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Nutritional composition, quality and hazards profiling of commercial post-larval feeds used in *Litopenaeus vannamei* hatcheries

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ABSTRACT

Nutritional profile and quality of commercial post-larval (PL) feeds (n=9), zoea feeds (n=2), mysis feeds (n=2) and artemia cysts (n=2) of different brands used in *Litopenaeus vannamei* hatcheries located in Andhra Pradesh on the east coast of India were assessed. The moisture content of PL feeds ranged between 6.48 and 11.04%. Average protein and fat content of PL feed was distinctly lower than zoea feed, mysis feed and artemia. Saturated fatty acids (SFA) were lower than unsaturated fatty acids (USFA) in all the hatchery feeds. Palmitic acid-C16 (7.09%) and tricosylic acid-C23 (6.29%), were the dominant SFA; oleic acid-C18:1 (38.92%) was the predominant monounsaturated fatty acid (MUFA); linolenic acid-C18:3 (9%) and docosahexaenoic acid DHA-C22:6 (8.53) were the prominent monounsaturated fatty acids (PUFA) recorded in PL feeds. Eicosapentaenoic acid EPA-C20:5 content in PL feed was low (1.15%). Essential amino acids (EAA) ranged between 22.3 and 41.53% in PL feeds. The monoamino dicarboxylic acidic amino acids (glutamic acid and aspartic acid) constituted nearly 35% of the total amino acids in PL feeds. The organochlorine pesticide, HepEpo was detected in one mysis feed sample at 7.05 ppb level. Chloramphenicol was detected in two PL feed samples at a high level of 112.5 ppb and 453 ppb. Cd content was more than 1ppm in PL, zoea and mysis feeds. Pb content was less than 1ppb in all the hatchery feeds. APC ranged between 20 and 560 cfu g^{-1}. Total Enterobacteriaceae, yeast and molds were detected only in one PL feed (20 cfu g^{-1}). The study revealed the need for good manufacturing practices (GMP) and screening of all feed components for antibiotics, pesticide residues and heavy metals.

Keywords: Hatchery feed, *Litopenaeus vannamei*, Nutrient composition, Residue profiling

Introduction

India is witnessing a phenomenal growth in *Litopenaeus vannamei* farming in recent years and is poised to attain further heights in production, particularly in Andhra Pradesh. The total vannamei shrimp production in India increased from 1,731 t in 2009-10 (MPEDA, 2010) to 10,000 t in 2010-11 (MPEDA, 2011). Phenomenal growth in the export of *Litopenaeus vannamei* was witnessed during 2012-13 to 2013-14 and the quantity exported increased from 91,171 t to 1,75,071 t (www.mpeda.com). Nutritionally balanced feed is a major input in shrimp farming. The quality and composition of feed has a major influence on the meat characteristics of cultured shrimp. The nutritional profile and quality of feed depends largely on the selection of feed ingredients, feed processing technology and conditions during storage and transport. Shrimp, like all animals, require proteins (amino acids), lipids (essential fatty acids), energy sources (lipids, protein, and carbohydrates), vitamins, minerals, oxygen, and water for their survival and growth. Formulated feeds are mixtures of different feed ingredients mixed in set proportions to provide the desired quantities of nutrients. Common ingredients used in commercial shrimp feeds include fish meal, squid meal, soybean meal, shrimp head meal, wheat flour, lecithin, starch, vitamin and mineral mixtures. Cultured shrimp forms a significant part of shrimp exports, both in terms of quantitiy and value. However, export rejections of shrimp due to the presence of hazards, particularly antibiotic residues is a major problem (Rapid Alert System for Food and Feed - http://ec.europa.eu/food/food/rapidalert/index_en.htm). Knowledge of the nutritional profile and quality of commercial feeds used in *L. vannamei* hatcheries is critical for wholesome shrimp production that meets export quality requirements. In the present study, commercial post-larval (PL) feeds (n=9), zoea feeds (n=2), mysis feeds (n=2) and artemia cysts (n=2) routinely used in *L. vannamei* hatcheries, located in Andhra Pradesh on the east coast of India were examined for nutrient composition and quality.

Materials and methods

Post-larval feeds (n=9), zoea feeds (n=2), mysis feeds (n=2) and artemia cysts (n=2) of different brands commercially available and routinely employed in *L. vannamei* hatcheries located in Andhra Pradesh were collected aseptically from hatcheries and transported to laboratory for nutritional, chemical and microbiological analyses. Moisture, protein, fat, ash, calcium, potassium, sodium and iron were determined as per standard methods (AOAC, 1990). Total carbohydrate was estimated by deduction method. Phosphorus was determined colorimetrically (Fiske and Subbarow, 1925). Cadmium, copper, zinc and lead were analysed following AOAC (2000) using atomic absorption spectrophotometer (Varian Spectra AA 220, Australia). Aerobic plate count (APC), total Enterobacteriaceae count (TEC) and total yeast mould count (TYM) were determined as per BAM (1995). Data were statistically analysised (Snedecor and Cochran, 1967).

