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Efficacy of compounded feeds prepared from fermented mantis shrimp on growth performance of *Peneaus indicus* post larvae

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ABSTRACT

Ten compounded feeds incorporating 20 - 100 % of mantis shrimp (Oratosquilla nepa), *fermented using* Bacilles licheniformis *and* Beauveria sp. *respectively and containing 32 to 49 % crude protein were fed to post larvae of* Penaeus indicus *(initial average weight 0.19 ± 0.06 g) for a period of 45 days . A significant (P< 0.05) increase in body weight was recorded in all*

the B . licheniformis *treatment groups as compared to control post larvae . The group of post larvae maintained on the diet with 80 % incorporation of mantis shrimp accorded the best growth in terms of average body weight (563.16 %). The FCR for these feeds ranged from 1.56 to 2.08 as compared to a FCR of 5.0 on the control feed. Survival in all the treatments was in the range of 86 to 98% and not significantly different from the control.*

Feeds containing 20 to 100% mantis shrimp fermented using Beauveria *sp. elicited a poor growth response as compared to feeds prepared from mantis shrimp fermented using* B. licheniformis. *At the 60 % incorporation however, a slightly better response was obtained wherein a 316 % increase was observed in comparison to 213% in case of control animals . The feed conversion ratio and survival rates also did not show any significant (P > 0.05) variation in comparison to the control group.*

Overall growth performance and carcass yields show the superiority of fermented mantis shrimp and prove its efficacy as a feed ingredient in shrimp feeds.

Introduction

In the context of increasing global demand for prawns and promotion of prawn culture the key factor becomes the provision of appropriate feed , which constitutes around 50 – 80 % of the total operational cost in aquaculture . Scarcity of high quality feed ingredients , their location specificity and cost remain critical factors in the large scale production of practical feed .

Fishery wastes (trash fish , mantis shrimp , squid waste , prawn heads and peels) along with other agro- industrial wastes are highly attractive for explotation on account of their protein , chitin, carbohydrate and cellulose contents and also for the presence of certain pigments and flavours . Most of these however , require a pre- treatment in some physical or chemical form , and are therefore uneconomical on a large scale . The use of marine micro - organisms to convert protein , carbohydrate , chitin and other low – cost , agro - industrial wastes into foodstuffs, rich in protein employing solid state fermentation seems to be promising and presents a novel and

cheaper method of compounding nutritious feeds for larval and grow-out stages of shrimp . Therefore, in the present investigation Solid State Fermentation (SSF) technology was employed for the production of microbial protein as well as protein enrichment of mantis shrimp *(Oratosquilla nepa*) using one strain of bacteria *B. licheniformis* and one strain of fungi *Beauveria* sp . in order to evaluate it's suitability for aquafeed formulation. The fermented material was incorporated at varying concentrations into a formulated feed base and fed to post larvae of *P. indicus* for a period of 45 days in order to evaluate it's effect on growth and survival and overall suitability in aquafeeds .

Materials and methods

Solid State Fermentation of mantis shrimp was carried out following the method suggested by Ramesh and Lonsane (1987) . Necessary changes were however , made in the medium composition and methodology after optimization of process parameters .

Microorganisms

Bacillus sp BTM 01 (*B. licheniformis*) and Fungi (*Beauveria* sp.) isolated from brackish water samples using ZoBell's marine agar for the former and mycological agar and streptomycin in sea water (35 ppt.) for the latter were maintained as agar slants and subcultured every week .

Preparation of solid substrate

Fresh mantis shrimp was collected from the Cochin Fisheries Harbour and transported immediately to the laboratory in polythene bags , sorted off adhering debris, washed well three to four times in running tap water and dried at 70 ° C for 24 h in an oven . The dried material with a moisture level of < 10 % was powdered in a laboratory pulveriser and sieved through a sieve of 200 μ to obtain uniform particle size. The pH was determined and so also the moisture content by using one g samples in duplicate. The solid substrate was dispensed as 5 g aliquots in petriplates and in 250 ml conical flasks and adjusted to the desired level of moisture content

(between 30-60 %) with sea water adjusted to the optimum pH (pH 8.0 and 12.0 for bacteria and fungi respectively). The flasks and petriplates along with their contents were autoclaved at 121 ° C for 60 min. and cooled down to room temperature .

