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Response of *Macrobrachium rosenbergii* (de Man) Juveniles to Fish Silages as Substitutes for Fish Meal in Dry Diets

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Abstract

A feeding trial was conducted for 60 days to study the use of fish silages as substitutes for fish meal in the juveniles diets of *Macrobrachium rosenbergii* (de Man). Two hundred and twenty five juveniles (mean body weight of 112.8 mg±0.01) were equally distributed (15 juveniles per 50 L plastic tub) in 3 experimental groups having 5 replicates each following complete randomised design. Three different isocaloric (estimated digestible energy 290 Kcal $100g^{-1}$) and isonitrogenous diets were prepared with either fish meal (FM), or acid fish silage (AFS) or fermented fish silage (FFS) keeping crude protein (CP) level of the diets at 35%, in which 15% CP was contributed by FM, AFS or FFS. Feed was given to each group at 5% of their body weight twice daily (10 A.M. and 10 P.M.). At least 50% of the water in each experimental tub was replaced with chlorine free borewell water. Water quality viz. water temperature, dissolved oxygen, free carbon dioxide, total hardness, total alkalinity, ammonia and nitrate at every alternate day. The shrimps fed diets containing FFS showed although not statistically different ($p>0.05$), but better weight gain (72.68%), specific growth rate (0.91% day^{-1}), feed conversion ratio (3.46), and protein efficiency ratio (0.85) than those fed FM and AFS. Carcass composition and survival of the juveniles were not affected ($P>0.05$) by the diets.

Introduction

Fish meal constitutes a major share in the formulated feed for any carnivorous or shrimp species as a protein source. But, there is an urgent need to reduce the current total dependence of the aquaculture industry upon this expensive and finite commodity of uncertain supply in the future as, more than 80% of the world's fish meal supply are being utilized by the livestock sector and the remaining few are available to other sectors including fisheries (Tacon 1995). Fish silage, which is produced as a result of self digestion, generally in an acidic medium, which in turn prevents the multiplication of the putrefying bacteria and enhances the activity of endogenous, protein digesting enzymes (De Silva and Anderson 1995), may be an ideal alternative to fish meal. Fish silages have many advantages such as easy to prepare on low as well as large scale basis, low investment, no chilling storage of fish is required prior to

ensiling, ecofriendly and produces an almost microbiologically sterile product (Summer 1977, Raa and Gildberg 1982). The production of fish silage for incorporation in pigs, poultry and mink diets is a common practice particularly in Denmark, Poland and Norway (Jackson et al. 1984). Moreover, incorporation of fish silage is considered as a cheap way of exploi- ting fishery waste as aquafeed (Asgard and Austreng 1981). Several workers have found fish silage to be superior to fish meal in their experiments while replacing fish meal with fish silage at different levels (Srinivasan et al. 1985; Ali et al. 1994, Valencia et al. 1994, Cisse et al. 1995, Jagannatha et al.1996, Ximenes et al. 1996).

However, till now, no attempt has been made to use silage based diets as feed for *M. rosenbergii*, which is currently gaining commercial importance. So, an attempt has been made in this study to evaluate utilization of different fish silages (acid fish silage and fermented fish silage) as alternatives to fish meal in the post larval diets of *M. rosenbergii*.

Materials and Methods

Preparation of fish meal and fish silages

Mixed raw fishes such as *Pseudosciaena diaecanthus, Otolithus cauvieri, Polynemus heptadactylus, Otolithus biauritus, Otolithus argenteus, Sardinella fimbriata* were collected fresh and kept frozen at 10ºC overnight before processing.

FISH MEAL (FM)

The FM was prepared by wet reduction method. About 1 kg of the thawed raw fish was boiled in a pressure cooker for 20 min, screw pressed and dried in an oven at 70ºC overnight. Finally, it was pulverized to powder form (60 micron size) to be used in the diet.

ACID FISH SILAGE (AFS)

Five hundred grams thawed and minced raw fish was mixed with 95% formic acid and 95% sulphuric acid at 1.5% each (Jackson et al. 1984) along with 125 mg butylhydroxytoluene (BHT) and stored at room temperature (28 to 31ºC) in a plastic container for 15 days with stirring at eight hour interval (6, 14, 22 hrs) by an automatic shaker.