Total lipid was extracted (Folch *et al*., 1957) and fatty acids were analysed according to the method of Metcalfe *et al.*(1966), using Gas Liquid Chromatography. Amino acid profile of the feeds was determined as per Ishida *et al.* (1981) using High Performance Liquid Chromatography (Shimadzu LC 10AS). Tryptophan was determined spectrophotometrically (Sastry and Tammuru, 1985). Organochlorine pesticides (OCP) in feed samples were extracted as per the method described in AOAC (2005). For OCP determination a Varian Gas Chromatograph system (CP-3800) equipped with Electron Capture Detector and CP Sil 8 CB capillary column (30 m, ID of 0.25 mm and 0.25 μm film thickness) was used. The gas chromatography oven was programmed as follows: initial temperature 170˚C, increased to 250˚C at 4˚C per min and held at 250˚C for 10 min. Antibiotic residues (chloramphenicol, nitrofuran metabolites, sulfonamides, oxytetracycline and chlortetracycline) in feed samples were extracted as per the method described in USFDA (2008). For antibiotic residue analyses, an MDS-SCIEX API 2000 MSMS system equipped with Atomic Pressure Ionization, Perkin Elmer Series 2000 HPLC and Merck C18 RP 5μ 250 x 4 mm column was used.

Results and discussion

Proximate composition of L. vannamei post-larval (PL) feeds

Results of the proximate composition of nine commercially available *L. vannamei* PL feeds is given in Table 1. Moisture content of PL feeds ranged between 6.48% and 11.04% with a mean moisture content of 8.23%. Only one PL feed had moisture content above 10%. Increased moisture content supports growth of microorganisms and makes the feed toxic (Gomez *et al*., 1997). Irrespective of the larval stage, shrimp need to consume adequate quantities of protein for maintenance and growth. The daily requirement of protein for young shrimp is higher than the larger shrimp owing to their faster percentage of weight gain. Commercial feeds are formulated to meet dietary protein requirements as the cost of feed is dictated largely by the quantity and quality of protein included in the feed. Protein content of the PL feeds ranged between 20.2% and 50.6% with an average protein content of 43.43%. One PL feed had a very low protein content of 20.2% and another had about 35%; rest of the PL feeds had protein content above 40%. Recommended protein requirements for shrimp sized 0.002 to 0.25 g, 0.25 to 1 g, 1 to 3 g and >3 g are: 50%, 45%, 40% and 35% of the total protein, respectively (Akiyama *et al*., 1991; Wyk, 1999). Even though majority of the *L. vannamei* hatchery feeds meet the protein requirements, few feeds were deficient in protein which invariably affects growth rate. Lipids provide a concentrated energy source and supply of essential fatty acids and also serve as vehicle for absorption of the fat-soluble vitamins A, D, E, and K. Lipids also serve as precursors for metabolic regulators such as prostaglandins, thromboxanes, and prostacyclins that play vital roles in maturation, molting, and growth. There was relatively wide variation in the fat content of PL feeds. The fat content of PL feeds ranged between 6.68% and 17.41% with a mean fat content of 11.86%. Ash content of PL feeds ranged between 8.08% and 15.81%. Recommended lipid requirements for shrimp sized 0.002 to 0.25 g, 0.25 to 1 g, 1 to 3 g and >3 g are 15%, 9%, 7.5% and 6.5% respectively, in total feed (Akiyama *et al*., 1991; Wyk, 1999). Carbohydrate content ranged between 11.48% and 56.77%. Carbohydrates are the most economical source of energy, have protein sparing effect and also provide proper pellet stability.

Comparison of proximate composition of L. vannamei PL feeds with other hatchery feeds

Proximate composition of PL feed was compared with zoea feed, mysis feed and artemia used in hatcheries

Table 1. Proximate composition (%) of commercially available *L. vannamei* post- larval feeds

PL feeds	Moisture	Protein	Fat	Ash	Carbohydrate
1	7.33	48.84	9.58	13.92	20.33
2	7.7	47.21	9.96	12.97	22.16
3	7.21	44.14	7.94	10.89	29.82
4	7.59	20.2	6.68	8.76	56.77
5	9.39	49.48	17.41	10.93	12.79
6	6.48	47.15	17.32	8.08	20.97
7	8.01	48.42	16.25	9.47	17.85
8	11.04	50.6	11.07	15.81	11.48
9	9.32	34.79	10.57	13.27	32.05
Mean±SD	8.23±1.42	43.43±9.96	11.86±4.08	11.57±2.59	24.91±13.75

(Fig. 1). The mean moisture content of PL feeds was lower than that of artemia (10.64%) and zoea feed (8.87%). Average protein content of PL feed (43.43%) was distinctly lower than zoea feed, mysis feed and artemia (> 49%). Fat content of PL feed (11.86%) was lower than mysis feed (19,3%) and zoea feed (16.21%). Artemia had very low fat content (0.35%). Similarly ash content was lower in artemia (3.94%) compared to zoea, PL and mysis feeds.