Inoculum preparation

Bacteria : A loopful of 24 h old agar slant culture of *B. licheniformis* was first grown in 10 ml of ZoBell's marine broth for 18 h at room temperature (28 ± 2 ° C). One ml of this culture was then transferred asceptically to 50 ml nutrient broth and incubated on a rotary shaker at 150 rpm for 18 h at room temperature . Cells were harvested by centrifugation (10,000 rpm for 15 min. at 4 ° C) and then were made to 10 ml using sterile physiological saline (0.85 % NaCl) after repeated washings . This prepared cell suspension was used as inoculum for fermentation .

Fungi : 20 ml of sterile physiological saline containing 0.1% Tween was added to each fully sporulated (2 week old) slant culture (raised on Bennet's agar prepared with aged sea water) by means of a sterile pippete . The spores were scraped using an inoculating needle under strict asceptic conditions and the spore suspension obtained was adjusted to the desired concentration using sterile physiological saline.

Inoculation and incubation

The prepared inocula were adjusted to a concentration of 2 mg dry cell equivalent in one ml of cell suspension for bacteria and 2 ml of spore suspension per flask (inoculum level selected randomly) for fungi and added to the sterilised moist media in flasks . The contents were mixed thoroughly and incubated in a slanting position at 35 ° C and 28 ° C for bacteria and fungi respectively with 60 – 70 % relative humidity for 48 to 72 h period . After fermentation , the contents were dried to a constant moisture level and proximate composition analysed .

Feed preparation : A set of five feeds each were formulated by incorporating 20, 40, 60 , 80 and 100 % of mantis shrimp fermented using *B . licheniformis* and designated as B MS 1, B MS 2 , B MS 3 ,

B MS 4 and B MS 5 respectively and a set of five feeds each were formulated by incorporating 20 , 40, 60 , 80 and 100 % of mantis shrimp fermented using *Beauveria* sp and designated as MS 1, MS 2, MS 3, MS 4 and MS 5 respectively . The diet devoid of fermented material designated as C served as control . The % incorporation of the other feed ingredients in the feed base and their composition are given in Table 1 . All powdered and weighed ingredients excluding tapioca flour were mixed together and thoroughly blended .Tapioca was gelatinised in hot water and then mixed with other ingredients . The prepared dough was extruded through a 1mm die and the feed pellets broken manually into smaller pieces of 3-5 mm length . The pellets were sun dried to less than 10 % moisture and stored in labelled plastic containers at room temperature (28 ± 2 ° C).

Table 1. Percentage incorporation of fermented mantis shrimp * and other feed ingredients used for feed formulation.

Ingredients (g/ 100 g dry diet	Diets					
	C	MS1	MS2	MS3	MS4	MS5
Mantis shrimp*	0	20	40	60	80	100
Shrimp meal	25	05	-	-	-	-
Fish meal	20	20		05	-	-
Soyabean meal	20	20	20	05	-	-
Groundnut oilcake	15	15	15	15	-	-
Tapioca powder	12.5	12.5	12.5	12.5	12.5	3.0
Oil [1]	04	04	04	04	04	04
Vitamin mixture [2]	02	02	02	02	02	1.5
Mineral mixture [3]	01	01	01	01	01	01
Cholesterol	0.5	0. 5	0.5	0.5	0.5	0.5

* Mantis shrimp fermented using *B. licheniformis* or *Beauveria* sp.

[1] Oil – mixture of a 1: 1 combination of vegetable and fish oil.

[2] Vitamin mixture (mg/ 100 g dry diet)- para aminobenzoic acid 5.55 ; inositol 22.06 ; nicotinic acid 22.21 ; Ca pantothenate 33.31; pyridoxine – Hcl 6.66 ; riboflavin 4.44 ; thiamine – Hcl 2.22 ; menadione 2.22 ; Betacarotene 5.55 ; tocopherol 11.10 ; calciferol 6.66 ; Na – ascorbate 110.3 .

[3] Mineral mixture (g /kg dry diet) K_2PO_4 1.008 ; Na_2HPO_4-$7H_2O$- 2.167; $Ca(H_2PO_4)$ $2H_2O$ - 2.671 ; $CaCo_3$ -0.978 ; Ca-lactate -1.663 ;KCl -0.282; $MgSO_4$ $7H_2O$ - 0.048 ; $MnSO_4$ $6H_2O$ - 0.0108 ; $CuCl_2$ -0.0015 ;KI- 0.0023; $CoCl_2 6H_2O$ - 0.0141 ; Celufill -0.0216 .

