Project Number:	1730-352-0502-E
Project Title:	Development of Digestibility Matrix for Soybean Meal (SBM) in the Pacific White Shrimp
Organization:	Auburn University
Principal Investigator:	D. Allen Davis

Project Status: What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.

Objectives:

1. Identify soy crushers that utilize soy from multiple countries of origin and obtain samples for chemical analysis and biological testing.
2. Determine digestibility coefficients for a range of soybean meals offered to the Pacific white shrimp.
3. Conduct a standardized growth trial to evaluate relative biological value as measured by growth and protein deposition.
4. Correlate possible biochemical compositions with digestibility values.

OVERVIEW:

Twenty-five test diets (both digestibility and growth diets) were formulated (Table 1a, 2a, 2b) using 24 different soybean meal (SBM) from different sources, while auburn university soybean meal was used as the control (Diet 1). All soybean meal received from different sources were analyzed by Dr Hans Stein University of Illinois (Ref 1410-523-5311)for proximate composition, gross energy, trypsin inhibitor level, essential and non-essential amino acid profiles, different fiber and mineral contents (Table 1b-1f). Digestibility and growth trials were conducted with *L. vannamei* (pacific white shrimps) at the E.W. Shell research station, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn AL, USA). Various parametric and non-parametric analyses were applied to the data to identify possible correlations and relations. Digestibility values were exceptionally low so this data is not usable and will need to be re-evaluated (see unforeseen circumstances)

MATERIALS AND METHODS

Diet preparation

Twenty-five grow out diets were formulated with using 24 different soybean meal (SBM) from different sources, while the control diet (Diet 1) was prepared using auburn university SBM (Table 1a, 2a, 2b). The digestibility diets were formulated according to the ratio of 70:30 basal diet and tested ingredients and 1% chromic oxide (Cr_2O_3) was included as inert marker. The test diets were prepared in the feed laboratory of Auburn University, Auburn, AL, USA using standard practices. In short, pre-ground dry ingredients and oil were weighted and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot water (~30% by weight) was then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (50 °C) to a moisture content of less than 10% and stored at 4 ^{0}C.

Culture system of growth and digestibility trials

The digestibility and growth trials were conducted in a semi-closed recirculation system at separate times. The system used for growth trials were consisted of 60-L aquaria connected to a common reservoir tank (800-L). Water quality was maintained by recirculation through an Aquadine bead filter (0.2 m2 media, 0.6 m × 1.1 m) and vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp. centrifugal pump. During the feeding period, dissolved oxygen (DO), temperature and salinity were monitored twice daily (0830 and 1630) using an YSI 650 multiparameter instrument (YSI, Yellow Springs, OH). Water samples were taken twice a week to measure total-ammonia nitrogen (TAN) and nitrite levels. All water quality parameters (DO, TAN, nitrite levels, temperature, salinity, pH) were maintained within acceptable ranges for Pacific white shrimp.

Digestibility trials

Eight shrimp/tank (~10.2 g mean weight) were stocked into a recirculating system similar to above but, with 130-L aquaria connected to a common reservoir tank (800-L) Six replicate groups of shrimp were offered each diet and the resulting fecal pellets from every two tanks were pooled into three replicate samples. Animals were allowed to acclimate to the experimental diet for at least three days before the fecal collection was initiated and given a resting period of two days with commercial shrimp diets in between two sets of digestibility diets. Feces were collected four times per day during a 2-3 day period. Each day, the first collection was discarded and the subsequent three collections were rinsed with distilled water, oven-dried (90 °C) and stored in sealed plastic containers at −20 °C for further analysis. Dry matter was determined by placing representative portions of each sample in an oven at 105 °C until constant weight was obtained. Gross energy of diets and fecal samples was analyzed with a semi micro-bomb calorimeter (Model 1425, Parr Instrument Co., Moline, IL, USA). Chromic oxide was determined by the method of (McGinnis and Kasting, 1964) in which, after a colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy Co., Rochester, NY, USA) at 540 nm. Protein was determined by micro-Kjeldahl analysis (Ma and Zuazaga, 1942). The apparent digestibility coefficients for dry matter (ADMD) protein (APD) and energy (AED) of diets (D) were calculated according to Cho et al. (1982) as follows:

$$\text{ADMD (\%)} = 100 - \left[100 \times \left(\frac{\%\ \text{Cr2O3 in feed}}{\%\ \text{Cr2O3 in feces}}\right)\right]$$

$$\text{ADP and ADE (\%)} = 100 - \left[100 \times \left(\frac{\%\ \text{Cr2O3 in feed}}{\%\ \text{Cr2O3 in feces}} \times \frac{\%\ \text{nutrients in feces}}{\%\ \text{nutrient in feeds}}\right)\right]$$