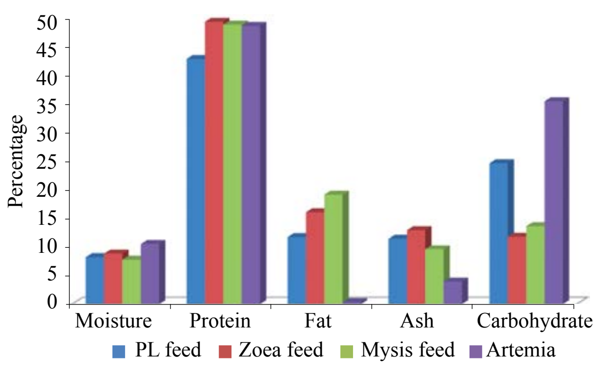

Fig. 1. Comparison of proximate composition of *L. vannamei* hatchery feeds

Mineral composition of L. vannamei PL feeds in comparison with other hatchery feeds

PL feeds had higher content of phosphorus compared to calcium (Table 2). The calcium to phosphorus ratio in PL feeds ranged between 1: 1.2 to 1: 6.8 with an average ratio of 1 : 3.5. Phosphorus is the expensive and problematic mineral in PL feed. It's biological availability varies with the source, with the water-soluble forms having higher availability to shrimp. Phosphorus must be provided in feed as it is poorly absorbed by the shrimp from the water (Davis and Gatlin, 1991). Phosphorus along with calcium are essential for exoskeleton formation and metabolic functions. Sodium to potassium ratio ranged between 1 : 0.66 and 1 : 2.4 with a mean ratio of 1 : 0.93. Low levels of potassium and or magnesium were related to poor shrimp survival (Davis *et al*., 2005). PL feeds had appreciable quantities of iron. Calcium content of PL feeds (444 mg 100 g^{-1}) was higher than mysis feed (242 mg 100 g^{-1}), zoea feed (171 mg 100 g^{-1}) and artemia (91.5 mg 100 g^{-1}). Phosphorus content of PL feeds (1572 mg 100 g^{-1}) was lower than mysis feed (1936 mg 100 g^{-1}). Sodium content was higher in PL feed (886 mg 100 g^{-1}) than other feeds but potassium content of PL feed (827 mg 100 g^{-1}) was lower than zoea feed (1129 mg 100 g^{-1}) (Table 3). Iron content was nearly five times higher in PL feeds compared to other hatchery feeds.

Fatty acid composition of L. vannamei hatchery feeds

SFA was lower than USFA in all the hatchery feeds (Fig. 2). PL feed (22.35%) had higher SFA than mysis feed (7.25%) and zoea feed (12.48%). Amongst the unsaturated fatty acids, MUFA content was distinctly higher than PUFA content in PL, zoea and mysis feeds. However, the MUFA and PUFA contents were similar in artemia.

Percentage occurrence of different fatty acids in L. vannamei PL feeds

Palmitic acid-C16 (7.09%) and tricosylic acid-C23 (6.29%), were the dominant SFA in PL feed (Table 4). Oleic acid-C18:1 (38.92%) was predominant MUFA. Linolenic acid-C18:3 (9%) and docosahexaenoic acid DHA-C22:6 (8.53) were the prominent PUFA. Eicosapentaenoic acid EPA-C20:5content in PL feed was low (1.15%). There was a wide variation, both in the type and quantity of each

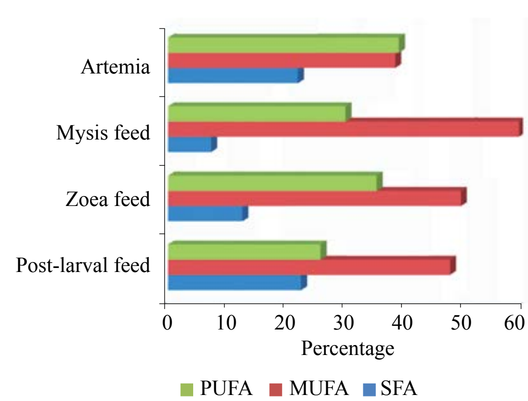

Fig. 2. Fatty acid composition of *L. vannamei* hatchery feeds

Table 2. Mineral composition of *L. vannamei* post-larval feeds

Sl. No.	Calcium (mg 100 g^{-1})	Phosphorus (mg 100 g^{-1})	Sodium (mg 100 g^{-1})	Potassium (mg 100 g^{-1})	Iron (ppm)
1	245	1671.1	1064	967	560.9
2	297	1899.71	1084	1163	2854.52
3	398	1721.49	397	430	511.1
4	177	1346.67	505	536	366.95
5	229	1457.93	1145	763	572.88
6	316	1342.48	460	723	493.31
7	253	1454.25	316	760	553.46
8	1145	1315.28	1651	1524	2831.26
9	936	1942.78	1356	578	543.31
Mean±SD	444±347.7	1572.41±243.23	886.4±478.9	827.1±343.15	1031.97±1028.55

