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Effect of molasses-fermented water hyacinth feed on growth and body composition of common carp, *Cyprinus carpio*

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Abstract
The present study was conducted to study the effect of molasses-fermented water hyacinth feed on growth and body composition of common carp, *Cyprinus carpio*. The experiment was conducted in FRP pool (1.5x1.0x0.75 m), consisted of 5 treatments (D1, D2, D3, D4 and D5) with 3 replicates each. Fermented water hyacinth (FWH) was incorporated into test diet, as an energy source, at different levels (@ 0 (D1), 40 (D2), 80(D3), 120 (D4) and 160 (D5) g/kg test diet) as a replacement for equal amount of rice bran fish were fed 3-5% of body weight for 120.All the water quality parameters were within the range for carp culture. Net weight gain (NWG) and Specific growth rate (SGR) were maximum in (24.63 g and 0.79) in D5 and minimum (16.12 g and 0.60) in D1 and the differences in NWG and SGR among treatments were significant ($P \leq 0.05$). FCR were recorded significantly ($P \leq 0.05$)better in D5 (1.18) and maximum in D1 (1.28).FWH supplementation in common carp improved flesh quality in terms of total protein and total lipid content significantly ($P \leq 0.05$), as higher flesh protein and lipid content was recorded in fish fed on FWH supplemented feeds. Molasses fermented WH can be replaced with rice bran up to 40 % (of RB) in the diets for common carp fingerlings. Fermented water hyacinth form an abundant alternative natural un-utilized resource for less expensive fish feed and higher fish yield to enhance farmer's income.

Keywords: Fermented water hyacinth, feed, *Cyprinus carpio*, growth, body composition

Introduction
Common carp (*Cyprinus carpio*) belongs to the order Cypriniformes and the family Cyprinidae, which is considered the largest family of freshwater fish. It generally inhabits freshwater environments, especially ponds, lakes and rivers, and also rarely inhabits brackish-water environments [4]. It is widely distributed in almost all countries of the world but is very popular in Asia and some European countries [21, 30]. Because of its high popularity, its distribution has been widely extended by human introduction. Common carp is the third most widely cultivated and commercially important freshwater fish species contributing 9% of the world's total finfish aquaculture production [13]. Common carp is a very well-known benthivorous fish that has larger bottom–up effects than other benthivorous fish. It consumes benthic organisms, such as water insects, larvae of insects, worms, molluscs and zooplankton. Inconsistent supply and soaring prices of conventional pelleted fish feed has relentlessly compelled the development of low-cost aquaculture system suitable for farmers in the developing countries. In order to maintain sustainable growth of fish, along with fertilization, supplementary feeding is aprerequisite in aquaculture, therefore, it should be approached through diet formulation and/or feeding strategy. Supplementary feed is one of the key inputs in aquaculture for elevating production and constitutes more than 60% of the input cost. Considering the ever increasing cost of conventional feed ingredients, it is essential to identify locally available nutrient rich low cost non-conventional feed resources (NCFR). Among various NCFR used in aquaculture, aquatic plants like water hyacinth could be good alternate feed resource due to its increased nutritive value and fast growth rate. Several studies have been carried out to evaluate the incorporation of different non-conventional animal and plant proteins and energy sources for herbivorous fishes for varying results [11]. However, formulating economic fish feeds using non-conventional, locally available feed resources pose a major task for farmers, in general and for fish nutritionist in particular.
Water hyacinth, WH (*Eichornia crassipes*) is a wild freshwater fern belonging to the family

Pontederiaceae. It is an erect, free-floating, stoloniferous, perennial herb [8, 25] and lives at the air-water interface forming two distinct canopies; leaf canopies comprising above-water structures and root canopies comprising below water structures [10]. This plant is native to South America, but has been naturalized in many tropical and subtropical regions of the world. It grows and reproduces (by seeds and by daughter plants) at very high rates; yielding up to 100–400 Mt/ha/year [18]. WH is considered the world's worst aquatic plant. It forms dense mats that block navigation and interfere with irrigation, fishing, recreation and power generation [17]. These mats also prevent sunlight penetration and aeration of the water, leading to oxygen deficiency, competitively exclude submersed plants and reduce biological diversity [12]. Despite its low protein content, WH contains 30–60% nitrogen-free extract (NFE) and between 20% and 30% crude fiber [15]. Several studies have evaluated WH as a food source for domestic animals [9, 28, 29]. Some uses are reported for this weed such as compost making, paper industry, biogas plants, cattle feed, furniture making, and waste water treatments. Most of the studies indicate that only low levels of WH can be incorporated into fish feeds. The relatively high fiber content in WH may limit its use in common carp feed because these fish lacks the ability to secrete cellulose, the main cellulose digesting enzyme compare to maltose and lactose [1]. Fermentation of WH may reduce its fiber contents and improve its nutritive value for fish. However, the effect of fermentation processing upon WH nutritive value for common carp remains uncertain. Therefore, the study was conducted to evaluate the effects of molasses-fermented water hyacinth feed on growth and body composition of common carp, *Cyprinus carpio*.

