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Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry byproduct meal

Arul Victor Suresh [a,*], K.P. Kumaraguru vasagam [a,1], Sergio Nates [b]

[a] Integrated Aquaculture International, 1st Floor No. 6 Block A Bangunan Lim Seng, Simpang 628, Jalan Tutong, Bandar Seri Begawan, BF 1120, Brunei Darussalam
[b] Fats and Proteins Research Foundation, 801 North Fairfax Street, Suite 205, Alexandria, Virginia 22314, USA

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ABSTRACT

Properties of two kinds of poultry byproduct meal (PBM), blood meal (BLM), hydrolyzed feather meal (HFM), anchovy fishmeal (AFM), fish hydrolysate (FHD), squid liver meal (SLM) and krill meal (KRL) as feeding effectors (attractants and palatability factors) were studied in juvenile blue shrimp *Litopenaeus stylirostris*. Biochemical analyses of the ingredients revealed that BLM and HFM had low levels of free amino acids (FAA), nucleotides, taurine, protein solubility and small peptide; whereas, KRL had high levels of all except protein solubility. The biochemical profile of PBM was only moderately inferior to that of AFM. FHD had high levels of FAA, protein, and taurine, but low levels of nucleotides and small peptides. Attractability and palatability assessments in shrimp were fairly consistent with the biochemical profile. A 6-week feeding trial using feeds formulated with 20% PBM, no fishmeal and 3% of BLM, AFM, FHD, SLM, or KRL showed that attractability of the feeds was improved by SLM and KRL, but palatability and growth were improved only by KRL.

1. Introduction

Successful replacement of fishmeal in shrimp feeds requires feed formulators to source alternative sources of nutrients and other functional factors that are present in fishmeal and critical to shrimp performance in aquaculture. The key nutrients are essential amino acids, particularly methionine and lysine, highly unsaturated fatty acids, phospholipids, and cholesterol. The key functional factors are attractability and palatability. Shrimp depend on chemosensory systems to identify, locate and ingest food (see reviews by Lee and Meyers, 1997; Derby and Sorensen, 2008). Feed ingredients of aquatic animal origin such as fishmeal, krill meal, shrimp meal and squid meal are rich in chemical compounds such as free amino acids (FAA), nucleotides, amines and nucleosides that are readily recognizable to the chemosensory systems of shrimp in the process of locating and ingesting food. Feed ingredients of terrestrial animal origin such as meat and bone meal, poultry byproduct meal, feather meal, and blood meal are believed to contain lower levels of the chemical compounds, but studies evaluating the attractability and palatability aspects of the ingredients in shrimp are limited.

The present study reports evaluation of the attractability and palatability aspects of selected ingredients of aquatic and terrestrial animal origin. Three aspects are covered: (1) the levels of soluble protein, small chain peptides, FAA, taurine and nucleotides in four terrestrial (petfood-grade poultry byproduct meal (PBP); feed-grade poultry byproduct meal (PBF); hydrolyzed feather meal (HFM); and spray-dried blood meal (BLM)) and four aquatic animal protein ingredients (anchovy fishmeal (AFM); fish hydrolysate (FHD); squid liver meal (SQL); and krill meal (KRL)), (2) attractability and palatability of the above ingredients in the blue shrimp, *Litopenaeus stylirostris*; and (3) growth performance of *L. stylirostris* fed diets containing high levels of poultry by-product meal supplemented with each of the aquatic animal proteins and BLM.

2. Materials and methods

The study was organized in two phases. In Experiment A, eight protein ingredients were chemically characterized and tested in shrimp for attractability and palatability using bland feed. In Experiment B, a set of practical diets containing high levels of poultry by-product meal and marine protein ingredients was tested for attractability, palatability and growth performance.

* Corresponding author at: 5661 Telegraph Road, Suite 3A, St. Louis, MO 63129, USA. Tel.: +1 314 293 5500; fax: +1 314 293 5525.
E-mail addresses: victors@integratedaquaculture.com (A.V. Suresh), drvasagamguru@gmail.com (K.P. Kumaraguru vasagam).
[1] Present address: Research Department of Zoology, V.O. Chidambaram College, Tuticorin, Tamilnadu, India.

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Table 1
List and source of test ingredients used in this study.

Ingredient	Origin, supplier
Poultry byproduct meal, petfood grade (PBP)	USA. Valley Proteins, USA
Poultry byproduct meal, feed grade (PBF)	USA. Valley Proteins, USA
Feather meal, hydrolyzed (HFM)	USA. Griffin Industry, USA
Blood meal, spray-dried (BLM)	USA. Griffin Industry, USA
Fishmeal, anchovy (AFM)	Peru, Alicorp Nicovita, Peru
Fish hydrolysate (FHD)	France, Sopropeche, France
Krill meal (KRL)	Antarctic, Aker Biomarine, USA
Squid liver meal (SQL)	Korea, PT. CJ Feed, Indonesia

2.1. Acquisition of ingredients and feed formulation

The list and source of ingredients tested in the study are shown in Table 1. Two sets of diets were formulated and used. The first set of diets (Table 2), coded with letter A in the prefix, was used in the assessment of attractability and palatability of test ingredients (Experiment A). A bland control feed was formulated to have ingredients of plant origin namely soybean meal, wheat flour, wheat gluten and alginate. Treatment diets were formulated to contain one of the test ingredients at the expense of soybean meal.

The second set of diets (Table 3), coded with letter B in the prefix, was used in the growth trial and in assessment of attractability and palatability (Experiment B). The control diet had pet food-grade poultry byproduct meal at 21.4% and no fishmeal. Each treatment feed had 3% of AFM, FHD, SQL, KRL or BLM added at the expense of poultry byproduct meal and wheat flour. A reference diet containing 23.3% of AFM was also formulated. All the diets in the second set (for growth trial) were formulated to meet the basic nutrient requirements for penaeid shrimp, using Feedsoft Professional 3.1 (www.feedsoft.com). The ingredient composition of the test diets is presented in Table 3.