Table 3. Comparison of mineral composition of *L. vannamei* hatchery feeds

Feeds	Calcium (mg 100 g^{-1})	Phosphorus (mg 100 g^{-1})	Sodium (mg 100 g^{-1})	Potassium (mg 100 g^{-1})	Iron (ppm)
Post-larval feed	444±347.7*	1572.41±243.23	886.44±478.9	827.1±343.15	1031.97±1028.55
Zoea feed	171±38.18	1090.2±175.74	762.5±57.28	1129.5±64.35	272.47±129.75
Mysis feed	242.5±64.35	1936.33±343.8	502.5±590.4	1004±346.48	238.02±168.96
Artemia	91.5±4.95	697.08±510.69	178.5±136.48	330.5±78.49	236.2±89.62

*Mean±SD

Table 4. Occurrence of different fatty acids (% of total fatty acid) in *L. vannamei* post-larval feeds

	L. vannamei post-larval feeds									
Fatty acid	1	2	3	4	5	6	7	8	9	Mean±SD
C14:0	6.02	3.81	2.77	3.82	5.3	5.65	6.84	2.21	6.14	4.73±1.63
C15:0	1.41	0	0	1.05	0	1.21	0	0	1.33	0.56±0.67
C16:0	0	15.09	14.23	18.36	0	0	0	16.13	0	7.09±8.48
C18:0	4.93	0	0	4.38	2.65	5.4	3.92	6.18	5.67	3.68±2.33
C23:0	2.96	2.28	2.18	2.98	15.94	2.43	14.6	9.59	3.65	6.29±5.59
C16:1	4.53	3.04	3.08	4.28	4.44	7.14	5.97	3.23	5.77	4.61±1.43
C18:1	49.44	36.92	38.15	38.63	14.08	56.9	16.82	38	61.38	38.92±16.01
C20:1	4.99	0	0	3.68	11.12	3.55	9.21	0	2.01	3.84±4.04
C18:2	14.68	0	0	0	19.46	0	24.03	0	0	6.46±9.97
C18:3	2.35	12.74	11.01	16.85	0	7.98	4.49	20.71	4.89	9.0±6.9
C20:4	1.38	0	0	0	0	1.2	0	0	1.57	0.46±0.69
C20:5	0	0	0	0	8.96	1.39	0	0	0	1.15±2.96
C22:6	1.63	20.94	22.68	1.7	12.43	3.2	10.85	0	3.35	8.53±8.64
Others	5.68	5.17	5.91	4.27	2.6	3.94	3.27	3.96	4.23	4.33±1.09

type of fatty acid in the PL feeds. The SFA, pentadecanoic acid-C15 and stearic acid-C18 were not detected in 56% of PL feeds and stearic acid-C18 was absent in 22% PL feeds. Amongst the MUFA, gadoleic acid-C20:1 was not detected in 33% PL feeds. In PUFA, arachidonic acid-C20:4 and timnodonic acid-C20:5 were absent in 67% and 78% PL feeds, respectively. Linolenic acid-C18:3 and DHA-C22:6 were absent in one PL feed. The poly unsaturated fatty acids, linoleic, linolenic, EPA and DHA are considered essential for growth, survival and better feed conversion ratio in shrimp and the recommended levels of these fatty acids are 0.4, 0.3, 0.4 and 0.4% of the feed, respectively (Kanazawa and Teshima, 1981). Kavitha *et al*. (2003) reported that ingredients of plant origin had higher proportion of C16:0, C18:1 and C18:2 fatty acids and lower EPA and DHA.

