Utilization of Fruit Processing Wastes in the Diet of *Labeo rohita* Fingerling

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ABSTRACT : A feeding trial was conducted for 60 days to study the utilization of fruits processing wastes as feed ingredient in the diet of *Labeo rohita* fingerlings. One hundred and sixty fingerlings (av. body weight, 1.65 g±0.03) were equally distributed in four experimental groups having 4 replicates each. Four different experimental diets were prepared by replacing wheat flour and rice bran with either orange (T2) (*Citrus quarantium*), pineapple (T3) (*Ananas* spp. and *Pseudananas* spp.) or sweet lime (T4) (*Citrus sinensis*) wastes to the basal diet along with the control (T1, without any fruit wastes) keeping the CP level at around 40%. The water quality parameters like DO, CO_2, pH, total alkalinity, total hardness, ammonia and water temperature were recorded within the optimum range. The diet containing 25% pineapple wastes (T3) showed significantly higher growth in terms of SGR (1.50), FCR (2.09) and PER (1.19) than the other groups. However, growth of T4 and T2 groups were not significantly different than the control group (T1). Protease activity (17.17 unit/mg protein), protein digestibility (91.57%) and carbohydrate digestibility (41.62%) were not significantly different among the different groups. Survival of the fingerlings were not significantly different among the experimental groups. It concludes that waste of orange, pineapple and sweet lime can be used at 25% level as a substitute of wheat flour and rice bran in the diet of *Labeo rohita*. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1661-1665)***

Key Words : Fruit Waste, *Labeo rohita*, Orange, Pineapple, Sweet Lime

INTRODUCTION

Carps are the commercially cultured fresh water fishes in Asia. In India *Catla catla* (Ham Buch), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) are highly preferred fishes. The culture practices that are in operation for these species are mostly semi-intensive with varying levels of supplemental feeding. Although the technique of carp culture has been standardized to some extent in India, there remains much scope for economic production through better management practices and utilization of unconventional feeds by replacing the conventional ingredients.

Fruit processing wastes and vegetable wastes are the potential source of energy in urban areas, which should be exploited to use as ingredients in fish feed. In India, over 35 million tones of fruits and vegetables are processed annually and this resulted in about 10 million tones of wastes (Maheswari et al., 1984). This wastes from fruit processing operation constitutes a large untapped source of energy and proteins. Most of these wastes are merely dump in fields, which causes pollution. Possible uses of these wastes in animal feed preparation have been suggested by some workers (Patel et al., 1972). Utilization of these huge wastes generally escapes the attention of animal nutritionist, especially in case of fish feed. Very little emphasis has been given to the use of this fruit-processing wastes, which is very cheap and easily available, but somewhat high in fibre. But some fishes mainly herbivorous fishes can utilize this wastes, though carnivorous fish may not. Moreover, undigested or partially digested fibre can also enhance the pond fertilization. In view of above, this experiment was carried out on the fingerlings of *Labeo rohita* (Ham.) to study the utilization of different fruit processing wastes as a feed ingredient or a substitute of conventional ingredients in the diet.

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MATERIALS AND METHODS

Experimental animal and experimental design

The experiment was conducted over a period of 60 days at Central Institute of Fisheries Education, Mumbai, India. The fingerlings of *Labeo rohita* (Ham.) employed in the present experiment were obtained from Khapoli Fish Seed Farm, Maharashtra. The experiment was set up in four distinct experimental groups, each group having four replicates, in 16 uniform size plastic tubs (50 L capacity each). Each of the tub was stocked with 10 fingerlings (average body weight 1.65 g±0.03) and arranged in four rows, following a completely randomized design (CRD). Round the clock aeration was provided to all the tubs, with a 2 HP air blower. Experimental tubs were cleaned manually by siphoning all the water along with faecal matter and left over feed daily. The siphoned water was replaced by an equal volume of fresh chlorine free borewell water.

