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STUDIES ON THE EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS OF SOYABEAN MEAL ON GROWTH, SURVIVAL AND CARCASS COMPOSITION OF *LITOPENAEUS VANNAMEI* (BOONE, 1931)

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ABSTRACT

Present study was undertaken to study Soybean meal (SBM) of different protein levels (25%, 30% and 35%) were used in the experiment. The experiments were conducted for 56 days in aquaria. Triplicates were maintained for each of the treatment. Feed was given 3 times a day @ 5% of body weight. The growth performance of *L. vannamei* was found to be best when fed with diet SBM30 (7.41gm) and the highest average weight gain of 0.79gm was achieved by the 56th day. Specific growth rate was highest (1.770) and survival rate (70%) was best in shrimps fed on SBM30. The shrimps fed on SBM30 diet showed the best FCR of 3.28. Carcass composition of *L. vannamei* showed the highest protein content (76.9%), highest ether extract (2.3%) and crude fibre content (4.2%). Ash content (7.8%) was high in shrimps fed with SBM30 diet and low during initial sampling (3.7%).

Key words: *Litopenaeus vannamei*, Soyabean meal (SBM), Growth, Survival, Carcass composition.

INTRODUCTION

In India, aquaculture industry is growing at an alarming rate surpassing some major hurdles like disease outbreak and pollution during its development. The Shrimp culture received maximum importance in the human consumption due to its unique taste, high nutritive value and persistent demand in world market in general. Since feed constitutes 50 – 60 % of the total variable costs of production in aquaculture, it would be highly desirable to develop an efficient and cost effective feed to improve the profitability, though price is the key reason to look for alternatives. Protein is the primary and most expensive component of shrimp diets. One factor considered for reducing shrimp production costs and increase producers profitability is incorporation of good quality less expensive shrimp feed. Tacon (1989) and Zaldivar (2002) reported that the nutritional value of Soybean meal is lower compared with fish meal for penaeid shrimp. However, it is relatively inexpensive and has acceptable protein and amino acid content, making it a potential substitute in practical diets. It is accepted that soyabean meal is available world wide and is cheaper than fishmeal. Reigh *et al.* (1990) and Cabanillas-Beltran *et al.* (2001) have proved that soyabean meal is a high quality protein supplement and is well digested by most crustacean. *Litopenaeus vannamei* has high growth rate, adoption to various culture systems, high market value, excellent response to compounded feed and high resistance against diseases, *L. vannamei* has become the primary culture shrimp through domestication.

The experiment was basically designed to determine the effect of replacement of fishmeal with plant protein sources at different economically viable ratios. Hence, the objectives of the study were to evaluate the effects of Soybean meal (SBM) with varying dietary protein ratios on growth performance of *L. vannamei*.

MATERIALS AND METHODS

The study was conducted during 2010-2011 in the Department of Aquaculture, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh. *Litopenaeus vannamei* (1500 numbers) were obtained from CP Hatchery, Nellore, brought to the research centre in plastic bags having 5.00 ppt saline water and were acclimatized slowly to fresh water within a period of one week and kept in fresh water FRP tanks for 20 days till their use in the study. During this period the seed were fed with control diet.

Twelve aquariums tanks (60x30x40 cm) were stalked on iron racks and placed in a secured place to avoid direct sunlight. Tanks were covered all the sides with black paper to avoid algal growth in the tank. Water in the aquariums was aerated and filters were used for filtering the water. Bore well water was taken into FRP tank and allowed to aerate for 48 hours. Aerated water was used for filling aquariums and allowed 24 hours for filter before introducing the shrimps into aquariums. Ten numbers of

Shrimps (average weights 2.73-2.75gm; average lengths 6.0-6.5 cm) were introduced in each aquarium and triplicates were maintained for each treatment (SBM25%, SBM30%, SBM35%) including control. Regular water exchange (50%) was done every day. Left over feed, excreta and other debris was siphoned off from the bottom of the tank without disturbing the shrimps.

