FINAL REPORT

Project Title: Attractability and Palatability of Rendered Animal Proteins to Blue Shrimp, *Litopenaeus stylirostris*

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ABSTRACT

Attractability and palatability of four rendered protein ingredients of terrestrial animal origin, namely Petfood grade poultry byproduct meal, (PBP), Feed grade poultry byproduct meal (PBF), Spray-dried blood meal (BLM) and Hydrolyzed feather meal (HFM), were studied by (1) quantification of biochemical compounds that are known attractants and palatability factors in aquatic animals; and (2) assessing attractability, palatability and growth in shrimp. Four protein ingredients of aquatic animal origin, namely Anchovy fishmeal (AFM), Fish hydrolysate (FHD), Squid liver meal (SQL) and Krill meal (KRL) were also subjected to the same study for comparative purposes, and to identify ingredients that can restore the attractability and palatability factors that are possibly diminished due to the use of rendered protein ingredients in feeds. There were vast differences among the ingredients in the levels of all biochemical markers of attractability and palatability, namely soluble protein, free amino acids, taurine, nucleotides, and small-size peptides. All ingredients of aquatic animal origin had relatively high levels of all analyzed compounds when compared to ingredients of terrestrial animal origin. Among ingredients of terrestrial animal origin, PBP and PBF had higher levels of the analyzed compounds when compared to HFM and BLM.

Two sets of feeds were prepared for assessment of attractability and palatability in the Pacific blue shrimp, *Litopenaeus stylirostris*. First set was composed of a base of plant-origin ingredients such as soybean meal, wheat flour, wheat gluten and alginate. To the basal composition, terrestrial animal byproducts or aquatic animal ingredients were added at 10% or 3%, respectively. The only exception was AFM which was tested at both 10%, and 3% inclusion levels. Attractability assessment found that 27.1% of the shrimp were attracted to the control feed in 10 minutes while 30, 32.9, 35.7, 41.4, 42.9, 45.7, 47.1, 55.7, and 58.6% of the shrimp were attracted to the feeds containing FHD, AFM (3%), HFM, KRL, AFM (10%), BLM, SQL, PBP, and PBF, respectively. Palatability was assessed by measuring feed consumption in one hour and expressed as mg feed/g shrimp biomass. It was 2.6 for the control feed, and 4.4, 5.7, 6.3, 7.0, 7.6, 7.7, 8.4, and 11.4 for feeds containing AFM (3%), BLM, FHD, PBP, HFM, AFM (10%), PBF, SQL and KRL, respectively.

The assessments were repeated with a second set of feeds. The control feed in the second set had 21.4% PBP and no fishmeal. To the basal composition 3% of AFM, FHD, SQL, KRL or BLM were added to make treatment feeds. A reference feed containing 23% of AFM and no PBP was also formulated. Percentage of shrimp attracted to the control feed in 5 minutes was 40% while the percentage of shrimp attracted to the feeds containing AFM, BLM, FHD, KRL or SQL in the same time period was 36.7, 40.0, 48.9, 53.3, and 61.1%, respectively. The reference feed attracted 53.3% of shrimp. Palatability of the control feed was 10.4, while that of feeds containing BLM, SQL, FHD, AFM, or KRL was 10.6, 11.1, 11.3, 11.7, and 13.1, respectively. The palatability of the reference feed was 15.5.

The second set of feeds was evaluated in a 42-day growth trial in triplicate microcosm tanks. *L. stylirostris* weighing 1.6 g were stocked at 50/m^2 and fed continuously for 12 hours using a belt feeder. Survival ranged from 85.3 to 92.67% and did not differ among treatments. FCR ranged from 1.41 to 1.48 and did not differ among treatments. Weekly weight gain of shrimp fed the control feed was 1.81

g/week. Weight gain of shrimp fed diets containing BLM, SQL, FHD, or AFM was 1.54, 1.62, 1.67, or 1.7 g/week, respectively. Weight gain of shrimp fed the reference diet was 2.00 g/week.

The present study establishes that poultry byproduct meals at inclusion rate up to 10% do not adversely impact the attractability and palatability of shrimp feeds, but at inclusion rate exceeding 20%, especially when such inclusion occurring at the expense of fishmeal, there is likely to be a slight negative impact on attractability and palatability as well as growth. To a large extent, the negative impact can be offset by the low level inclusion of a highly effective attractant and palatability enhancer like krill meal. Blood meal, on the other hand, has a negative impact on palatability and growth even at an inclusion level of 3%. Among the various biochemical indicators analyzed, abundance of low molecular weight (< 1000 dalton in size) peptides seemed to indicate ingredients that impart considerable attractability and palatability to shrimp feeds.

Introduction:

One of the limitations in using high levels of terrestrial animal proteins such as meat and bone meal, poultry byproduct meal, and blood meal in shrimp feeds is the concern that the ingredients have low levels of attractants and palatability factors that are abundant in proteins of aquatic animal origin such as fishmeal, shrimp meal, and squid meal (Cruz-Suarez et al. 2007). Inadequate levels of attractants and palatability factors in the feeds cause reductions in feed intake which in turn cause poor growth and yield. Characterization of the attractability and palatability aspects of rendered animal proteins provides shrimp feed manufacturers with valuable tools to utilize the ingredients better. For example, by knowing the levels at which ingredients rich in attractability and palatability factors are effective complements to terrestrial animal proteins, the inclusion level of the proteins in feed could be increased. Quantifying the factors in each ingredient help formulators reach a targeted level of attractability and palatability in the finished feed, thereby allowing more flexibility in formulation which typically results in lower cost of feed.

Our study was aimed at achieving the following objectives:

(1) To quantify the levels of commonly recognized factors of attractability and palatability such as soluble protein, small chain peptides, free amino acids, taurine and nucleotides in four terrestrial animal protein ingredients, namely 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, namely anchovy fishmeal (AFM); fish hydrolysate (FHD); squid liver meal (SQL); and krill meal (KRL).

(2) To assess the attractability and palatability of the above ingredients in the blue shrimp, *L. stylirostris*; and

(3) To measure performance of shrimp fed diets containing high levels of a selected terrestrial animal protein, in this case, PBP, and each of the aquatic animal protein.