FERMENTED FISH SILAGE (FFS)

Multi-species whole fish (500 g) were thawed, minced and mixed with 15% (W/W) molasses, water (10%) and BHT 125 mg to make a thick slurry. The slurry was boiled in a pressure cooker for 30 min and cooled at room temperature following the method of Durairaj et al .(1985). Pure culture of *Lactobacillus plantarum* collected from the National Dairy Research Institute, Karnal, India

was cultured in pasteurized milk added to the cooled slurry at 3.5%. Slurry was kept in an air tight wide mouth bottle to make it completely anaerobic as suggested by Kompiang et al. (1979). The bottle was kept for 15 days at room temperature with regular stirring as described earlier.

After 15 days, both the silages were removed from containers and boiled for 10 min to stop the enzymatic activity. Finally, these were dried in an oven at 60°C overnight and then pulverized.The quality aspects of both the silages and fish meal were assessed by determining pH (Fagbenro and Jauncey 1995), total volatile base nitrogen (TVBN)(Conway and Byrene 1993) and α-amino nitrogen (Pope and Stevens 1939)

Feed preparation

All other ingredients (except groundnut oil cake and rice bran) were kept the same for all the three experimental diets except for FM in T1 group, AFS in T2 group and FFS in T3 group (Table 1). Equal amount of crude protein (15%) was contributed by FM, AFS or FFS to the respective diets. Accordingly, different ingredients were adjusted to make all the three diets isocaloric and isonitrogenous. All the ingredients, except vitamin and mineral, were mixed with the required amount of water to make dough, which were steam cooked for 20 min. in a pressure cooker. Vitamin-mineral premix were added to the doughs after cooling and passed through an extruder having a die of 2 mm size (Double Twin Screw Extruder, Basic Technology Private Ltd., Calcutta, India) at barrel screw speed, 430 rpm; feeding rate, 90 rpm; barrel temperature, 120°C and cutter speed, 1100 rpm. Pellets, thus obtained, were dried in an oven at 60°C overnight and stored in room temperature.

Rearing

The feeding trial was carried out at the Digestive Physiology, Nutrition and Feed Laboratory of Central Institute of Fisheries Education, Mumbai for a period of 8 weeks from 4th January, 1998 to 5th March, 1998. The experiment was set up in three experimental groups, having 5 replicates each, in 15 uniform size plastic tubs (50 L capacity) following a Completely Randomized Design (CRD). Each tub was stocked with 15 juveniles of *M. rosenbergii* of uniform size (0.1050 to 0.1162 g). A uniform volume of 30 L fresh water was maintained in each tub with continuous aeration. Water in each tub was replaced with 50% fresh chlorine free water daily till the completion of the feeding experiment. Physico-chemical parameters of water (temperature, dissolved oxygen, free CO_2, total hardness, total alkalinity, ammonia, nitrate were checked every other day following the methods described by APHA (1985).

Feeding trial

Feeding ration was adjusted based on daily observations of feed intake of the juveniles and adjusted at 5% of the body weight per day of the juveniles for the initial 15 days and subsequently reduced by 1% for every fortnight till the

end of the experiment. Total ration was divided into two parts; about 1/3 of the total feed was given at 10 a.m. and the remaining amount at 10 p.m. At the end of the experiment, all juveniles (in bulk) from each tub were separately weighed, sacrificed and stored at -20ºC for analytical work.

Feed and tissue analysis

Each diet and tissue sample of the different experimental groups in triplicates were analyzed for moisture, total ash, and crude protein following AOAC (1980) while ether extract and crude fibre (feed only) were determined by using Soxtech (Model STZ, 104 extraction unit, Tecator) and Fibretec system (Model M,1017 hot extraction, Tecator), respectively. Total carbohydrate content of the tissues were calculated by subtracting CP% + EE% + ash% from 100. Whereas NFE was calculated by subtracting CP% + EE% + CF% + ash% from 100.

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

Ingredients	Diets		
	T1	T2	T3
Fish meal	20.0	-	-
Acid fish silage	-	20.95	-
Fermented fish silage-	-	23.2	
Prawn head meal	10.0	10.0	10.0
Acetes meal	5.0	5.0	5.0
Groundnut oil cake	24.0	24.05	23.80
Wheat flour	10.0	10.0	10.0
Rice bran	11.0	10.	8.0
Tapoica	10.0	10.0	10.0
Cod liver oil + ground nut oil (1:2)	4.0	4.0	4.0
Vitamin mineral mixture * 1	3.0	3.0	3.0
Sodium alginate	1.0	1.0	1.0
Sodium hexametaphosphate	2.0	2.0	2.0
Total	100.0	100.0	100.0
Crude protein *2	35.01	34.75	34.85
Fat *2	7.65	7.89	8.25
Crude fibre *2	6.97	7.15	6.52
NFE *2	35.71	35.27	35.63
Ash *2	14.66	14.94	14.75
Digestible energy (Kcal kg $^{-1}$) *2	2902.20	2894.14	2901.23

