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AN EVALUATION OF THE SUITABILITY OF CROAKER FISH (*Otolithes* sp.) WASTE PRODUCTS SILAGES IN FEED *Macrobrachium rosenbergii* (de Man)

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ABSTACT

Waste product from croaker fish (*Otolithes* sp.), was eco-friendly utilized by ensiling with lactic acid bacteria (*Lactobacilli plantarum*), 4% (w/w) sulphuric acid and 4% (w/w) mixture of organic acids (formic acid and propionic acid in 1:1 ratio). These prepared silages were incorporated in juvenile of freshwater prawn *Macrobrachium rosenbergii* and feeding trials were conducted for a period of 60 days. Three different isonitrogenous diets were prepared with either bio-fermented fish waste silage, and sulphuric acid and mixture of organic acids (formic acid and propionic acid in 1:1 ratio) maintaining crude protein level of diets at 35 %. Biochemical composition, its nutritive evaluation and advantages of bio-fermented fish waste silage comparison with sulphuric acid and mixture of organic acids are discussed in this paper. The juveniles fed diets containing bio-fermented fish waste silage showed not statistically different (P> 0.05), but better weight gains(%), FCR, protein efficiency ratio (PER), feed efficiency ratio FER and net protein utilization than sulphuric acid and mixture of organic acids (formic acid and propionic acid in 1:1 ratio).

Key words: Waste utilization, *Macrobrachium rosenbergii,* Lactic acid bacteria, bio-fermented silage, sulphuric acid silage, formic acid and propionic acid silage.

INTRODUCTION

Semi-intensive and intensive farming systems for carnivorous fish and shrimp are currently depending on the use of fishmeal as the sole or major source of dietary protein and lipid in the aqua-feed. The predictions of fishmeal requirement in aquafeeds were 2.115 and 2.831 million tonne in the years 2000 and 2010 respectively (Barlow, 2000) [1]. The possibility that fishmeal availability could act as a major constraint to the development of the aquaculture industry.

Fishmeal production by scientific method is costlier, because the concentration of stick water in the process needs more effort. In the case of traditional method, the stick water released from fish during boiling is simply wasted resulting in heavy nutrient loss. Further, the traditional fishmeal preparation will be difficult particularly during glut seasons, if sunlight is poor. The other alternative way of utilizing trash fishes is the production of fish silage which does not release any off-odour during preparation. The method is simple, cost effective and easy to adopt and hence the silage preparation may be studied in detail and can be adopted at local level. Several workers have found that fish silage is superior to fish meal in the experiments (Ali *et al*., 1994[2]; Srinivasan *et al*.; 1985[3]; Viola *et al*., 1986[4]; Ali and Sahu 2002[5]).

The giant freshwater prawn, *Macrobrachium rosenbergii* is the one of the few species receiving attention owing to the large size and easy availability of seed. One potential use of fishery by-products is to convert them into fish feed having promising future to fulfill the increasing demand of fish meal. Fishery by-products processed by fermentation to enhance microbial stability could represent a practical, economical and eco-friendly alternative protein source to traditional fishmeal (Raa and Gildberg 1982)[6].

In India, the use of fish silage is a new concept and need to utilize as a source of protein in fish feeds. Keeping it in view, an attempt has been made to evaluate utilization of different fish silages in diets of *Macrobrachium rosenbergii* (bio-fermented fish waste silage, and sulphuric acid and mixture of organic acids (formic acid and propionic acid in 1:1 ratio) from Dhoma fish waste.