In the experiment, formulated feed was used for feeding. The feeds for the experiment were formulated with different protein concentrations. Control feed having 30% protein was prepared using de-oiled rice bran and groundnut oil cake. Vitamin and mineral mixture @1% was added. Experimental feeds were prepared with Soybean meal having 25%, 30%, 35% protein concentrations (Table 1&2). Other ingredients used in the experimental feeds were de-oiled rice bran and groundnut oil cake. Vitamin and mineral mixture @ 1% was also added to experimental feeds. All the ingredients used in feeds were obtained from local markets and were estimated for proximate composition (AOAC, 1995).

CRUDE PROTEIN

Nitrogen content of the sample was estimated by Kjeldahl method and the crude protein was estimated by multiplying nitrogen percentage by a constant factor 6.25.

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

ETHER EXTRACT

Ether extract was estimated by soxhlet apparatus using petroleum ether as a solvent.

$$\text{Ether extract (\%)} = \frac{\text{Weight of initial sample} - \text{Weight after extraction}}{\text{Weight of initial sample}} \times 100$$

ASH

Ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace heated at 600^0C for 6 hours.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

CRUDE FIBRE

Crude fibre was estimated by treating the moisture and fat free sample successively with dilute acid (1.25%) and alkali.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of crude fibre}}{\text{Original weight of sample}} \times 100$$

WEIGHT INCREMENT

Weight increment (gm) = Final body weight (gm) – Initial body weight (gm).

SPECIFIC GROWTH RATE

$$[(L_n \text{ FBW} - L_n \text{ IBW}) / \text{day}] \times 100$$

FBW -- Final body weight
IBW -- Initial body weight
Ln -- Logarithm

SURVIVAL RATE

$$\text{Survival (\%)} = \frac{\text{Total number of fish survived}}{\text{Total number of fish stocked}} \times 100$$

FOOD CONVERSION RATIO (FCR)

$$\text{Feed Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

Table.1: Feed formulation of control diet and crude protein content (%) in feed ingredients

Sl. No	Ingredient	Protein (%)	Control T1	SBM 25 T2	SBM 30 T3	SBM 35 T4
1	Soybean meal	37.3	-	40.50	60.54	80.80
2	Groundnut oil cake	38.4	50.0	10	10	10
3	Deoiled rice bran	12.5	49.0	48.50	28.46	8.20
4	Vitamin and mineral Mixture	-	1	1	1	1

Table 2: Proximate composition of the various ingredients used in formulated feeds

Sl. No.	Ingredient	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)
1	Soybean meal	9.8	37.3	4.6	9.2	5.6
2	Groundnut oil cake	8.8	38.4	5.6	7.3	7.6
3	Deoiled rice bran	7.7	12.5	3.9	22.5	15.8

RESULT AND DISCUSSIONS

A large amount of experimental work has been performed on the requirements of protein to the *L. vannamei*. A wide range of practical formulations developed by feed manufacturers have been identified, emphasizing in the beginning, on water stability of pellets, based on farmer's recriminations.

In the present study important water quality parameters such as Dissolved oxygen, Temperature, pH, Total alkalinity and total hardness were observed weekly at every sampling in all aquaria. During the experimental period the dissolved oxygen values varied between 5.23-8.42ppm (Table 3). The highest value of 8.42ppm was recorded in SBM25 and the lowest value of 5.23ppm was

recorded in SBM30, SBM35 (14th day) during the entire experiment. The range of temperature values recorded in the experiment varied between 29.3 ^{0}C and 31.6^{0}C (Table 4). The temperature ranges were within the optimal ranges for shrimp culture (Van Wyk *et al.*, 1999). The pH values observed were within the optimal range for shrimp culture (Table 5). In the present experiment the variations in the pH are low and this can be attributed to higher total alkalinity observed during the experiment (Pandey and Shukla, 2005). The Total alkalinity values ranged between 160 and 300 mg/l (Table 6). Alikunhi, (1957) stated that in highly productive waters the alkalinity had to be over 100 mg/l. Pandey and Shukla (2005) stated that stagnate water in low rainfall areas during summer season are likely to have high total alkalinity values and all the ponds above 90 mg/l of total alkalinity have been found to be productive. The Total hardness values ranged between 226 and 237mg/l (Table 7). As per the classification of waters based on the total hardness, the waters are hard waters (Boyd, 2000). Water quality parameters remained stable throughout the experimental period Alikunhi, K.H. (1957).

