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Bio-enriched feed for false clown fish *Amphiprion ocellaris*

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The development, production, and use of cost-effective aquatic feeds, whether in the form of live natural food organisms or manufactured compound aquafeeds, is central to the successful operation of all intensive aquaculture systems, including the production of marine ornamentals. A 49 day feeding trial was conducted using two test diets (bio-enriched feed with carotene and bio-enriched feed without carotene) and a control diet in the hatchery produced 90 days old juveniles of false clown fish *Amphiprion ocellaris*. Mixed oil cakes and wheat flour (soybean meal: wheat flour: groundnut oilcake: sesame oil cake; 4:3:2:1) fermented for 72 h using a phytase producing bacterial strain *Bacillus licheniformis* MTCC 6824 isolated from mangrove swamp was used for bio-enrichment. The crude protein and crude fat contents of the diets was 42 and 9% respectively. Feeding experiments were carried out in 45 L cylindro-conical fiber glass tanks with a continuous aeration and a hiding pot for the fish. Three replicates of each treatment and control were set up for the trial. Tanks had an artificial light source on for 8 hours and off for 16 hours per day. Complete water changes were done at weekly intervals, and water quality parameters like pH and temperature were checked daily. The control diet was a commercial ornamental diet (Alini, Fish Food manufactured by Tropical Industry), purchased from a local aquarist. Feeding to satiation was conducted by first offering a predetermined amout of food. If the food had been consumed entirely in five to ten minutes, additional feed was provided. If residual feed remained, feeding was terminated for that particular feeding and the amount of food provided during the next feeding was decreased. The goal was to provide the minimum amount of food necessary. The treatment with diets containing B-carotene gave better growth (3.63 ± 0.3 cm; 0.63 ± 0.04g) and colour retention than the control diet (2.64 ± 0.4 cm; 0.44 ± 0.2 g) and that with no cartotene (2.83 ± 0.1 cm; 0.35 ± g). No mortality was observed during the feeding trial in any of the treatments. Cent percent survival was observed during the feeding trial.

Fermented raw materials as fishmeal substitutes in aquafeeds

Partial replacement of dietary fishmeal protein with bacterial (*Bacillus coagulans*) fermented soybean meal or mixed oil cakes for *Penaeus monodon* juveniles as well as post larvae were found to be successful after the feeding trials under laboratory conditions. The fermented soybean meal (FSBM) obtained after 48 h and bacterial fermented ingredient mix (BFI) after 36 h had better nutritional profile than unfermented raw materials. Biological parameters calculated to evaluate the quality of diets include, weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU, %) growth rate (GR, %), apparent dry matter digestibility and apparent nutrient digestibility of protein and lipid).

Feeding Trial I (50 days)

Five experimental shrimp diets with crude protein level of 35% were formulated by replacing 20, 40, 60, 80 and 100% of fishmeal with fermented soybean meal and a 25% fishmeal based diet was formulated as control. The feed evaluations were conducted in circular plastic containers containing 35 L of 20 ppt water, provided with continuous aeration. *P. monodon* (PL_{30} of mean weight 0.01 G ± 0.06) post larvae were obtained from a local shrimp hatchery at Cochin. The shrimp were fed daily at the rate of 12 g 100^{-1}g body weight throughout the trial and the daily ration divided

Table 1: Growth response and feed utilization efficiency of shrimp fed with diets containing various replacement levels of fishmeal by FSMB for 50 days

Index	Diets and Percentage Replacement of f fishmeal					
	Control	20	40	60	80	100
Mean wt. gain (g)	0.77 ± 0.14	0.82 ± 0.23	1.14 ± 0.3	0.62 ± 0.16	0.53 ±0.14	0.46 ±0.06
SGR (%D^{-1})	1.53	1.22	2.12	1.06	0.86	0.73
FCR	1.75	1.58	1.55	1.89	2.42	2.46
PER	1.63	1.43	1.44	1.27	1.25	1.15
Apparent protein digestibility	76.48	81.13	81.30	79.20	79.26	74.35
ANPU	10.43	11.23	17.43	10.77	9.48	8.66
Apparent fat digestibility	92.16	94.90	93.80	90.10	87.19	86.32
ADMD	49.47	53.76	57	56.80	57.695	55.29

SGR-Specific Growth Rate; FCR-Feed Conversion Ratio; PER-Protein Efficiency Ratio; ANPU-Apparent Net Protein Utilization; ADMD-Apparent Dry Matter Digestibility

Table 2: Results of the 42 day feeding trial using BFI incorporated diets

Index	**CF**	**F1**	**F2**	**F3**	**F4**
Mean wt. gain (g)	0.15	0.19	0.51	0.57	0.63
Feed conversion ratio	3.30	3.04	1.75	1.59	1.58
Protein efficiency ratio	0.78	0.91	1.55	1.68	1.71
Apparent protein digestibility	57.68	72.49	76.90	83.54	86.50
Apparent protein utilization	10.29	12.12	21.17	24.06	25.65
Apparent fat digestibility	79.31	85.62	90.23	91.08	94.55

into two dosages and fed at 0900 h (40%) and at 1600 h (60%). The significant finding from this experiment is that substitution of FM upto 40% with FSBM in the diet for *P. monodon* had significantly (P<0.05) improved the growth performance of juvenile tiger shrimp and that fishmeal replacement up to 60% did not affect the shrimp growth or survival. However, fishmeal replacement by FSBM at levels exceeding 60% resulted in reduced performance (Table-1).

Feeding Trial II (42 days)

Four experimental diets @ 38.5% crude protein were formulated by incorporating BFI at 5, 15, 25 and 35%, designated as F1, F2, F3 and F4 respectively by replacing fishmeal in the same proportion. A diet with 35% fishmeal devoid of BFI served as the control (CF). The feeding trials were conducted in 50 L circular perspex tanks containing 35 L of 15ppt water. Each treatment consisted of three replicate groups of *P.monodon* post larvae (10 shrimp per tank; mean weight: 30 ± 0.09mg) along with the control groups, maintained in a closed system with continuous aeration. The shrimp post larval groups fed diet F4, with 100% fishmeal substitution using BFI, exhibited the maximum weight gain (0.63 ± 0.03g), which was significantly higher (P<0.05) than those fed with other diets as well as the control, the best FCR (1.58), PER (1.71), apparent protein and fat digestibility (86.50 and 94.55 respectively (Table-2)). The improved performance of diets incorporated with BFI suggests the significance of bacterial enrichment of nutrients to easily available forms by SSF through the production of enzymes.