Materials and Methods

Experimental design

The study was carried out in outdoor FRP pools (1.5 x 1.0 x 0.75 m) at the Fish Farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The experiment consisted of 5 treatments (D1, D2, D3, D4 and D5) with 3 replicates each. Test diet was prepared using rice bran, soy meal and mustard meal in the ratio 20:13:15. Fermented water hyacinth was incorporated into test diet, as an energy source, at different levels (@ 0 (D1), 40 (D2), 80(D3), 120 (D4) and 160 (D5) g/kg test diet) as a replacement for equal amount of rice bran (Table 1). Mineral mixture and vitamin mixture were added @ 1% of feed prepared. Carboxy methyl cellulose was added as binder @ 2% of prepared feed and adequate water was added. Fish were stocked @ 10 fingerlings/FRP pool and Fish were fed with supplementary diets D1 – D5@ 5% of fish body weight (BW) daily for the first month and @ 3% BW for the rest of 90 days. Amount of feed was adjusted at every sampling according to increase in fish weight for 120 days (May to September).

Preparation of fermented water hyacinth (FWH) leaf meal

Water hyacinth was collected from nearby water bodies. All other ingredients were purchased from the local market. Fermented water hyacinth leaf meal was prepared according to [12]. Water hyacinth was collected from nearby water bodies and brought at Fish Farm, College of Fisheries, GADVASU. Initially, leaves and leaf stalks were separated from roots and chopped into small pieces (1–2 cm) and were sun-dried until water content dropped to 50%. Than 5% sugar cane molasses and 2 ml ortho phosphoric acid/kg were added to sun-dried water hyacinth with continuous mixing and were stored for 50 days in covered plastic containers at room temperature. Fermented material was oven dried at 60^0C for 24 h and finely grinded and stored in labelled plastic bag.

Experimental diets

Five experimental diets were formulated with varying percentage of fermented WH (Table 1).The moisture, crude protein, lipid and ash content in the test diets (Table 1) and fish body composition were analyzed, according to standard procedures of Association of Official Analytical Chemist [2].

Table 1: Proximate composition of experimental diets and ingredients (on dry matter basis)

Proportion of ingredients in experimental diets (%)					
Ingredients	**D1 (Control)**	**D2 (10% WH)**	**D3 (20% WH)**	**D4 (30% WH)**	**D5 (40% WH)**
Fermented WH	-	4	8	12	16
Rice bran (RB)	40	36	32	28	24
Soy meal	26	26	26	26	26
Mustard oil cake	30	30	30	30	30
CMC	2	2	2	2	2
Mineral mixture	1	1	1	1	1
Vitamin mixture	1	1	1	1	1
Proximate composition of experimental diets (%)					
Crude protein	31.48	33.91	33.22	34.63	35.25
Crude fat	2.56	1.86	1.72	1.56	1.43
Crude fiber	22.96	22.56	24.26	24.78	25.12
Ash	9.13	9.75	9.78	10.11	11.21
NFE	33.87	31.98	31.02	28.92	26.99

Water parameters

Water parameters such as temperature, pH, dissolved oxygen, total hardness, total alkalinity, ammonia nitrogen, nitrite nitrogen and nitrate nitrogen were were analyzed forthnightly according to standard methods [3].

Growth parameters

Fish sampling was done at monthly intervals to record fish growth in terms of total body length and body weight (BW). A random sample of 10 fish from each pond was collected to record length and weight. Total length gain (TLG), net weight gain (NWG), specific growth rate (SGR) and condition factor (K- valve) of fish for each treatment were calculated [16].

TLG = Final total length (TL) (cm) –Initial total length (TL) (cm)

$$\text{NWG} = \text{Final body weight (g)} - \text{Intial body weignt (g)}$$

$$\text{Weight gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \text{X100}$$

$$\text{SGR (\% weight gain day}^{-1}) = \frac{\ln \text{final BW (g)} - \ln \text{initial BW (g)}}{\text{Culture days}} \times 100$$

$$\text{Condition Factor (K)} = \frac{\text{Body weight(g)}}{\text{(Body length)cm}^3} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Statistical analysis

Statistical analysis of the data was performed with a statistical package (SPSS 16.0, SPSS Inc., and Richmond, CA, USA). Values were presented as means ± standard error of the mean.One way ANOVA was applied to work out of effect of fermented water hyacinth leaf meal on pond productivity and gut content analysis of fish ($P \leq 0.5$), followed by Duncan's multiple comparison to determine significant differences among the treatments.