MATERIALS AND METHODS

The trash fish *Otolithes sp.* (Dhoma) waste (by-product), after the edible meat extraction, was used for preparation fish waste meal and fish silage. Bio-fermented silage (A) was prepared by pure culture of lactic acid bacteria (*Lactobacilli plantarum* NCIM 2083(ATCC 8014) was added and mixed with 500g of fish waste and Malesses 75g (@15%w/w), Water 50g (@ 10%) and BHT 0.125 g (@250ppm). Sulphuric acid silage (A) were prepared by adding 95% sulphuric acid (4 % wt/v) and Organic acid silage were prepared by adding a mixture of 95% formic acid 2% (w/w) and 2% (w/w) propionic acid silage (C (1:1 ratio), in 3 groups containing minced fish waste 500 g of each. Antioxidant were added BHT 0.125g (@250 ppm) in the mixture to avoid the fat oxidation.

The proximate composition of all feed ingredients and prawns were analyzed standard methods (AOAC, 1995)[7].

The feed formulations were made to keep the crude protein and lipid content around 35 % and 8% respectively. All the ingredients were kept same in all the three experiments for Bio-fermented silage A group, sulphuric acid silage in B group and silage prepared from a mixture formic acid and propionic acid in group C. Equal proportions of protein were supplemented from Bio-fermented silage A group, sulphuric acid silage in B group and silage prepared from a mixture formic acid and propionic acid in group C in fish silage to the corresponding feeds. Accordingly inclusion of different ingredients were adjusted to make all the feeds isonitrogenous. All the ingredients except vitamin and mineral mixture (Table-2), were mixed thoroughly in a mixer blender and required amount of water and oil were added. The feed mix was put in air tight polythene packets and kept for one hour for proper conditioning. Then the conditioned feed mixture was sieved and vitamin-mineral premix and vitamin C (Celin tablet, Glaxo) were added. Finally, it was passed through an extruder having a die of 1 mm size (Double twin screw extruder machine, BTPL, West Bengal) at barrel screw speed of 350 rpm, and die plate temperature 90 °C. Pellets were put in airtight polythene packets till their use.

Cultural experiment

The experiment was conducted over a period of 60 days, in the Wet-Lab of Department of Aquaculture, Central Institute of Fisheries Education, Mumbai.

The experiment was set up in five distinct experimental groups each group having four replicates in 20 uniform size plastic tubs (50 l capacity each and bottom diameter 37.5 cm).15 juveniles of *Macrobranchium rosenbergii* uniform size were stocked in each tub. The average weight of the juveniles in each tub ranged from 1.33 to 1.34 gms at the time of stocking. The feed was given @ 5% of body weight of juveniles and was reduced and @ 3 % by at the end of the experiment. The water quality parameters viz, temperature, pH, dissolved oxygen, free carbon dioxide, carbonate hardness, ammonia-N, nitrite-N, nitrate-N and alkalinity were estimated by using standard methods APHA, 1998[8].

Growth evaluation

Percentage weight gain (PWG), Specific growth rate (SGR),Feed conversion ratio (FCR), Feed Efficiency Ratio (FER), Survival % were calculated. Data were processed for Analysis of Variance (ANOVA) and significance were tested by the method described by Snedocor (1966)[9].

RESULTS AND DISCUSSION

Proximate composition of all the dried silages in (Table -1) and other all ingredients in

Table - 1: Proximate composition of bio-fermented silage A, sulphuric acid silage B and organic acid silage C * (% Dry wt. Basis)

S. No.	Nutrients	Bio-fermented silage(A)	(4%) Sulphuric acid silage (B)	(4%)Organic acid silage*(C)
1.	Moisture	71.73±0.20	68.04±0.32	67.18±0.29
2.	Crude Protein	44.24±0.03	49.51±0.36	47.36±0.01
3.	Ether Extract	18.52±0.07	17.13±0.09	18.26±0.04
4.	Nitrogen Free Extract	10.36±0.11	7.20±0.04	8.10±0.02
5.	Total Ash	27.13±0.06	25.84±0.36	25.96±0.22

*Organic acid silage were prepared by adding mixture of 95% formic acid propionic acid in to 1: 1 (W/W) ratio.

