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Nutritional Evaluation of Animal Fleshing as a Fish Meal Replacer in *Labeo rohita*

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Abstract: The present study explores the unexploited application of abundantly available proteinaceous solid waste material, animal fleshing (PTSW) from tannery as a feed protein source for fish cultures. Growth profile study of *Labeo rohita* was conducted for 8 weeks to assess the effects of diets supplemented with PTSW. Experimental diets includes differentially processed PTSW (raw, chemically treated with hydrogen peroxide and fermented PTSW) while diet with fish meal alone served as the reference feed. The fish were characterized by mean final weight; percent weight gain, feed conversion ratio and survival rate. The study concluded that there were no significant differences ($p<0.05$) in the characteristics of fish cultures fed on replacement diets. Hence, replacement of fishmeal with PTSW will be a best approach to ease out solid waste disposal problem for environmentalist and also beneficial as an economic feed in aquaculture.

Key words: Proteinaceous Tannery Solid Waste (PTSW), fish feed, growth parameters, ratio, fish cultures, animal fleshing

INTRODUCTION

Leather industry which processes on an average 10,000 kg of skins day^{-1} produces 5,500 kg of solid waste day^{-1}. The pollution emission includes about 2,500 kg of tanned solid waste, 3,500 kg of non-tanned solid waste which are proteinaceous in nature and about 1000 kg of substrate that is lost in the wastewater (Maia, 1998). Fleshing is the flesh material of the limed skins generated during fleshing operation. Animal fleshings (PTSW) discharged from the tanning industry are processed by chemical/thermal process which is energy intensive and a time-consuming process (Bajza and Markovic, 1999).

This in turn creates solid waste disposal problem in India as well as emission of greenhouse gases like methane and carbon dioxide (Maire and Lipsett, 1980). Fleshing contains proteins as a major constituent which can replace fish meal as it contains essential amino acids for fish growth.

Global aquaculture production has become two fold during the last decade there by facilitating its rapid growth in animal food production (FAO, 2002). Asian countries contribute a major share of fish production (FAO, 2007) of which India is one of the leading countries in freshwater aquaculture production. In 1998, the total production of freshwater fishes was 1.7 million ton of which 87% was contributed by carps (FAO, 1999). Nutrition is the major expensive component in the aquaculture industry, representing >50% of operating costs. For more than a decade, there has been pertinent search in identifying alternatives to fishmeal for use within aqua feeds (Tacon, 1993; FAO, 2002). For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide nutritious diet for proper growth and quality protein production. The lack of quality feed leads to the failure of aquaculture to meet the challenge of narrowing the gap between fish supply and demand.

The two main constraints concerned with feeds are their price and efficacy (Ako and Tamaru, 1999). Fish feed is an expensive input both from an economic and ecological point of view. Economically as the cost of fish feed accounts for >50% (El-Sayed, 1999; De Silva, 1993) and ecologically because fish feed contains fishmeal that have high protein content are obtained from wild fish catches.

Approximately, 4-5 ton of whole fish are required to produce 1 ton of dry fishmeal. While the annual production of fish meal is stabilized at 6-7 million ton, aquaculture is expanding at a rate of 11% year^{-1} (Allan, 2004).

The best approach in feed formulation is to use high-quality feedstuffs that meet the nutritional and energy requirements of the aquaculture species. The quality of different feedstuffs is greatly dependant on the amino acid profile in their protein. Because of the increasing cost of high quality fish meal required for aqua feed and due to declining stocks of fish from capture fishery and

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competition for feed in animal husbandry, there is scarcity for good quality proteins. Therefore, numerous feed trials with locally available and cheap sources of feed ingredients have been carried out. In many tropical regions of Asia and Africa, wastes from crops, agro-industry and animal production are used according to their nutritional composition, volume and pollution risk in animal feeds including fish feed either as feed ingredients, supplementary feeds or as pond fertilizers (Ravishankar and Keshavanath, 1986; Wohlfarth and Hulata, 1987; Subosa, 1992; Tacon, 1993).