The apparent digestibility coefficients of dry matter (ADMDI), protein (APDI) and energy (AEDI) of the test ingredients (I) were calculated according to Bureau and Hua (2006) as follows,

$$\text{ADMDI} = \text{ADMDD} + \left[(\text{ADMD} - \text{ADMDref. diet}) \times \left(\frac{0.7 \times \text{Dref}}{0.3 \times \text{Dingr}}\right)\right]$$

$$\text{APDI} = \text{APDD} + \left[(\text{APDD} - \text{APDDref. diet}) \times \left(\frac{0.7 \times \text{Dref}}{0.3 \times \text{Dingr}}\right)\right]$$

$$\text{AEDI} = \text{AEDD} + \left[(\text{AEDD} - \text{AEDDref. diet}) \times \left(\frac{0.7 \times \text{Dref}}{0.3 \times \text{Dingr}}\right)\right]$$

Dref= % nutrient (or KJ/g gross energy) of basal diet (dry weight)
Dingr = % nutrients (or KJ/g gross energy) of test ingredient (dry weight)

Growth trials

Dietary treatments were randomly assigned to tanks and each trial was conducted using a double blind experimental design. Growth trial was conducted in two phases. First growth trial was conducted with 14 treatments with assigning 4 replicates for diet 2-14 while 8 replicates were assigned for control diet (Diet 1). Twelve treatments were tested during the second growth trial, each with five replicates including the control diet and diet 15-25. Ten Pacific white shrimp were stocked per tank with mean initial weight of 0.23±0.02 g during trial one and 0.67±0.02 g during trial two. Shrimp were offered tested diets four times daily. Daily ration of feed was calculated based upon an estimated weight gain and expected feed conversion ratio (FCR) of 1.8. Shrimp were counted weekly and the feed was adjusted each week based on survival and observation of the feeding responses of shrimp. Growth trial-1 was conducted for 6-weeks while trial-2 was conducted for 5 weeks considering the survival of shrimps. At the conclusion, shrimp were counted and group-weighed. Mean final weight, final biomass, percent survival, and feed conversion ratio were determined.

Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). Data from individual growth trials were analyzed separately using one-way ANOVA followed by the Tukey pairwise comparison test to evaluate significant differences ($p < .05$) among treatment means (Table 3a & 3b). Thermal Growth Coefficient (TGC) for every single diet was calculated with the objective of combining the growth data from trial 1 and 2. TGC values of different SBM were further standardized by calculating the "percentage TGC" reference to the TGC of control diet of relevant trial. Standardized TGC values were analyzed using one-way ANOVA followed by the Tukey pairwise comparison test to evaluate significant differences ($p < .05$) among treatment means (Table 3c).

With the objective of reducing the dimensions and grouping different SBM sources, Principle component analysis (PCA) and a Cluster analysis was performed using the chemical characteristics of SBM (Table 4; Figure 1). For the PCA and Cluster analysis, whole data set was standardized by calculating z scores (standard scores) to avoid the different units and scales of measurements while some of the variables which were balanced during the formulations were excluded from the analysis. Multiple linear regression was performed to identify the relationships between TGC with principle components selected from PCA (Table 5). Correlation coefficient analysis was utilized to identify the relationships between TGC and major variables representing the principle components, which has significant impact on TGC (Table 6).

RESULTS

Digestibility trial

The 25 soy samples were included in a digestibility trial. We have completed Cr, Energy, Amino acids and a portion of the kjeldahl (protein) analyses. Once the Cr, Energy and AA results were completed we started calculating apparent nutrient (Dry Matter, Energy, Protein and AA) digestibility (ADMD, AED, APD, AAAD) based on Cr, Energy and protein (sum of AA and the initial Kjeldahl analysis). Unfortunately, digestibility coefficients for the diets were extremely low which resulted in corresponding low ingredient digestibility values. In previous trials we have found that the sum of AA and Kjeldahal Nitrogen resulted in similar digestibility values for protein. Hence, these results were calculated confirming the poor response is not an analytical error in protein determinations. We also repeated a number of the diet fecal samples for Cr to confirm that it was not a marker analysis issue.