(Table -2) is shown before preparing the feed. In case fish bio-fermented silage (A), (4%w/w) sulphuric acid silage (B) and a mixture of 2% (w/w) formic acid and 2% (w/w) propionic acid silage (C) after 15th days of ensiling shows a slight variation in dry matter (DM) content but differences were not significant (P > 0.05). Wood *et al.* (1987) [10]found 74.4% CP (DW basis) in formic acid preserved fish silage and 71.6% for low temperature dried fish meal. Ali *et al.* (1994)[2] found 61.86, 56.54 and 56.82% crude protein content in fish meal, formic acid silage and sulphuric acid silage respectively. Hoq *et al.* (1995) [11] noticed the crude protein for raw silage, dried silage and laboratory made fish meal as 14%, 52.9% and 64%, respectively. Goddard and Al-Yahyai[12] (2001) reported that protein contents of a mixture (1:1) of formic acid and propionic acid silage ranges from 66.9 to 72.5% (DW basis). Jagannatha *et al.* (1996)[13] found the ether extract as 32% (DW) and 10.4% (WW, 32% on DW basis, 67.5% moisture) for fish meal and fermented fish viscera silage, respectively.

The bio-fermented silage A contained lower protein content than sulphuric acid B (4% w/w) silage, due to the addition of molasses and slight dilution by lactic acid produced and other fermented products during the fermentation process. Similar observations were reported by Raa and Gildberg, (1982)[6]; Fagbenro and Jauncey, (1995)[14] and Ali and Sahu, (2002)[5]. The higher lipid content of bio-fermented silage A than those of sulphuric acid B (4%) and a mixture of 2% (w/w) formic acid and 2% (w/w) propionic acid silage C may be due to extraction of lactic acid as well along with fats during ether extraction (Fagnenro and Jauncey, 1995)[14].

Table - 2: Proximate composition of ingredients used for diet preparation

S. No.	Nutrients	Moisture (%)	Crude Protein(%)	Ether Extract(%)	Crude Fiber(%)	Nitrogen Free Extract (%)	Total Ash(%)
1.	Rice flour	9.52±0.05	7.09±0.39	1.37±0.06	0.79±0.10	79.99±0.63	1.26±0.04
2.	Wheat flour	8.42±0.16	11.81±0.39	2.45±0.28	6.07±0.11	69.10±0.16	2.15±0.14
3.	Ground Nut oil cake	8.34±0.08	41.15±0.20	8.02±0.04	13.36±0.02	24.00±0.01	5.16±0.01
4.	Prawn head meal	5.28±0.01	41.93±0.20	6.19±0.23	10.44±0.57	12.02±0.73	24.40±0.86
5.	Soybean meal	7.32±0.05	43.12±0.20	1.30±0.04	6.98±0.06	34.20±0.04	7.34±0.06
6.	Tapioca	6.54±0.02	1.58±0.39	0.51±0.25	2.39±0.06	87.03±0.41	2.01±0.09

Table - 3: Ingredients and their combination (%) in experimental diets

S. No.	Ingredients	(%) in different experimental diets A	B	C
1.	Bio-fermented silage	25.0	-	-
2.	Sulfuric acid silage (Inorganic acid)	-	24.0	-
3.	A mixture of formic and propionic acid silage(Organic acid)	-	-	24.0
4.	Prawn head meal	10.0	10.0	10.0
5.	Soya meal	25.0	23.0	24.5
6.	Groundnut oil cake (GOC)	18.5	18.0	18.0
7.	Rice flour	6.0	7.0	6.5
8.	Wheat flour	7.5	9.0	9.0
9.	Tapioca	2.0	3.0	2.0
10.	Cod liver oil & Sunflower oil	3.0	3.0	3.0
11.	**Vitamin and Mineral mix (EmixPlus*)**	**2.0**	**2.0**	**2.0**
12.	Carboxymethyl cellulose (CMC)	1.0	1.0	1.0
	Total (%)	100	100	100

*** Emix Plus**

Marketed in India by, Everest Pharma,
11, BMC shopping Centre, Mitha Nagar,
Goregaon (West), Mumbai- 400090.

