

Effect of co-dried silage from fish market waste as substitute for fish meal on the growth of the Indian major carp *Labeo rohita* (Hamilton, 1822) fingerlings

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ABSTRACT

Silage prepared by acid digestion of wastes from fish market and co-dried with rice bran was used as ingredient in experimental diet for rohu (*Labeo rohita*) fingerlings as source of animal protein by partial or complete replacement of fish meal. Four isonitrogenous and isocaloric diets were formulated replacing 0, 50, 75 and 100% of fish meal (FM) by fish silage co-dried with rice bran (CFS). The results of the study for six weeks indicated that, the control group that was fed with feed (0% replacement) made from FM showed highest weight increment with lower feed conversion ratio (FCR) and higher specific growth rate followed by the group fed diet made with 50% replacement of fish meal by CFS. The specific growth rate and feed conversion ratio between 100% and 50% FM-based diets were not significantly different ($p < 0.05$), whereas it was significantly low ($p > 0.05$) with 0% and 25% FM-based diets. The replacement of 50% FM by CFS in *L. rohita* fingerlings diets did not significantly affect the growth and feed utilisation parameters. Whereas, the higher replacing levels of FM by CFS (75 or 100%) in diets significantly ($p < 0.05$) reduced the growth and feed utilisation parameters.

Keywords: Animal protein, Feed conversion ratio, Fish silage, *Labeo rohita*, Specific growth rate

Introduction

The cost of feed represents the largest expense in fish farming. Fish meal (FM) is the preferred dietary protein source for many farmed fish and shrimp species, and is valued for its amino acid balance, vitamin content, palatability and unidentified growth factors (Tacon, 1993). However, the production of fish meal is capital and energy-intensive; hence it is not manufactured in many developing countries, where it is imported at prohibitive costs which is uneconomical to feed manufacturers. Periodic scarcity and high prices of fish meal have encouraged the evaluation of alternative feedstuff as source of protein. A large number of studies have been carried out in recent years on alternative ingredients that can be used instead of fish meal, which is the primary ingredient in formulated fish feed. Several studies have been carried out to identify alternative protein sources which would allow for a reduction in the cost of feed (Cheng *et al.*, 2003; Portz and Cyrino, 2004). Particular attention has been given to the use of waste products from the fish processing industry for production of both fish meal and fish silage. Work on fish silage as a fish feed ingredient started in 1980s with Hardy *et al.* (1984), who reported low growth in salmon. This is also considered as an eco-friendly procedure because it involves recycling of organic material that is considered to be usable waste. Wassef (2003) reported that fish silage can replace fish meal completely in tilapia. Fermented fish silage was tested by Hanafy and Ibrahim (2004) in tilapia and African catfish, who reported that 25 – 50% fish meal can be replaced. The use of fish processing waste by way of making fish silage for the feed industry has the potential

of reducing the cost of producing fish feed by approximately 15 to 20% (Yildirim *et al.*, 1999; Gullu and Guzel, 2003; Turker and Buyukhatipoglu, 2006). Vidotti *et al.* (2003) investigated the efficiency of fish silage in replacing fish meal in pacu diets and their results indicated that 75% fish meal can be replaced.

Fish silage is defined as a liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic acid-producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish (FAO, 2007). The advantages of making fish silage from fish processing waste include reduced cost of feed, elimination of storage costs for fresh fish feed, and production of feed that is not only easy for fish to digest but is also close in nutritional value to natural feed (Raa and Gildberg, 1982; Tatterson and Windsor, 1982; Raa *et al.*, 1983). Fish-silage is a liquid product and to ensure an optimal use in animal feeds its water content has to be reduced. A process for reducing the water content is co-drying (Lopez, 1990; Dong *et al.*, 1993), whereby dry feedstuffs (fillers) are added to wet fish silage to absorb solubilised proteins and some of the moisture. Co-drying has been reported to improve the nutritional properties of the blends and to change the physical properties, which facilitates their preparation as conventional feedstuffs (Stone *et al.*, 1984). Cost and cash-flow analyses described by Aagard *et al.* (1980) and Balogun and Oyeyemi (1986) have demonstrated that co-dried fish-silage-based feeds provide an economic return better than that obtained with dry fish-meal-based feeds on a small-scale production. It has been reported that fish silage blended with fillers can also replace fish meal since diets with fish ensilage were nutritionally adequate, particularly

in that the essential amino acid content, and the protein contents were highly digestible; and also there were minimal losses of nutrients because the diets were water-stable and rapidly consumed (Fagbenro and Jauncey, 1995). On the other hand, the formulation of fish feeds necessitates the inclusion of high protein feedstuffs as filler for wet fish silage.