Given the same basal diet was also used with different ingredients and with different shrimp but produce reasonable results we can conclude it is not the diets. Albeit, contamination of the fecal samples by feed is a potential explanation, analytical results do not support this hypothesis. We feel that this is due to some health issue of the shrimp resulting in impaired digestion. Based on the analytical result, calculations for digestibility are not reasonable. I will happy to provide a summary or the raw data but as the data is not reliable it is not presented. It is suggested that this trail need to be repeated, we are currently collecting new fecal samples

Growth trial

Growth performances

At the conclusion of the culture period of trial-1, no significant differences were detected in final mean weight, weight gain, percentage weight gain and thermal growth co-efficient (TGC) between shrimp fed the various diets while FCR differed significantly between the diets (Table 3a). The diet-8 incorporated with SBM45537 resulted the numerically largest FCR (1.97) while the lowest was recorded from diet-4 and 5 with the FCR values of 1.60 and 1.64 respectively. Survival, final weight and weight gain were ranged from 80 to 98%, 5.1-5.9g, and 4.8-5.7g respectively.

At the end of trial-2, significant differences were detected for final mean weight, weight gain, percentage weight gain, survival and thermal growth coefficient (TGC) between shrimp fed with various diets (Table 3b). Diet-21 incorporated with SBM4550 showed the largest numerical values for final mean growth, weight gain and percentage weight gain respectively with 6.33g, 5.66g and 851%.

According to the statistical analysis between the percentage TGC values of all the experimenting SBM (reference to TGC of control SBM), significant differences were found between the SBM sources. The diet 21 incorporated with SBM4550 showed the largest numerical values for TGC while lowest numerical value for TGC was noted from diet 17 consist with SBM45536.

Grouping information base on Cluster analysis

According to the dendrogram generated through the cluster analysis, 24 SBM sources were separated in to five major groups, which was clearly observed in score plot of PCA as well. SBM consisted in diet 2-11 and 14- 19 were grouped together while SBM of diet 12, 13, 23, 24, 25 were clustered into an another group. Three individual clusters were observed comprised with diet-20, diet-21 and diet-22 alone.

Principle component analysis

Principle component analysis (PCA) of chemical characteristics of SBM sources and their loadings are presented in Table 4. Collectively, first five PCs explained 83% of total sample variance. According to the loading values, PC1 was represented by sucrose and iron while PC2 was represented by sodium, sulphur, non-phytate phosphorous, zinc and phosphorous. Phosphorous in phytic acid, total phytic acid, ADF, NDF, fructose, phosphorous and raffinose were in-charged in PC3 while raffinose in PC3 showed a negative loading value presenting its negative influence on TGC.

Multiple linear regression

The results of multiple linear regression of TGC on first five PCs are presented in Table 5. p-value for the whole model was less than 0.05 while only PC2 and PC3 had significant positive impacts on TGC. Combined the results of principle component analysis and multiple linear repression, we may conclude that the phosperous, non-phytate phosperous, sodium, sulfur, zinc, phosperous in phytic acid, total phytic acid, fructose, ADF and NDF has a positive attribute for the TGC while raffinose has a negative impact on TGC.

Pearson correlation coefficients

Pearson correlation coefficients of TGC with raffinose, ADF, NDF, phosphorous, phosphorous in phytic acid, total phytic acid, non-phytate phosphorous, sodium, sulfur and zinc are presented in Table 6. Out of those parameters, only phosphorous, phosphorous in phytic acid and total phytic acid levels positively correlate with TGC. Though raffinose showed a negative correlation with TGC, it's not significant (p value= 0.086 > 0.05).

Conclusion

Two growth trials were completed that compared the biological value (growth response) of *L. vannamei* offered high soy feed formulations. The growth response was not as different as expected; however, there were clear difference. As in previous project we used principle component analysis and standardization techniques to correlate a response of growth to characteristics of the meals. In this case the strongest correlation was to phosphorus and phytate. As both are correlated it is probably the level of phytate that is the driver as this will effect both mineral and protein availability. Raffinos was also weekly correlated with the growth response which is logical and supported by other research. One theory for the limited response is that the meals were source with to similar a composition. Using both cluster analysis and principle component analysis we looked at how related the meals were and identified two major groups and three sources that acted differently. There is no clear association of one group or the other with performance. Although, the data on digestibility is not reliable, there were considerable difference between samples. Hopefully with revised data we may see better trends. A master of science thesis will be developed around the data and we will continue to work with more advanced statistics to help identify drivers of the growth response.