Table 3: Dissolved Oxygen content (ppm) in water of aquaria with *L. vannamei* (whiteleg shrimp)

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	7.51	8.42	8.02	8.05
07	5.92	5.61	5.93	6.00
14	6.23	6.02	5.23	5.23
21	5.42	7.24	6.41	5.24
28	5.64	5.62	5.65	5.62
35	5.64	5.65	5.61	6.01
42	5.66	6.46	5.24	5.64
49	5.63	5.63	5.63	5.62
56	5.64	6.02	6.00	5.63

Table 4: Temperature (°C) of water in aquaria with *L. vannamei* (whiteleg shrimp)

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	29.5	29.5	29.5	29.5
07	30.4	30.4	30.4	30.4
14	29.3	29.3	29.3	29.3
21	30.2	30.2	30.2	30.2
28	29.6	29.6	29.6	29.6
35	30.8	30.8	30.8	30.8
42	31.6	31.6	31.6	31.6
49	29.3	29.3	29.3	29.3
56	30.4	30.4	30.4	30.4

By replacing fishmeal with Soybean meal, shrimp production costs may be reduced and shrimp body cholesterol will be reduced, Soybean meal diet had the lowest hepatopancereas cholesterol content, and yet does not affect the growth performance. Soybean meal and soy oil is widely available and produced through sustainable practices, their use in shrimp feed should be encouraged. As human living standards improve, the demand for seafood, such as shrimp will increase (Cheng and Hardy, 2004). Soybean meal above 50 % in diets for crustaceans and peneaid shrimps was discouraged because of low palatability and pellets stability (Akiyama, 1990; Lim and Dominy, 1992; Floreto *et al.*, 2000). Lawrence *et al.* (1986) found that animal protein sources could be included at levels as low as 7% in shrimp's diets and recommended 40-50% Soybean levels. However, some researches included shrimp–head meal in their test diets, which is an attractant that may also improve the diet palatability. Lim and Dominy (1990) reported a significant decline in feed intake of *L. vannamei* with dietary Soybean meal concentration exceeding 42%. This may be the cause of the poor results we obtained with diets containing more than 41% Soybean meal.

Table 5: p^H of the water in aquaria with *L. vannamei* (whiteleg shrimp)

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	8.5	8.5	8.4	8.5
7	8.4	8.5	8.5	8.5

14	8.4	8.3	8.4	8.4
21	8.5	8.5	8.5	8.5
28	8.5	8.5	8.5	8.5
35	8.5	8.5	8.5	8.4
42	8.4	8.4	8.4	8.4
49	8.4	8.4	8.4	8.4
56	8.5	8.3	8.4	8.4

Table 6: Total alkalinity (mg/l) of water in aquaria with *L. vannamei* (whiteleg shrimp):

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	280	300	290	300
7	250	220	230	230
14	200	170	190	200
21	210	210	220	220
28	210	210	220	220
35	210	200	220	210
42	170	160	160	170
49	170	160	170	160
56	210	210	220	210