The objective of this study was to make use of waste from the fish markets and to provide the fish feed industry with an ingredient for feed that is less expensive than fish meal. This will also make a contribution to protect the environment by utilising organic waste that could pollute the environment. The physical properties of the co-dried silage diets and their effects on growth performance of fingerlings of Indian major carp, *Labeo rohita* was investigated.

Materials and methods

Preparation of co-dried silage (CFS)

Entrails of fish collected from the Agartala fish market was brought to the laboratory where these were washed and chopped with knife. Fish market wastes comprised mainly of entrails of rohu (*Labeo rohita*), catla (*Catla catla*) and to some extent that of common carp (*Cyprinus carpio*). Acid silage was prepared by thoroughly mixing the chopped fish entrails with 90% formic acid (2%, w/w) and concentrated sulphuric acid (2%, w/w). Sulphuric acid was employed to accelerate hydrolytic activity of the enzymes present in the fish. Formic acid was used to inhibit microbial growth. During the initial liquefaction period, the silage was stored in covered 5 l PET jars and stirred occasionally. Fat was regularly removed as floating scums during ensilation in order to prevent excessive autoxidation. The room temperature during liquefaction ranged from 26 to 31 °C. To prevent mould growth, the surface of the silage was sprayed with 1% (v/w) potassium sorbate solution. Following complete liquefaction, after five days, the liquid silage was neutralised with sodium hydroxide. Liquid silage was co-dried with rice bran (1:1, dry weight basis) and oven-dried at 45 °C for 48 h following the method suggested by Goddard and Al-Yahyai (2001).

Experimental diets

Four diets were formulated (Table 1) to replace 0 (D1), 50 (D2), 75 (D3) and 100% (D4) of FM by CFS (based on protein content of FM and CFS as 50% and 16% respectively). All diets were formulated to be isonitrogenous (27% protein) and isocaloric (300 kcal ME 100 g diet⁻¹). Co-dried fish silage and the basic ingredients were finely ground and mixed thoroughly in domestic grinder. The finely mixed ingredients were made in to a dough (by adding water at 30% of total ingredient weight) of good consistency and were steam cooked for 10 min with intermediate stirring. It was then passed through a hand pelletizer (fitted with 3 mm die plates) to obtain pellets of 3 mm dia. The wet pellets were oven-dried at 45 °C for 18 h (to <10% moisture) and subsequently broken into very small size (<3 mm dia), this being the particle size suitable for the experimental fish. Dry pellets were kept in sealed polyethylene bags and stored in

a dry place. pH and water stability of the diets were estimated in triplicate, as described below.

pH: Distilled water was added to 5 g samples of milled diets and made to 50 ml, and the mixture was centrifuged at 8000 rpm for 10 min using centrifuge (Remi, India). The supernatant was used for pH determination.

Water stability: Triplicated 50 g samples of pre-screened pellet crumbles of each diet were placed on a plastic sieve and slowly immersed in a 40 l glass aquarium containing deionised water at 27 °C for 10 min (Fagbenro and Jauncey, 1995). The sieve was removed and the crumbles were allowed to drain for 1 min, oven-dried at 105 °C for 2 h, cooled in a desiccator and reweighed. Water stability was calculated as the percentage difference in sample weight after re-weighing and expressed as percentage loss of dry matter (% LDM).

Table 1. Ingredients composition (%) of the experimental diets*

Ingredients	Experimental diets (g 1000 g ⁻¹)			
	D1	D2	D3	D4
Fish meal (FM)	100	50	25	0
Co-dried silage (CFS)	0	150	250	300
Mustard oil cake	200	200	200	200
Soyabean flour	200	200	200	200
Wheat bran	300	200	125	100
Rice flour (binder)	198	198	198	198
Mineral mix	01	01	01	01
Vitamin mix	01	01	01	01

* D1 = (FM: CFS = 100: 0), D2 = (FM: CFS = 50: 50)

D3 = (FM: CFS = 25: 75), D4 = (FM: CFS = 0: 100)

Experimental design

Fingerlings of *Labeo rohita* collected from the fish farm of the College of Fisheries, Lembucherra were brought to the laboratory and acclimatised in a plastic pool environment over a period of seven days. During the acclimatisation period, the fish were fed with commercial fish feed containing 25% protein. Compressed air was continuously supplied through air stones to all the rearing tanks. The feeding trial was carried out for a period of six weeks in plastic pools (1.5 m dia with water depth 0.4 m) at a stocking density of 50 rohu fingerlings per pool with two replications. Feeding was done twice daily in equal meals @4% of fish body weight, throughout the experimental period. Everyday morning the bottom of the pool was cleaned and 50% water was exchanged at weekly intervals. Water samples were analysed weekly for temperature, dissolved oxygen, pH and ammonia-N. Weekly samplings were conducted to weigh the fish from each tank and the quantity of feed given was adjusted accordingly.