All diets were produced in the feed preparation facility at Shrimp Nutrition Research Center (SNRC), Brunei. All ingredients except the vitamin premix, soy lecithin and fish oil were mixed in a vertical mixer for a few minutes. The mixture was then ground through a rotor beater mill (SR 300, Retsch, Germany) which used a 0.25-mm screen to pass through the ground materials. The ground meal was returned to the vertical mixer and mixed with about 40% water. The wet mash was then autoclaved at 105 °C for 5 min. The autoclaved mash was cooled and mixed with the vitamin premix, soy lecithin, fish oil and 10–15% water in the vertical mixer. The resulting dough was extruded through a 2-mm die in a meat grinder to produce noodle-like long strands of feed. The strands were dried in a forced-air draft oven set at 50 °C for 3–4 h. The dried strands were broken into 4–5-mm-long feed particles in a food processor, and stored in tightly-sealed plastic containers at 20 °C until used.

Table 3
Feed formulations and analyzed proximate composition (g 100 g^{-1} as is) of feeds used in the growth trial and assessment of attractability and palatability in experiment B.

Ingredient (g 100 g^{-1})	Feed[a]						
	B-CNL	B-AFM	B-KRL	B-SQL	B-FHD	B-BLM	B-REF
Soybean meal	40	40	40	40	40	40	40
Wheat flour	28.2	27.4	27.4	26.8	27.4	27.2	26
Monocalcium phosphate	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Wheat gluten	3.3	2.5	2.5	3.1	2.5	2.5	2.5
Fish oil	1	1	1	1	1	1.2	2.1
Lecithin, fluid	2	2	2	2	2	2	2
Vitamin premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Alginate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cholesterol	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Poultry byproduct meal, petfood grade	21.4	20	20	20	20	20	0
Fishmeal, anchovy	0	3	0	0	0	0	23.3
Krill meal	0	0	3	0	0	0	0
Squid liver meal	0	0	0	3	0	0	0
Fish hydrolysate	0	0	0	0	3	0	0
Blood meal, spray-dried	0	0	0	0	0	3	0
Proximate composition							
Moisture	6.56	5.81	5.49	4.79	5.11	5.67	5.26
Crude protein	40.08	40.47	41.55	41.01	41.57	41.28	42.93
Crude fat	7.92	7.88	7.37	7.83	8.39	8.06	7.60
Crude fiber	1.91	1.88	2.12	2.03	1.98	1.81	2.02
Ash	7.84	8.17	8.07	7.94	7.89	7.75	9.10

CNL, control; BLM, AFM, Fishmeal (anchovy); KRL, Krill meal; SQL, Squid liver meal; FHD, Fish hydrolysate; Blood meal (Spray-dried); REF, Reference.
[a] Feed names with prefix 'B' indicate the experiment phase as 'B'.

2.2. Biochemical analysis of ingredients and feeds

Test ingredients and test diets were analyzed by methods of AOAC (2005) for determination of moisture (AOAC 930.15), crude protein by combustion (AOAC 990.03), crude fat (by acid hydrolysis, AOAC 954.02), crude fiber (AOAC 978.10), and ash (AOAC 942.05). Test ingredients were analyzed for soluble protein, nucleotides, taurine, FAA and size distribution of peptides. Protein solubility was determined by measuring crude protein (AOAC 990.03) in an aqueous extract of the sample. Nucleotides were analyzed by reversed phase HPLC as per Ryder (2000). Taurine was determined using HPLC (AOAC 994.12). FAA were determined using a modification of the standard amino acid analyses using HPLC (AOAC 994.12). The modification involved the use of distilled water instead of hydrochloric acid in the hydrolysis of proteins. Size distribution of peptides was analyzed by HPLC size exclusion chromatography as per Aksnes et al. (2006). All the analyses except peptide size distribution were performed at the

Table 2
Feed formulations used in the assessment of attractability and palatability of test ingredients in experiment 'A'.

Ingredient (g 100 g^{-1})	Feed[a]									
	A-CNL	A-PBP	A-PBF	A-HFM	A-BLM	A-FMH	A-FML	A-KRL	A-SQL	A-FHD
Soybean meal (46% crude protein)	72	62	62	62	62	62	69	69	69	69
Wheat flour	25	25	25	25	25	25	25	25	25	25
Wheat gluten	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Alginate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poultry byproduct meal, petfood Grade	0	10	0	0	0	0	0	0	0	0
Poultry byproduct meal, feed grade	0	0	10	0	0	0	0	0	0	0
Feather meal, hydrolyzed	0	0	0	10	0	0	0	0	0	0
Blood meal, spray-dried	0	0	0	0	10	0	0	0	0	0
Fishmeal, anchovy	0	0	0	0	0	10	3	0	0	0
Krill meal	0	0	0	0	0	0	0	3	0	0
Squid liver meal	0	0	0	0	0	0	0	0	3	0
Fish hydrolysate	0	0	0	0	0	0	0	0	0	3

CNL, control feed; PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.
[a] Feed names with prefix 'A' indicate the experiment phase as 'A'.

Nestle Purina Analytical Laboratory, USA. Peptide size distribution analysis was performed at the W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University School of Medicine, USA.

2.3. Test shrimp

All shrimp used in the trial were domesticated, high-health *L. stylirostris* derived from the Aquaculture Development Center (ADC) of the Department of Fisheries in Brunei Darussalam. The shrimp were transported from ADC to the Shrimp Nutrition Research Center at the post-larval stage and grown in a 5000-liter circular outdoor, nursery tank using a commercial shrimp feeds. When they reached required sizes they were used in various trials. Average body weight sizes of 1.5 g, 1.5–2 g, and 10 g were used in growth, attractability and palatability trials, respectively. The shrimp were in intermolt stage when selected for attractability and palatability trials.

2.4. Attractability and palatability trials

2.4.1. Assessment of attractability

Three rectangular, glass tanks each with multiple chambers were used to assess attractability of feeds to the shrimp. Each tank was constructed of clear glass, 90 × 30 × 30 cm in length, width and height, and had an acclimatization chamber at one end and three feeding chambers at the other end (Fig. 1). A movable glass shutter separated the acclimatization and feeding chambers. Each feeding chamber had an opening to allow free access of shrimp to the feed placed in the chamber. The tanks were set up in an unlit room that received diffused natural daylight, and all assessments were conducted at the same time of the day commencing at 9 am.