Animal proteins are preferred due to their superior nutritional qualities such as protein content and amino acid profile. Because of the continuing demand of seafood, aquaculture production intensifies animal fleshing, generated from pre-tanning operations constitute about 50-60% among tannery solid wastes that are highest in protein (50.9%) and collagen (3.5%) content. The best way to utilize them is to recover, the value added by-products that may have commercial value (Raju *et al*., 1997; Bajza and Vrucek, 2001). Taylor was successful in isolating protein products from chrome shavings using protease. The present study focuses on, the formulation of aqua feed with chemically treated PTSW and fermented metabolites as a sole source of protein and to investigate the growth responses based on this diet. Worldwide awareness to reduce the pollution load and wise solid waste management was the motivating factor to carry out this study. The inclination towards this study was to evaluate the suitability of animal fleshing as a novel ingredient in diets of *Labeo rohita*. Hence, the focal theme of the present study was to investigate the effect of inclusion of PTSW as protein source in the formulation of fish feed and its effect on growth and survival.

MATERIALS AND METHODS

Laboratory feeding trial: *Labeo rohita* of initial weight 5±0.93 g (mean±SD) was obtained from a private fish farm, Chennai, India. The fish was transferred into 200 L fibre tank. They were acclimatized for 15 days in the tanks with control feed before, conducting the experiment. The tanks were aligned and kept under natural photoperiod (12 h light: 12 h dark). The fish were fed with a standard diet at 3% of their body weight in two divided doses daily during the experiment (6 days a week for 8 weeks). Triplicate samples of fish from each tank were collected every 2 weeks and collectively weighed and the ration was adjusted each time accordingly.Water quality parameters were checked every week in accordance with the standards of the American Public Health Association (APHA\AWWA\WEF, 1998). Water quality parameters were maintained uniform throughout the experimental period. pH was in the range 6.6-7.9, temperature, 26-30°C; alkalinity, 112-117.5 mg L^{-1}; DO, 6.7-7.2; chloride, 3.03-3. About 13 mg L^{-1}; total hardness, 105-109 mg L^{-1}; sulphates, 3.2-3.

About 4 mg L^{-1}; nitrates, 12.2-17.9 mg L^{-1}; ammonia-N, 1.84-5.2 mg L^{-1} and free carbon dioxide, 2-8 mg L^{-1}. Chemicals and reagents were purchased from Merck, India.

Diet formulation and preparation: Fish feed preparation involved extruding a mixture of proteinaceous tannery solid wastes and feed additives into usable form. The animal fleshing was collected from a leather industry processing raw cow skins to finished leather located in Chennai, Tamil Nadu. The experimental diets were formulated to be isonitrogenous and isolipidic. The processing of PTSW material involved either chemical treatment with H_2O_2 or fermentation with bacterial strain isolated from candidate species or else used in the native form (raw AF).

The animal fleshing was treated with hydrogen peroxide for 1 h. About 3% hydrogen peroxide solution was added to 200 L tank. Each 1 mL added 30 L^{-1} will increase total peroxide levels by 1 mg L^{-1}. About 15 mg L^{-1} for 48 h^{-1} is meant to be a fish safe concentration. In the experimental feed, PTSW and in the control feed fish meal serves as the sole protein source. Sunflower oil and groundnut oil cake were used as lipid sources (10%). Wheat flour and rice bran were used as carbohydrate sources (approximately 14%). Appropriate quantities of dry ingredients were weighed, ground and mixed in a food processor. The dough was extruded to about 3 mm diameter and dried overnight in a hot air oven at 50°C. The dried product was stored in freezer at -20°C until further use (Table 1). The quantity of feed given was readjusted every 15th day after weighing the fish. To

Table 1: Amino acid composition of PTSW

Amino acid	Composition (mg g^{-1})
Glycine	0.255±0.005
Serine	0.088±0.008
Histidine	0.407±0.012
Threonine	0.171±0.040
Alanine	0.044±0.017
Arginine	0.069±0.012
Tyrosine	0.179±0.022
Valine	0.097±0.200
Methionine	0.065±0.015
Phenylalanine	0.038±0.004
Isoleucine	0.009±0.001
Leucine	0.065±0.002
Lysine	0.110±0.200

Table 2: Composition of aqua feed formulation

Experimental diets	Rice bran (g)	Wheat flour (g)	Oil cake (g)	Puffed rice (g)	Mono sodium glutamate (g)	Sunflower oil (g)	Protein source (35 g)
RF	20	20	10	13	1	1	Raw
Control	20	20	10	13	1	1	Fish meal
CF	20	20	10	13	1	1	(H_2O_2 treated PTSW)
HF	20	20	10	13	1	1	(Fermented PTSW)

determine the feed consumption any leftover feed was collected 6 h after each feeding and weighed after cooker drying.