Sample preparation from fruit-processing wastes

Fruit-processing wastes are the residues of fruits that are obtained after extracting the juice. For the experiment, three

Table 1. Composition of experimental diets (% DM basis)

Ingredients	Diets			
	T1	T2	T3	T4
Fish meal	32	32	32	32
Groundnut cake	38	38	38	38
Wheat flour	15	-	-	-
Rice bran	10	-	-	-
Orange waste	-	25	-	-
Pineapple waste	-	-	25	-
Sweet lime waste	-	-	-	25
Cod liver oil	1	1	1	1
Sunflower oil	1	1	1	1
CMC[1]	1	1	1	1
Vitamin-min mix.[2]	2	2	2	2
Total	100	100	100	100

[1] Carboxy methyl cellulose (Sdfine chemical ltd., India)

[2] Vitamin and mineral mix (Suplevite-M, Sarabhai Chemicals, Baroda, India); a 2.5 kg pack contained vitamin A, 5,000,000 IU; vit D_3, 1,000,000 IU; vit B_2, 2 g; vit E, 750 IU; vit K, 1 g; cal.pantothenate, 2.5 g; nicotinamide, 10 g; vitamin B_{12}, 6 g; choline chloride 150 g; calcium, 750 g; manganese, 27.5g; iodine, 1 g; iron, 7.5 g; zinc, 15 g; copper, 2 g; cobalt, 0.45 g; vit C, 300 mg/ kg diet (Celein tablets.Glaxo India ltd).

different types of fruit wastes (which are easily available almost through out the year) were collected from several juice centers of Mumbai. The fruits were orange (*Cirtus qurantium*), pineapple (*Ananas* spp. and *Pseudananas* spp.) and sweet lime (*Citrus sinensis*). About 2 kg of each fruit wastes were collected and sundried for one week continuously. After one week, it was oven dried and then pulverized to make into powder form of size 250 μ.

Feed preparation

All the ingredients were analysed for their proximate composition (Table 2) before preparing the feed. The ingredients were same for all the groups, except orange waste in T2 group, pineapple wastes in T3 group and sweet lime wastes in T4 group, whereas T1 was the control group without any fruit waste. All the ingredients (Table 1) were thoroughly mixed along with required amount of water and kept for one hour in a air tight polythene bag for conditioning after which it was passed through an extruder having a die of 2 mm size (Twin Screw Extruder, Basic Technology Private Ltd., Calcutta-12) at barrel screw speed of 430 rpm, feeding rate of 90 rpm, barrel temperature of 95°C and cutter speed of 1100 rpm. Pellets thus obtained were dried at 60°C in an oven and put in airtight container till their use.

Feeding trial

Initially feed was given at 5% of the total biomass for the 15 days and subsequently feeding rate was adjusted by reducing 1% at every fortnight interval till the end of the experiment. Daily ration was divided into two equal parts, one part was given in the morning hours at 9 am, and the other part in the evening hours at 9 pm.

Water quality parameters, viz. water temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness were recorded weekly once. Total ammonia were recorded on alternate days (APHA, 1985). Body weight was taken at 15 days of interval to assess the growth of the fish in terms of specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU).

Protease activity and *in vitro* digestibility

Protease activity of different experimental fish diets was assayed by Peterson (1977) method and calculated as follows:

$$\text{Protease activity (units/ml)} = C \times 1.000 / t \times V$$

where, C=μg/ml of casein hydrolysed

t =Duration of incubation

V=volume of enzyme (μg)

To assay protein digestibility of different experimental diets, the following reaction mixture were set up (Rick ,1974)

i) Casein sample: 0.18 ml 1% casein+0.7 ml phosphate buffer+0.02 ml enzyme (homogenate of haepatopancrease)

Table 2. Proximate composition of the feed ingredients and experimental diets (% DM)

Ingredients	Moisture	CP	EE	CF	Ash	NFE*
Fish meal	4.85	66.00	7.01	2.9	11.55	12.54
Groundnut oil cake	5.17	42.00	5.51	6.61	5.65	40.23
Wheat flour	5.55	12.05	3.50	7.91	4.09	72.50
Rice bran	5.68	13.01	1.78	16.01	12.05	57.15
Orange waste	5.49	11.87	5.06	13.19	5.86	64.02
Sweet lime waste	5.51	10.62	5.50	13.45	6.01	64.42
Pineapple waste	5.61	11.98	4.59	14.25	5.21	63.97
Experimental diets						
T1	5.12	40.25	6.59	6.68	9.76	36.72
T2	4.96	40.16	7.10	7.21	9.55	35.98
T3	5.11	40.34	7.02	7.43	9.35	35.86
T4	5.23	39.99	7.13	7.34	9.61	35.93

* Crude protein, fat, crude fibre and ash were analysed on a dry matter basis; NFE (%) was calculated as (100-%CP-%EE-%CF-%Ash).