Attractability of feed was tested for one feed at a time. Ten randomly selected shrimp of 1–2 g size were stocked into the acclimatization chamber, and allowed to acclimatize for 1 h. After 1 h, 1 g of the feed to be tested was placed in one of the three chambers. Ten minutes after the placement of the feed, the movable glass shutter was raised to allow access of shrimp to the feed. At 1, 2, 5, 10 and 15 min following the raising of the shutter, the number of shrimp in the feeding chamber was counted and recorded. Each feed was tested seven, randomly selected times. Percentage of shrimp in feeding chamber at different time intervals was calculated from the data collected.

2.4.2. Assessment of palatability

Ten, sloped-bottom, cylindrical fiberglass tanks of 50-L were used to assess palatability of feeds to the shrimp. The tanks were located indoor under clear plastic fiber roofing that allowed penetration of daylight. Each tank had a 25-mm drain at the bottom that was operated by using a valve. The tanks were filled and stocked with seven shrimp of approximately 10 g each. The shrimp were allowed to acclimate for 1 h after which aeration to the tank was stopped, the bottom of the tank was flushed to remove any feces, and 2 g of test feed was introduced into the tank. The shrimp were given 60 min to consume the feeds. At the end of 60 min, the shrimp were removed from the tank and mass weighed. The uneaten feeds were removed from the drain by opening the valve, and collected on mesh netting. Feces, if any, were carefully removed using a rubber aspiration bulb. The feeds were then dried in a forced-air draft oven at 60 °C for 8 h. Leaching loss of each feed was determined by keeping the feed under water for 60 min in the tanks that were used for palatability determination. The tanks were not stocked with shrimp at the time of leaching loss determination. The feeds were recovered, dried and weighed to calculate the leaching loss. This loss was used as a correction factor and was applied to arrive at an estimate of uneaten feed. Feed consumption was calculated as mg feed/gram of shrimp. Each feed was tested in nine randomly selected times. All assessments were conducted at the same time of the day commencing at 10 am.

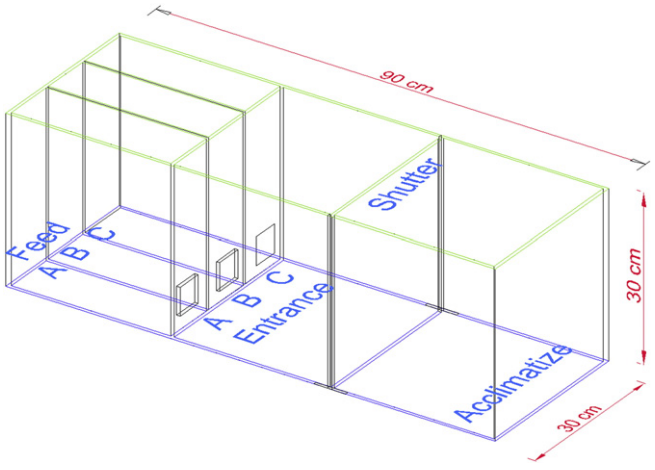

Fig. 1. Drawing of glass tank used for evaluation of feeds for attractability.

2.5. Growth trial in microcosm tanks

Growth trials with second set of test diets were performed in 21 self-cleaning microcosm tanks of 1827-L water holding capacity. The microcosm tanks were independent, self-circulating units in which all the water movement was driven by airlift and gravity. The system has been described in detail in Kumaraguru vasagam et al. (2009). The tanks were located indoors under clear plastic fiber roofing that allowed penetration of daylight. Each dietary treatment was assessed in triplicate tanks. Each tank was randomly stocked with 50 shrimp of about 1.5 g size and reared for 42 days. Ten shrimp were sampled every 7 days for weight. Feeding rate was based on a standard, shrimp size-based feeding chart. The daily ration was divided into two parts: 40% for feeding in the morning (08:00 H); and the remainder (60%) for feeding in the evening (16:30 H). The feed was placed on a belt-feeder (Zeigler Brothers, USA) and delivered in a continuous manner. Water quality conditions were maintained within the following ranges: temperature, 28–31 °C; salinity, 27–30‰; pH, 7–8.2; dissolved oxygen, >5 mg/L. At the end of the trial, the following parameters of performance were tested statistically: final shrimp weight, weight gain, survival, feed consumption and feed conversion ratio.

2.6. Statistical analysis

Mean values of attractability, palatability and growth performance were subjected to One-way Analysis of Variance (ANOVA) followed by LSD for multiple comparison analysis to test if there were any significant differences ($P<0.05$) among treatment means. In attractability assessment, the earliest time at which statistically significant differences ($P<0.05$) occurred among treatment means within a given set of feeds was taken as the point at which multiple comparisons of treatments (LSD) were performed. The above statistical analyses were performed using Analyze-It ® software.

3. Results

3.1. Analysis of ingredients

Proximate and taurine composition of the test ingredients are presented in Table 4. FAA composition is presented in Table 5. Protein solubility, peptide size distribution and nucleotide distribution are given in Figs. 2–4, respectively. Considerable differences were observed among ingredients in their composition of all analyzed

Table 4
Proximate composition (g 100 g^{-1} as is) and taurine content of test ingredients.

Nutrients	PBP	PBF	HFM	BLM	AFM	FHD	KRL	SQL
Moisture	5.9	3.5	4.6	6.6	9.4	6.8	8	11.3
Crude protein	66.2	55.5	83.9	90.6	66.1	72.9	59.4	65.6
Crude lipid	14.4	15.1	7.2	2.6	7.3	17.1	18.5	7.4
Ash	12.1	22.7	5.6	1.9	16.3	5.3	11	15.2
Taurine (mg/kg as is)	4463	2118	477	304	5046	7147	5381	7378

PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.

Table 5
Free amino acid composition (FAA, g 100 g^{-1} as is) of the test ingredients.