Results

Water quality

During the course of the experiment, the temperature ranged from 28.1 to 34.5 °C, pH was around 8.0, dissolved oxygen varied from 5.56-8.78 mgL^{-1}. Ammonia nitrogen and nitrite nitrogen were less than 1 mgL^{-1}, while nitrate was less than 0.5 mg/L.According to our results, the estimated values of hardness and alkalinity were found within the rangeof 201.33-240.67 $mgL^{-1}$and 224.48-295.66 mgL^{-1} respectively (Table 2). All these parameters were within the range for carp culture as suggested by [5, 6, 7, 19]. This shows that fermented WHsupplemented diet has no adverse effect on water quality.

Table 2: Water quality parameters in different treatments during experiment*

Parameters	Treatments (with different levels of FWH)				
	D1	D2	D3	D4	D5
Water temperature (°C)	$31.49^a \pm 1.74$	$31.07^a \pm 0.36$	$30.61^a \pm 0.35$	$31.51^a \pm 0.35$	$30.81^a \pm 0.36$
pH	$7.89^a \pm 0.25$	$7.99^a \pm 0.06$	$8.02^a \pm 0.05$	$8.03^a \pm 0.03$	$7.85^a \pm 0.30$
Dissolved oxygen (mgL^{-1})	$7.13^b \pm 1.02$	$7.51^{ab} \pm 0.15$	$7.71^a \pm 0.17$	$7.28^{ab} \pm 0.19$	$7.36^{ab} \pm 0.20$
Total hardness (mgL^{-1})	$211.96^b \pm 22.16$	$222.44^a \pm 4.65$	$228.52^a \pm 2.28$	$227.19^a \pm 2.50$	$226.96^a \pm 2.51$
Total alkalinity (mgL^{-1})	$278.11^a \pm 18.98$	$259.22^a \pm 4.62$	$248.77^a \pm 5.04$	$246.11^a \pm 3.62$	$273^a \pm 4.82$
Ammonia nitrogen (mgL^{-1})	$0.065^a \pm 0.005$	$0.066^a \pm 0.007$	$0.066^a \pm 0.005$	$0.061^a \pm 0.005$	$0.050^a \pm 0.004$
Nitrite nitrogen (mgL^{-1})	$0.071^a \pm 0.05$	$0.077^a \pm 0.01$	$0.071^a \pm 0.01$	$0.069^{ab} \pm 0.01$	$0.062^b \pm 0.01$
Nitrate nitrogen (mgL^{-1})	$0.26^a \pm 0.12$	$0.31^a \pm 0.03$	$0.29^a \pm 0.02$	$0.34^a \pm 0.04$	$0.31^a \pm 0.03$
Orthophosphate (mgL^{-1})	$0.30^b \pm 0.04$	$0.29^b \pm 0.04$	$0.31^{ab} \pm 0.04$	$0.34^{ab} \pm 0.04$	$0.39^a \pm 0.04$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly ($P \leq 0.05$)

Effect of molasses-fermented water hyacinth feed on growth and survival

The work on utilization of fermented water hyacinth leaf meal in aquaculture especially with common carp is meager. However, comparing the present results with those studies may be inappropriate due to the differences in fish species and sizes, rearing systems, culture conditions and diet composition. The growth of fish was assessed in terms of total length (TL) and body weight (BW) at monthly intervals during the culture period. At the end of the experiment, total length gain (TLG), net weight gain (NWG), specific growth rate (SGR) and condition factor (K-value) of fish for each treatment was calculated.