Materials & Methods:

Acquisition and characterization of ingredients:

The source and proximate composition of ingredients tested in the research are shown in Table 1. Most ingredients were acquired in 2008 and stored in tightly sealed plastic containers at 20^{o}C.

The following analyses were performed on samples of ingredients tested in the study:

(a) Proximate composition analyses 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)

(b) Free amino acids 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.

(c) Taurine was determined using HPLC (AOAC 994.12).

(d) Protein solubility was determined by measuring crude protein (AOAC 990.03) in an aqueous extract of the sample.

(e) Nucleotides were analyzed by reversed phase HPLC chromatography as per Ryder (2000).

(f) Size distribution of peptides was analyzed by HPLC size exclusion chromatography as per Aksnes et al. (2006).

Analyses (a)-(e) were performed at Nestle Purina Analytical Laboratory, USA and analysis (f) was performed at W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University School of Medicine, USA.

Feed formulation, preparation and composition:

Two sets of feeds were used in the study. The first set of feeds, coded with letter A in the prefix, was used in the assessment of all test ingredients for attractability and palatability using a control feed formulated with plant origin ingredients, namely soybean meal, wheat flour, wheat gluten and alginate. In each treatment feed, one of the test ingredients was included at the expense of soybean meal (Table 2). Inclusion level of the test ingredient varied. All terrestrial animal proteins were included at 10%. FHD, SQL and KRL were included at 3%, while AFM was included at both 3% and 10%.

The second set of feeds, coded with letter B in the prefix, was used in the growth trial. The feeds were also assessed for attractability and palatability. The control feed in this set had petfood-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 feed containing 23.3% of AFM was also formulated. The feeds were formulated to meet certain nutrient specifications as shown in Table 3. The formulas and actual nutrient composition of the feeds are presented in Table 4.

All ingredients except vitamin premix, soya lecithin and fish oil were mixed in a vertical mixer for a few minutes. The mixture was then ground through a mill to pass through a 0.25 mm screen. The mixed meal was returned to the vertical mixer and mixed with about 40% water. The wet meal was then autoclaved at 105^{o}C for 5 minutes. The autoclaved meal was mixed with 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^{o}C for 3-4 hours. 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^{o}C.

Shrimp:

All shrimp used in the trial were domesticated, high-health *L. stylirostris* derived from 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 acclimated in a 5000 liter circular outdoor, nursery tank. When they reached about 1-1.5 grams they were stocked in indoor fiberglass tanks and used in various trials.

Assessment of attractability:

A rectangular, glass tank with multiple chambers was used to assess attractability of feeds to the shrimp. The glass tank was 90x30x30 cm in length, width and height, and had an acclimatization chamber at one end and three feeding chambers at the other end (Figure 1 a &b). 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.

Attractability of feed was conducted one feed at a time. Randomly selected ten numbers of shrimp of 1-2 g size were stocked into the acclimatization chamber, and allowed to acclimatize for one hour. After one hour, one gram 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 minutes 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, and subjected to 1-way Analysis of Variance (ANOVA) per time interval within a set of feeds. 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 treatment means were made using Fisher's Least Significant Differences (LSD) method.

Assessment of palatability:

Sloped-bottom, cylindrical fiberglass tanks of 50-L (Figure 2) were used to assess palatability of feeds to the shrimp. Each tank had a 25 mm drain at the bottom that can be 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 one hour after which aeration to the tank was stopped and two grams of test feed were introduced into the tank. The shrimp were given 60 minutes to consume the feeds. At the end of 60 minutes, 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. They were then dried in a forced-air draft oven at 60^{o}C for 8 hours. Leaching loss of each feed under water for 60 minutes was separately estimated, and an appropriate correction factor was applied to arrive at an estimate of uneaten feed. Feed consumption was calculated as mg feed/gram of shrimp. Each feed was tested for palatability nine, randomly selected times. One-way ANOVA followed by LSD for multiple comparison analysis was used to test if there were any significant differences in palatability among feeds within a given set of feeds.

Growth trial:

Microcosm tanks made of fiber glass, and having a capacity of 1827 L, were used for testing growth of shrimp on the second set of experimental feeds. The microcosm tanks are independent, self-circulating units in which all the water movement is driven by airlift and gravity. Cross-sectional view of the tank is shown in Figure 3. The system has been described in detail in Kumaraguru vasagam et al. (2009).

Each microcosm tank was stocked with 50 shrimp of about 1.5 g size and reared for 42 days. Ten shrimp were sampled every seven 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 for feeding in the evening (16:30 H). The ration was placed on a belt-feeder and delivered in a continuous manner. At the end of the trial, following parameters of performance were measured and used in statistical analyses: final shrimp weight, weight gain, survival, feed consumption and feed conversion ratio. One-way ANOVA followed by LSD for multiple comparison analysis was used to test if there were any significant differences in performance among shrimp receiving different feeds.

Results:

Analysis of ingredients:

Table 5 presents the values of various indicators of attractability and palatability in the test ingredients. Considerable differences were observed among ingredients in the analyzed parameters. BLM was different from all other ingredients by having undetectable levels of nucleotides and free amino acids, 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 free amino acids. HFM's protein solubility and taurine content were also low. The two grades of poultry byproduct meals had similar peptide size distribution, but PBP had higher levels of nucleotides, and taurine than PBF.

PBP, PBF and AFM had similar peptide size distribution, but AFM had higher levels of free amino acids, nucleotides and taurine. FHD had the highest protein solubility and free amino acid 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 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. It had the highest level of AMP among all ingredients.

Among free amino acids, 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.