Chemical analyses

Moisture, fat, protein, fibre and ash content of fish market wastes, liquid silage, co-dried silage and experimental

diets were analysed in triplicate samples according to AOAC (2005) methods. Moisture was determined after oven-drying at 105 °C to constant weight and ash by incinerating the dried residue for 24 h at 550 °C in a muffle furnace. Total nitrogen (N) was determined by the micro-Kjeldahl procedure and crude protein was estimated as N X 6.25. Crude lipid was determined after extraction of dried samples with petroleum ether. Crude fibre was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H₂SO₄ and 1.25% NaOH.

Growth performance and feed utilisation parameters

Growth performance and feed utilisation parameters were estimated using the following equations:

Mean weight gain = Mean final weight (g) – Mean initial weight (g)

$$\text{Weight gain percent (WG \%)} = \frac{(W_f - W_i) \times 100}{W_i}$$

$$\text{Specific growth rate (SGR, \% per day)} = \frac{(L_n W_f - L_n W_i) \times 100}{T_f - T_i}$$

where, W_i = initial average weight of experimental fish

W_f = Final average weight of experimental fish

T_i = Initial number of fish

T_f = Final number of fish

L_n = Natural logarithm

Feed conversion ratio (FCR) = Feed ingested (g) / Weight gain (g)

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 11.0 for windows). Analysis of variance (one way ANOVA) was performed to determine the differences between means from diet treatments (p=0.05). The tests for differences were done by using Duncan's Multiple Comparison Test. Significance of differences was defined at p<0.05.

Results and discussion

Properties of fish wastes, silage, co-dried silage and diets

Biochemical composition of fish market wastes, silage and silage co-dried with rice bran is presented in Table 2. From the proximate analysis of viscera it was found that it had 68.42% moisture, 15.59% crude protein, 12.81% fat and 2.97% ash. Fresh viscera from *Catla catla* consisted of 60.6% moisture, 27.8% fat, 8.5% protein and 3.2% ash (Bhaskar *et al.*, 2007). Factors affecting the proximate composition of the fresh material include age, sex, body weight, season, feeding aspects *etc.* (Sikorski and Kolakowski, 2000).

The silage production process is simple, practical and economical, not requiring expensive equipments and procedures, such as those used in the production of fish meal. Acidification and storage of visceral mass caused minor variations in moisture, protein and ash contents. The biochemical composition of silage estimated (average) in this study was: moisture 69.38%, crude

Table 2. Biochemical composition of silage and co-dried silage*

Parameter	Fish market wastes	Silage	Co-dried silage
Moisture (%)	68.42 ± 2.76	69.38 ± 2.87	4.06 ± 1.89
Crude protein (%)	15.59 ± 1.49	14.33 ± 0.94	16.1 ± 1.42
Ash (%)	2.97 ± 0.38	4.06 ± 1.16	3.33 ± 0.48
Lipid (%)	12.81 ± 1.57	2.47 ± 0.88	5.58 ± 1.11

*Values are mean ± SD, n=3

protein 14.33%, lipid 2.47% and ash 4.06%. Alwan *et al.* (1993) reported that the chemical composition (g 100 g⁻¹) of silage varied with the type of raw material; who recorded moisture percentage in the range, 72.6 to 80.3 (mean 76.4%); oil 0.3 to 7.1% (mean 3.8%); ash 3.5 to 7.2% (mean 5.1%); protein 14.4 to 16.1% (mean 14.7%) while studying chemical and microbiological aspects of fish market wastes. The silage had lower (p<0.05) protein content less than the fish viscera due to presence of scales, bones *etc.* in the viscera, during ensilation, and as a result ash content in the silage was found higher (p<0.05) than the viscera. Lower fat content in the silage was due to frequent removal of floating scum of fat during ensilation which otherwise, would create rancidity problems. Upon complete liquefaction after 5 days, pH of the silage reached less than 4.5, which indicated the stability of silage at ambient temperatures reported by Fagbenro and Olusoji (1997). To prevent spoilage during storage of dry feed ingredients and finished feed, it should contain less moisture (<10%), as microorganisms require unbound water to grow and levels greater than 12% can support bacterial, mold and yeast growth (Hardy and Barrows, 2002). In co-dried silage, the moisture content was 4.06% ± 1.89 which is ideal to prevent spoilage during storage.