Comparison of fatty acid profile of L. vannamei hatchery feeds

The fatty acid profile of PL feed was compared with zoea feed, mysis feed and artemia used in hatcheries and the results are depicted in (Table 5.) The saturated fatty acids, myristic acid-C14, stearic acid-C18 and tricosylic acid-C23 were present in all the hatchery feeds. However, lauric acid-C12 and margaric acid-were observed only in artemia and pentadecylic acid-C15 was noticed only in PL feeds. Palmitic acid-C16 was not detected in mysis feed. Among MUFA, palmitoleic acid-C16:1 and oleic acid-C18:1 were detected in all hatchery feeds but myristoleic acid-C14:1 was detected only in artemia. Gadoleic acid-C20:1 was detected in all feeds except artemia. Oleic acid-C18:1 is the predominant MUFA in all hatchery feeds. The PUFAs, lionoleic-C18:2, linolenic-C18:3 and EPA-C20:5 were present in all the hatchery feeds but oleic acid-C18:1, arachidonic acid-C20:4 and DHA-C22:6 were not detected in artemia. DHA-C22:6 content was relatively high in zoea feed (17.49%) and mysis feed (12.68%). In PL feed, linolenic acid-C18:3 (9%) and DHA-C22:6 (8.53%) were the predominant PUFA. C18:3 (29.49%) was the most dominant PUFA in artemia.

Table 5. Comparison of fatty acid (%) profile of *L. vannamei* hatchery feeds

Fatty acid	Post-larval Feed	Zoea feed	Mysis feed	Artemia
C12:0	0	0	0	0.58±0.82
C14:0	4.73±1.63	2.14±0.09	1.90±0.62	2.42±1.01
C15:0	0.56±0.67	0	0	0
C16:0	7.09±8.48	5.27±7.45	0	8.87±12.54
C17:0	0	0	0	0.68±0.96
C18:0	3.68±2.33	1.96±2.76	4.82±1.22	5.60±1.27
C23:0	6.29±5.59	3.12±4.4	0.53±0.75	3.645±5.16
C14:1	0	0	0	2.26±0.71
C16:1	4.61±1.43	3.42±0.62	2.95±0.01	3.80±0.42
C18:1	38.92±16.01	40.48±0.03	54.51±19.3	32.09±4.94
C20:1	3.84±4.04	5.3±3.74	1.48±2.09	0
C18:2	6.46±9.97	5.96±8.42	10.42±0.11	6.95±0.81
C18:3	9.0±6.9	7.49±6.14	1.13±1.6	29.49±2.1
C20:4	0.46±0.69	0.61±0.86	2.02±0.98	0
C20:5	1.15±2.96	3.49±4.94	3.56±5.03	2.35±3.7
C22:6	8.53±8.64	17.49±5.08	12.68±13.43	0

The Amino acid profile of L. vannamei PL feeds

Essential amino acids (EAA) ranged between 22.3 and 41.53% in PL feeds (Table 6). Larger quantities of low-quality proteins with inadequate levels of essential amino acids are required, than high-quality proteins to produce the same amount of growth. If one of the essential amino acids is limiting, it can prevent the building of protein. Amino acid requirements of a species closely resemble the amino acid composition of their muscle tissue and feed formulation for shrimp, are based on this concept (Lim and Persyn, 1989; Akiyama *et al*., 1991). Feed formulations are aimed to meet the minimum requirements of all ten amino acids using a variety of plant and animal proetin sources. The type and amounts of these different feed ingredients influence final amino acid composition of the feed. Arginine, valine, histidine, isoleucine, leucine, lysine, threonine, tryptophan, methionine and phenylalanine requirement for shrimps have been reported (Akiyama *et al*., 1991). The recommended levels of arginine, lysine and histidine are 5.8%, 5.3% and 2.1% of the total protein, respectively (Akiyama *et al*., 1991; Millamena *et al*., 1998; Fox *et al*., 2006). Phenyl alanine (6.86%), valine (6.7%), threonine (5.99%) and isoleucine (5.6%) were the major essential amino acids present in PL feeds. Glutamic acid

Table 6. Amino acid (%) profile of *L. vannamei* post-larval feeds

	L. vannamei post- larval feeds									
Amino acid	1	2	3	4	5	6	7	8	9	Mean±SD
Threonine	6.2	6.8	6.2	4.5	6.8	6.5	7.1	4.9	4.9	5.99±0.97
Valine	6.1	7	7.2	2	7.3	8.5	9.3	6.7	6.2	6.7±2.04
Methionine	1.8	1.8	3.3	0	2.7	2.8	2.4	2.7	2.8	2.26±0.98
Isoleucine	5.6	6.3	7.2	4.4	6.2	4.8	5.4	5.4	5.4	5.6±0.84
Leucine	10.1	0	0	0	0	0	0	0	0	1.12±3.37
Pheny alanine	6.1	6.9	8	5.2	6.3	7.6	8.7	5.7	7.2	6.86±1.14
Histidine	4.4	4.8	5	3.7	3.4	4.6	0	0	0	2.88±2.22
Tryptophan	1.23	2.23	1.35	2.5	3.71	3.15	2.56	1.97	2.54	2.36±0.79
Σ EAA	41.53	35.83	38.25	22.3	36.41	37.95	35.46	27.37	29.04	33.79±6.19
Aspartic acid	12	13.9	12.2	9.2	15.4	14.1	14.9	12.9	11.9	12.94±1.89
Serine	5.6	6.1	6.1	4.4	6.8	6.8	5.6	5.2	6.1	5.86±0.76
Glutamic acid	22.2	25.6	24.8	18.2	22.1	20.9	18	19.5	29.5	22.31±3.78
Proline	7.1	6	5.8	3.5	6.2	7.5	9.1	7.8	9.7	6.97±1.87
Glycine	3.3	4.1	3.8	2.6	5.2	4	5.6	5.7	3.4	4.19±1.09
Alanine	5.7	6.3	6.7	5.3	9.2	9.8	10.3	9.5	6.7	7.72±1.95
Tyrosine	3.8	4.4	3.8	1.6	2.4	2.2	3.7	2.6	3	3.06±0.92
Σ NEAA	59.7	66.4	63.2	44.8	67.3	65.3	67.2	63.2	70.3	63.04±7.49