Proximate analysis: The proximate analysis of fish feed were carried out in duplicates. In feed ingredients crude protein (NX 6.25) was determined by Kjeldahl method after acid digestion, moisture content gravimetrically after drying for 4 h at 105°C, ash content after incineration in a muffle furnace at 550°C for 16 h, lipid was extracted by petroleum ether extraction in a Soxhelt apparatus. Amino acid content of PTSW was determined using reverse phase HPLC as described by Cohen and Michaud (1993). Table 2 shows the proximate composition and amino acid content of the formulated fish feed.

Analysis of the growth parameter: Growth was monitored at 15th day intervals by collectively weighing each group of fish. Fish were starved overnight before taking the weight growth and nutrient utilization were monitored and analyzed in terms of weight gain, SGR, PER, FCR and survival rate.

Statistical analysis: Stastical analysis of data was performed by Analysis of Variance (ANOVA) using Microsoft software stastica followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Protein intake by fish is important to provide the amino acids required for synthesis of new tissues (growth and reproduction) or replacing worn out protein (maintenance). The total protein content of raw PTSW was minimum compared to chemical treatment or fermented metabolite. This may be due to firmly bound and intact structure of proteins or due to heavy cross linkage. The fleshing was pretreated with hydrogen peroxide as it destroys the harmful micro organisms present in the fleshing. Hydrogen peroxide is very powerful bactericide and viricide that possess no undesirable residues, improves feed efficiency and is environmentally beneficial. Rach *et al.* (1997) reported that several fish species such as fathead minnow *Pimephales promelas*, brown trout *Salmo trutta*, bluegill

Table 3: Proximate composition of fish feed

Proximate composition (%)	Control feed (C)	Raw PTSW feed (RF)	H_2O_2 treated feed (CF)	Fermented feed (HF)
Protein	35.8±0.24	35.2±0.19	34.4±0.23	36.7±0.87
Dry matter	94.6±0.02	95.8±0.08	94.2±0.03	95.3±0.05
Lipid	10.3±0.21	10.5±0.24	11.6±0.67	12.7±0.87
Moisture	5.4±0.08	4.2±0.02	5.8±0.07	4.7±0.05
Ash	3.8±0.67	3.5±0.70	2.4±0.58	3.9±0.78

Table 4: Growth profile of *Labeo rohita* fed PTSW diets

Mean values	RF	Control	CF	HF
Initial weight (g)	5.67 ± 0.25^{c}	5.24 ± 0.34^{a}	5.36 ± 0.87^{b}	5.32 ± 0.65^{b}
Final weight (g)	13.24 ± 0.78^{a}	13.46 ± 0.67^{ab}	13.68 ± 0.56^{b}	13.87 ± 0.43^{c}
Survival rate (%)	98	98	97	97
SGR (%)[1]	1.41 ± 0.342^{a}	1.57 ± 0.233^{b}	1.56 ± 0.162^{b}	1.59 ± 0.709^{c}
Weight gain (%)	133.5 ± 0.097^{a}	156.8 ± 0.702^{b}	155.2 ± 0.239^{b}	160.7 ± 0.143^{b}
FCR[2]	1.18 ± 0.89^{b}	1.09 ± 0.49^{a}	1.08 ± 17^{a}	1.05 ± 0.63^{a}
PER[3]	2.52 ± 0.34^{a}	2.74 ± 0.42^{b}	2.77 ± 0.33^{b}	2.85 ± 0.31^{c}

Results are means of triplicate treatments±SE. Means in the same row with different superscripts are significantly ($p<0.05$) different. [1]Specific Growth Rate (SGR) = [ln final BW (g) - ln initial BW (g)] ×100/time (days). [2]Feed Conversion Ratio (FCR) = Dry feed consumed (g)/wet BW gain (g). [3]Protein Efficiency Ratio (PER) = Wet weight gain (g)/protein consumed (g)

