Fishery Technology
2011, Vol. 48(2) pp : 149 - 154

Effect of Chitin and Chitosan on the Physico-chemical Quality of Silage Based Fish Feed

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Silage made from the filleting waste of rohu, *Labeo rohita* was used for the preparation of palletized fish feed. Three sets of feeds were prepared *viz.*, control and two lots incorporated with chitin and chitosan respectively. Chitin and chitosan incorporated feeds were prepared along with the control. The biochemical and physical properties *viz.*, durability, stability, sinking rate and leaching of the feeds were studied. The loss during stacking was found to be minimum in samples incorporated with chitin and chitosan while higher loss was observed in the case of control, indicating that chitin and chitosan have better binding property ($p<0.05$). The stability of feed was significantly higher ($p<0.05$) in the case of chitin and chitosan incorporated samples when compared to control indicating that both chitosan and chitin improved the stability when added at 2% level in feed. Pellet durability was also better in chitosan incorporated samples. Incorporation of chitin and chitosan was found to reduce the leaching of the pelletized feed. The control feed showed maximum leaching of protein (5.04 mg $100g^{-1}$) after 15 min in water but in the case of chitosan incorporated feeds leaching of protein for the corresponding period was marginal (0.82 mg $100g^{-1}$). The feeds contained essential amino acids indicating that high quality fish feed can be prepared from fish waste silage.

Key words: Silage, fish feed, chitin, chitosan, stability, durability, leaching

The aquaculture sector has been and continues to be predominant in developing countries, particularly in Asia, which accounts for more than 85% of the global production (Sena & Brian, 2009). Feed is the largest production cost for commercial aquaculture and improving feed efficiency in industrial systems is a priority (Rosamond *et al.*, 2000). The aquaculture industry not only utilize fish feeds sourced from farm locations but also consider the discards and wastes from post harvest activities. The technology of fish feed production involves combination and blending together of feed ingredients (based on a formula) into nutritionally balanced and economically sound diet. Fish meal is the preferred source of animal protein in feed. The production of fish meal from fish waste or from whole fish is both capital and energy intensive. The development of feeds utilizing sustainable alternatives to fish meal and fish oil which provide economic and nutritional performance at par with the commercial feeds is essential to enhance the production through aquaculture. According to Gabriel *et al.* (2007), there is a need to formulate diets with least possible cost by careful selection of ingredients that are cheap but rich in nutrients for optimum fish growth. Silage, made from trash fish and fishery waste can be used as a substitute for fish meal. Production of fish silage from processing waste is a safe, economically advantageous and environment friendly process. Chitin and chitosan are known to be non-toxic, biodegradable and biocompatible natural biopolymers. Nair (1986) observed increase in average live weight and dressed weight with minimum wastage in broiler chicken fed on diet containing 0.5% chitin compared to the chitin free diet. The objective of the present study is to evaluate the biochemical quality and properties of feed prepared from fish silage incorporated with chitin and chitosan.