There were no significant differences in initial body length and body weight between the treatments (Table 3). One way ANOVA showed that fermented water hyacinth leaf meal significantly affected that final length and final weight of the common carp ($P \leq 0.05$). At the termination of an experiment, total length gain (TLG) was maximum (2.83 cm) in D5 and minimum (2.28 cm) in D1 and significant difference ($P \leq 0.05$) was observed among the treatments. Similarly, BW (g) was found to be maximum (40.18 g) in D5 and minimum (31.22 g) in D1. The overall results of the present study revealed that fish growth in terms of net weight gain (NWG) and specific growth rate (SGR) was significantly higher in all fermented water hyacinth leaf meal treatments($P \leq 0.05$) and were found to be maximum(24.63 g and 0.79) in D5 and minimum (16.12 g and 0.60) in D1.Fermented water hyacinth containing diets might have liberated nutrients resulted in better growth performance in FWH treatments compared to control (D1) as it contain more nutrients than control diet.It is in agreement with the study of Mohapatra and Patra [23]reported that water hyacinth has been successfully used as a non-conventional feed ingredient and its inclusion @15% in fish feedwas found to be optimum for the maximum growth of common carp, *Cyprinus carpio* whereas, Keshavanath [20] reported better body weight gainin rohu (*Labeorohita*), grass carp (*Ctenopharyngodon idella*) and common carp (*C. carpio*) fed with diets incorporated with the leaf meal powder at 50% level. Which was quite less compared to present study as it was consumed in raw form contrast to fermented water hyacinth meal used in the present study. Water hyacinth was used as a replacement for fish meal for rearing of common carp fry, *C. carpio* with compounded artificial diets at different levels and it showed better results in a diet consisting of up to 40% water hyacinth content [22]. While, in the present study values of the fermented water hyacinth leaf meal were found to be better compared to control in terms of growth. However, ideal combination of fermented water hyacinth leaf meal was observed in T5 (40% of rice bran replacement with FWH i.e. 160g/Kg feed).

Food consumption was adjusted according to the monthly sampling; it was increased@ 3% of total body weight for subsequent months. ANOVA showed that water hyacinth leaf meal significantly affected that specific growth rate ($P \leq 0.05$).Food conversion ratio (FCR) was significantly affected by fermented water hyacinth leaf meal (Table 4). FCR at D5 was significantly lower than the other treatment and control ($P \leq 0.05$). FCR of fish was 1.28, 1.27, 1.26, 1.22 and 1.18 in D1, D2, D3, D4 and D5 treatments. The K-value of fish ranged from 1.23 to 1.34 and the values were found to significantly different in all the treatments ($P \leq 0.05$). The higher K-value in treatment D5 revealed that FWH supplementation improved the health condition of fish. As K-value reflects proportional body weight gain with length gain and serves as an index to assess health condition of fish [14, 24].

Protein efficiency ratio (PER) of fish in D1, D2, D3, D4 and D5 treatments was 1.27, 1.23, 1.29, 1.39 and 1.46 respectively. The differences in PER among treatments shows that common carp fish was significantly affected by the supplementation of fermented water hyacinth ($P \leq 0.05$). The trend showed that PER was found to be increasing with increase in percentage of fermented water hyacinth.

Survival

At the end of experiment, all the tanks were drained out completely to harvest all the fish for calculating the % survival of fish in different treatments. The average survival observed in all treatments ranged between 93.33 and 96.66% (Table 3). It was not significantly affected by supplementation of fermented water hyacinth in diets ($P \geq 0.05$).

Table 3: Growth performances and feed utilization efficiencies in common carp fingerlings fed on fermented water hyacinth diets for 120 days*.

Parameters	Treatments (with different levels of FWH)				
	D1	D2	D3	D4	D5
Avg. initial LG. (cm)	$11.35^{a}\pm0.28$	$11.47^{a}\pm0.21$	$11.56^{a}\pm0.20$	$11.64^{a}\pm0.27$	$11.57^{a}\pm0.05$
Avg. final LG. (cm)	$13.63^{d}\pm0.05$	$13.79^{cd}\pm0.03$	$13.87^{c}\pm0.11$	$14.11^{b}\pm0.01$	$14.40^{a}\pm0.06$
TLG (%)	$2.28^{a}\pm0.24$	$2.32^{a}\pm0.24$	$2.30^{a}\pm0.28$	$2.47^{a}\pm0.28$	$2.83^{a}\pm0.83$
Avg. initial WT. (g)	$15.09^{a}\pm0.43$	$15.16^{a}\pm0.19$	$15.35^{a}\pm0.17$	$15.08^{a}\pm0.05$	$15.54^{a}\pm0.20$
Avg. final WT. (g)	$31.22^{e}\pm0.24$	$32.53^{d}\pm0.40$	$33.76^{c}\pm0.20$	$36.80^{b}\pm0.20$	$40.18^{a}\pm0.59$
NWG (%)	$16.12^{d}\pm0.58$	$17.37^{c}\pm0.37$	$18.41^{c}\pm0.05$	$21.72^{b}\pm0.17$	$24.63^{a}\pm0.45$
Weight gain (%)	$107.23^{d}\pm6.90$	$114.62^{cd}\pm3.06$	$119.96^{c}\pm1.23$	$143.96^{b}\pm1.09$	$158.51^{a}\pm2.45$
SGR	$0.60^{d}\pm0.02$	$0.63^{cd}\pm0.01$	$0.65^{c}\pm0.004$	$0.74^{b}\pm0.003$	$0.79^{a}\pm0.007$
FCR	$1.28^{a}\pm0.007$	$1.27^{a}\pm0.013$	$1.26^{a}\pm0.001$	$1.22^{b}\pm0.007$	$1.18^{c}\pm0.006$
K-value	$1.23^{b}\pm0.02$	$1.24^{b}\pm0.01$	$1.26^{b}\pm0.02$	$1.30^{ab}\pm0.01$	$1.34^{a}\pm0.03$
PER	$1.27^{b}\pm0.04$	$1.23^{b}\pm0.02$	$1.29^{b}\pm0.005$	$1.39^{a}\pm0.008$	$1.46^{a}\pm0.01$
Survival (%)	$96.66^{a}\pm3.33$	$96.66^{a}\pm3.33$	$93.33^{a}\pm3.33$	$93.33^{a}\pm3.33$	$96.66^{a}\pm3.33$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly ($P \leq 0.05$)