Proximate composition of the co-dried silage indicated that the fillers (rice bran) had absorbed some of the moisture which facilitated drying, and had given satisfactory co-dried products with about 95% of dry matter. Protein and lipid content of co-dried silage was higher than liquid silage (Table 2). Almost isoproteic (protein content ~27%) feed was prepared with co-dried silage as partial or complete replacement of fish meal. Water stability (% loss of dry matter) and pH of the feed was in the range of 5.2-5.8 and 6.4-6.6 respectively. Water stability value determines the duration of availability of pellet to fish. The low water stability value (<10%) of feed observed in this study (Table 3) indicated that it was adequate when being fed to major carp in ponds and would reduce losses of nutrients through leaching as suggested by Wood *et al.* (1985) and New (1987). Co-drying of liquid fish silage with cereals has a number of advantages over the drying of whole silage. The drying time is reduced, the texture of the co-dried product when dry is softer depending on the amount of cereal added, and the nutrient density of the co-dried mixture can be adjusted to meet the requirements of feed formulation (Goddard and Al-Yahyai, 2001). Previous studies on sun-dried mixtures of fish silage and rice bran have demonstrated their nutritional value as replacements for fish meal in aquafeeds for Indian carps (Ali *et al.*, 1994) and tilapia (Goddard and Al-Yahyai, 2001, Goddard *et al.*, 2003).

Table 3. Physical properties and proximate composition of the experimental diets*

Parameters	Experimental diets (g 1000 g ⁻¹)			
	D1	D2	D3	D4
Physical properties				
pH	6.5	6.5	6.6	6.4
Water stability (% LDM)	5.8	5.6	5.3	5.2
Proximate composition (determined on dry matter basis, g 100 g ⁻¹)				
Dry matter	92.34	92.76	92.18	91.83
Ash	7.33	8.27	7.84	7.32
Crude protein	26.65	26.84	26.63	27.65
Lipid	7.56	8.21	7.94	8.63
Crude fibre	7.58	6.78	7.03	6.87
N-free extract	43.22	42.66	42.74	41.36
Estimated metabolisable energy (ME)#	305.0	309.0	306.0	310.0

*D1 = (FM: CFS = 100: 0), D2 = (FM: CFS = 50: 50) D3 = (FM: CFS = 25: 75), D4 = (FM: CFS = 0: 100)

Metabolisable energy (ME) was estimated based on the physiological values of fish: protein, 4.5 k cal g⁻¹; carbohydrate, 3.3 k cal g⁻¹; fat, 8.0 k cal g⁻¹ (Brett and Groves, 1979)

Growth study of *Labeo rohita* fingerlings

Deep tube-well water was used in this study for rearing fish. The water temperature in the experimental pools varied between 26.8 - 30.2 °C during the study period, with an average of 28.6 ± 1.3 °C (Table 4). The pH, dissolved oxygen and ammonia-N of water recorded were in the range of 7.2 - 8.1, 5.6 - 6.7 ppm and 0.01 - 0.03 ppm respectively during the experimental period. Water quality parameters recorded were found to be within the acceptable limits for fish growth and health. Fish became accustomed to the diets within the first week.

Table 4. Water quality parameters recorded in the rearing tanks during the experimental period

Water quality parameters	Range
Temperature	26.8 - 30.2 °C
Dissolved oxygen	5.6 - 6.7 ppm
pH	7.2 - 8.1
Ammonia-N	0.03 ppm

The average mean weight gain, weight gain percent, specific growth rate (SGR) and feed conversion rate (FCR) estimated are given in Table 5. Rohu fingerlings fed on diet made with fish meal (0% replacement) gained higher ($p < 0.05$) body growth, followed by diet with 50%, 75% and 100% replacement of fish meal respectively. The overall growth pattern of fingerlings also remained higher ($p < 0.05$) for 100% fish meal based diet. The specific growth rate obtained with the experimental diet with fish meal was significantly higher ($p > 0.05$) compared to the other silage-based diets, however, there was no significant difference between diets D3 and D4. A comparison of the average FCR at the end of the study revealed that the lowest value (that is, the best food utilisation) was obtained with the fish meal based diet (D1), while the highest value was found in the 100% silage-based diet (D4). The FCR values for D1 and D2 (50% silage) were found to be close to each other and not significantly different. A comparison of FCR values indicates that the control group and 50% silage group utilised the feed better than the other groups.