Free amino acids	PBP	PBF	HFM	BLM	AFM	FHD	KRL	SQL
Aspartic acid	0.07	0	0	0	0	0.05	0	0
Threonine	0	0	0	0	0	0.06	0	0
Serine	0.06	0	0	0	0	0	0	0
Glutamic acid	0.08	0	0	0	0.08	0.19	0	0.07
Proline	0.05	0	0	0	0.07	0.06	0.36	0
Glycine	0.06	0.07	0	0	0.05	0.13	0.32	0
Alanine	0.1	0.16	0	0	0.16	0.3	0.06	0.16
Valine	0	0.08	0	0	0.06	0.12	0.12	0.05
Methionine	0	0	0	0	0	0.08	0	0
Isoleucine	0	0	0	0	0	0.07	0	0
Leucine	0	0.08	0	0	0.08	0.24	0	0.06
Tyrosine	0	0	0	0	0	0.06	0	0
Phenylanine	0	0	0	0	0.05	0.09	0	0
Histidine	0.05	0.05	0	0	0.48	0.08	0	0.68
Lysine	0.06	0.06	0	0	0.08	0.18	0	0.08
Arginine	0.06	0	0	0	0.05	0.05	0.39	0
Total FAA[a]	0.59	0.5	0	0	1.16	1.76	1.25	1.1

PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.
[a] FAA, free amino acid.

parameters. BLM was different from all other ingredients by having undetectable levels of nucleotides and FAA, and 83% of its peptides being larger than 10 kDa in size. It also had the lowest protein solubility and taurine content. While HFM had 63% of its peptides in the size range below 10 kDa, it had low levels of nucleotides and no FAA. HFM's protein solubility and taurine content were also low. The two grades of poultry byproduct meals had almost similar peptide size distribution, but PBP had higher levels of nucleotides and taurine than PBF. Peptide size distribution of AFM was also similar to PBP and PBF but AFM had higher levels of FAA, nucleotides and taurine.

FHD had the highest protein solubility and FAA content among all ingredients, and high taurine content, but low levels of nucleotides. Though FHD had 71% of its peptides smaller than 10 kDa, the proportion of peptides smaller than 1 kDa was the lowest among marine origin ingredients, and lower than those of poultry byproduct meals. KRL had the highest proportion of peptides smaller than 10 kDa. About 75% of KRL's peptides were smaller than 1 kDa. KRL also had the highest level of nucleotides. SQL had the highest level of taurine, and a high proportion of peptides smaller than 1 kDa.

AFM and SQL were rich in the nucleoside, inosine and its monophosphate form (IMP). Inosine was the dominant nucleoside in PBP and PBF, but among monophosphate forms adenosine monophosphate (AMP) dominated. Similar trend was present in FHD too. Krill had relatively low levels of all nucleosides, but very high levels of the monophosphate forms.

Among FAA, alanine was present in all ingredients except HFM and BLM. Free valine was present in all aquatic animal protein ingredients, but not in any terrestrial animal protein. Free histidine was dominant in AFM and SQL. FHD had considerable quantities of free glutamic acid, glycine, alanine, valine, methionine, leucine and lysine. KRL had high levels of proline, glycine and arginine.

3.2. Assessment of attractability and palatability of test ingredients (Experiment A)

Results of the attractability and palatability of the series 'A' diets are presented in Table 6. Statistical analysis of the attractability data showed significant difference ($P<0.003$) among test diets at the 10th minute. Multiple comparison of means at 10th minute showed that feeds A-PBF, A-PBP, A-KRL, A-SQL, A-FMH and A-FML attracted more shrimp consistently while the feeds CNL and FHD elicited poor response by shrimp. Feeds A-PBF and A-PBP were more attractive to shrimp than any of the feed tested. Visible behavioral differences among shrimp offered different feeds were apparent immediately after they were provided access to the feed.

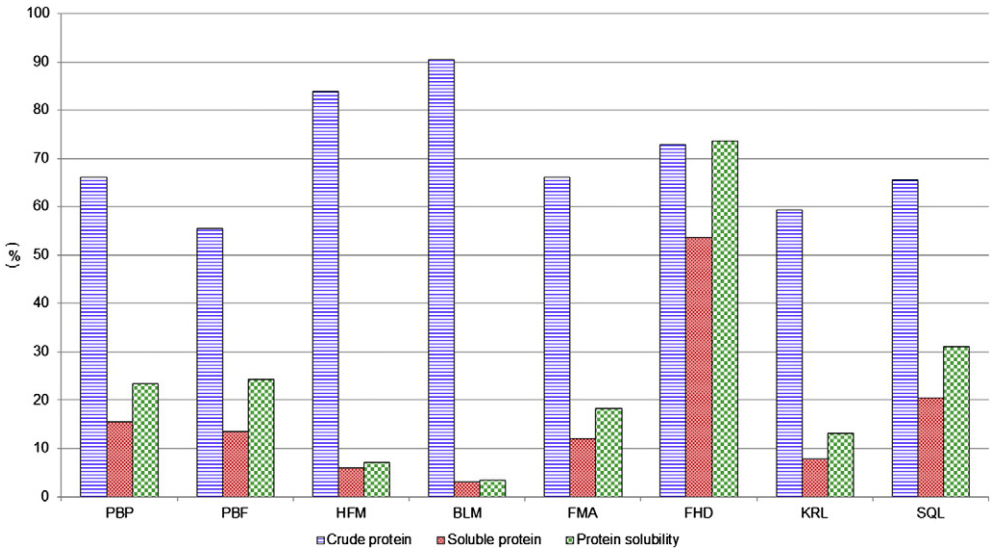

Fig. 2. Solubility of proteins from test ingredients in water, PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.