Animal experiments

Post larval *P. indicus* , having an average initial length of 3.1 ± 0.3 cm and an average initial weight of 0.19 ± 0.06 g , procured from a commercial shrimp hatchery , were used for the feeding experiments. The larvae were acclimatized to laboratory conditions for a week prior to starting the feeding trial during which period they were maintained on the control feed . A set of post larvae were sacrificed and their body composition analysed before the start of the feeding experiment.

The post larvae were housed in 10 litre plastic tubs each provided with individual aeration . Each treatment was carried out in triplicate with twenty post larvae present in each replicate . Feeding was carried out at the rate of 20 % of the body weight in two divided doses at 10 and 18 h daily .The salinity of the water used was maintained at 35± 1 ppt ; temperature at 28.0 ± 2.0 ° C and dissolved oxygen at 5.8 ± 0.4 ml / lit. throughout the experimental duration of 45 days . One third water exchange was carried out daily while complete water exchange was done every third day . Left over feed and faecal matter were removed daily . At the termination of the experiment post larvae from each group were weighed and their carcasses dried , powdered and analysed for their proximate composition.

Analytical methods

Moisture , crude protein , crude fat , crude fibre and ash contents in fermented mantis shrimp , feed ingredients, prepared feeds and carcasses were determined by standard procedures of A. O . A . C . (1990). Water analysis was carried out as per the method out lined by APHA (1980).Water stability of the feeds was determined by the method of Jayaram and Shetty (1981). RNA content of the fermented mantis shrimp was measured by the method of Fleck & Munro (1962) and DNA by the method of Burton (1956). Data obtained in the feeding experiments were subjected to statistical analysis ((Snedecor and Cochran, 1973).

Results and discussion

The use of microorganisms to convert carbohydrates , lignocelluloses and other industrial wastes into protein rich food and feedstuffs has been well documented(Barbesgaard, 1977; Toyama, 1976 ; Mudgett, 1986 and Ghildyal *et., al.*,1981) . Fishery wastes on account of their high protein and polyunsaturated fatty acid contents, pigments and flavours offer great potential in aquafeed formulation. However, their high perishability under normal ambient conditions remains a hurdle in their maximum utililisation . Mantis shrimp was therefore collected and subjected to fermentation separately using both bacteria *B . lichenifórmis* and fungi *Beauveria* sp . respectively . In the bacterial fermentation apart from the mild change of colour obtained after fermentation was complete (48 h) , no other major physical change in appearance was observed . In the case of fermentation with fungi which took 72 h there was pronounced spore formation leading to a visible change in colour and a strong mouldy odour . Protein content increased in both the fermentations though the increase was higher in case of bacterial fermentation as compared to the fermentation with fungi . The protein content increased from an initial value of 32.40 % to 44.60

upon fermentation with *B. licheniformis* while an increase of 37.65% was recorded upon fermentation with *Beauveria* sp. Increase in protein content of banana meal for poultry feeding from 6 to 16 % was recorded (Leon , 1988) and in cassava from an initial value of 1.28 g / 100 g dry matter to 14.32 g / 100 g dry matter (Balagopalan and Padmaja , 1988). All other nutrients showed a desirable decrease in both the fermentations, which was indicative of the ability of the microorganisms to carry out bioconversion (Sridhar and Chandrashekar, 1985). The present work on fermentation of mantis shrimp - a fishery waste , is probably one of the first reported and there is no similar work to compare results .

The nucleic acid content of *B . licheniformis* fermented mantis shrimp was 2.2 % and in *Beauveria sp* fermented mantis shrimp it was 0.8 % . Both these values are within the safe limits prescribed for nucleic acids in single cell proteins (Litchfield , 1968).

The results of the proximate composition analysis of the five feeds prepared incorporating 20 to 100 % of mantis shrimp fermented with *B . licheniformis* as well as the five feeds prepared incorporating 20 to 100 % of mantis shrimp fermented with *Beauveria* sp. are presented in Table 2 A & B. The protein content of the former ranged between 40 to 49 % and the latter between 32 to 40 % . The control diet had a protein content of 47 % . Crude fat ranged from 10 to 11 . 36 % ; crude fibre from 2.94 to 3.46 % and ash from 14.98 to 17.31 % respectively for the bacterially fermentated feeds while for the diets prepared using mantis shrimp fermented with fungi crude fat ranged between 10.96 to 11.41 %, fibre from 2.42 to 3.00 % and ash from 14.92 to 17.09 % . All feeds met the nutritional specifications of shrimp feeds .