TABLE 1A: CODES FOR DIFFERENT SOYBEAN MEAL USED IN THE EXPERIMENT

Diet Number	Ingredient Code
1	AU Soy
2	45531
3	45532
4	45533
5	45534
6	45535
7	45536
8	45537
9	45538
10	45539
11	45540
12	45541
13	45542

Diet Number	Ingredient Code
14	45543
15	45544
16	45545
17	45546
18	45547
19	45548
20	45549
21	45550
22	45551
23	45552
24	45553
25	45554

TABLE 1B: CHEMICAL ANALYSIS[1] (PROXIMATE COMPOSITION, GROSS ENERGY AND TRYPSIN INHIBITORS) OF THE DIFFERENT SOYBEAN MEAL USED IN DIETS OF PACIFIC WHITE SHRIMP, *LITOPENAUS VANNAMEI*

Soybean meal Sample key	Proximate composition (%)					GE, kcal/kg	Trypsin Inhibitors/ mg (TIU)
	Dry Matter	Moisture	Ash	Crude Protein	Fat		
AU[2]	88.14	11.86	5.78	43.7	1.03	4394	
45531	89.37	10.63	6.44	45.85	1.25	4191	3.32
45532	89.77	10.23	6.58	46.40	1.53	4213	3.05
45533	89.42	10.58	6.42	45.35	1.39	4194	3.00
45534	89.70	10.30	6.36	45.78	1.10	4204	3.37
45535	89.40	10.60	6.48	45.92	1.07	4185	2.13
45536	88.93	11.07	6.99	47.50	0.86	4168	1.98
45537	88.85	11.15	6.96	46.62	1.40	4190	2.09
45538	89.51	10.49	7.06	47.87	1.37	4210	1.25
45539	89.01	10.99	7.01	47.16	1.38	4209	2.57
45540	89.43	10.57	6.90	47.43	3.47	4238	2.19
45541	88.19	11.81	6.77	47.31	1.45	4163	2.92
45542	88.26	11.74	6.39	48.02	2.13	4232	2.67
45543	90.01	9.99	7.45	51.08	0.83	4241	4.27
45544	88.08	11.92	6.42	50.29	2.55	4302	4.62
45545	87.55	12.45	6.46	51.02	1.55	4231	2.93
45546	88.59	11.41	6.45	47.70	1.55	4173	3.17
45547	88.66	11.34	6.12	47.79	1.88	4190	2.91
45548	89.68	10.32	6.41	49.94	2.00	4254	1.25
45549	87.83	12.17	7.34	47.02	1.44	4075	2.70
45550	87.77	12.23	7.43	45.48	1.51	4042	3.47
45551	88.53	11.47	8.60	48.06	1.47	4113	4.37
45552	88.82	11.18	6.84	49.07	1.83	4189	5.27
45553	87.23	12.77	5.60	50.96	0.87	4146	2.90
45554	88.72	11.28	6.59	50.63	0.63	4175	3.95

TABLE 1C: INDISPENSABLE AMINO ACID PROFILE[1] (AS IS BASIS) OF THE SOYBEAN MEAL USED DIETS OF PACIFIC WHITE SHRIMP, *LITOPENAUS VANNAMEI*