The superior performance of control fish group fed with the fish meal based diet (D1) is attributed to the fact that the nutritional value of FM-protein approximating almost exactly to the nutritional requirements of the experimental fish *Labeo rohita* fingerlings (Jatomea *et al.*, 2002). When 50% of FM protein was replaced by CFS protein, it did not result in any significant effect on growth parameters (mean weight gain, WG%, SGR and FCR) while the highest replacing levels (75 or 100%) significantly affected these parameters. The availability of amino acids in the co-dried silage-based diets may be low because of the destruction of amino acids as a result of the Maillard reaction (reaction between alpha-amino groups and sugar aldehyde groups). Fish silage contains free amino acids and thus becomes susceptible to the Maillard reaction which causes reduction in biological activity of lysine and other amino acids, particularly during co-drying with a feed stuff which has high carbohydrate content (Baltes, 1982). The silage might also have contained some residual sugar after liquefaction/fermentation (Kompang *et al.*, 1980), and this could have enhanced the formation of Maillard reaction products (Kies, 1981). Such a loss of essential amino acids might have also contributed to the overall growth and poor protein utilisation obtained in *L. rohita* fingerlings fed with silage-based diets. Fagbenro and Jauncey (1995) reported that fermented fish-silage co-dried with protein feedstuffs was found to be a suitable protein supplement in case of juvenile catfish (*Clarias gariepinus*) which can provide up to 50% of dietary protein without affecting feed efficiency, fish growth or health.

In the present study, the lower substitution level of FM by CFS (50%) supported better growth rates in *L. rohita* fingerlings in comparison with the higher levels (75 and 100%). Berg and Storebakken (1996) reported a similar effect for low inclusion levels (15%) using fish silage in Atlantic salmon diets. Similarly, Soltan *et al.* (2008) reported that replacing 50% of fish meal by dried fermented fish silage in diets of African cat fish (*Clarias gariepinus*), did not significantly affect the growth and feed utilisation parameters. Nwanna and Daramola (2001) found that, replacing FM by shrimp head silage in Nile tilapia diets up to 15% showed best response in terms of growth, while higher replacement levels (20, 25 or 30%) resulted in

Table 5. Stocking size and growth of *Labeo rohita* fed on experimental co-dried fish silage-based diets for six weeks

Diets	Initial weight (g) (Mean ± SD)	Final weight (g) (Mean ± SD)	Mean weight gain (g)	WG (%)	SGR (% per day)	FCR	Percent survival rate
D1	6.8 ± 0.21	13.0 ± 3.94	6.27 ^{bc}	92.7 ± 4.56 ^c	1.57 ± 0.2 ^{bc}	1.75 ^a	94.64 ± 3.87
D2	7.3 ± 0.19	13.2 ± 3.87	5.89 ^b	80.1 ± 4.33 ^b	1.40 ± 0.1 ^b	1.86 ^a	95.31 ± 3.89
D3	7.3 ± 0.19	12.8 ± 3.07	5.42 ^a	73.7 ± 5.21 ^a	1.31 ± 0.1 ^a	2.05 ^b	94.56 ± 4.16
D4	7.3 ± 0.19	12.5 ± 2.76	5.12 ^a	69.7 ± 3.87 ^a	1.26 ± 0.1 ^a	2.14 ^{bc}	95.58 ± 4.17

*Values bearing different superscripts in the same column are significantly different ($p < 0.05$)

poor growth response. In another study, Fagbenro and Jauncey (1998), observed that dried fermented fish silage has potential use as protein feedstuff in *C. gariepinus* diets. Tacon (1993) demonstrated that, abalone viscera silage can be used to replace FM in diets for abalone without significant differences in growth.

Results of the present study indicated that, replacement of 50% FM by CFS in the diets of *L. rohita* fingerlings did not significantly affect the growth and feed utilisation parameters, while the higher replacing levels of FM by CFS (75 or 100%), significantly reduced ($p < 0.05$) all the growth and feed utilisation parameters. It can be concluded that, fish meal can safely be replaced with silage from fish market wastes (co-dried with ricebran) up to 50% level in the diets of *L. rohita* fingerlings without any negative effect on the growth and feed utilisation parameters.

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