Table Proximate composition of the control and experimental diets (prepared from mantis shrimp feeds fermented with *B. licheniformis*) used for the feeding trails .

Nutrient	Diets (% dry matter)					
	C	BMS1	BMS2	BMS3	BMS4	BMS5
Dry matter	92.22	91.42	93.42	91.16	90.48	92.12
Crude protein	46.81	48.14	49.79	52.14	54.29	56.95
Ether extract	10.78	6.48	8.42	6.96	7.63	7.21
Crude fibre	2.13	2.06	2.21	2.33	2.41	2.56
NFE *	23.93	28.96	24.67	23.51	20.82	19.27
Ash	16.35	14.36	14.91	15.06	14.85	14.01

* Nitrogen free extractives calculated as 100 – (% crude protein + % crude lipid + % crude fibre + % ash + % moisture)

Table 2 B. Proximate composition of the control and experimental diets (prepared from mantis shrimp feeds fermented with *Beauveria sp.* fungi) used for the feeding trails .

Nutrient	Diets (% dry matter)					
	C	MS1	MS2	MS3	MS4	MS5
Dry matter	92.22	90.16	90.47	92.48	91.86	90.07
Crude protein	46.81	40.25	36.84	32.48	31.68	37.04
Ether extract	10.78	11.41	10.96	11.02	11.38	11.02
Crude fibre	2.12	2.86	2.42	2.54	2.81	3.00
NFE *	23.93	28.39	33.64	39.08	39.00	34.02
Ash	16.35	17.09	16.14	14.88	15.13	14.92

* Nitrogen free extractives calculated as 100 – (% crude protein + % crude lipid + % crude fibre + % ash + % moisture) .

The water stability of these feeds is presented in Figure 1A & B . The initial dry matter (DM) content of these feeds was above 95 % and only 2 -4 % loss was observed within one hour . From 2-5 h most feeds exhibited an additional loss of 8 % in DM at the rate of approximately 2 % / h . No cracking or physical disintegration of the

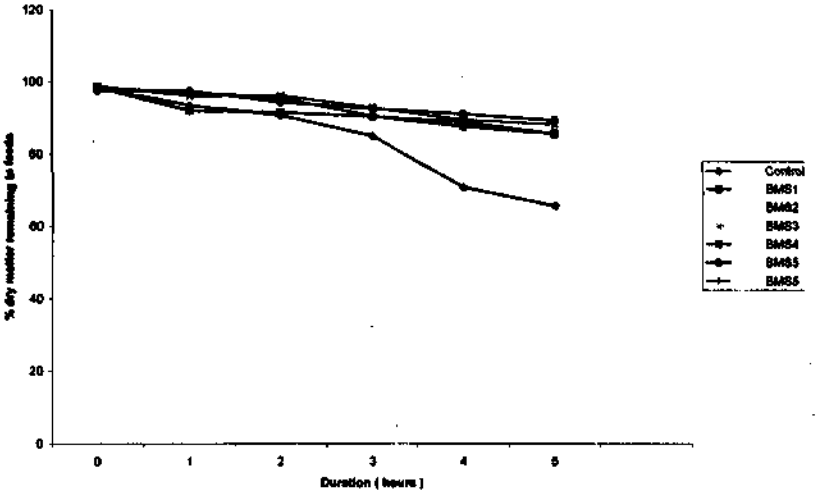

Fig. 1A Water stability of the control and experimental feeds prepared using *B.licheniformis*

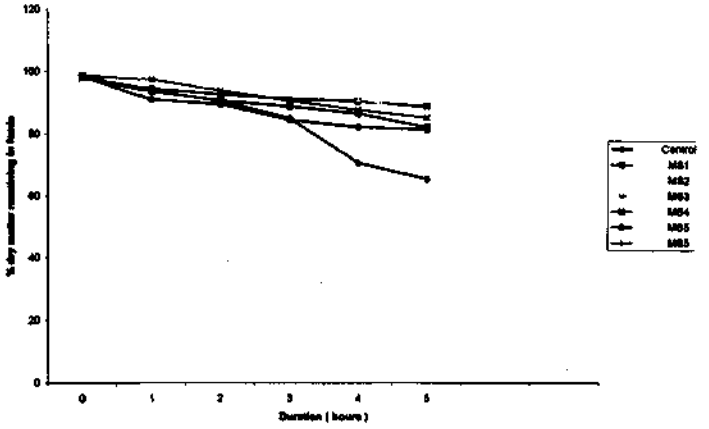

Fig. 1B Water stability of the control and experimental feeds prepared using *Beauveria sp.*

feed pellets was observed , though desirable softening was observed in all feeds and the post larvae readily accepted the pellets.