*-1Vitamin and mineral mix (Suplevite-M, Sarabhai Chemicals, Baroda, India); a 2.5 kg pack contained vitamin A, 5000000 I.U.; Vit D3 ,1000000 I.U.; Vit B2 2 g; Vit E,750 I.U.;Vit K, 1 g; Cal. pantothenate, 2.5 g; Nicotinamide,10 g; Vitamin B12, 6 g; Choline chloride 150 g; calcium ,750 g; manganese, 27.5 g; Iodine,1 g; Iron, 7.5 g; Zinc; Copper, 2 g; Cobalt, 0.45 g; Vit C was supplemented separately in the form of Celein tablets, Glaxo Company, India @ 300 mg·kg-^{1} diet.

*2 crude protein, fat, crude fibre, ash were analyzed on dry matter badsis; NFE was calculated as (100-% CP-% EE-% CF-% NFE-% Ash); digestible energy was calculated assuming coefficiencies as 2 kcal·g^{-1} for carbohydrate of legumes; 3 kcal·g^{-1} for carbohydrates of non-legumes; 4.25 kcal·g^{-1} for animal proteins; 3.8 kcal·g^{-1} for plant proteins and 8 kcal·g^{-1}for fats.

Growth, feed conversion ratio, protein efficiency ratio and survival

Body weights of the juveniles were taken at 15 days interval to assess the growth performance in terms of percentage weight gain, specific growth rate, feed conversion ratio (FCR) and protein efficiency ratio (PER). Survival of the juveniles was calculated from the total number of juveniles present at the end of the experiment compared to the initial number x 100.

Statistical analysis

Mean values of the growth, FCR, PER and survival of the *M. rosenbergii* juveniles fed diets were statistically analyzed to see the significant difference, if any, through one way ANOVA as described by Snedecor (1961).

Result and Discussion

Physico-chemical parameters of water

Water quality parameters, except water temperature recorded during the experimental period were found to be within the normal range for *M rosenbergii* (New 1976; Smith et al. 1978, 1981; Sandifer and Smith 1985; Vasques et al. 1989) (Table 2). Water temperature during the experimental period was within the range of 21.1ºC to 25.5ºC which was lower than the required as Raman (1967) suggested the optimum temperature for *M. rosenbergii* to be 29 to 30ºC.

Proximate composition of the FM, AFS and FFS

Proximate composition of the FS, AFS and FFS is given in table 3. High crude protein ($P<0.01$)) content of the FM might be due to coagulation of total protein during cooking and removal of lipid along with some suspended solids during pressing as suggested by Windsor (1982). However, low CP ($P<0.01$) content of the fermented fish silage was probably due to the presence of molasses, sugars and slight dilution by lactic acid and other fermented products as reported by Raa et al. (1982). The lipid content of FM was lower than those of the fish silages because of cooking and subsequent pressing, which might have

Table 2. Average range of water parameters for the different treatments during the 60 days experimental period.

Treatments	Temperature (ºC)	pH	DO ($mg \cdot L^{-1}$)	Total alkalinity ($mg \cdot L^{-1}$)	Total hardness ($mg \cdot L^{-1}$)	Ammonia ($mg \cdot L^{-1}$)	Nitrate ($mg \cdot L^{-1}$)
T1	21.5-25.5	7.2-7.5	6.80-7.68	168-220	180-200	0.11-0.15	0.14- 0.20
T2	21.5-25.5	7.0-7.5	6.90-7.55	170-220	182-200	0.11-0.16	0.14- 0.20
T3	21.5-25.5	7.0-7.5	6.98-7.30	170-205	180-200	0.11-0.16	0.14- 0.20

*Free CO_2 during this period was nil.

resulted in removal of certain amount of lipid along with the stick water. The higher lipid content (P<0.01) of FFS than those of AFS and FM may be due to extraction of lactic acid as well along with fats during ether extraction (Fagbenro et al. 1995). The ash content of the FFS was higher (P<0.01) than the others which is probably due to incidental entry of impurities through molasses.