Assessment of attractability and palatability of all test ingredients in a basal formula comprised of plant-origin ingredients only (Assessment A):

Figure 4 shows the response of shrimp to feeds formulated with plant-origin ingredients, and either 3% of aquatic animal proteins or 10% terrestrial animal proteins (the only exception was that AFM was tested at both 3% and 10% inclusion levels). Visible behavioral differences among shrimp offered different feeds were apparent immediately after they were provided access to the feed. Feeds containing PBF at 10%, PBP at 10%, KRL at 3%, SQL at 3%, and AFM at 10% attracted more shrimp consistently while the CNL feed containing none of the test ingredients and the feed containing FHD at 3% elicited poor response by shrimp. Statistical analysis showed significant difference ($P<0.003$) among feed treatments at the 10^{th} minute (Table 6). Multiple comparison of means at 10^{th} minute showed that feeds containing PBF at 10%, PBP at 10%, SQL at 3% and BLM at 10% were more attractive to shrimp than the feed not containing any tested ingredients (A-CNL), and the feed containing 3% fish hydrolysate. Feeds containing PBF at 10% and PBP at 10% were also more attractive to shrimp than those containing HFM at 10%, KRL at 3%, and AFM at 3% or 5%.

There was significant difference ($P<0.0001$) among feeds in their palatability to shrimp (Table 7). Feed consumption in the first 60 minutes 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 (Feed A-CNL), their feed consumption increased 2-4 times when one of the test ingredients was incorporated in the feed. Inclusion of KRL at 3% resulted in the highest consumption. Inclusion of SQL at 3%, PBF at 10%, AFM at 10%, PBP at 10%, and HFM at 10% resulted in an increase of feed consumption by about 3 times when compared to A-CNL. Inclusion of FHD at 3% and BLM at 10% resulted in about twice the increase in feed consumption when compared to A-CNL. There was no significant increase in feed consumption when AFM was included in the feed at 3%.

Assessment of attractability and palatability of selected test ingredients in feeds containing high level of poultry byproduct meal (Assessment B):

Figure 5 shows the response of shrimp to feeds formulated with high level of poultry byproduct meal and 3% of AFM, FHD, KRL, SQL or BLM. The response of shrimp to a reference feed containing high level of fishmeal is also included. Statistical analysis showed significant difference ($P<0.04$) among feed treatments at the 5^{th} minute (Table 8). Multiple comparison of means at 5^{th} minute showed that there was no difference between the control feed, and those feeds that contained AFM or BLM at 3%. Shrimp found the feeds containing KRL or SQL to be more attractive.

There was significant difference ($P<0.008$) among feeds in their palatability to shrimp (Table 9). However, multiple comparisons showed no significant difference between feeds that contained PBP

only and those that contained PBP and 3% of AFM, FHD, SQL, KRL or BLM. The shrimp found the reference feed to be more palatable than other feeds.

Performance of shrimp on feeds containing high level of poultry byproduct meal:

Table 10 shows growth, survival, FCR and yield of shrimp fed diets containing high level of PBP with and without the attractants and palatability enhancers. The reference feed contained 23% fishmeal and no PBP. 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. 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 control diet registered a weekly weight gain of 1.81 g. In comparison, shrimp fed the reference diet registered a weight gain of 2.0 g/week, but there was no statistically significant difference between the two feeds. Shrimp fed diet with PBP and KRL had a weight gain of 1.86 g/week. Shrimp fed diets containing PBP and AFM, FHD, or SQL, all registered numerically lower weight gain when compared with those fed control diet, but the differences were not significant. The lowest weight gain was observed in shrimp fed the diet with BLM, and there was significant difference between this diet and the control diet, the diet with krill meal and the reference diet.

Discussion:

The present study establishes that poultry byproduct meals by themselves at high inclusion levels (10-20% in feed) provide a degree of attractability and palatability to the feed. In Attractability Assessment A, poultry byproduct meals included in a formula composed of plant origin ingredients at 10% inclusion level scored the highest in terms of attractability. It is noteworthy that feeds containing poultry byproduct meals performed superior to feed containing 10% AFM. In Palatability Assessment A, feeds with 3% KRL or SQL scored higher than those with 10% poultry byproduct meals. However, the palatability of feeds with 10% PBF or PBP was not significantly different from that of the feed with 10% AFM. In fact, palatability of the feed with 10% PBF was numerically superior to that of the feed with 10% AFM. On the other hand, the control feed in Assessment B which had about 21% PBP scored lower on attractability and palatability. While performance of the control feed was not significantly different from that of the feed that containing 3% AFM, comparison of it to that of the Reference feed which contained 23% AFM, showed that the attractability and palatability of the feed were negatively impacted. So, it can be said with fair confidence that poultry byproduct meal at inclusion rate up to 10% do not adversely impact the attractability and palatability of shrimp feeds, but at higher inclusion rates such inclusion occurring at the expense of fishmeal is likely to have negative impact on attractability and palatability as well as growth.

To a large extent, the negative impact of replacing large quantities of fishmeal with poultry byproduct meals can be offset by the low level inclusion of a highly effective attractant and palatability enhancer like KRL. Among the four attractant and palatability enhancers of aquatic animal origin tested in the present study, KRL scored the highest because it performed consistently high in all except one of the assessments of attractability and palatability, and the growth trial. This finding is consistent with the findings of Smith et al. (2005). Smith et al. (2005) evaluated preference of the shrimp, *Penaeus*

monodon, to feeds containing squid meal, fish hydrolysate, crustacean meal, krill meal, krill hydrolysate or betaine and found that feeds containing crustacean meal or krill meal were preferred by the shrimp. Feeds containing crustacean meal or krill meal at 5% also provided higher shrimp weight gain than feeds containing fish hydrolysate or krill hydrolysate in the feeding trial. The basal feed used by the authors had 25% meat meal and 20% lupin meal, but it also had 17% fishmeal and 5% squid meal, whereas the control feed in our study had 21% petfood-grade poultry byproduct meal, 40% soybean meal and no protein ingredients of aquatic animal origin.

It was BLM's relatively high performance in the Attractability Assessment A that prompted testing it in Assessment B and growth trial at 3% inclusion. However, it adversely affected the attractability and palatability of the feed as well as had a significant negative effect on shrimp growth. Studies investigating blood meal in shrimp feeds are limited. While one investigation showed that ring-dried blood meal inclusion at 10% had no effect on growth of 3-4 g *L. vannamei*, two studies have shown that its inclusion depressed growth in *Farfantepenaeus californiensis* and *F. paulensis* (Hertrampf & Piedad-Pascual, 2000). It is speculated that the poor performance of animals fed high levels of blood meal is due to the extremely high level of leucine present in blood meal causing antagonistic effects on isoleucine metabolism (Tacon and Akiyama, 1997). In the only study assessing attractability and palatability aspects of rendered animal byproducts, Nunes et al. (2006) reported that inclusion of meat and bone meal or blood meal at 3% in a gelatin feed made the feed more attractive than the 100% gelatin feed. Meat and bone meal was more effective than blood meal, but both ingredients were less effective than fishmeal.