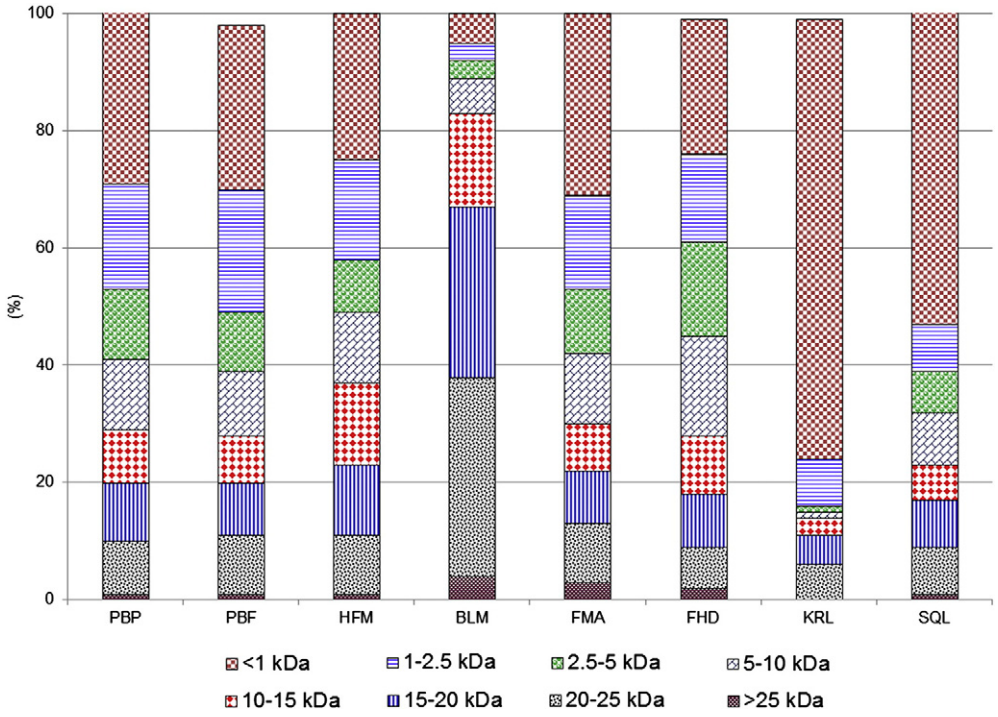

Fig. 3. Distribution of nucleotides in test ingredients, PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.

There was also significant difference ($P<0.0001$) among feeds in their palatability to shrimp (Table 6). Feed consumption in the first 60 min by shrimp was used as the measure of palatability. While shrimp ate only 2.6 mg feed/g biomass of the feed containing none of the test ingredients (A-CNL), their feed consumption increased 2–4 times when one of the test ingredients was incorporated in the feed. Feed A-KRL showed the highest consumption (11.44 mg/g shrimp). Feed A-SQL, A-PBF, A-FMH, A-PBP, and A-HFM resulted in an increase of feed consumption by about three times when compared to A-CNL. There was no significant ($P<0.001$) increase in feed consumption between A-FML and A-CNL. Dry matter loss of the test feeds ranged from 6.3 to 10.6% and was adjusted in the estimation of feed consumption.

3.3. Assessment of attractability and palatability of test feeds containing high level of poultry byproduct meal supplemented with different protein ingredients (Experiment B)

Results of the attractability and palatability of the series 'B' diets are presented in Table 7. Statistical analysis showed significant difference

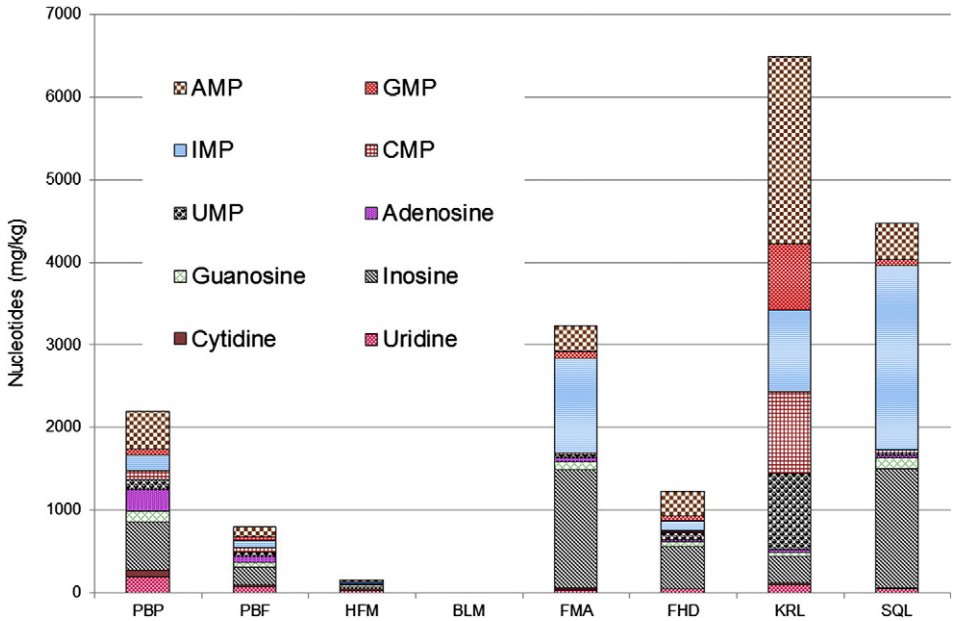

Fig. 4. Frequency distribution of peptides by molecular weight in test ingredients, PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.

Table 6
Attractability and palatability of feeds formulated to have individual ingredients in Experiment A.

Feed*	Attractability (%)†		Palatability (mg/feed/g shrimp biomass) ††	
	Mean	SE	Mean	SE
A-CNL	27.1[a]	4.2	2.6[a]	0.4
A-PBP	55.7[bc]	6.5	7[bd]	1.0
A-PBF	58.6[bc]	5.1	8.35[bd]	1.0
A-HFM	35.7[ab]	5.3	7.6[bd]	1.0
A-BLM	45.7[b]	4.8	5.69[bc]	0.8
A-FMH	42.9[ab]	6.1	7.71[bd]	0.6
A-FML	32.9[ab]	7.1	4.44[ac]	0.4
A-KRL	41.4[ab]	2.6	11.44[e]	0.8
A-SQL	47.1[b]	6.1	8.46[bd]	0.8
A-FHD	30[a]	8.5	6.26[bc]	0.8

Means in the same column sharing same letters in superscript are not significantly different ($P<0.05$).
*Feed names with prefix 'A' indicate the experiment phase as 'A'.
CNL, control feed; PBP, Poultry byproduct meal (Petfood grade); PBF, Poultry byproduct meal (Feed grade); HFM, Feather meal (Hydrolyzed); BLM, Blood meal (Spray-dried); AFM, Fishmeal (anchovy); FHD, Fish hydrolysate; KRL, Krill meal; SQL, Squid liver meal.
†Percentage shrimp in feeding chamber containing test feed 10 min after the shrimp were provided free access to feed.
††Palatability, as measured by feed consumption (mg feed/g shrimp biomass) in 60 min.