Effect of molasses-fermented water hyacinth feed on body composition of fish

The results of proximate body composition of fish before and after the experiment are presented in Table 4. There were marked differences between body composition of common carp fish as results indicated that fermented water hyacinth supplementation improved flesh quality in terms of total protein and total lipid content ($P \leq 0.05$), as higher flesh protein and lipid content was recorded in fish fed on FWH supplemented feeds. This contrasts the findings of El-Sayed [12]who found that body composition of Nile tilapia fed on the tests diets showed no significant differences in the protein, and lipid content, while it was on agreement in ash content of fish. This may be because of the different feeding habit of common carp and Nile tilapia and comparatively longer duration of the experiment in the present study.

In the present study, fish fed on diet without FWH had higher level of carbohydrates in fish flesh compared to fish feed on FWH supplemented diets. FWH supplementation in common carp feed, had no significant effect on the ash content in the flesh. Whereas, FWH supplementation in common carp feed reduced the moisture content significantly with higher percentage of moisture content found in Diet1 ($P \leq 0.05$).

Table 4: Initial and final body composition of common carp (on wet weight basis) in different treatments at the termination of experiment*.

Parameters	Initial	Treatments (with different levels of FWH)				
		D1	D2	D3	D4	D5
Protein	13.02±0.36	$13.32^{d}\pm0.36$	$14.41^{bc}\pm0.37$	$14.15^{cd}\pm 0.35$	$15.21^{b}\pm 0.17$	$16.72^{a}\pm 0.27$
Lipid	1.78± 0.02	$1.88^{b}\pm 0.02$	$1.97^{b}\pm 0.05$	$2.18^{a}\pm 0.04$	$2.14^{a}\pm 0.01$	$2.18^{a}\pm 0.03$
Carbohydrate	2.09± 0.04	$2.19^{ab}\pm 0.04$	$2.30^{a}\pm 0.01$	$2.22^{a}\pm 0.04$	$1.94^{b}\pm 0.04$	$2.06^{ab}\pm 0.05$
Ash	1.75± 0.03	$1.65^{a}\pm 0.03$	$1.64^{a}\pm 0.04$	$1.59^{a}\pm 0.02$	$1.56^{a}\pm 0.02$	$1.71^{a}\pm 0.03$
Moisture	81.36± 0.32	$80.96^{a}\pm 0.32$	$79.68^{b}\pm 0.35$	$79.86^{b}\pm 0.30$	$79.15^{b}\pm 0.13$	$77.33^{c}\pm 0.41$

*Values (Mean±S.E.) with different alphabetical superscripts in a row differ significantly ($P \leq 0.05$)

The FWH supplementation in common carp diets produced higher growth and better body composition than the control diet. Fermented water hyacinth containing diets might have liberated nutrients resulted in better growth performance in FWH treatments compared to control (D1) as it contain more nutrients than control diet.

Conclusion

Better growth performance is always the aim of aquaculture farmers. According to results from the present study, fermented water hyacinth (FWH) can partially replace conventional energy sources (rice bran) in the compound diet of common carp. Significantly high growth and improved flesh quality were recorded in common carp fed with diet D5 (40% of RB replacement with fermented WH i.e. 16% of total feed) for 120 days. It also suggested that fermented water hyacinth showed impact on growth of fish at high levels (>30%) compared to low levels (<20%). Molasses fermented WH can be replaced with rice bran up to 40 % (of RB) in the diets for common carp fingerlings. In developing countries like India, fish farmers are unable to buy costly fish feed, fermented water hyacinth form an abundant alternative natural un-utilized resource for less expensive fish feed and higher fish yield to enhance farmer's income.

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