Extensive characterization of test ingredients was undertaken in this study to quantify biochemical compounds in ingredients that are recognized as chemoattractants and feeding stimulants for aquatic animal species (Lee and Myers, 1997). These included soluble protein, small chain peptides, free amino acids, taurine and nucleotides. Vast differences occurred in the levels of the compounds among test ingredients. HFM and BLM had extremely low levels of soluble protein, free amino acids, nucleotides, taurine and smaller size peptides in consistent with their poor performance as attractants and palatability factors. PBP and PBF had relatively higher levels of soluble protein, free amino acids, nucleotides, taurine and smaller size peptides in consistent with their relative better performance as attractants and palatability factors. The abundance of free amino acids, nucleotides, taurine and smaller size peptides was as expected in protein ingredients of aquatic animal origin. But, taurine and free amino acid levels did not correspond with attractability and palatability performance of the ingredients. FSD, and SQL had high levels of both, yet their performance was the poorest. The two ingredients also had vastly higher levels of soluble proteins than the better performing AFM or KRL.

Cordova-Murueta and Garcia-Carreno (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 the higher inclusion rates of 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. We found that among ingredients of aquatic animal origin tested in our study, FHD had the lowest level of peptides in the 500-1000 Daltons range. On the contrary, KRL having the highest level of the smaller peptides performed the

best. Interestingly, KRL had the lowest protein solubility among proteins of aquatic animal origin. Williams et al. (2005) showed that the insoluble fraction of krill meal and shrimp meal that contain growth factors for *P. monodon*.

Conclusions:

The present study establishes that poultry byproduct meals have attractability and palatability factors, but at levels slightly lower than those of fishmeal. At inclusion rate up to 10%, inclusion of poultry byproduct meals does not adversely impact the attractability and palatability of shrimp feeds. At higher inclusion rates, especially when such inclusion occurs at the expense of fishmeal, there is likely to be a slight negative impact on attractability and palatability as well as growth. To a large extent, the negative impact can be offset by the low level inclusion of a highly effective attractant and palatability enhancer like krill meal. Blood meal, on the other hand, has a negative impact on palatability and growth even at an inclusion level of 3%. Among the various biochemical indicators analyzed, abundance of low molecular weight (< 1000 dalton in size) peptides seemed to indicate ingredients that impart considerable attractability and palatability to shrimp feeds.

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Table 1: Source and proximate composition of ingredients tested in the study

Ingredient	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Origin, Supplier
Poultry byproduct meal, Petfood grade	5.9	66.2	14.4	12.1	USA, Valley Proteins, USA
Poultry byproduct meal, Feed grade	3.5	55.5	15.1	22.7	USA, Valley Proteins, USA
Feather meal, Hydrolyzed	4.6	83.9	7.2	5.6	USA, Griffin Industry, USA
Blood meal, Spray-dried	6.6	90.6	2.6	1.9	USA, Griffin Industry, USA
Fishmeal, anchovy	9.4	66.1	7.3	16.3	Peru, Alicorp Nicovita, Peru
Fish hydrolysate	6.8	72.9	17.1	5.3	France, Sopropeche, France
Krill meal	8.0	59.4	18.5	11.0	Antarctic, Aker Biomarine, USA
Squid liver meal	11.3	65.6	7.4	15.2	Korea, PT. CJ Feed, Indonesia

Table 2: Feed formulas used in the assessment of all test ingredients for attractability and palatability in a basal diet comprised of plant-origin ingredients only

Ingredient (%)	A-CNL	A-PBP	A-PBF	A-HFM	A-BLM	A-FMH	A-FML	A-KRL	A-SQL	A-FHD
Soybean meal, 46%	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

Table 3: Selected nutrient specifications used in the production of feeds for the feeding trial and assessment of attractability and palatability

Nutrient	Minimum	Target Range
Crude protein, %	40	40-41
Crude fat, %	7.5	7.5-8
Available phosphorus, %	0.5	0.5-0.55
Total arginine	2.3	2.3-2.5
Total lysine	2.0	2.0-2.4
Total sulfur amino acids	1.3	1.3-1.4
Total threonine	1.3	1.3-1.5
Cholesterol	0.14	0.14-0.17
Phospholipids	1.3	1.3-1.6
Total n3-Essential Fatty Acids*	0.5	0.5-1

* Sum of Linolenic acid (C18:3n3), Eicosapentaenoic acid (C20:5n3), Docosahexaenoic acid (C22:6n3)

Table 4: Formulas and analyzed nutrient composition of feeds used in the growth trial and assessment of ingredients for attractability and palatability (NA = Not analyzed)

Ingredient (%)	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
Analyzed values (% as is)							
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
Phosphorus	1.52	1.55	NA	NA	NA	NA	1.70
Cholesterol	0.14	0.16	NA	NA	NA	NA	0.19
Amino acids							
Arginine	2.48	2.49	NA	NA	NA	NA	2.53
Histidine	1.10	1.06	NA	NA	NA	NA	1.29
Isoleucine	1.44	1.48	NA	NA	NA	NA	1.55
Leucine	2.76	2.73	NA	NA	NA	NA	3.00
Lysine	1.95	1.99	NA	NA	NA	NA	2.39
Methionine	0.61	0.63	NA	NA	NA	NA	0.71
Cystine	0.73	0.70	NA	NA	NA	NA	0.70
Phenylanine	1.81	1.75	NA	NA	NA	NA	1.92
Tyrosine	1.21	1.16	NA	NA	NA	NA	1.33
Threonine	1.33	1.33	NA	NA	NA	NA	1.41
Tryptophan	0.42	0.43	NA	NA	NA	NA	0.47
Valine	1.60	1.69	NA	NA	NA	NA	1.69
Fatty acids							
Linoleic acid (C18:2)	2.29	2.22	NA	NA	NA	NA	1.89
Linolenic acid (C18:3n3)	0.19	0.19	NA	NA	NA	NA	0.20
Eicosapentaenoic acid (C20:5n3)	0.06	0.08	NA	NA	NA	NA	0.29
Docosahexaenoic acid (C22:6n3)	0.06	0.08	NA	NA	NA	NA	0.25