Single cell proteins have many advantages over other conventional feed ingredients (Tacon , 1990) and have been successfully used in animal and poultry feeds but their use in aquaculture so far has been limited to a few studies incorporating bacterial biomass indirectly or directly into finfish and shellfish feeds (Yasuda and Taga , 1980; Gatesoupe ,1980 , 1989 ; Mohamed , 1996 and Sridhar and Chandrashekar, 1996).The results of feeding *P. indicus* post larvae the feeds prepared incorporating mantis shrimp fermented using *B. lichenifformis* are given in Table 3 .

Table 3. Growth performance of *P.indicus* post larvae reared on B. licheniformis fermented mantis shrimp feeds for 45 days.

	Experimental Diets					
Parameters	C	BMS1	BMS2	BMS3	BMS4	BMS5
Final body* length (cm)	4.7a (± 0.004	5.9 b (±0.010)	6.8c (± 0.009)	6.1 b (±0.011)	6.9 c (± 0.006)	6.1 b (±0.014)
Final body** weight (g)	0.595a (± 0.034)	0.82b (±0.04 8)	0.96b (± 0.020)	0.99b (± 0.120)	1.26c (± 0.052)	0.86b (±0.032)
Average gain in length(%)	51.61	90.32	119.35	96.77	122.58	96.77
Average gain in weight (%)	213.16	331.58	405.26	421.05	563.16	352.63
Specific growth rate(%)	0.9	1.4	1.71	1.78	2.38	1.49
Feed conversion ratio (FCR)	5.0 a	2.0 b	1.93 b	2.08b	1.56b	1.68b
Survival (%)	80a (± 2)	86a (± 2)	96a (± 4)	94a (± 6)	98a (± 2)	90a (± 4)

* Initial body length = 3.1 ± 0.3 cm

** Initial body weight = 0.19 ± 0.06 g.

Each mean ± standard deviation in parenthesis is based on measurements from triplicate tanks . Row means having the same superscripts are not significantly different ($P < 0.05$) .

There was a significant ($P < 0.05$) increase in body weight in all the treatment groups in comparison to control post larvae with group BMS 4 (80 % incorporation of mantis shrimp) giving the best growth in terms of weight gain (563.16 %) . The feed conversion ratio ranged from 2.08 on feed BMS 3 to 1.56 on feed BMS 4 and was significantly lower ($P < 0.05$) than the control FCR of 5.0 . Survival in all treatments was in the range of 86 to 98 % and not significantly different from that of the control group where 90 % survival was obtained .

The results of the feeding trial incorporating varying levels of mantis shrimp fermented using *Beauveria* sp. are presented in Table 4. These feeds elicited a poor growth response as compared to feeds prepared using *B . lichenifórmis* fermented mantis shrimp in terms of both increase in weight and length . At the 60 % incorporation (MS 3) however, a slightly better response was obtained wherein a 316 % increase in body weight was observed in comparison to an increase of 213 % in case of control post larvae. The feed conversion ratio and survival rates of all experimental groups also did not differ much from those of the control post larvae. The most probable explanation for this result with mantis shrimp fermented using *Beauveria* sp. may be due to the fact that the fungi was not that effective in breaking down chitin which comprises a very high percentage in mantis shrimp as effectively as *B. lichenifórmis* . However, as there was an increase in the protein content of mantis shrimp upon fermentation with *Beauveria* sp. standardisation of process parameters such as increase in the time for fermentation , fermentation with other fungal species having chitinolytic properties and/ or combination with a chitinolytic bacterial strain will definitely yield superior results in terms of growth performance of aquatic culture animals.

Table 4. Growth performance of *P. indicus* post larvae reared on *Beauveria* fermented mantis shrimp feeds for 45 days.