Soybean meal Sample key	Indispensable Amino Acids (%)										
	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl alanine	Threonine	Tryptophan	Valine	Total
AU[2]	3.39	1.23	2.19	3.60	2.94	0.68	2.37	1.79	0.69	2.39	21.27
45531	3.31	1.26	2.07	3.33	2.86	0.61	2.22	1.58	0.65	2.13	20.02
45532	3.38	1.30	2.15	3.45	2.94	0.63	2.30	1.67	0.66	2.23	20.71
45533	3.33	1.28	2.11	3.40	2.88	0.61	2.25	1.63	0.64	2.17	20.30
45534	3.24	1.28	2.09	3.36	2.91	0.63	2.20	1.66	0.65	2.16	20.18
45535	3.36	1.24	2.08	3.39	2.91	0.64	2.23	1.68	0.68	2.16	20.37
45536	3.31	1.35	2.19	3.62	3.04	0.65	2.41	1.82	0.70	2.29	21.38
45537	3.23	1.33	2.18	3.61	2.97	0.63	2.40	1.78	0.69	2.27	21.09
45538	3.32	1.34	2.13	3.56	2.88	0.62	2.39	1.76	0.69	2.23	20.92
45539	3.33	1.36	2.16	3.64	3.04	0.65	2.42	1.83	0.71	2.24	21.38
45540	3.36	1.36	2.26	3.66	3.04	0.64	2.43	1.78	0.70	2.36	21.59
45541	3.22	1.30	2.23	3.59	2.91	0.61	2.41	1.72	0.66	2.30	20.95
45542	3.30	1.34	2.25	3.61	2.97	0.63	2.42	1.74	0.69	2.32	21.27
45543	3.56	1.41	2.39	3.83	3.14	0.66	2.60	1.86	0.68	2.48	22.61
45544	3.52	1.36	2.41	3.89	3.14	0.65	2.57	1.87	0.73	2.47	22.61
45545	3.55	1.41	2.46	3.96	3.15	0.68	2.68	1.87	0.72	2.51	22.99
45546	3.45	1.40	2.32	3.75	3.15	0.67	2.49	1.84	0.72	2.41	22.20
45547	3.40	1.38	2.24	3.68	3.06	0.64	2.42	1.77	0.70	2.34	21.63
45548	3.63	1.44	2.30	3.79	3.21	0.69	2.51	1.85	0.76	2.40	22.58
45549	3.40	1.38	2.26	3.68	3.05	0.66	2.43	1.78	0.68	2.31	21.63
45550	3.30	1.32	2.14	3.52	2.96	0.62	2.31	1.72	0.68	2.24	20.81
45551	3.42	1.39	2.29	3.73	3.08	0.66	2.42	1.82	0.65	2.38	21.84
45552	3.42	1.38	2.21	3.58	3.03	0.62	2.39	1.73	0.66	2.28	21.30
45553	3.71	1.46	2.41	3.92	3.25	0.68	2.62	1.90	0.70	2.49	23.14
45554	3.63	1.44	2.35	3.82	3.18	0.67	2.55	1.85	0.69	2.44	22.62

TABLE 1D: DISPENSABLE AMINO ACID PROFILE[1] (AS IS BASIS) OF THE DIFFERENT SOYBEAN MEAL USED IN DIETS OF PACIFIC WHITE SHRIMP, *LITOPENAUS VANNAMEI*

Soybean meal Sample key	Dispensable Amino Acids (%)									Sum of Amino Acids (%)
	Alanine	Aspartic Acid	Cysteine	Glutamic Acid	Glycine	Proline	Serine	Tyrosine	Total	
AU[2]	2.03	5.33	0.63	8.53	1.98	2.40	2.00	1.59	24.49	45.76
45531	1.79	4.78	0.62	7.77	1.75	2.06	1.86	1.57	22.2	42.22
45532	1.91	4.96	0.65	8.01	1.87	2.16	1.95	1.6	23.11	43.82
45533	1.86	4.86	0.63	7.87	1.82	2.07	1.95	1.55	22.61	42.91
45534	1.90	4.94	0.63	7.95	1.87	2.10	2.01	1.26	22.66	42.84
45535	1.91	4.96	0.65	8.02	1.9	2.13	2.06	1.57	23.20	43.57
45536	2.05	5.12	0.62	8.21	1.97	2.26	2.15	1.72	24.10	45.48
45537	2.00	5.02	0.60	8.07	1.92	2.20	2.07	1.67	23.55	44.64
45538	1.99	5.03	0.61	8.05	1.95	2.24	2.09	1.69	23.65	44.57
45539	2.03	5.15	0.62	8.30	1.93	2.26	2.2	1.73	24.22	45.60
45540	2.04	5.16	0.60	8.30	1.99	2.19	2.08	1.72	24.08	45.67
45541	1.98	5.11	0.59	8.16	1.98	2.22	2.08	1.62	23.74	44.69
45542	2.02	5.17	0.62	8.20	2.00	2.24	2.04	1.68	23.97	45.24
45543	2.17	5.50	0.65	8.78	2.10	2.37	2.18	1.80	25.55	48.16
45544	2.15	5.50	0.61	9.00	2.07	2.36	2.35	1.76	25.80	48.41
45545	2.16	5.50	0.66	8.98	2.10	2.44	2.26	1.82	25.92	48.91
45546	2.09	5.35	0.64	8.60	2.04	2.39	2.13	1.78	25.02	47.22
45547	2.02	5.19	0.62	8.34	1.97	2.30	2.05	1.67	24.16	45.79
45548	2.11	5.43	0.66	8.92	2.04	2.40	2.17	1.74	25.47	48.05
45549	2.02	5.24	0.61	8.46	1.98	2.21	2.09	1.62	24.23	45.86
45550	1.95	5.03	0.61	8.10	1.92	2.19	2.03	1.62	23.45	44.26
45551	2.05	5.33	0.64	8.61	2.07	2.36	2.21	1.66	24.93	46.77
45552	1.98	5.22	0.61	8.31	1.99	2.27	2.06	1.69	24.13	45.43
45553	2.14	5.66	0.64	9.11	2.12	2.51	2.27	1.74	26.19	49.33
45554	2.10	5.53	0.64	8.88	2.08	2.45	2.21	1.75	25.64	48.26