Table 5: Protein solubility, nucleotides concentration, free amino acid levels, taurine and peptide size distribution of test ingredients

	PBP	PBF	HFM	BLM	AFM	FHD	KRL	SQL
Protein solubility (%)	23.5	24.4	7.18	3.48	18.3	73.7	13.1	31.2
Nucleotides (ppm)								
Uridine	196	71	22	< 10	40	51	101	51
Cytidine	81	33	23	< 10	19	< 10	26	12
Inosine	589	205	31	< 10	1440	516	312	1440
Guanosine	130	62	20	< 10	90	55	49	140
Adenosine	259	74	19	< 10	47	21	40	35
UMP	123	45	< 10	< 10	32	84	919	24
CMP	108	60	< 10	< 10	28	29	991	32
IMP	182	88	22	< 10	1150	122	988	2230
GMP	71	41	< 10	< 10	83	58	798	67
AMP	461	127	27	< 10	312	292	2270	443
Taurine (ppm)	4463	2118	477	304	5046	7147	5381	7378
Free amino acids (%)								
Aspartic acid	0.07	< 0.05	< 0.05	< 0.05	< 0.05	0.05	< 0.05	< 0.05
Threonine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.06	< 0.05	< 0.05
Serine	0.06	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Glutamic acid	0.08	< 0.05	< 0.05	< 0.05	0.08	0.19	< 0.05	0.07
Proline	0.05	< 0.05	< 0.05	< 0.05	0.07	0.06	0.36	< 0.05
Glycine	0.06	0.07	< 0.05	< 0.05	0.05	0.13	0.32	< 0.05
Alanine	0.1	0.16	< 0.05	< 0.05	0.16	0.3	0.06	0.16
Valine	< 0.05	0.08	< 0.05	< 0.05	0.06	0.12	0.12	0.05
Methionine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.08	< 0.05	< 0.05
Isoleucine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.07	< 0.05	< 0.05
Leucine	< 0.05	0.08	< 0.05	< 0.05	0.08	0.24	< 0.05	0.06
Tyrosine	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.06	< 0.05	< 0.05
Phenylanine	< 0.05	< 0.05	< 0.05	< 0.05	0.05	0.09	< 0.05	< 0.05
Histidine	0.05	0.05	< 0.05	< 0.05	0.48	0.08	< 0.05	0.68
Lysine	0.06	0.06	< 0.05	< 0.05	0.08	0.18	< 0.05	0.08
Arginine	0.06	< 0.05	< 0.05	< 0.05	0.05	0.05	0.39	< 0.05
Peptide size distribution (%)								
MW>25kDa	1	1	1	4	3	2	0	1
20>MW<25kDa	9	10	10	34	10	7	6	8
15>MW<20kDa	10	9	12	29	9	9	5	8
10>MW<15kDa	9	8	14	16	8	10	3	6
5>MW<10kDa	12	11	12	6	12	17	1	9
2.5 >MW< 5 kDa	12	10	9	3	11	16	1	7
1 >MW< 2.5 kDa	18	21	17	3	16	15	8	8
500Da>MW<1kDa	30	28	25	5	31	23	75	54

Table 6: Percentage shrimp in feeding chamber containing test feed 10 minutes after the shrimp were provided free access to feed in Attractability Assessment A (see Table 2 for formulas)

Feed	Mean	SE
A-CNL	27.1[a]	4.21
A-FHD	30[a]	8.45
A-FML	32.9[ab]	7.14
A-HFM	35.7[ab]	5.28
A-KRL	41.4[ab]	2.61
A-FMH	42.9[ab]	6.06
A-BLM	45.7[b]	4.81
A-SQL	47.1[b]	6.06
A-PBP	55.7[bc]	6.49
A-PBF	58.6[bc]	5.08

Means sharing the same letters in superscript are not significantly different (P<0.05)

Table 7: Palatability, as measured by feed consumption (mg feed/g shrimp biomass) in 60 minutes, of feeds in Palatability Assessment A (see Table 2 for formulas)

Feed	Mean	SE
A-CNL	2.6[a]	0.44
A-FML	4.44[ac]	0.41
A-BLM	5.69[bc]	0.76
A-FHD	6.26[bc]	0.80
A-PBP	7[bd]	0.97
A-HFM	7.6[bd]	1.03
A-FMH	7.71[bd]	0.61
A-PBF	8.35[bd]	1.04
A-SQL	8.46[bd]	0.78
A-KRL	11.44[e]	0.84

Means sharing the same letters in superscript are not significantly different (P<0.05)

Table 8: Percentage shrimp in feeding chamber containing test feed 5 minutes after the shrimp were provided free access to feed in Attractability Assessment B (see Table 4 for formulas)

Feed	Mean	SE
B-AFM	36.7[a]	6.01
B-CNL	40.0[a]	5.00
B-BLM	40.0[a]	5.77
B-FHD	48.9[ab]	7.16
B-REF	53.3[b]	6.24
B-KRL	53.3[b]	6.87
B-SQL	61.1[b]	2.61

Means sharing the same letters in superscript are not significantly different (P<0.05)

Table 9: Palatability, as measured by feed consumption (mg feed/g shrimp biomass) in 60 minutes, of feeds in Palatability Assessment B (see Table 4 for formulas)

Feed	Mean	SE
B-CNL	10.37[a]	1.065
B-BLM	10.64[a]	0.572
B-SQL	11.06[a]	0.935
B-FHD	11.34[a]	1.040
B-AFM	11.67[a]	1.113
B-KRL	13.09[a]	0.911
B-REF	15.53[b]	1.253

Means sharing the same letters in superscript are not significantly different ($P<0.05$)