Each –250 gm contains of Emix Plus

Viatmin- A	5,50,000 IU	Iron	750 mg
Viatmin-D_3	1,10,000 IU	Zinc	1500 mg
Viatmin-B2	220 mg	Copper	200 mg
Viatmin-E	75 mg	Cobald	45 mg
Viatmin-K	100 mg	Calcium	50 g
Viatmin- B6	100 mg	Phosphous	30 g
Viatmin- B12	0.6 mg	L-lysine	1 g
Calcium Pantothenate	250 mg	DL-Methionine	1 g
Niacinamide	1 g	Selenium	5 g
Choline chloride	15 g	Satwari Carriers	250 mg q.s.
Maganese	2700 mg	Iodine	100 mg

(Lactobacillus 120 million units and yeast culture 3000 crore units) and Vitamin C was supplemented separately in the form of Celein tablets, Glaxo company, India @ 100 mg kg^{-1} diet).

The ash content of the bio-fermented silage A was higher than the others, which is probably due to incidental entry of impurities through molasses. Similar results were obtain by Ali *et al.* (1994)[2] found 24.39, 11.36 and 12.79% ash content in fish meal, formic acid silage and sulphuric acid silage respectively.

Cultural experiment

Physico-chemical parameters of water

Physico-chemical parameters of water such as except water temperature (^{0}C), water pH, dissolved oxygen, free carbon dioxide, total alkalinity, total hardness, total ammonia and Nitrite (NO_2-N) and Nitrate (NO_3 –N) recorded during the

Table - 4: Proximate composition of experimental diets

S. No.	Treatment	Moisture (%)	Crude Protein(%)	Ether Extract(%)	Nitrogen Free Extract(%)	Crude Fiber(%)	Total Ash(%)
1.	Bio-fermented silage (A)	5.11 ±0.01	35.04 ±0.39	8.70 ±0.02	30.02 ±0.41	6.73 ±0.02	14.40 ±0.02
2.	Sulphuric acid silage (B)	5.41 ±0.07	34.85 ±0.20	8.51 ±0.04	30.31 ±0.22	6.49 ±0.03	14.45 ±0.02
3.	A mixture Formic and propionic acid silage(C)	5.53 ±0.03	34.65 ±0.00	8.45 ±0.04	30.07 ±0.07	6.77 ±0.02	14.54 ±0.03

Table - 5: Physico-chemical parameters of the water during experimental period of 60 days of Treatment – A, B, C, D and E

Treat-ments	Mean water (°C) Temp.	Mean pH	Mean Dissolved oxygen	Mean CO_2	Mean total Alkalinity (ppm)	Mean total Hardness (ppm)	Mean Nitrate (ppm)	Mean Nitrate (ppm)	Mean NH_3 (ppm) Before Water Ex.	After Water Ex.
A	25.146	8.146	6.346	ND	228.538	193.395	0.047	0.141	0.054	0.108
	0.194	±0.073	±0.048	-	±8.674	±2.536	±0.003	±0.007	±0.003	±0.004
B	25.146	8.146	6.277	ND	227.846	193.846	0.045	0.143	0.053	0.102
	0.194	±0.073	±0.048	-	±9.006	±2.759	±0.002	±0.007	±0.003	±0.004
C	25.146	8.146	6.315	ND	228.923	194.462	0.044	0.143	0.057	0.108
	0.194	±0.073	±0.044	-	±8.214	±2.812	±0.002	±0.007	±0.002	±0.003

experimental period were found to be within the normal range for *M. rosenbergii* (New and Singkholka,1982[15]; Smith *et al*., 1976;[16] and 1981[17]). The optimum temperature range for *M. rosenbergii* was reported to be in the range of 29°C to 31°C (Raman, 1967[18]).

Proximate composition of experimental diets

All the experimental diets (i.e. diet containing bio-fermented silage A, sulphuric acid silage B and a mixture of formic acid and propionic acid silage C) were analysed for their proximate composition crude protein, lipid, nitrogen free extract (NFE), crude fibre and ash content. The proximate composition of all the experimental diets were is given below.