Growth performance of *Litopenaeus vannamei* (whiteleg shrimp) fed on different experimental diets shows highest weight gain in the treatment of SBM30 (Table 9). An overall study indicated that the SBM30 recorded total weight increment of 4.66gm in the 56 days experimental period. This was followed SBM35 (4.46gm). Davis *et al.* (2002) tested *L. vannamei* juveniles fed on different processed pea meals at 5%, 10%, and 20% of inclusion level, and found that when it was extruded or micronized, their protein and energy content were highly digestible without apparent adverse effects on growth or survival. Soybean meal at 45% incorporation of a diet with 25% crude protein showed an optimum in terms of weight gain (Lawrence *et al.*, 1995). The lower weight gain (Table 10), which resulted from feeding the diet containing 35% protein, is probably due to the low energy to protein ratio of the diet, which would cause shrimp to utilize protein as a source of energy. The 35% protein diet was probably due more to the protein to energy ratio of the diet. The protein requirement for maximum weight gain, 30% protein diet was found to induce superior growth in juvenile and sub adult *L. vannamei* as compared to 25% and 35% protein diets. Smith *et al.* (1984) studied the response of three sizes of *L. vannamei* (4.0, 9.8 and 20.8gm) fed diets containing 22%, 29%, and 36% crude protein for period of 30 days. Dietary protein contain only affected weight gain for 4.0gm shrimps, with a significant increasing weight gain corresponding to the increasing dietary protein content. Kureshy and Davis (2002) reported that juvenile shrimps fed with 32% protein diet had significantly higher weight gain compared to juveniles fed with 16% protein diet. In the present study also juveniles of *L. vannamei* fed with 30% protein showed highest weight gain than 25% and 35% of plant protein source. Soybean meal above 50 % in diets for crustaceans and peneaid shrimps was discouraged because of low palatability and pellets stability (Akiyama, 1990; Lim and Dominy, 1992; Floreto *et al.*, 2000). Lawrence *et al.* (1986) found that animal protein sources could be included at levels as low as 7% in shrimp's diets and recommended 40-50% Soybean levels. The growth data was subjected to analysis of variance (ANOVA). The statistical analysis has shown that F- value is found to be significant among Treatments. Since F- value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classifications. The Treatment SBM30 is found to be significantly superior when compare to other Treatments.

Table 7: Total Hardness (mg/l) of water in aquaria with *L. vannamei* (whiteleg shrimp):

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	226	229	227	230
7	227	228	229	230
14	229	228	228	229
21	230	230	231	229
28	230	229	232	234
35	231	230	229	231

42	231	231	230	229
49	233	235	231	230
56	234	235	236	237

Table 8: The percentage of survival of *L. vannamei* (whiteleg shrimp) fed with different experimental diets

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
7	100.0	100.0	100.0	90.0
14	90.0	90.0	100.0	80.0
21	80.0	90.0	90.0	80.0
28	80.0	80.0	90.0	700
35	70.0	80.0	80.0	70.0
42	60.0	70.0	80.0	60.0
49	60.0	60.0	80.0	50.0
56	50.0	60.0	70.0	50.0

Table 9: Growth performance of *L. vannamei* (whiteleg shrimp) fed with different experimental diets

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	2.72 ± 0.19	2.75± 0.40	2.75± 0.50	2.75± 0.30
7	3.01± 0.10	3.15± 0.25	3.20± 0.20	3.16± 0.06
14	3.37± 0.42	3.55± 0.05	3.66± 0.16	3.60± 0.30
21	3.80± 0.10	3.99± 0.10	4.16± 0.02	4.07± 0.03
28	4.19± 0.01	4.44± 0.14	4.69± 0.18	4.57± 0.10
35	4.55± 0.10	4.94± 0.10	5.28± 0.18	5.15± 0.15
42	5.03± 0.13	5.44± 0.23	5.91± 0.40	5.71± 0.12
49	5.40± 0.17	5.97± 0.41	6.62± 0.10	6.46± 0.10
56	5.90± 0.10	6.52± 0.22	7.41± 0.21	7.21± 0.11

Survival percentages of *L. vannamei* shrimp in various experimental Treatments are presented in Table 8. The survival percentage throughout the period of experiment was lowest for the control, SBM35, and highest for SBM30.

