight relationship and condition factor for T_5 (control)

3.9 Proximate composition of stinging catfish

The results of proximate composition showed that there was no significant difference in moisture and ash contents in all the treatments however there was significantly higher crude protein in T_2 17.04(±0.98) and T_4 16.76(±0.78), crude lipid in T_5, T_4 and T_3treatments. In addition, carbohydrate content was significantly higher in T_1 than rest of the treatments (Table 5).

Table 5: The proximate composition of experimental stinging catfish fry (% moisture basis)

Parameters	T_1(with 100% wheatgrass)	T_2(with 75% wheatgrass)	T_3(with 50% wheatgrass)	T_4 (with 25% wheatgrass)	T_5(0% wheatgrass as Control)
Moisture	76.15(±0.9)^a	76.36(±0.65)^a	76.21(±0.85)^a	76.52(±0.55)^a	76.18(±0.85)^a
Crude protein	13.69(±0.75)^c	17.04(±0.98)^a	16.49(±0.55)^{ab}	16.76(±0.78)^a	15.17(±0.9)^b
Crude lipid	2.90(±0.90)^b	3.50(±0.55)^{ab}	3.95(±0.60)^a	4.00(±1)^{ab}	4.60(±0.7)^a
Ash	2.38(±0.68)^a	2.61(±0.65)^a	2.70(±0.75)^a	2.64(±0.7)^a	2.68(±0.7)^a
Carbohydrate	4.88(±0.95)^a	0.49(±0.15)^b	0.50(±0.1)^b	0.08(±0.01)^b	1.37(±0.65)^b

***Significant level indicates rightward

3.10 Cost benefit analysis of different feeds

The feed cost was calculated according to the market price of the ingredients available in the local market. The price of the feed in different treatments increased with the inclusion of fishmeal. The control feed cost was the highest price where 100% fish meal was used and the lowest price was with T1 treatment where no fish meal and 100% wheatgrass powder was used.

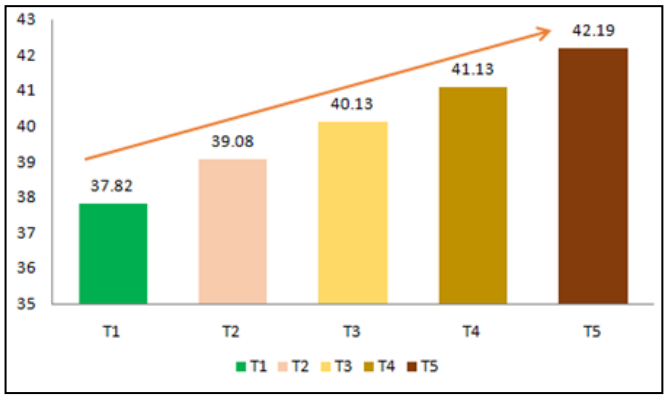

Fig 10: The price (Tk/kg) of different feeds in various treatments

4. Discussion

The present empirical study was apprehended to see the potentiality of sprouted young wheatgrass powder over fishmeal in stinging catfish fry rearing feed. Total production of wheatgrass powder was 208.85g and around 15.27% powder was retained from the live wheatgrass where Chouhan and Mogra (2014) [8] obtained 10% wheatgrass powder from live wheatgrass by shade drying. The production of the stinging catfish fry range was 732.86 to 1183.67kg per hectare per 60 days that supports the findings of Khan *et al.* (2003) [3] where they showed 2080 to 3364 kg/ha/4months production depending on stocking density. The overall production was lower in the present study may be due to higher stocking density. However, the highest SGR found in T1 (0.77) followed by control (0.73) and lowest SGR found in T2 (0.46) treatment. Nath *et al.* (2014) [7] showed 0.29 SGR in wheatgrass based feed for Asian catfish. Siddiqui *et al.* (2014) [9] also found 2.79 SGR for stinging catfish when fed soyabean meal based feed. The higher SGR value in this experiment gained may be due to feed having adequate nutrients. The range of % crude protein was 13.69(±0.75) to 17.04(±0.98) in proximate composition of stinging catfish. Siddiqui *et al.* (2014) [9] found 15.36±0.02 % protein in proximate composition of stinging catfish after feeding soyabean meal based feed. The higher protein content in the fish muscle fed with wheatgrass based feed indicates the vitamins of wheatgrass have positive impact on fish growth. During this 60 days experiments the water quality parameters like pH, Dissolved Oxygen (DO), temperature and Ammonia (NH_3), Nitrite (NO_2), Phosphate (PO_4^-) and Sulphate (SO_4^{2-}) showed conformity with the recommended range of David *et al.* (1969) [10], Hossain (2009) [11].

5. Conclusion

The study has successfully developed wheatgrass culture, wheatgrass powder and fish feed formulation technique and showed its potentiality as fishmeal supplementary ingredients for rural Bangladesh. Besides this, the financial benefit gained by this wheatgrass based feed which can produce on demand, cheapest and easily available. Therefore, considering the nutrient profile of wheatgrass powder, digestibility, formulation of feed, cost of fish feed and easy to culture wheatgrass at home condition, the replacement of fish meal with wheatgrass powder is recommended at fish farming.

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