Effect of diets on Growth parameters

Body weight gain

At the end of the experiment, the growth responses, better weight gain significant (P< 0.05) were recorded in diet A, B and C in fish silages are reported in Table 6. Diet A (containing Bio-fermented fish silage) gives significantly (P< 0.05) the highest weight gain, FCR, SGR, PER and NPU.

The juveniles of different treatments gained weight with in the range of 1.56±0.04 and 1.64±0.07 %. The body weight gain (Table 6) was expressed in percentage to avoid the initial variation in body weight and presented. The highest weight gain was achieved by A (122.28%) treatment where as the lowest was achieved by C treatment (116.68%). However, the differences of the mean percentage weight gain of different experimental treatments were not statistically significant (P > 0.05).

At the end of the experiment, not significant (P >0.05) (Table 6) but better weight gains (%) were recorded for the bio-fermented silage A based diets than the both sulphuric acid silage B and a mixture of formic acid and propionic acid silage C. Quality of protein depends upon the amino acid make up of the respective protein. So definitely the superiority of bio-fermented silage may be due to better amino acid makeup compared to other diets. Which is in agreement with the findings of Viana *et al*, (1996) [19] and Ali and Sahu, (2002)[5]. Jagannatha *et al*. (1996) [16] reported fermented fish and poultry offal silages to be nutritionally superior to fish meal when fed to *Cyprinus carpio*. Fish silage is known to be a good attractant (Hardy and Masumoto, 1991[20]), since larger amounts of soluble protein and free amino acids are present. Srinivasan *et al.* (1985[3]) found double weight gain in *Cyprinus carpio* and *Cirrhinus mrigala* with the fish silage based diet as compared to control diets containing mustard oilcake and rice bran.

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 fish meal (Gallagher 1993)[21] Comparatively, diet containing bio-fermented silage A was better ($P > 0.05$) than those of (4%) sulphuric acid silage B and a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) C (Table 6). This may be because of the higher rate of proteolysis in (4%) sulphuric acid silage, (4%) a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) than bio-fermented silage as confirmed by its higher values of TVB-N and a-amino-nitrogen. After 15 days of ensiling, both the lactic acid and sulfuric acid silages retained a high percentage of large peptides. The difference in pH during the ensiling process (pH 4.38 for lactic acid silage; pH 3.2 for (4%) sulfuric acid silage and a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) pH 3.60) probably affected the type and rate of enzymatic activity, as has been reported by Raghunath and McCurdy (1990)[22] for silage prepared from acidified rainbow trout viscera. Compared to the lactic acid silage after15 days ensiling, the 15-day-old sulfuric acid silage had greater percentage of lower molecular weight peptides and free amino acid than lactic acid. The lactic acid silage contended large peptide with a molecular weight distribution from 500 to 1200 daltons than the sulfuric acid silage (Dong *et al.,* 1993[23]). In present study lower growth response was found in *M. rosenbergii* on a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) base diet C, as compared to bio-fermented silage containing diet A and sulfuric acid silage containing diet B was due to depress proteolytic activity by formic acid suggested by Rungruangsak and Utne (1981) [24] and Ali *et al.* (1994)[2].

Approximately, 80% of the protein in acid preserved silage becomes solubilized after one week at a temperature of 23 to 30 ^{0}C (Hoq *et al.,* 1995)[11]. Therefore, the acidic fermentation silage contained higher levels of essential amino acids in 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 acids (Dong *et al.,*1993[23]). The essential amino acids, if prematurely absorbed, may be irreversibly further metabolized and not available for protein synthesis.