(22.31%) and aspartic acid (12.94%) were the predominant non-essential amino acids. The monoaminodicarboxylic acidic amino acids (glutamic acid and aspartic acid) constituted nearly 35% of the total amino acids in PL feeds. The selective predominance of acidic amino acids over basic amino acids might be attributed to the composition of feed ingredients. Plant sources of protein were reported to be of limited use in aquaculture feeds due to low EAA content (Fox *et al*., 2006). Histidine was absent in 33% feeds. Lysine, arginine and histidine levels in feed have important bearing on the growth rate of shrimp (Millamena, 1998, 1999). Feed ingredients of plant origin were reported to be deficient in methionione and lysine (Adelizi *et al*., 1998; Kavtitha *et al*., 2003). Fox *et al*. (2006) observed that methionine, lysine and arginine are probably the most limiting amino acids in shrimp feeds. Low levels of lysine, arginine coupled with higher level of glutamic acid suggests that plant based feed ingredients dominated the PL feed formulation.

Comparison of amino acid profile of L. vannamei hatchery feeds

EAA were higher in artemia (49.75%) and zoea feed (41.92%) compared to PL feed (33.79%) and mysis feed (31.33%) (Fig. 3). Phenyl alanine, valine (6.7%), threonine (5.99%) and isoleucine (5.6%) were the major essential amino acids in PL feeds (Table 7). Glutamic acid (22.31%) and aspartic acid (12.94%) were the predominant non-essential amino acids. Sulphur containing amino acid, methionine was relatively higher in mysis feed. Tryptophan was higher in zoea feed and artemia. Isoleucine and the imino acid proline were lower in mysis feed. Tyrosine was low in zoea and mysis feed. Alanine to proline ratio was distinctly higher in mysis feed (2.6) and zoea feed (2.3). This ratio was 1.1 in PL feed and artemia.

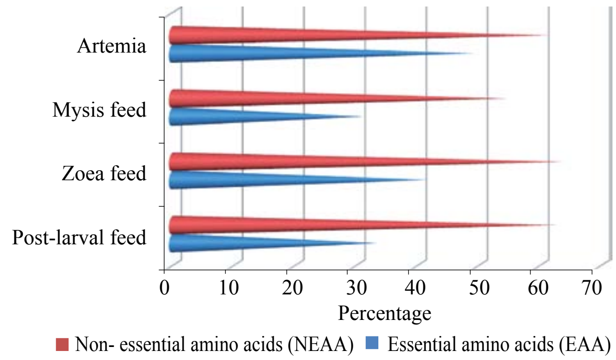

Fig. 3. Comparison of amino acid profile of *L. vannamei* hatchery feeds

Organochlorine pesticides in L. vannamei PL feeds

L. vannamei hatchery feeds were tested for 13 organochlorine pesticides of which 12 pesticides namely α-BHC, β–BHC, γ–BHC, Hep, Aldrin, ppDDE, Dieldrin, opDDD, Endrin, ppDDD, opDDT and ppDDT were not detected in any of the feeds. However, the organochlorine pesticide, HepEpo was detected in one mysis feed sample at 7.05 ppb level (Table 8). Heptachlor is a synthetic insecticide that gets converted to heptachlor epoxide. There are no natural sources of heptachlor and heptachlor epoxide. The source of HepEpo could be contaminated feed ingredients, probably of plant origin. Muscle tissues from a wide range of fish species were reported to contain organochlorine pesticides including chlordane, dieldrin and heptachlor epoxide (Roach and Runcie, 1990).

Antibiotic residues in L. vannamei hatchery feeds

L. vannamei PL, zoea, mysis feeds and artemia cysts were tested for chloramphenicol, nitrofuran metabolites, sulfonamides, oxytetracycline and chlortetracycline (Table 9.)