($P<0.04$) among feed treatments at the 5th minute. Multiple comparison of means at 5th minute showed that there was no difference between feed B-CNL and B-FML or B-BLM. Shrimp found the feeds B-KRL, B-REF and B-SQL to be more attractive.

There was also significant difference ($P<0.008$) among diets in their palatability to shrimp (Table 7). However, multiple comparisons showed no significant difference between feeds containing PBP (B-CNL, B-BLM, B-SQL, B-FHD, B-AFM and B-KRL). However the feed B-REF was significantly higher than the rest of the diets.

3.4. Performance of shrimp on feeds containing high level of poultry byproduct meal

Data on the growth performance of shrimp fed diets containing high level of PBP with and without the attractants and palatability enhancers are presented in Table 8. Significant difference among treatments was observed only in the final weight ($P<0.03$) and weekly weight gain ($P<0.03$) of the shrimp. Shrimp fed the B-BLM registered a lowest weekly weight gain (1.54 g) which was not significantly different from those achieved by shrimp fed B-CNL, B-AFM, B-FHD and B-SQL. Shrimp fed B-REF registered the maximum weight gain (2.0 g/week), but it was not statistically significant from that of shrimp fed B-KRL. Survival exceeded 86% in all feed treatments and did not differ among treatments. Similarly there was no difference in FCR or yield among the various treatments.

Table 7
Attractability and palatability of feeds formulated to contain high level of poultry byproduct meal with or without 3% of attractants and palatability enhancers in Experiment B.

Feed	Attractability (%)†		Palatability (mg feed consumed/g shrimp biomass) ††	
	Mean	SE	Mean	SE
B-CNL	40.0[a]	5.0	10.37[a]	1.1
B-AFM	36.7[a]	6.0	11.67[a]	1.1
B-KRL	53.3[b]	6.9	13.09[a]	0.9
B-SQL	61.1[b]	2.6	11.06[a]	0.9
B-FHD	48.9[ab]	7.2	11.34[a]	1.0
B-BLM	40.0[a]	5.8	10.64[a]	0.6
B-REF	53.3[b]	6.2	15.53[b]	1.3

Means in the same column sharing same letters in superscript are not significantly different ($P<0.05$).
†Percentage shrimp in feeding chamber containing test feed 5 min after the shrimp were provided free access to feed.
††Palatability, as measured by feed consumption (mg feed/g shrimp biomass) in 60 min.
CNL, control; BLM, AFM, Fishmeal (anchovy); KRL, Krill meal; SQL, Squid liver meal; FHD, Fish hydrolysate; Blood meal (Spray-dried); REF, Reference.

4. Discussion

Response of shrimp to feeding effectors (collective term for attractants, feeding incitants and stimulants (Smith et al., 2005)) has been studied well in the past due to its relevance in understanding crustacean feeding behavior and marine ecology, and applications in aquaculture (Zimmer-Faust, 1989; Holland and Borski, 1993; Coman et al., 1996; Sanchez et al., 2005; Smith et al., 2005; Nunes et al., 2006; Grey et al., 2009). Marine animal sources and/or chemical compounds have been used primarily as test materials in almost all studies. Sanchez et al. (2005) included casein, a milk protein, in the experimental diets with the expectation that it would serve as a bland ingredient, i.e. an ingredient without any substantial feeding stimulation effect, in diets for the Pacific white shrimp, *Litopenaeus vannamei*. The findings of the study, however, led them to speculate that casein at high levels of inclusion may play the role of a feeding effector. Nunes et al. (2006) included meat and bone meal and blood meal in the validation phase of the study evaluating several feed attractants and stimulants in *L. vannamei*. They found blood meal to be among the least stimulatory ingredients, and meat and bone meal to be similar to fish soluble, but inferior to fishmeal and squid meal as sources of attractants and palatability factors. To our knowledge, no other studies have been reported on the attractability and palatability aspects of terrestrial animal protein byproducts to shrimp. This is the first time that a detailed investigation of commercially available feed ingredients of terrestrial animal protein origin with respect to chemoattraction and palatability to shrimp is reported.

The first part of our investigation involved chemical characterization of terrestrial animal byproducts and comparison of the chemical characteristics with marine origin ingredients, namely fish meal, fish hydrolysate, krill meal and squid liver powder. Derby and Sorensen (2008) noted in their review that feeding behavior of crustaceans is most effectively stimulated by small, water-soluble, nitrogen-bearing compounds, particularly amino acids and nucleotides.

We found considerable differences among terrestrial animal byproducts in chemical composition. Blood meal and hydrolyzed feather meal had no or very low levels of FAA and nucleotides. The protein of the ingredients was not highly soluble in water and was composed predominantly of large peptides. Taurine content of the two meals was the lowest among all tested ingredients. In contrast, poultry byproduct meals had high levels of FAA and nucleotides. The protein was soluble, and had a high proportion of small peptides. Taurine content was high. There were notable differences among the two types of poultry byproduct meal. The higher protein, petfood-grade poultry byproduct meal had more nucleotides and taurine than the feed-grade byproduct meal.

The differences among various terrestrial animal byproducts in their chemical composition may be attributed to the nature of the animal tissues from which they originate and methods of processing. Blood meal is made by drying whole blood which includes blood cells and plasma. Since plasma is a carrier of nutrients, one would expect the presence of FAA in it. Yet the absence of any measurable FAA in it requires further investigation. The lack of nucleotides in it may be because cells account for only 45% of vertebrate blood and a majority of the cells are erythrocytes lacking nuclei. Feathers are composed of structural proteins, mainly β-keratins, and are therefore abundant in amino acids such as proline, serine, and cystine that are important in determining protein structure. Feathers are supplied with blood

Table 8
Growth, survival, feed conversion ration and yield of shrimp fed diets containing high level of poultry byproduct meal with or without 3% of attractants and palatability enhancers for 42 days.