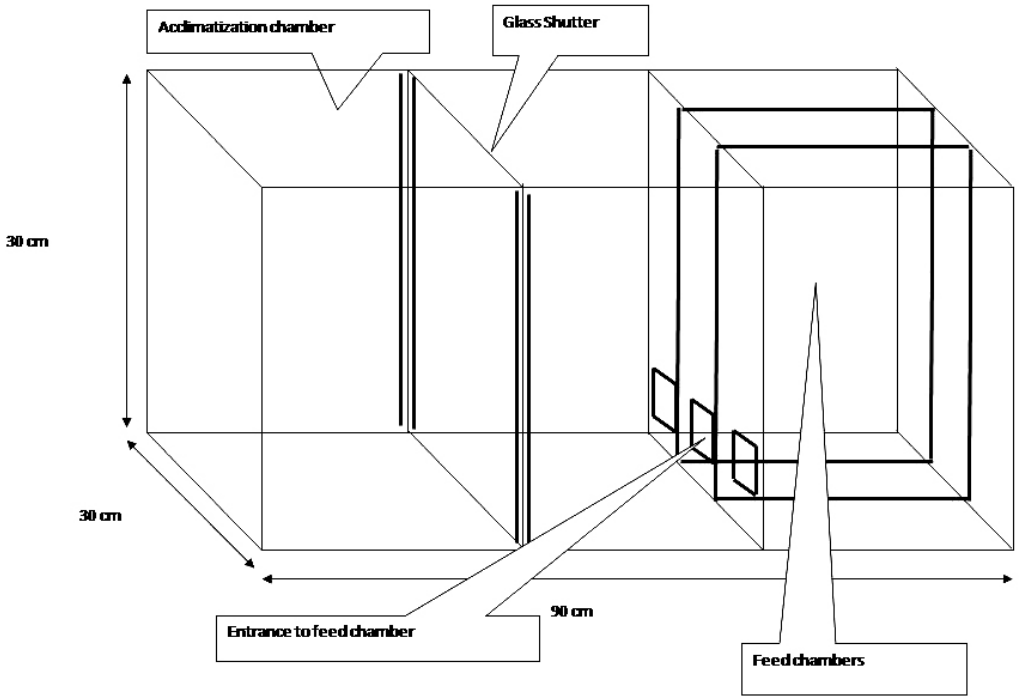
Table 10: Growth, survival, feed conversion ration and yield of shrimp fed diets containing high level of poultry byproduct meal with or without 3% of attractants and palatability enhancers for 42 days (See Table 4 for feed formulas)

	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 sharing the same letters in superscript in a row are not significantly different ($P<0.05$)

FIGURES

(a)



(b)

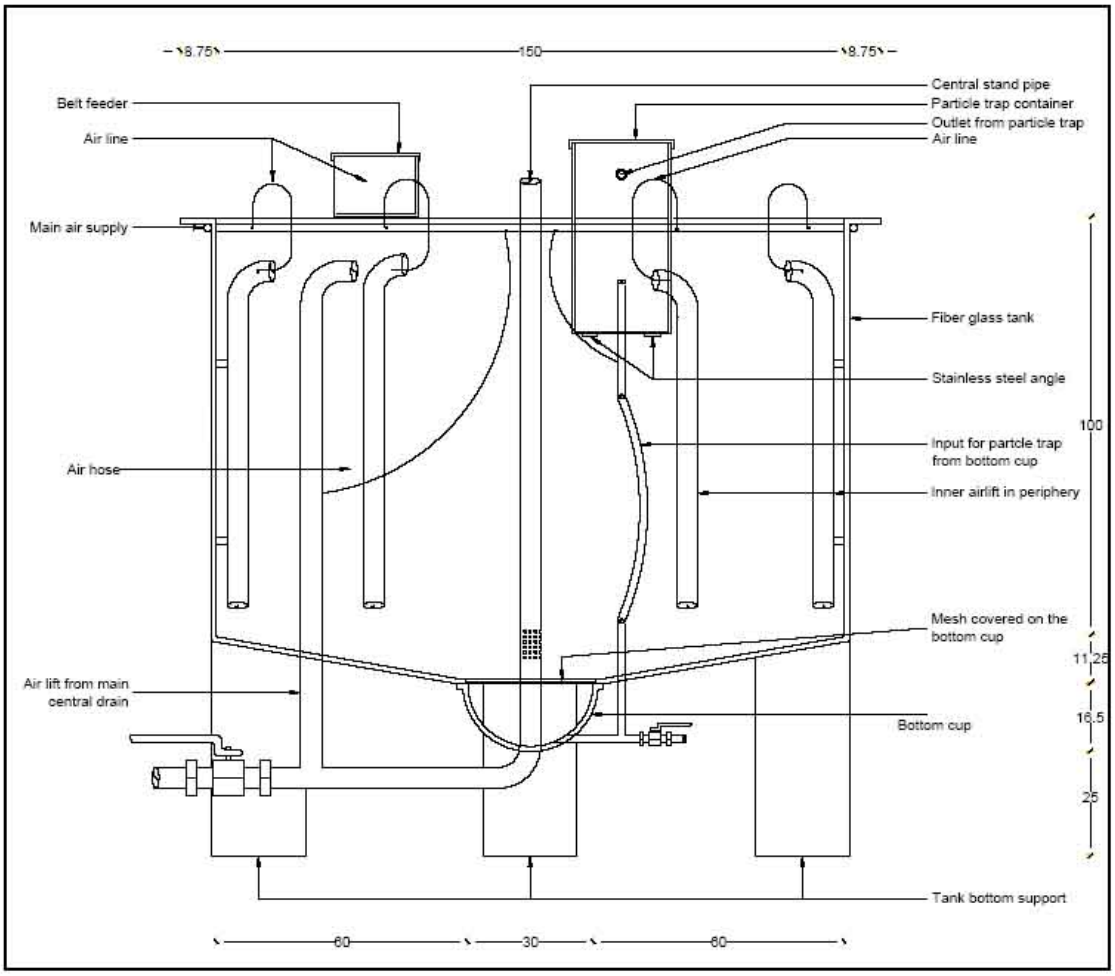
Figure 1: (a) Diagram and (b) photograph of tank used for evaluation of feeds for attractability