Silage quality

pH

The pH of the AFS and FFS recorded on alternate days during 15 days ensiling showed a declining trend and a stable pH of 4.3 was achieved on the 6th day. Ahmed et al. (1995) got a microbiologically safe pH of about 4.2 in 3 to 5 days with 10% and 12.5% molasses treated samples. The silage at pH 4.5 and above is always susceptible to spoilage caused by *Clostridium botulinum, Staphylococcus aureas* and fungus (Anon 1971). Moreover, silage at 4.5 and above pH can be used directly without neutralisation in feed (Lo et al. 1993)

TOTAL VOLATILE BASE NITROGEN (TVBN)

The total volatile base nitrogen (TVBN) values recorded for the AFS, FFS after ensiling and for the FM were 79.80, 33.60 and 30.15 mg·100 g^{-1}, respectively (Table 4). The TVBN value of *Lactobacillus plantarum* fermented silver belly fish after one month of ensiling was reported to be 36 mg·100g^{-1} (Durairaj et al. 1985). Haaland and Njaa (1989) recorded TVBN value in the form of NH_3 as 112 mg 100g^{-1} on the 14 th day of ensiling the capelin fish using 1.4% formic acid. According to Connel (1980) TVBN more than 100 to 200 mg·100 g^{-1} on dry weight basis of salted dried fish could indicate spoilage. Krishnaprasad (1995) found the TVBN value as 41.33 mg·100 g^{-1} in oven dried fish at 45ºC after 40 hours of drying. Thus, AFS, FFS and FM prepared for the experiment was not spoiled by any means.

Table 3. Proximate composition of fish meal, acid fish silage and fermented fish silage (% DM basis) used as ingredients in the experiment.

Ingredients		Dry matter				
± SE	Moisture protein ± SE	Crude extract ± SE	Ether fibre ± SE	Crude ± SE	Ash Free Extract	Nitrogen ± SE
FM	48.90[a] ±0.03	75.70[a] ±0.62	8.32[a] ±0.05	1.25[a] ±0.01	10.66[a] ±0.06	4.07[a] ±0.07
AFS	56.20[b] ±0.21	72.12[b] ±0.59	9.25[b] ±0.09	2.75[b] ±0.03	12.68[b] ±0.07	3.20[b] ±0.07
FFS	69.90[c] ±0.27	65.55[c] ±0.48	10.52[c] ±0.08	3.46[c] ±0.02	13.79[c] ±0.08	6.68[c] ±0.06

Columns containing different superscripts are significantly different (P < 0.01).

α-AMINO NITROGEN

The α-amino nitrogen values recorded for the AFS and FFS at 15th day of ensiling and for FM were 560, 56 and 37.8 mg·100 g^{-1}, respectively. Slightly higher results were reported for AFS (638 mg·100 g^{-1}; Haaland and Njaa 1989) and for cooked FFS (62.3 mg·100 g^{-1}; James.1966). Krishnaprasad (1995) recorded an α-amino nitrogen value of 89.92 mg·100g^{-1} in oven dried bombay duck (a fish meal) at 45ºC for 40 h.

Effect of diets on growth

Although insignificant (P>0.05) better weight gain were recorded for the silage based diets than the FM (control) at the end of the experiment (Table 5), which is in agreement with the findings of Ximenes et al. (1996). While working on *C. carpio* fry Srinivasan et al. (1985) found double weight gain with the fish silage based diet as compared to FM (control). The better growth performance of the silage based diets may be due to the presence of comparatively higher amounts of free amino acids and active hydrolytic enzymes than the FM (Gallagher 1993). Comparatively FFS was better (P>0.05) than AFS. This may be because of the higher rate of proteolysis in AFS than FFS as confirmed by its higher values of TVBN and α-amino nitrogen (Table 4). Approximately, 80% of the protein in acid preserved silage becomes solubilized after one week at a temperature of 23 to 30ºC (Haq et al. 1995). Therefore, the AS contained higher levels of essential amino acids in the free form which are available for immediate absorption. In order to be nutritionally available, silage had to contain the majority of the nitrogen fraction as intact protein or peptides rather than as free amino

Table 4. TVBN and a-amino nitrogen values (mg·100 g^{-1}) of fish meal, acid fish silage and fermented fish silage and after 15 days of ensiling.

Treatments	TVBN ±SE	α-amino nitrogen ±SE
AFS	79.80^{a} ± 11	560.0^{a} ± 14
FFS	33.60^{b} ± 9	56.0^{b} ± 2
FM	30.15^{b} ± 6	37.8^{b} ± 10

Figures bearing different superscripts in a column differ significantly (P<0.01).