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Materials and Methods

Fish silage was prepared from the filleting waste of freshwater carp, rohu (*Labeo rohita*) by adding formic acid at 3.5% level. Chitin and chitosan were prepared from the shell waste of Indian white prawn by the method of Nair & Madhavan (1974) and the properties were analyzed. The ingredients for the feed were procured from a local market in Cochin. Three lots (I, II and III) of feeds were prepared by carefully mixing the ingredients (Table 1) with water to form the dough. In lot II and III chitin and chitosan were added at 2% level respectively and lot I was kept as control. The dough was steamed for 15 minutes and on cooling, it was made into pellets of 5 mm diameter using a semi automatic pelletizer. It was then vacuum-dried and packed for further study. Chitin nitrogen was determined by Micro-Kjeldahl method (AOAC, 2000). Viscosity of chitosan was measured as per AOAC (2000). Molecular weight and degree of deacetylation of chitosan sample was determined as per the methods of Rutherford & Austin (1978) and Muzzarelli & Rocchetti (1985) respectively. The pellet durability index of the feed during stacking was determined by shaking 10 g feed taken in 50 ml conical flask for 10 minutes using a cyclo mixer. It was sieved using 1mm size mesh and loss estimated (Anon.1994). For the determination of pellet durability index in water, 10 g feed was kept in water for different time intervals (5, 10 and 15 min) in a sieve of 0.5 mm mesh size. The mesh was taken out and the remaining feed was carefully collected, dried and weighed for calculating the loss (Anon.1994). The stability of the feed was estimated by observing the withering off in water. Ten gram of feed pellets of 10 mm length was kept in 50 ml water in a beaker and the intactness was visually evaluated for 15 min at 5 min interval and a score was assigned based on a ten point hedonic scale (10 point for fully intact pellet). Sinking rate was calculated as the rate of sinking of pellets in water. Feed pellets of 0.5 cm length were put into an aquarium tank of 30 cm depth and the sinking rate was (cm sec^{-1}) recorded with the help of a stopwatch and the average of 5 observations was noted. For estimation of leaching rate, 10 g of the feed was taken in thimbles made of filter paper and kept in 50 ml of water for 5, 10 and 15 min. The protein leached out was estimated by Biuret Method (Varley *et al.*, 1980)

Total nitrogen, crude fat, ash and moisture content of the feed were estimated as per standard methods (AOAC, 2000). Total amino acids in the samples were determined as per Ishida *et al.* (1981).

Statistical analysis was carried out using SPSS, version 10. One way ANOVA was performed and the level of significance was studied at 5%.

Results and Discussion

The properties of chitin and chitosan incorporated in the feed are given in Table 2. Chitin prepared had low ash content with high purity (98%). The chitosan had a viscosity of 158 cP and degree of deacetylation of 79.1%. The properties of chitin and chitosan vary considerably depending on the source of raw material and production process. The degree of deacetylation of chitosan depends on the process temperature and alkali concentration. The molecular weights of chitin and chitosan are very

Table 1. Percentage composition of ingredients used for experimental feed

Ingredients	Lot I*	Lot II**	Lot III***
	% composition		
Fish silage	20	20	20
Wheat flour	25	25	25
Ground nut oil cake	24	24	24
Rice bran	30	28	28
Chitin	-	2	-
Chitosan	-	-	2
Vitamin-mineral mix	1	1	1

* Control feed, ** Feed with 2% chitin, *** Feed with 2% chitosan

different and severe degradation takes place during deacetylation step which involves the harsh treatment with alkali at high concentration and at high temperature (Wu *et al.*, 1978).

Table 2. Properties of chitin and chitosan used for the experimental feed

	Chitin	Chitosan
Ash (g %)	1.69	2.45
Chitin nitrogen (g %)	7.0	7.28
Viscosity (cP)	—	158
Molecular weight (Da)	—	1.0217×10^6
Degree of deacetylation (%)	—	79.1

Proximate composition of silage and feeds developed is given in Table 3. The silage composition was similar to fish composition except that the moisture content was little higher. Consequently the other components like protein, ash and fat were at a lower level. Since the feed developed was vacuum dried, the moisture content was very low in all the samples. No noticeable difference was observed in moisture levels among the samples. Other parameters like, ash, fat and fibre did not show significant difference.