	B-CNL		B-AFM		B-FHD		B-KRL		B-SQL		B-BLM		B-REF	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Initial mean weight (g)	1.59	0.03	1.68	0.06	1.67	0.02	1.64	0.02	1.63	0.06	1.59	0.04	1.62	0.02
Final mean weight (g)	12.43^{ab}	0.81	11.87^{ab}	0.46	11.71^{ab}	0.27	12.82^{bc}	0.29	11.38^{ab}	0.43	10.84^{a}	0.22	13.60^{bc}	0.70
Weekly weight gain (g)	1.81^{ab}	0.14	1.70^{ab}	0.09	1.67^{ab}	0.05	1.86^{bc}	0.05	1.62^{ab}	0.07	1.54^{a}	0.04	2.00^{bc}	0.12
Survival (%)	89.33	5.93	92.00	1.15	86.00	5.29	92.67	5.46	85.33	7.06	93.33	3.53	86.67	4.67
FCR	1.41	0.10	1.48	0.08	1.42	0.04	1.42	0.03	1.48	0.06	1.46	0.05	1.41	0.08
Yield (kg/m^2)	0.28	0.00	0.28	0.01	0.25	0.01	0.30	0.02	0.25	0.03	0.26	0.01	0.30	0.01

Means in the same row sharing the same letters in superscript are not significantly different ($P<0.05$).
*Feed names with prefix 'B' indicate the experiment phase as 'B'.
CNL, control; BLM, AFM, Fishmeal (anchovy); KRL, Krill meal; SQL, Squid liver meal; FHD, Fish hydrolysate; Blood meal (Spray-dried); REF, Reference.

only during the early stages of their development, and they become metabolically inactive when fully grown. For these reasons, it is only natural that they have undetectable levels of nucleotides and FAA. Hydrolysis in the process of producing "Hydrolyzed Feather Meal" involves cooking of the feathers in high temperature and pressure which results in changes in the amino acid composition of the feathers, particularly cystine (Moritz and Latshaw, 2001). So, while changes in protein structure during hydrolysis of the feathers are evident, it seems unlikely that small peptides and FAA are produced.

Poultry byproduct meal is rendered from poultry carcasses including meat, bone, undeveloped eggs and offal that are unfit for human consumption. As muscle tissues predominate in the rendered raw material, the meal is high in nucleotides and taurine. Autolysis prior to rendering results in FAA and small peptides. The key difference between petfood-grade poultry byproduct meal and feed grade poultry byproduct meal is the higher inclusion of meat than bone in the former, and this is reflected in the higher levels of nucleotides and taurine in the petfood-grade poultry byproduct meal.

When compared to prime-grade anchovy meal, petfood-grade poultry byproduct meal has 13% lower taurine, 46% lower nucleotides and 97% lower FAA. Yet, both ingredients had similar levels of small peptides and protein solubility. Based on the chemical composition data, and subsequent attractability and palatability assessments and shrimp performance in feeding trial, one can place petfood-grade poultry byproduct meal as only moderately inferior to fishmeal in terms of attractability and palatability.

Among ingredients of marine animal origin, we found notable differences in biochemical composition. As expected, fish hydrolysate had high levels of FAA. It had the second highest level of taurine among all ingredients. Its protein solubility was the highest, but contrary to expectation it had the lowest proportion of small peptides among all ingredients of marine animal origin. It also had very low level of nucleotides. Fish hydrolysate's lower effectiveness as a source of attractants and palatability factors was evident in the animal trials as well. Smith et al. (2005) reported that fish hydrolysate inclusion at 2% in the feed did not significantly improve feed intake, growth or FCR of black tiger shrimp, *Penaeus monodon*. The base feed used by Smith et al. (2005) had 17% fishmeal, 25% meat meal, 5% squid meal, and 20% lupin meal. Grey et al. (2009) reported that three of the four different salmon hydrolysates tested in the study were effective feeding effectors in *P. vannamei*. Only feed intake was measured in the study and the base feed was composed of only wheat flour and soybean four. Inclusion of the hydrolysates was at 5% on dry matter basis. No other feeding effector was tested in the study for comparative purposes. So, the findings of Grey et al. (2009) are limited in their interpretation to a broad range of shrimp feed formulas. The reason for unexpected poor performance of fish hydrolysate as a feeding effector in shrimp requires further investigation.

Squid liver meal, a byproduct of squid processing, is made by hydrolyzing squid viscera, then condensing the hydrolyzate and drying it on a carrier such as soybean meal (Hertrampf and Piedad-Pascual, 2000). The ingredient had the highest level of taurine and high levels of nucleotides, FAA, and small peptides. Krill meal, obtained from whole krill *Euphasia* spp., was found to have a desirable profile of chemicals that are known feeding effectors. Though it had very low protein solubility, it had the highest proportion of small peptides among all ingredients. It was rich in nucleotides, FAA, and taurine as well.

The second part of our investigation involved assessment of attractability and palatability of the ingredients in both a bland diet and in a complete diet. Assessment of attractability involved an adaptation of the methods previously used by other researchers (Sanchez et al., 2005; Smith et al., 2005; Nunes et al., 2006; Grey et al., 2009). The methods principally involve measurement of shrimp's orientation to, or presence on, or consumption of, one of the two feeds offered. Contrary to others, we measured shrimp response to each ingredient separately and independently, and compared the responses using statistical tools. The shortest time taken for the shrimp to discriminate among attractants was determined statistically, and the response to each attractant at that time slot was compared. The rationale behind this approach was to reduce the likelihood of palatability playing a role in determining the number of shrimp that is found on the feed. Similarly, we used a cylindrical shaped tank for determining palatability so that the feed can be found as quickly as the feed entered the tank thereby reducing the likelihood of attractability playing a role on feed intake.