Table 3. Growth performances (mean ±SE) of different experimental groups

Parameters	Experimental groups			
	T1	T2	T3	T4
Initial B.wt (g)	1.66^{a}±0.02	1.66±0.01	1.63±0.01	1.64±0.02
Final B.wt (g)	3.90±002	3.89±0.02	4.02±0.06	3.81±0.02
Weight gain%	135.75^{a}±1.99	134.52^{a}±0.88	145.32^{b}±3.48	131.51^{a}±1.92
SGR	1.43^{a}±0.01	1.42^{a}±0.01	1.50^{b}±0.02	1.40^{a}±0.01
Feed intake (g)	5.00±0.04	4.96±0.03	5.01±0.03	4.84±0.02
FCR	2.20^{a}±0.03	2.22^{a}±0.02	2.09^{b}±0.03	2.23^{a}±0.03
PER	1.13^{a}±0.02	1.13^{a}±0.01	1.19^{b}±0.02	1.12^{a}±0.01
NPU	17.15^{a}±0.90	16.71^{a}±0.65	17.87^{a}±0.48	15.71^{b}±0.58
Survival (%)	100.00±0.00	100.00±0.00	100.00±0.00	97.5±2.89

Mean values with same superscript in the row are not statistically significant ($p>0.05$).

SGR: [(in final B.wt-in initial B.wt.)/60 days]×100. FCR: feed given (g)/weight gain (g).

PER: weight gain (g)/protein intake (g). NPU (%): (final fish protein %×body weight)-(initial fish protein %×body weight)/protein intake×100.

ii) Feed sample: 18 mg feed+0.88 ml buffer+0.02 ml enzyme
iii) Casein blank: 0.18 ml casein+0.72 ml buffer
iv) Feed blank: 18 mg feed+0.90 ml buffer
v) Enzyme blank: 0.88 ml buffer+0.02 ml enzyme

All the tubes were incubated for 30 min at 37°C in a shaker water bath. After incubation 0.4 ml of 10% chilled TCA solution was added to all the tubes, mixed well and allowed to stand for 30 min in ice. After complete precipitation all the tubes were centrifused at 10,000 rpm for 10 min. The supernatant was collected and double amount of 0.5 N NaOH was added with continuous shaking. Finally 1.5 ml of diluted Folin's reagent was added and the absorbance was measured at 691 nm against a tyrosine standard after 10 min. The amount of tyrosine released due to the hydrolysis of the substrate by the enzyme was obtained from the standard curve, which is directly correlated with the digestibility of the substrate.

To assay carbohydrate digestibility of different experimental diets, the following reaction mixture were set up (Rick and Stegbauer, 1974)

i) Starch sample: 0.4ml 1% starch+1.56 ml phosphate buffer+0.04 ml enzyme (homogenate of haepatopancrease)
ii) Feed sample: 40mg feed+1.96 ml buffer+0.04 ml enzyme
iii) Starch blank: 0.4 ml 1% starch+1.60 ml buffer
iv) Feed blank: 40 mg feed+2.0 ml buffer
v) Enzyme blank: 1.99 ml buffer + 0.04 ml enzyme

All the tubes were incubated for 30 min at 37°C in a shaker water bath. After incubation equal volume of DNS reagent was added to all the tubes and heated for 5 min in boiling water followed by cooling in chilled water for 30 min. All the tubes were centrifused at 3,000 rpm for 10 min. The supernatant was collected and the absorbance was measured at 546 nm against a maltose standard. The rate of hydrolysis of carbohydrate in the feed was than compared with that of starch after blank correction, which is an index of digestibility.

In vitro protein and carbohydrate digestibility of feed was determined by using the method given by and. respectively.

Bio-chemical analysis of feed and tissues

Feed and feed ingredients were analysed for the proximate contents viz., crude protein, ether extract, ash, crude fibre and NFE content as per the standard methods of AOAC (1980). Similarly tissues were analysed at the end of the experiment.

Statistical analysis

The data were statistically processed by one-way analysis of variance and the differences of the means were determined at 5% level using Duncan Multiple Range Test (DMRT) as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Physico-chemical parameters such as water temperature (22-26°C), water pH (7.2-7.5), dissolved oxygen (6.9-7.5 ppm), free carbon dioxide (0 ppm), total alkalinity (180-204 ppm), total hardness (180-195 ppm) and ammonia (0.50-0.55ppm) were found within the acceptable limit (Jhingran, 1992).