Lepomis machrochirus and channel catfish *Ictalurus punctatus* to lerate hydrogen peroxide concentrations up to 1000 mg L^{-1} for 45 min. Digestion of tannery solid waste by microorganism are found to be the suitable biotechnological process and will result in the breakdown of proteins and peptides and an increase in the amino acid content (Kumar *et al.*, 2008). The PTSW was hydrolyzed by the protease producing *Bacillus megaterium* LR1 isolated from the gut of *Labeo rohita*. The fermented PTSW was incorporated in the fish feed and growth parameters were analyzed. The best composition as shown in Table 3 was arrived after studying the survival probabilities of carp fish under the prevailed environmental conditions for the test period of 30 days. Fish were observed to be in good condition of health and survival was 100% in all groups. The allotted diets were well accepted and showed greater response to feeding as they fed voraciously within 10 min of the service. The results of growth performance and efficiencies of protein utilization of *Labeo rohita* fed with fermented PTSW and fish meal as the main sources of animal proteins in diets are shown in Table 3. The group of fish fed with fermented PTSW had higher values of growth performance and efficiencies of protein utilization. Fish fed with the control diet and fermented PTSW were not statistically different with the nutrient utilization values, FCR and PER ($p<0.05$) (Table 4). The data shows that the final Body Weight (BW), Feed Intake (FI), Feed Conversion Ratio (FCR), Weight Gain (WG) and Specific Growth Rate (SGR) were significantly influenced by PTSW inclusion. The amino acid present in PTSW namely Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, His, Lys and Arg could be regarded as the essential amino acids for fish muscle proteins. Protein Efficiency Ratio (PER) also increased in the fermented diet. Feed had no significant effect on survival and resulted in a higher production.

There was no feed-related mortality observed during the entire period of the experiment. This was also the case with the FCR in which an improving trend was observed with fermented PTSW formulated diets. This result agrees with the findings of Rodriguez-Serna *et al.* (1996) that animal by-product meal can be used as the sole protein source in commercial diets without affecting growth and feed utilization. Positive response to the PTSW formulated diets coincides with the results of Westgate (1979), Fowler (1990) and Brannon *et al.* (1996) where in Poultry By-product Meal (PBM) was used as the major protein source in fish diet. The present result agrees with Nengas *et al.* (1999) that a combination of poultry meat meal and feather meal improved growth of sea bream. Similarly, Bureau *et al.* (2000) obtained an increase in growth performance for rainbow trout fed diets containing a combination of feather meal and meat and bone meals as substitute for fish meal.

The results of these studies have shown the possibility of replacing fish meal protein with fermented PTSW in fish diets. The highly selective action of proteases on inter-and intra-peptide disulphide bonds by enzyme hydrolysis may be attributed to the increase in productivity values of fish fed diets containing hydrolyzed PTSW by increasing the feed uptake.

De Silva and Anderson stated that animal by-product meal particularly fish meal appears to contain unidentified growth factors. Similarly, PTSW contained essential amino acids which enhanced fish growth (Table 1). The net change in weight of the *Labeo rohita* was similar to control group.

This indicates that PTSW could substantially replace the control. The amino acid content of PTSW namely glycine, proline, hydroxy proline may have contributed to overall fish growth. The results obtained from the present studies were consistent with values obtained by other investigators based on similar techniques and methodology. Hence, it is advantageous to incorporate such abundantly available inexpensive feed ingredient as a complete replacer to fish meal to curtail cost of carp production.

CONCLUSION

Exploitation of PTSW as the substrate for the production of aqua feed would meet the need of the aqua industries as replacer of the fish meal and also simultaneously alleviate the endangering problems encountered with disposal of solid waste in an environmentally sound manner. To the best of the knowledge, this is the 1st time to formulate and incorporate PTSW in aqua feed. A low-cost technology for the production of fish feed pellets utilizing PTSW was developed.

The nutritional quality of the developed fish feed pellets was determined through a feeding experiment by comparing the growth rate of fish using the fish meal and the substituted PTSW in plastic aquariums. Production parameters were similar to those of control fish produced using similar management procedures.

This approach will definitely be an alternative measure for the utilization of proteinaceous tannery solid waste and will be a promising clean technology for the disposal of untanned solid leather waste. Even though further long-lasting experiments are needed, the preliminary results show that an appropriate feeding strategy with PTSW might improve dietary intake of nutrients constituting an option for improving the utilization of alternative protein sources.

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