This can partly explain why the nutritional value of microbial fermented silage is better than acidic fermented silage, since the former is kept anaerobically during storage, thus preventing oxidation. Another possible explanation why bio-fermented silage is nutritionally better than acidic fermentation silage is the presence of antimicrobial substances (bactericide), which has been reported in bacteria fermented silage (Wirahadikusumah 1969)[25]. The antagonistic properties of lactic acid bacteria are mainly due to the production of organic acids. It is the undissociated molecules of the organic acid that are responsible for the anti-microbial activity, and lowering the pH of the product increases the proportion of undissociated molecules of organic acid. High-molecular weight substances have been observed in fish silage with activity against gram positive bacteria (Raa and Gilderg, 1982[6] and Dong *et al*.,1993[23]).This may killed food entoxifying bacterial and helpful to avoid foods born entoxification.

Feed conversion ratio (FCR) and protein efficiency ratio (PER)

The mean values of feed conversion ratio (FCR) was statistically ($P> 0.05$) in different groups, but better feed conversion ratio (FCR) were recorded (Table 6) for diet containing bio-fermented silage A (2.67) than sulfuric acid silage B (2.73) than a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) base diet C (2.87) groups. Protein efficiency ratio (PER), feed efficiency ratios and net protein utilization (Table 6) of different groups were also statistically different (P > 0.05). Better results of protein efficiency ratio (PER) were recorded for diet containing bio-fermented silage C (1.07) than sulfuric acid silage D (1.06) than a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) base diet E (1.01) groups (Table 6). Ali *et al*. (1994)[2] reported that on the basis of observed growth rate, FCR, PER, NET protein utilization and apparent protein digestibility *Cirrihinus mrigala* fed diet with 75% replacement by silage preserved with 30 % sulphuric acid gave the best performance followed by diet with 75% replacement by silage preserved with 90 % formic acid. The rest of the diets with 50% and 100% replacement with both the silages showed varying but consistently better performance than the control (0% silage).

Highest growth rate and FCR (Tables 6) are obtained in bio-fermented silage containing diet A than sulfuric acid silage containing diet B than a mixture of (4%) formic acid and propionic acid silage (1:1 ratio) base diet C in decreasing manner for inclusion of in the respective diets are 25%, 24%, and 24% (Table 3.2) respectively.

Feeding behaviour of *M. rosenbergii* juveniles also play important role in food acceptance. *M. rosenbergii* have large chelae legs aided by the third maxillipeds which capture feed and convey feed to the mouth. It has chewing habit of holding feed pellet in the chelate legs. Better stability due to the presence of molasses in bio-fermented silage A containing diet made food available for long period in good condition for the prawn. Feed consistency is improved, effective for nutrient retention and leaching is prevented which leads less wastage. This may one of the reasons for better growth, FCR, protein efficiency ratio (PER) feed efficiency ratio (FER) net protein utilization in bio-fermented silage A containing diet as compared to other diets. Viola *et al*., (1986)[4] and Wood, (1987)[10] have made similar observations. They found that, the high physical stability of moist fermented tilapia silage pellets were effective for nutrient retention and did prevent protein losses through leaching. The advantage of using binders in fish silage feeds is obvious as the feed consistency is improved and wastage reduced (Viola *et al*., 1986)[4], thereby, ensuring optimum feed/nutrient uptake (Wood, 1980)[26]. The binding capacity of molasses used in bio-fermented silage A preparation may also have contributed to the pellet stability, in addition to the high pelletability of other feedstuffs (Wood, 1987)[10].

Percentage of survival

Percentage of survival of different treatments were recorded in the range of 83.33% (C treatment) to 88.33% (A treatment). The survival percentage of the juveniles of different treatments has been presented in Table 6. However, the survival percentages of different groups were not statistically significant (P>0.05). However, the mortality occurred was mainly handling stress during cleaning siphoning operation. Survival rate was not affected by feeding in any the experimental diets. Although the better survival percentage (88.33%) was achieved in bio-fermented silage containing diet A. Similar results were obtained by Ali and Sahu, (2002)[5].