Table 7. Comparison of amino acid (%) profile of *L. vannamei* hatchery feeds

Amino acid	Post-larval feed	Zoea feed	Mysis feed	Artemia
Threonine	5.99±0.97	7.1±2.69	6.95±0.64	5.9±0.28
Valine	6.7±2.04	7.55±1.34	4.7±5.23	7.25±0.64
Methionine	2.26±0.98	2.45±0.92	5.05±1.77	2.7±0
Isoleucine	5.6±0.84	6.34±0.76	3.55±5.02	6.45±0.49
Leucine	1.12±3.37	0	0	5.25±7.43
Pheny alanine	6.86±1.14	7.25±0.07	6.8±1,56	8.05±1.63
Histidine	2.88±2.22	3.2±4.53	2.6±3.68	0
Tryptophan	2.36±0.79	8.03±9.43	1.68±0.45	14.15±1.34
Σ EAA	33.79±6.19	41.92±0.87	31.33±6.18	49.75±7.28
Aspartic acid	12.94±1.89	14.4±0.42	12.65±1.91	14.15±1.34
Serine	5.86±0.76	7.5±0.57	7.2±1.98	8.8±1.13
Glutamic acid	22.31±3.78	22.25±6.86	20.55±1.06	16.75±2.33
Proline	6.97±1.87	4.25±6.01	2.9±4.1	6.95±0.64
Glycine	4.19±1.09	4.1±1.56	2.35±0.49	3.05±0.07
Alanine	7.72±1.95	9.75±5.16	7.45±1.63	7.75±0.64
Tyrosine	3.06±0.92	2.15±1.63	1.85±1.91	4.85±0.21
Σ NEAA	63.04±7.49	64.4±7.64	54.95±10.96	62.3±5.94

Table 8. Organochlorine pesticides in *L. vannamei* hatchery feeds

Organochlorine pesticide	Zoea feed		Mysis feed		Post-larval feed									Arte mia	
	1	2	1	2	1	2	3	4	5	6	7	8	9	1	2
α- BHC	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
β –BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
γ –BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hep	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HepEpo	ND	ND	7.05 ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ppDDE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
opDDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ppDDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
opDDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ppDDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

*ND : not detected

Nitrofuran metabolites, sulfonamides, oxytetracycline and chlortetracycline were not detected in any of the hatchery feed samples. However, chloramphenicol was detected in two PL feed samples at a high level of 112.5 ppb and 453 ppb. The use of antibiotics in aquaculture may cause development of antibiotic resistance among pathogens infecting cultured animals and humans (Holmstrom *et al*., 2003). Chloramphenicol is used as a prophylactic in spawning tanks and also in larval tanks as prophylactic for three successive days at 4 ppm after each major moult (Treece and Fox, 1993). Uddin and Kader (2006) reported that 40% of the *Penaeus monodon* hatcheries in Bangladesh used chloramphenicol, 25% hatcheries used erythromycin, 20% used prefuran and 15% used oxytetracycline in broodstock maintenance to prevent possible bacterial infections after eye stalk ablation. Usage of chloramphenicol is banned in aquaculture in India (www.mpeda.com) but the presence of high level of chloramphenicol in the post-larval feeds (112.5 ppb and 453 ppb) could be attributed either to predetermined addition, to promote the efficacy of the feed in the post-larval tanks to combat mortality, or to inadvertent incorporation through feed ingredients.The absence of nitrofuran metabolites, sulfonamides, oxytetracycline and chlortetracycline and selective presence of chloramphenicol need further investigations. Feed manufacturers should adopt good manufacturing process and screen all feed ingredients for the presence of banned antibiotic residues.

Heavy metals in hatchery feeds

The *L. vannamei* hatchery feeds were tested for the presence of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and cobalt (Co) (Table 10). Cd content was more than 1 ppm in PL, zoea and mysis feeds. Pb content was less than 1 ppm in all the hatchery feeds. Cd, Pb, Cu and Zn content was relatively lower but Co content was higher in artemia when compared to PL, zoea and mysis feeds. Frias-Espericueta *et al*. (2001) reported that exposure of *L. vannamei* to total Cd content of 1, 2.5, 5 and 10 ppm, recorded mortalities of 20, 56.6, 60 and 93.3%, respectively and 100% mortality occurred in 15, 20 and 25 ppm at 96, 72 and 12 h of exposure, respectively. Cd causes structural and functional disruptions by binding with SH-containing ligands in the membrane and other cell constituents (Landis and Yu, 1999). Cd can uncouple oxidative phosphorylation and impair the cell energy metabolism. Body concentrations of non-essential metals like Cd do not appear to be regulated by crustaceans and hence tend to accumulate to high levels in the tissues leading to death (Zanders and Rojas, 1992). Moreover, the metabolic responses of crustaceans to heavy metal exposure appear to be strongly affected by the moulting cycle. In the present study, Cd was detected in all the *L. vannamei* hatchery feeds and this issue needs to be addressed in view of its harmful effects and quality concerns. The maximum limit of cadmium set by European Union in fish meat is 0.05 ppm (EC, 2005).