Growth parameter

Highest SGR (Table 3) was recorded in T3 group that was significantly different ($p<0.05$) than all other groups. However, growth rate of control group was not significantly different than the T2 and T4 groups. Better growth rate recorded in T3 group may be due to better utilization of pineapple waste present in this diet. Though no literature is available in this regard, it may correlate with the presence of a proteolytic enzyme `bromelain` in pineapple, which might be the factor for better utilization of protein (Murachi and Takahashi, 1970).

FCR of the different groups showed the same trend as

Table 4. Proximate body composition of *Labeo rohita* before and after the experiment (% Dry matter basis)

Diets	Moisture	Total carbohydrate	Protein	Fat	Ash
Initial	80.12±3.2	5.71±0.52	68.16±2.2	9.68±0.31	16.45±2.82
T1	78.26±2.2	6.19±0.12	67.16±5.12	8.00±0.31	18.65±2.26
T2	78.73±4.5	6.3±0.14	67.32±2.43	8.56±0.33	17.82±1.92
T3	78.59±3.2	6.8±0.16	67.86±4.44	7.94±0.46	17.42±1.25
T4	79.63±5.4	4.3±0.2	67.89±2.89	9.08±0.29	18.7±2.01

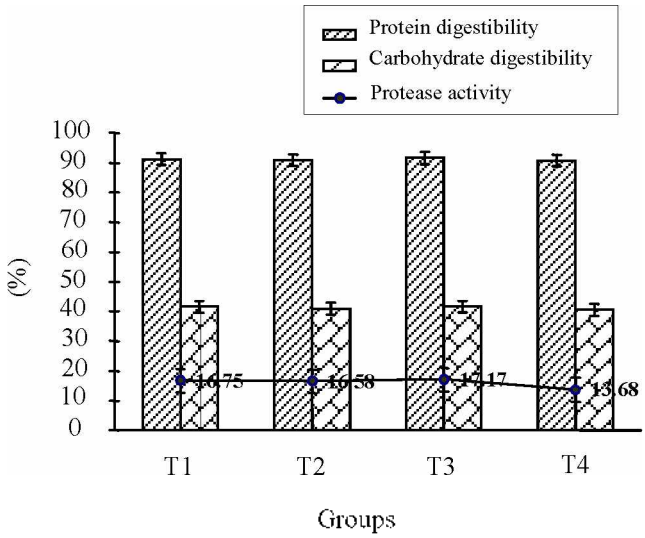

Figure 1. Protein digestility, carbohydrate digestibility and protease activity (unit/mg protein) of different experimental groups.

that of other parameters. Better efficiency was found in T3 group, may be due to the presence of 'bromelain' as discussed earlier. However, sweet lime and orange wastes incorporated in the diet showed similar FCR with control group indicating similar nutritional value of fruit processing wastes as that of rice bran and wheat bran.

Significantly highest ($p<0.05$) PER was found in T3 group containing pineapple waste, whereas lowest was recorded in T4 group containing sweet lime waste. This may be due to the higher amount of protein present in the pineapple waste and lowest protein in the sweet lime wastes.

Protease assay

The mean specific protease activity of different experimental groups (Figure 1) were found to be 16.75, 16.58, 17.17 and 13.68 unit/mg protein for T1, T2, T3 and T4 groups respectively. Though highest proteolytic activity was found in T3 group, it was not statistically significant from other groups. Since no information is available, it is difficult to compare the present results with other data.

***In vitro* protein and carbohydrate digestibility**

The apparent protein digestibility (%) of different experimental diets were found 91.21, 90.87, 91.57 and 90.72 for T1, T2, T3 and T4 groups respectively which were not statistically significant. The present result is in agreement with the previous report of Jafri and Anwar (1995) that plant proteins are very efficiently digested by Indian Major Carp. Similarly carbohydrate digestibility (%) found 41.61, 40.98, 41.62 and 40.57 for T1, T2, T3 and T4 respectively, were also not significantly different.

Survival

Survival of all the groups ranged between 97-100 %, which were statistically insignificant ($p>0.05$), indicates that any of the fruit processing wastes did not have any adverse/detrimental effect on health. Body tissue content of the fingerlings was least affected due to feeding of fruit processing wastes (Table 4).

CONCLUSION

From the above experiment it may conclude that vast amount of fruit processing wastes available have the potential to be used as a source of energy by replacing conventional ingredients like rice bran and wheat flour. Pineapple wastes have a significantly higher growth promoting effect in *labeo rohita* fingerlings compare to sweet lime and orange wastes. Fruit processing waste can be safely used at a level of 25% in the diet of *Labeo rohita* fingerlings.

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