The amino acid composition of the feed developed is given in Table 4. Since essential amino acids decide the quality of the feed,

Table 3. Proximate composition of silage and feeds developed

Parameters	Fish silage	Lot I*	Lot II**	Lot III***
	(% composition)			
Moisture	82.82	2.5	2.7	2.6
Ash	3.91	18.81	18.62	19.0
Fat(dwb)	2.17	6.69	5.67	4.69
Protein (TN x 6.25)	11.58	26.25	29.75	30.62
Crude fibre		18.0	17.11	13.5
Carbohydrate (by difference)	—	27.7	26.26	29.5

* Control feed, ** Feed with 2% chitin, *** Feed with 2% chitosan

Table 4. Amino acid composition of the experimental feed (g 100 g^{-1} Protein)

	Lot I *	Lot II **	Lot III ***
Asp	8.95	7.19	8.64
Thr	3.09	3.95	3.53
Ser	13.02	9.90	9.64
Glu	21.34	17.01	16.41
Pro	0.47	0.50	0.11
Gly	6.82	5.30	15.35
Ala	3.94	3.45	3.22
Cys	0.15	0.53	0.41
Val	0.74	0.15	0.16
Met	5.94	4.70	4.27
Ile	8.50	6.84	6.58
Leu	ND	ND	ND
Tyr	2.68	2.16	1.98
Phe	5.47	4.58	4.07
Try	0.78	0.68	0.52
His	2.94	2.38	2.23
Lys	6.79	5.60	4.87
Arg	-	-	-

* Control feed, ** Feed with 2% chitin, *** Feed with 2% chitosan

the information on the amino acid composition is essential in assessing the nutritional quality. In general, there was no significant difference in the amino acid content among the different samples The isoleucine content in the feeds found to be 8.5, 6.85 and 6.58% in lot I, lot II and lot III respectively will positively influence the growth of aquatic organisms. Wide variations in isoleucine content on feeds available in the market has been reported by Kavitha *et al.* (2003). Cysteine content was found to be lower in all the feed samples whereas methionine levels were found to be higher. Murthy & Varghese (1998) reported that cysteine can be synthesized from methionine. Kavitha *et al.* (2003) observed higher quantities of essential amino acids in the laboratory developed feeds when compared to commercial samples. Even though lysine is an essential amino acid, the higher content of it in feeds cannot be regarded as favourable as it is antagonist to arginine (Berge *et al.*, 2002). Although Lot I (control) had higher levels of many amino

acids, high leaching rate of the feed in water will significantly reduce the availability of amino acids to the fish during feeding.

Loss during stacking which is an important quality parameter for the feed shows the stability of feed against shakes and jerks during transportation and transfer. Minimum loss during stacking was observed in samples incorporated with chitin and chitosan (1.1% and 1.0% respectively) while a significantly higher loss ($p<0.05$) was observed in case of control (3.8%), showing that chitin and chitosan have better binding property. Sinking rate of the feeds indicate the speed at which the feed pellets move downwards. Since some fishes prefer floating feed, this parameter is an important quality criterion. In lot II viz., chitin incorporated feed the sinking rate was 6.33 cm sec^{-1} which was significantly different ($p<0.05$) from the control and chitosan incorporated samples which had 4.66 and 4.33 $cm.sec^{-1}$ sinking rates respectively. Sinking rate of the pellets increased on storage and was inversely proportional to moisture content of the feed (Menghe & Edwin, 2006).

Stability and durability of the feed developed are given in Table 5. The stability of feed was significantly higher in chitin and chitosan incorporated samples (lot II & Lot III) when compared to control indicating that both chitosan and chitin improved the stability when added at 2% level in feed. But chitosan incorporated samples showed higher stability than chitin incorporated feed. In all the three lots, the stability decreased during the course of immersion in water; but the rate of decrease was comparatively less in chitin and chitosan incorporated samples. The stability of the pellets is influenced by different factors like feed composition, nature of the ingredients, type of processing and the moisture content (Hastings, 1971). The degree of stability of the feed is dependant on the gelatinization during steam conditioning (Stivers, 1970). The incorporation of the feed with chitosan might have resulted in the increase of gelling since the silage contains acid which causes a partial dissolution of chitosan. Durability is probably the most important characteristic of pelleted feed. Poor pellet durability results in the generation of fines and the feed will not be available for fish. Durability can be predicted by determining the Pellet Durability Index which gives reference to how well pellets hold their integrity during packaging and handling. In the present study, pellet durability was found to be significantly higher in chitosan incorporated samples. Durability decreased with corresponding increase in immersion time in all the feed samples. However, after 15 minutes of immersion, durability of chitosan containing samples was significantly high ($p>0.05$) compared to other two samples. In control sample, durability was significantly less after 15 minutes of immersion. Higher durability of Lot II & III indicates that chitin and chitosan incorporation has resulted in better binding of the ingredients during the preparation of feed. Chevanan *et al* (2006) reported that the pellet durability of the extrudates meant for tilapia feeding was in