Table 9. Antibiotic residues in *L. vannamei* hatchery feeds

Antibiotic	Zoea feed		Mysis feed		Post-larval feed									Artemia	
	1	2	1	2	1	2	3	4	5	6	7	8	9	1	2
Chloramphenicol	ND	ND	ND	ND	ND	ND	453 ppb	ND	ND	ND	ND	112.5ppb	ND	ND	ND
Nitrofuran metabolites	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfonamides	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxytetracycline	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlortetracycline	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 10. Heavy metals (ppm) in *L. vannamei* hatchery feeds

Feeds	Cadmium	Lead	Copper	Zinc	Cobalt
Post- larval feed	1.36±1.40	0.25±0,25	37.91±20.79	102.49±24.82	87.49±39.8
Zoea feed	1.38±0.81	0.13±0.04	20.72±20.76	91.6±94.84	94.55±32.17
Mysis feed	1.31±0.47	0.55±0.52	23.91±19.62	106.71±106.05	89.32±7.45
Artemia	0.19±0.27	0.13±0.01	8.65±3.18	42.48±52.72	105.88±25.63

Microbiological quality of L. vannamei PL feeds and other hatchery feeds

Improper storing and handling of shrimp feed leads to bacterial and mould growth. Bacteria and mould growth occur in feeds stored in humid and moist locations. Aflatoxin producing moulds (*Aspergillus* spp.) damage shrimp health. Results of aerobic plate count (APC), total Enterobacteriaceae count (TEC) and total yeast mould count (TYMC) of the different feeds are presented in Fig. 4. APC ranged between 20 cfu g^{-1} and 560 cfu g^{-1} with a mean count of 145 cfu g^{-1}. Total Enterobacteriaceae were detected in only one PL feed at a count of 20 cfu g^{-1}. Similarly yeast and mould were detected in one PL feed at 20 g^{-1} level. The low moisture content of the pelleted feed might be the main factor for the low yeast mould content and total Enterobacteriaceae counts. Wojdat *et al*. (2005) observed that the average level of yeast and moulds in animal feed stuff samples ranged between 10^1 - 10^4 cfu g^{-1} and the number of Enterobacteriaceae in feeds for fattening animals were lower than 10 cfu g^{-1} in 85% of the samples examined, whereas in 4.6% of the samples the number of Enterobacteriaceae was 10 to 300 cfu g^{-1}. APC was less than 100 cfu g^{-1} in 55% of *L. vannamei* PL feed samples. However, 22% of the samples had relatively higher APC of >300 cfu g^{-1}; which might be mainly from spore forming bacteria that got deposited on the PL feeds during handling. Raghavan (2003) reported higher bacterial counts ranging between 10^3–10^5 cfu g^{-1} in the commercial shrimp feed samples.

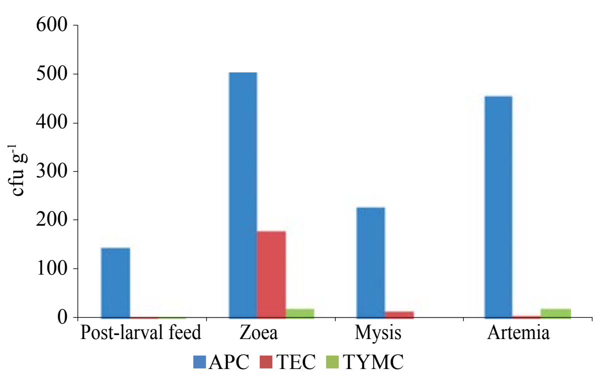

Fig. 4. Microbiological quality of *L. vannamei* post-larval and other hatchery feeds
APC - Aerobic plate count, TEC - Total Enterobacteriaceae count, TYMC - Total yeast mould count

Rajanna *et al*. (2004) reported APC values of 10^3 cfu g^{-1} in shrimp feeds which increased to 10^4 cfu g^{-1} during storage for 30 days. APC and TYM counts were high in zoea feed (510 cfu g^{-1}; 20 g^{-1}) and artemia (460 cfu g^{-1}; 20 g^{-1}) respectively. Among the hatchery feeds, PL feed had the lowest mean APC (145 cfu g^{-1}). Total Enterobacteriaceae count was relatively high in zoea feed (180 cfu g^{-1}) whereas in other feeds the mean count was less than 20 cfu g^{-1}.

The study revealed the need for regular monitoring of all types of feed employed in shrimp hatcheries for nutritional, biochemical and bacteriological quality with special emphasis on contaminants such as heavy metals, pesticides, insecticides and antibiotics. Good manufacturing practices (GMP) is the need of the hour for production of aquaculture feeds free from contaminants and other hazards.

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