We found that shrimp took only 10 min to discriminate among feeds containing different ingredients when a basal, bland feed of wheat flour and soybean meal was used. When the basal feed contained large quantities of poultry byproduct meal or fishmeal, the discrimination was even faster at 5 min. Inclusion levels for testing of ingredients in experiment A (bland base feed) were arbitrarily set for rendered animal byproducts at 10% and for marine animal products at 3%. Only fishmeal was tested at 3% as well as 10%, and the feed containing fishmeal at 3% had lower attractability and palatability. Smith et al. (2005) found that shrimp response to increasing doses of feeding effectors varied from ingredient to ingredient. While shrimp responded positively to increasing inclusion of krill from 1 to 5% by increased feed consumption, they responded negatively to increasing inclusion of squid meal. Córdova-Murueta and García-Carreño (2002) studied fish hydrolysate, krill hydrolysate and squid meal in *L. vannamei* feeds, and reported that inclusion of all three ingredients improved growth. However, fish hydrolysate and squid meal were most effective at the lower inclusion rate of 3% than at 9% and 15%. They found squid meal to have similar profile of low molecular weight proteins as fish hydrolysate, and suggested that low molecular weight proteins produced by enzymatic hydrolysis may promote growth at low levels, but deleterious at high levels. Nunes et al. (2006) noted that irrespective of the effectiveness of an ingredient as feeding effector, including it at optimum level in the feed is required for it to stimulate feeding.

The data from attractability and palatability assessments did not always relate to the biochemical composition of the ingredients. For example, blood meal registered higher attractability value than its biochemical profile would indicate. Both types of poultry byproduct meal were found to have similar attractability and palatability values, although the biochemical profile of petfood-grade PBM was superior to that of feed-grade PBM. Apart from such anomalies, the attractability and palatability data were valuable to understand the relative value of each ingredient in terms of their use as feeding effectors in shrimp feeds.

The third part of our investigation involved assessment of growth using selected attractants. The base, control feed consisted of high levels of poultry byproduct meal and no fishmeal. When compared to shrimp fed the reference feed which had high levels of fishmeal, shrimp fed the control feed grew 10% slower in 6 weeks. Three percent inclusion of feeding effectors other than krill meal made no difference in shrimp growth. Inclusion of krill meal improved growth by 3%.

The effectiveness of crustacean-origin ingredients, particularly krill, in improving feed intake and growth is well documented. Holland and Borski (1993) reported that feeds containing shrimp head offal extract improved palatability of the feeds to *L. vannamei* and that they were more effective than feeds containing squid extract. Smith et al. (2005) found that a crustacean meal sourced from Chile and krill meal improved growth significantly in *P. monodon*. The inclusion rate of either meal in the test feeds was 5%. Williams et al. (2005) reported that shrimp head meal and krill inclusion in a basal feed containing 31% fishmeal at 5, 10, or 15% improved *P. monodon* growth in a dose-dependent manner. They further showed that the growth stimulating factor was in the non-soluble protein fraction of the shrimp head meal. Interestingly, krill had the lowest protein solubility among ingredients of marine origin tested in our study.

Samocha et al. (2004) reported that krill meal inclusion did not improve attractability or palatability to *L. vannamei* of a diet formulated with high levels of coextruded soybean poultry byproduct meal. The inclusion level was 1% and it could have been too low to elicit a significant response. Sanchez et al. (2005) reported that 4% krill meal improved attractability of a feed containing 16% casein-based protein, but not that of a feed containing 45% casein-based protein. They speculated that casein itself at high doses be an attractant. They further reported that 4% krill meal inclusion in a feed containing 16% wheat gluten-based protein failed to attract shrimp in 1 h, but attracted shrimp in 2 h. They speculated that the gluten slowed down the release of attractants from the feed.

Limited effectiveness of fish hydrolysate as a feeding effector or growth enhancer for shrimp has been reported by Smith et al. (2005). The hydrolysate was included at 2%. Grey et al. (2009) reported the effectiveness of salmon hydrolysate as a feeding effector in *L. vannamei* at 5%. The most effective hydrolysate was in the liquid form and added at about 12%. They did not perform any growth assessment. Squid liver meal was evaluated in the study by Nunes et al. (2006) and found to be effective as a feeding effector for *L. vannamei* at 0.5–1% inclusion levels, but growth assessment was not conducted. Fishmeal at 3% inclusion level failed to elicit any significant growth effect indicating that factors present in fishmeal for growth are not sufficiently concentrated to be effective at low levels of inclusion. Inclusion of blood meal resulted in the lowest performance of shrimp. Inclusion of blood meal in commercial shrimp feeds is noted to result in lowered performance (Tim O'Keefe, Personal Communication) although one published trial did not show any negative effect of blood meal inclusion on shrimp growth (Dominy and Ako, 1988). As Tacon and Akiyama (1997) pointed out that blood meal is rich in leucine and is extremely low in isoleucine that may cause antagonistic effects leading to isoleucine deficiency.

One of the notable aspects of the present study is the lack of strong correlation between attractability and palatability characteristics of the diets and shrimp performance. The reference diet and the diet containing krill meal scored consistently high on attractability, palatability and growth whereas the diet containing blood meal scored consistently low on all three parameters. However, diets containing fish hydrolysate and squid liver powder scored moderately well in attractability and palatability but performed relatively poor in growth. The control diet and the diet containing low level fishmeal scored low in attractability and palatability, but moderately well in growth. Obvious explanation for this observation is that rapid identification and consumption of diets alone would not result in fast growth, but dietary nutrient levels and balance also influence growth performance. It follows then that evaluation of ingredients for their attractability and palatability alone would not provide sufficient knowledge for practical feed formulation, and is preferably accompanied by shrimp performance evaluation.

5. Conclusion

We found that poultry byproduct meal accorded considerable feeding effector properties to shrimp feeds at high levels of inclusion. It is probably only slightly inferior to fishmeal in terms of attractability and palatability. Blood meal and hydrolyzed feather meal, on the other hand, are not effective feeding effectors for shrimp. Fishmeal's functionality as a feeding effector to shrimp is applicable only at high levels of inclusion. Fish hydrolysate is not an effective feeding effector to shrimp at 3% inclusion level. Squid liver meal is an effective attractant at 3%, but does not improve shrimp growth. Krill meal at 3% is an effective attractant, palatability enhancer as well as growth enhancer in diets having no fishmeal and formulated with high levels of poultry byproduct meal. Among the various biochemical parameters analyzed in the present study, levels of small peptides and nucleotides in the ingredients closely correlated with the effectiveness of the ingredients as attractants and palatability enhancers for shrimp.

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