(a)

(b)

Figure 2: *Photographs of tank systems used for evaluation of feeds for palatability. (a) Tank set-up; (b) Valve to collect uneaten feeds*

(a)



(b)

Figure 3: (a) Cross-sectional diagram and (b) photograph of microcosm tank used in growth trial

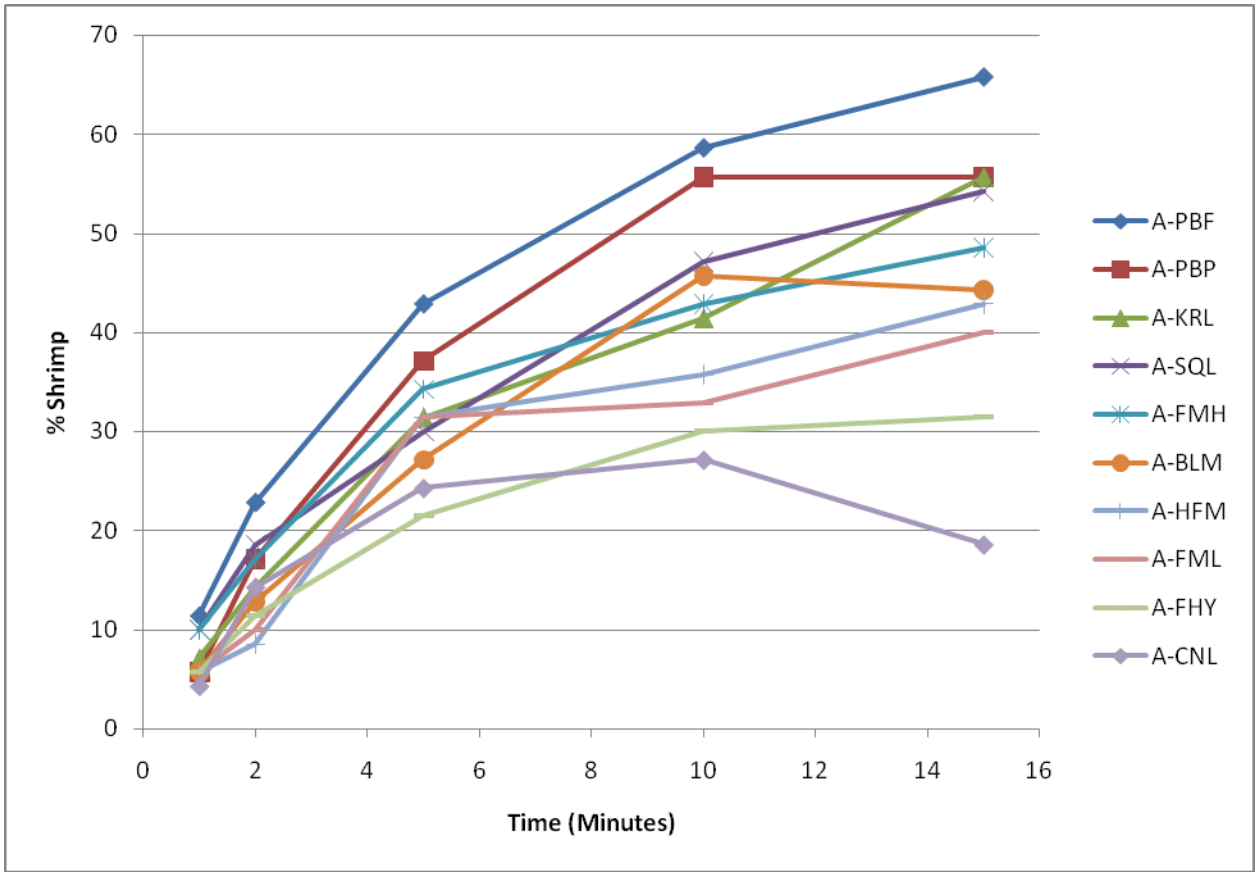


Figure 4: Percentage of shrimps in the feeding chamber containing test feed at 1, 2, 5, 10 and 15 minutes of providing shrimp free access to the feed in Attractability Assessment A (See Table 2 for feed formulas)