Table 5. Mean initial body weight (IBW), % weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival of prawns fed with the diets for 60 days.

Treatments	IBW (g·prawn^{-1}) ± SE	WG (%) ± SE	SGR (% day) ± SE	FCR ± SE	PER ± SE	Survival ± SE
FM	0.10±0.03	64.74±6.50	0.83±0.07	4.03±0.43	0.74±0.07	98.67±1.33
AFS	0.12±0.00	65.88±1.06	0.84±0.01	3.66±0.06	0.79±0.01	98.67±1.33
FFS	0.12±0.004	72.68±6.58	0.91±0.18	3.46±0.29	0.85±0.07	100.00±0.00
Significance	NS	NS	NS	NS	NS	NS

NS- Not significance
WG (%) = (Final wt.-initial wt.) X 100; SGR=[(In Final wt.-In initial wt.)/60 days]X 100
FCR= [Wt. gain (g)/feed given (g)]; PER=Wt. gain/.protein intake;
Survival = Initial stock/Final stock X 100

acids (Dong et al. 1993). The essential amino acids, if prematurely absorbed, may be irreversibly further metabolised and not available for protein synthesis (Griger 1947). Dong et al. (1993) observed greater percentage of lower molecular weight peptides and free amino acids in AFS as compared to lactic acid fermented silage. Again, previous works have shown that some of the toxic factors of silages are associated with the lipid fraction (Kompiang et al. 1980). This can partly explain why the nutritional value of microbiological on fermented silage is better than AFS since the former is kept anaerobically during storage, thus preventing oxidation. Another possible explanation why microbiological silage is nutritionally better than AFS is the presence of antimicrobial substances (bactericide) which has been reported in FS (Wirahadikusumah 1969).

FCR AND PER

Although the mean values of FCR and PER of different groups were not statistically different ($p>0.05$), better results were recorded for FFS and AFS groups than FM (Table 5). Similar results were also reported by Ayinla and Akande (1988) who got the least FCR (1.2) for silage based diet as compared to the FM based diet. Lapie and Bigueras Banitez (1992) got higher PER value for the diet having FM and fish silage at 1:1 ratio than the diet without having fish silage. Ali et al. (1994) reported better FCR and PER with diet having silage at different concentrations than the control (0% silage). Cisse et al. (1995) reported better FCR (1.79) and PER (1.46) for AFS than FM based diet. Ximenes et al. (1996) recorded better FCR (1.8) and PER (1.98) with silage based diet as compared to the FM based diet (FCR 2.0 and PER 1.85). Though weight gains of the silage based diets were not significantly higher ($P>0.05$) than the FM based diet, the improvement in FCR, PER and SGR for inclusion of FS and AS in the respective diets are 14.14%, 14.86%, 6.76%; 9.64%, 1.2%, respectively (Table 5).

Body composition

Though an increasing trend was observed in the final body crude protein and crude fat contents in the juveniles of *M. rosenbergii* total carbohydrate and ash decreased (Table 6). Thus, no marked dietary influence ($P>0.05$) was

Table 6. Proximate composition of tissues of juveniles of *M. rosenbergii* (%) after 60 days of feeding.

Treatments	Dry matter				
	Moisture ±SE	CP ±SE	Total carbohydrate ±SE	EE ±SE	Ash ±SE
Initial	75.17±0.06	63.12±0.03	16.07±0.03	4.83±0.01	15.98±0.01
FM	75..69±1.25	69.61±8.69	9.24±15.65	5.69±1.44	15.46±10.04
AFS	75.82±1.23	70.39±10.97	9.28±6.81	5.95±1.51	14.38±10.21
FFS	75.98±1.98	71.42±5.18	5.88±15.63	6.02±1.45	16.68±9.60
Significance	NS	NS	NS	NS	NS

NS - Not significance

observed on the biochemical composition of the juveniles. These results correspond to the reports of Ali et al. (1994); Fagbenro et al. (1995); Belal et al. (1995) who too did not find any significant difference in the biochemical composition of their respective experimental animals due to the inclusion of fish silages in their experimental diets.

In conclusion on the above findings it was revealed that the fish silage based diets showed encouraging results in the juveniles diets of *M. rosenbergii*. FFS can substitute FM which is otherwise cheaper, user friendly and eco-friendly. FFS and AFS when used at 21 to 23% level in the diets showed better performance than FM ($P>0.05$). However, the maximum inclusion level of the product may be studied further to develop an economically viable feed for *M.rosenbergii*.

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