Specific growth rates for *L. vannamei* (whiteleg shrimp) treated with different diets were calculated and presented in table 11. The specific growth rates by end of the experimental period (56 days) were calculated for all treatments. Control group had the lowest Specific Growth Rate of 1.382%. The highest value was in SBM30 (1.770%). These were followed by SBM25 (1.541) respectively. The Feed Conversion Ratio in different experiments of *L. vannamei* groups were calculated and presented in the table 12. The range for Feed Conversion Ratio observed during the period of experiment was 3.28 (SBM30) – 4.13 (control).

Table 10: Weight gain in *L. vannamei* (whiteleg shrimp) fed on different experimental diets

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
7	0.29	0.40	0.45	0.41
14	0.36	0.40	0.46	0.44
21	0.43	0.44	0.50	0.47
28	0.39	0.45	0.53	0.50
35	0.36	0.50	0.59	0.58
42	0.48	0.50	0.63	0.62
49	0.37	0.53	0.71	0.69
56	0.50	0.55	0.79	0.75

Table 11: Specific growth rates (%) in *L. vannamei* (whiteleg shrimp) fed on different experimental diets

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
Initial	2.72	2.75	2.75	2.75
Final	5.9	6.52	7.41	7.21
SGR	1.382	1.541	1.770	1.721

Table 12: Feed Conversion Ratio of *L. vannamei* (whiteleg shrimp) fed with different experimental diets

Treatment / Period (Days)	Control	Treatment SBM		
	T_1	T_2 SBM_{25}	T_3 SBM_{30}	T_4 SBM_{35}
7	3.63	2.75	2.48	2.69
14	3.27	3.10	2.78	2.86
21	3.09	3.17	2.91	3.03
28	3.76	3.45	3.09	3.19
35	4.42	3.45	3.13	3.10
42	3.66	3.80	3.28	3.22
49	5.10	3.94	3.26	3.27
56	4.13	4.14	3.28	3.36

Table 13: Proximate composition of *L. vannamei* carcass (whiteleg shrimp) fed with different experimental diets

Treatment / Components	Initial	Control	Treatment SBM		
			SBM 25	SBM30	SBM 35
Moisture	96.2	95.8	97.1	98.9	98.8
Crude protein	69.8	70.2	75.7	76.9	76.4
Ether extract	1.8	2.0	2.2	2.3	2.1
Crude fibre	3.7	3.9	4.0	4.2	4.1
Ash	7.2	7.4	7.6	7.8	7.7

The proximate composition of *L. vannamei* carcass was analyzed at the beginning of the experiment and also at the end of the experiment period (56th day) and presented in the table 13.Carcass analysis were given on shrimp receiving each diet at the beginning and at end or the experiment (Gauquelin *et al.*, 2007). The data on carcass composition in this study showed that carcass lipid content increased with increasing dietary lipid. Similar to the previously reported studies about fish and crustaceans. *L. vannamei* has been used as a model for many nutrition works and the extension of its culture, the range of ingredients selected, the rearing systems selected, prove the potential of this species to convert plant protein sources (Cuzon *et al.*, 2004).

The good production results observed in this study confirms the earlier work done by Amaya *et al.* (2007). Commercially manufactured feed and including plant proteins as replacements of animal protein sources, without adverse effects on shrimp performance or production economics. Further, the study provide important evidence that animal protein sources can be completely removed from shrimp feeds without negatively effecting shrimp growth. The present results can function as a reference for developing shrimp culture in fresh water conditions, particularly in regions where water chemistry is similar to that in the study area. Under these conditions, white shrimp culture can be cultivated without the need to increase salinity. Freshwater white shrimp culture could reduce pressure on coastal areas from this activity and provide disease incidence below that of marine culture systems. If culture systems were established in rural areas they could increase employment opportunities and aid in diversifying production.

The present study suggested that during the juveniles stages of *L. vannamei*, use of Soybean meal improved the survival rate, good growth, less FCR, but when you compare with cost of the feeds, the fishmeal is costlier than Soybean meal, so the use of fishmeal in earlier larval to juvenile stages preferred for the better survival rate and afterwards switch over to Soybean meal there by the profit of the farmer will increase.

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