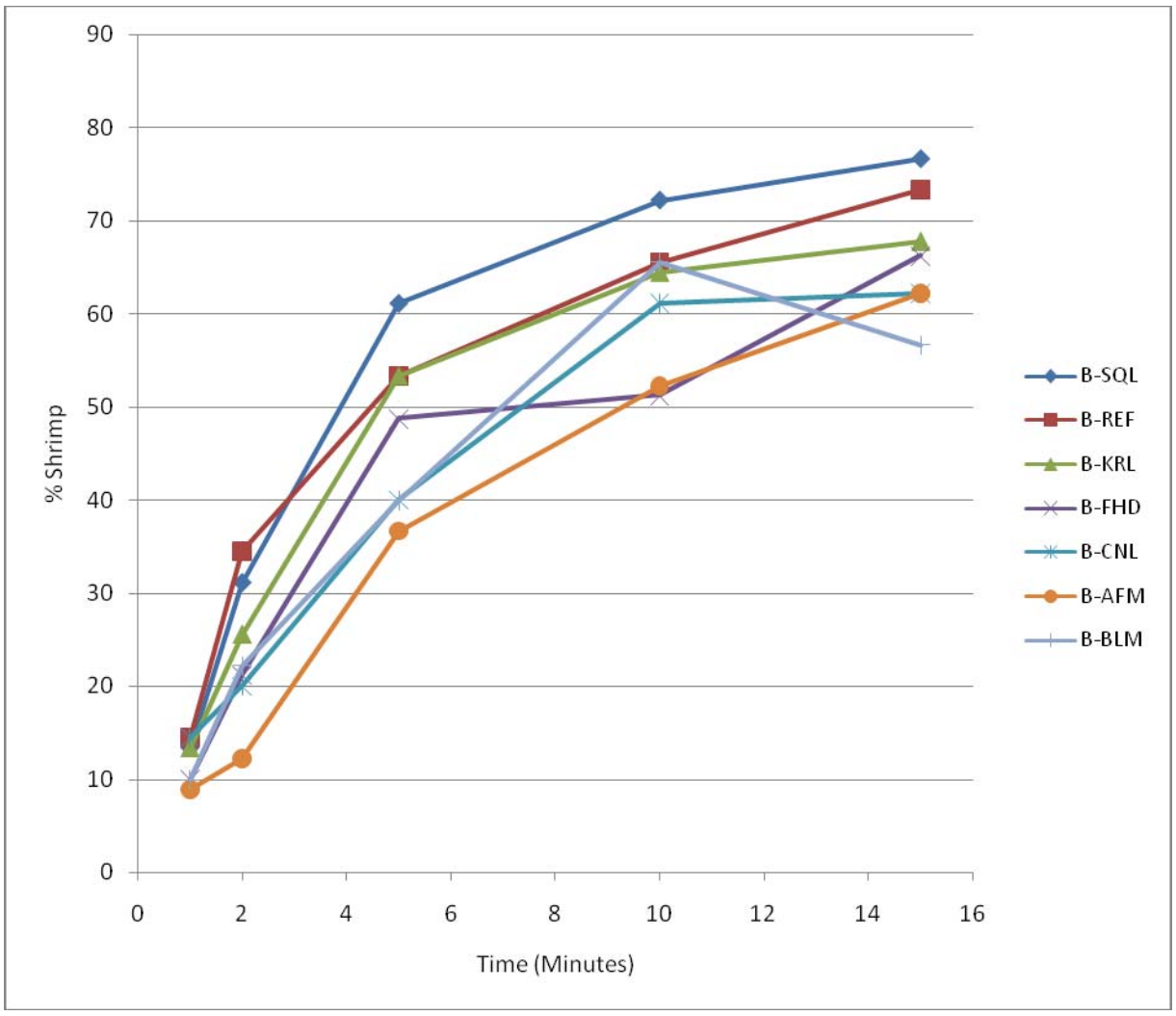


Figure 5: Percentage of shrimps in the feeding chamber containing test feed at 1, 2, 5, 10 and 15 minutes of providing shrimp free access to the feed in Attractability Assessment B (See Table 4 for feed formulas)

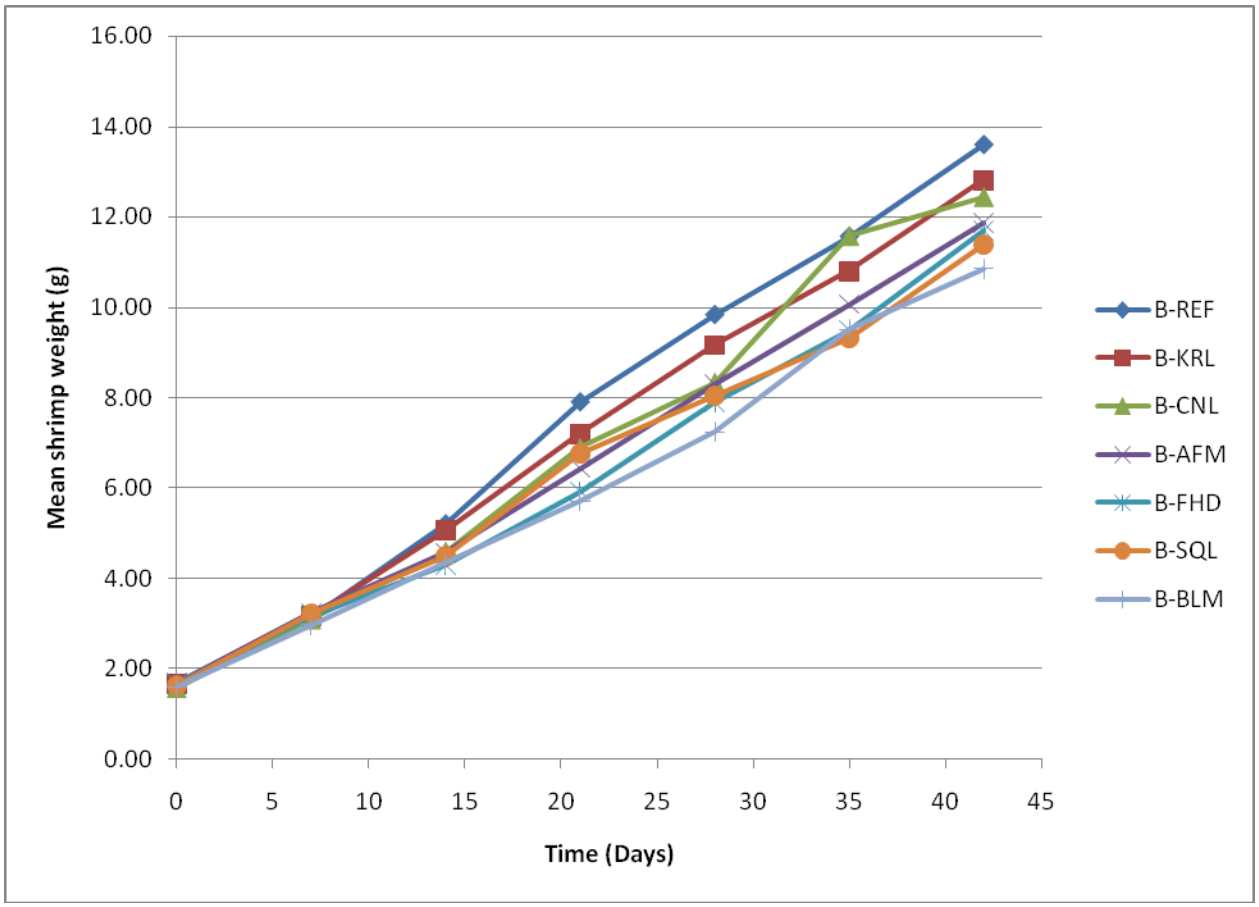


Figure 6: Growth of shrimp fed diets containing high level of poultry byproduct meal with or without 3% of attractants and palatability enhancers (See Table 4 for feed formulas)