Parameters	Experimental Diets					
	C	MS1	MS2	MS3	MS4	MS5
Final body* length (cm)	4.7 a (± 0.004)	5.0 a (±0.008)	5.1 a (± 0.012)	5.8a (±0.018)	4.9 a (± 0.018)	4.6 a (±0.002)
Final body** weight (g)	0.595 a (± 0.034)	0.62 a (±0.046)	0.64a (± 0.052)	0.79a (± 0.028)	0.595a (± 0.022)	0.570a (±0.038)
Average gain in length(%)	51.61	61.29	64.54	87.10	58.06	48.39
Average gain in weight (%)	213.16	226.32	236.84	315.79	213.16	200.00
Specific growth rate (%)	0.90	0.96	1.0	1.33	0.90	0.84
Feed conversion ratio (FCR)	5.0 a	3.14a	3.60a	3.00	4.22a	3.79a
Survival (%)	80a (± 2)	88a (± 4)	80a (± 6)	92a (± 8)	70a (± 3)	94a (± 6)

* Initial body length = 3.1 ± 0.3 cm
** Initial body weight = 0.19 ± 0.06 g
Each mean ± standard deviation in parenthesis is based on measurements from triplicate tanks. Row means having the same superscripts are not significantly different ($P < 0.05$).

The carcass body composition of *P. indicus* post larvae after feeding mantis shrimp fermented using *B. licheniformis* for a period of 45 days is elaborated in Table 5. There was an increase in the dry matter carcass content in all treatment groups (range 22.36 – 32.60 %) as compared to the DM content of 22.36 % in case of control animals. Protein content also was higher in all groups fed fermented mantis shrimp with BMS5 (100 % incorporation) recording the highest protein content of 71%. Though a moderate increase was recorded in the lipid content of all the animals fed fermented

mantis shrimp (5.13 - 6.02 %) it was statistically insignificant as compared to the lipid content of 4.76 % obtained in post larvae maintained on the control feed. A significant decrease was obtained in the ash content of all post larvae fed varying concentrations of fermented mantis shrimp (12.50 - 15.56 %) in comparison to 20.93 % ash content of the control post - larvae. This result can again be correlated to the beneficial changes under gone by mantis shrimp, otherwise high in ash during SSF. With regard to carcass composition of post larvae maintained on mantis shrimp fermented using *Beauveria sp.* (Table 5) the increase in DM content obtained in all the experimental groups was comparatively lower to that obtained on feeds fermented with *B. licheniformis* .The increase in protein content observed in these groups (64.96 % in MS1 with 20% incorporation to 71.66 % in MS5 with 100 % incorporation) was not in keeping with the results of the growth study. Likewise an increase in lipid content and decrease in ash contents of experimental groups in comparison to control as observed in the case of post larvae maintained on diets of mantis shrimp fermented using *B. licheniformis* was also obtained in post larvae fed the diets fermented with *Beauveria sp.* Growth performance and percentage carcass yields showed that use of the enriched material did not adversely affect the growth of *P. indicus* post larvae. The growth *per se* obtained in these post larvae fed with SCP enriched feeds was superior in comparison to control, feed intake was less which resulted in a reduction in feed usage and good FCR's.

Table 5. Carcass body composition of *P.indicus* post larvae reared on *B. licheniformis* and *Beauveria* fermented mantis shrimp feeds for 45 days.

Body Composition*	Control	BMS1	BMS2	BMS3	BMS4	BMS5	MS1	MS2	MS3	MS4	MS5
% dry matter	28.79	30.11	31.46	30.85	32.00	32.60	24.62	25.32	23.70	24.36	24.10
% moisture	71.21	69.89	68.54	69.15	68.00	67.40	75.38	74.68	76.30	75.64	75.90
% crude protein	64.96	70.46	69.39	69.98	70.09	71.00	69.48	66.28	71.17	70.48	71.66
% crude lipid	5.13	5.65	5.82	5.71	6.02	5.94	5.41	5.17	6.50	6.45	6.36
% ash	12.50	15.01	15.36	14.04	15.19	15.56	11.76	11.54	11.23	12.04	11.82

* Initial values- Dry matter-22.36%; moisture-77.64%; protein- 60.79%; Lipid- 4.76 %; ash-20.93 %.

BMS1to5 feeds formulated using mantis shrimp fermented with *B . licheniformis.* MS1to5 feeds formulated using mantis shrimp fermented with *Beauveria.*

Though one of the first works reported on the use of SSF for marine products , the results of the present study are quite encouraging and clearly indicate that mantis shrimp can be nutritionally enriched and preserved by solid state fermentation. Further experiments incorporating fermented mantis shrimp at lower levels will generate more data on the level of mantis shrimp incorporation yielding the most promising growth and survival in post larvae, juvenile and adult shrimp for future application in shrimp culture.

The studies carried out are preliminary and offer immense scope to provide beyond doubt that solid state fermentation, which is simple and economic, is the appropriate technology for the futuristic aquafeed industry.

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