Table 5. Durability and stability of the experimental feeds *

Time (min)	Lot I*	Lot II**	Lot III***	Lot I*	Lot II**	Lot III***
	Durability in water (% loss)			Stability (Organoleptic score)		
5	0.4±0.01[a]	0.41±0.01[a]	0.21±0.02[b]	5.16±0.28[a]	6.33±0.57[b]	7.2±1[c]
10	8.2±0.2[a]	6.4±0.1[b]	0.41±0.01[c]	3.83±0.76[a]	5.5±0.5[b]	6.83±0.73[c]
15	25.56±0.35[a]	11.0±0.2[b]	5.2±0.1[c]	3.16±0.28[a]	4.83±0.76[b]	5.5±0.5[c]

* Results are presented as mean ± standard deviation (SD) of 3 replications. Values in a row with same superscript are not significantly different ($P>0.05$).

* Control feed, ** Feed with 2% chitin, *** Feed with 2% chitosan

the range of 0.37 to 0.96% of three feed blends containing 28% protein.

Leaching of protein from the feed is given in Table 6. Excessive leaching makes the feed nutritionally deficient causing nutrients to accumulate in the pond water thus altering the water quality. Leaching of protein was found to be maximum in the control during the entire period of observation. Significantly lower levels of leaching were observed in lot III sample incorporated with chitosan. Within a period of 15 minutes, leaching of protein doubled in control samples whereas only a marginal increase was observed in chitosan incorporated samples. After 5 minutes, leaching of protein in control was found to be 6 times more than lot III. Increasing trends in the nutrient leaching with increasing time of immersion in water was reported by Falayi *et al.* (2003). Muylder *et al.* (2008) observed that addition of binders like gelatin, wheat flour or processing conditions influenced the leaching rates of proteins in pelleted fish feeds. Leaching of proteins is most probably a function of the protein solubility of the raw materials rather than a consequence of processing or the addition of binders (Muylder *et al.*, 2008). Leaching of protein is dependent on the size and nature of protein. Since silage which contains breakdown products of protein was used as protein source in feed, higher levels of leaching was expected. The control feed showed maximum leaching (5.04 mg $100g^{-1}$ feed) after 15 minutes but the marginal increase in leaching in Lot II & III samples for the corresponding period indicate the effect of chitin and chitosan in reducing the leaching of feed, chitosan being the most effective.

Table 6. Leaching of protein (mg $100g^{-1}$ feed) from the experimental feeds at different time intervals *

Time (min)	Lot I*	Lot II**	Lot III***
5	2.49±0.03[a]	1.58±0.05[b]	0.6±0.005[c]
10	4.16±0.02[a]	2.46±0.06[c]	0.83±0.15[b]
15	5.04±0.22[c]	2.46±0.01[b]	0.82±0.06[a]

* Results are presented as mean ± standard deviation (SD) of 3 replications. Values in a row with same superscript are not significantly different ($P>0.05$).
* Control feed, ** Feed with 2% chitin, *** Feed with 2% chitosan

The study indicated that incorporation of chitin and chitosan in silage based fish feed formulations can improve the keeping quality. The stability and durability of the feed was significantly improved by incorporating chitin and chitosan. Further studies are required to assess the effectiveness of the feed by feeding studies.

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