Body composition

Table 7 shows increase in protein content from initially 59.46% to 65.59, 65.95, 64.17 and 64.77 % in diet containing bio-fermented silage A, sulphuric acid silage B and a mixture of formic acid and propionic acid silage C respectively. 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 7). Ali and Sahu (2002)[5] reported similar observations in the biochemical composition of *M. rosenbergii* fed with diet containing fish meal, acidic fish silage and bacterial fermented fish silage. Thus, no marked dietary influence (P > 0.05) was observed to the reports of Ali *et al.* (1994)[2]; Fagbenro *et al.* (1994)[27]; Belal *et al.* (1995)[28] who, too did not find any significant difference in the biochemical composition of their respective experimental animals due to the inclusion of fish silage in their experimental diets.

In conclusion on the above findings it was revealed that, silages prepared with organic rather than inorganic acids are easier to handle (less corrosive), and may not require neutralization. Also, fish can readily metabolize organic acids. There are definite advantages to using bio-fermented (lactic acid bacteria) rather than adding organic or

Table - 6: Comparative statement showing different Growth Parameters the different groups fed with experimental diets during the experimental period of 60 days

S. No.	Growth Parameters	A	B	C
1.	Initial weight Prawn	1.34 ± 0.05	1.34 ± 0.07	1.34 ± 0.05
2.	Final weight Prawn	2.99 ± 0.13	2.93 ± 0.05	2.90 ± 0.09
3.	Body weight again	1.64 ± 0.07	1.59 ± 0.03	1.56 ± 0.04
4.	Body weight again %	122.28 ± 0.71	120.06 ± 7.63	116.68 ± 2.23
6.	Feed conversion ratio (FCR)	2.67 ± 0.04	2.73 ± 0.18	2.87 ± 0.04
7.	Feed efficiency Ratio (FER)	0.37 ± 0.01	0.37 ± 0.02	0.35 ± 0.00
8.	Protein Efficiency Ratio (PER)	1.07 ± 0.02	1.06 ± 0.06	1.01 ± 0.02
10.	Net protein Utilization (%)	70.42 ± 1.11	69.28 ± 4.18	65.17 ± 0.91
12.	Specific growth rate (SGR)	0.60 ± 0.02	0.59 ± 0.07	0.58 ± 0.04
11.	Survival %	88.33 ± 5.00	85.00 ± 3.19	83.33 ± 3.33

Table - 7: Biochemical composition of juveniles of *Macrobrachium rosenbergii* of different experimental groups (% DM basis)

S. No.	Treatment	Period (Day)	Moisture	Dry matter			
				Crude Protein	Ether Extract	Nitrogen Free Extract	Total Ash
1.	Initial Weight juveniles	0	79.32±0.08	59.46±0.39	4.30±0.01	19.16±0.36	17.08±0.04
2.	Bio-fermented silage (A)	60	75.17±0.09	65.95±0.20	5.57±0.06	12.60±0.10	15.88±0.04
3.	Sulphuric acid silage (B)	60	75.66±0.02	64.17±0.20	4.88±0.06	13.82±0.15	16.13±0.11
4.	A mixture Formic and propionic acid silage (C)	60	75.54±0.07	64.77±0.20	4.91±0.06	13.94±0.29	16.39±0.03

inorganic acids to ensile visceral by-product. Since the culture of lactic acid bacteria is easy to maintain and reproduce, the cost of the culture would be minimal compared to the cost of purchasing lactic acid, or any of the other common organic and inorganic acids suitable for silage preparation.

In present study bio-fermented silage based diet (A) has shown best results than other diets (treatments). Therefore, bio-fermented silage (A) is cheaper, user friendly and eco-friendly than sulphuric acid silage B (Inorganic acid silage) and a mixture of formic acid and propionic acid silage C (organic acid silage). Bio-fermented silage is suitable, inexpensive protein sources for juveniles diets of *M. rosenbergii*. The use of fish waste in aquafeed is potentially a profitable solution to a fish disposal problem by converting in bio-fermented silage.

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