TABLE 1E: PERCENTAGE COMPOSITION OF SUGARS & FIBER[1] OF THE DIFFERENT SOYBEAN MEAL USED IN DIETS OF PACIFIC WHITE SHRIMP, *LITOPENAUS VANNAMEI*

Soybean meal Sample key	Sugars, %						Fiber, %		
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Stachyose	ADF	NDF	Lignin
AU[2]									
45531	0.07	0.00	8.87	0.00	1.16	5.51	7.17	11.92	0.24
45532	0.07	0.00	9.54	0.00	1.12	5.75	4.37	7.79	0.07
45533	0.07	0.00	9.07	0.00	1.24	5.59	5.44	9.03	0.25
45534	0.07	0.00	8.97	0.00	1.13	5.66	5.85	9.94	0.21
45535	0.07	0.00	8.90	0.00	1.33	5.72	5.65	9.41	0.17
45536	0.06	0.00	8.05	0.00	1.34	5.50	3.3	6.27	0.08
45537	0.07	0.00	7.87	0.00	1.44	5.66	3.84	7.12	0.81
45538	0.12	0.07	7.50	0.00	1.66	4.77	4.41	9.37	0.28
45539	0.06	0.00	8.12	0.00	1.41	5.58	3.21	6.36	0.17
45540	0.07	0.00	6.77	0.00	1.60	4.96	3.92	7.28	1.14
45541	0.07	0.00	4.86	0.00	1.48	4.08	7.66	12.44	0.74
45542	0.08	0.00	4.81	0.00	1.47	3.58	5.68	9.69	0.30
45543	0.06	0.00	6.32	0.00	1.45	4.90	4.45	8	0.16
45544	0.07	0.00	6.20	0.00	1.88	4.69	3.04	4.88	0.13
45545	0.08	0.00	5.53	0.00	1.47	5.19	4.02	7.49	0.28
45546	0.08	0.00	8.29	0.00	1.93	6.46	3.39	6.72	0.09
45547	0.10	0.08	9.52	0.00	1.04	6.32	3.14	6.56	0.25
45548	0.07	0.00	8.52	0.00	1.12	6.69	3.12	6.88	0.33
45549	0.07	0.00	8.18	0.00	1.68	6.34	4.12	7.76	0.25
45550	0.06	0.00	8.71	0.00	1.51	6.72	4.74	8.49	0.09
45551	0.42	0.31	1.80	0.00	1.44	3.28	8.26	12.45	0.25
45552	0.00	0.00	5.09	0.00	2.15	5.66	6.35	10.04	0.38
45553	0.00	0.00	5.81	0.00	2.12	6.05	4.95	7.94	0.19
45554	0.00	0.00	6.10	0.00	2.23	5.43	6.18	9.58	0.20

TABLE 1F: COMPOSITION OF MINERALS[1] IN THE DIFFERENT SOYBEAN MEAL USED IN DIETS OF PACIFIC WHITE SHRIMP, *LITOPENAUS VANNAMEI*

Soybean meal Sample key	Minerals																
	Ca, %	P, %	P in PA, %	Total PA, %	Non-phytate P, %	Cr, ppm	Cobalt, ppm	Cu, ppm	Fe, ppm	Mg, %	Mn, ppm	Molybdenum, ppm	K, %	Se, ppm	Na, ppm	S, %	Zn, ppm
AU[2]	0.32	0.64						9.7		0.24							46.8
45531	0.20	0.66	0.52	1.85	0.14	19.8	< 0.2	7.74	120	0.25	31.1	2.72	2.08	< 4	9.45	0.42	44.6
45532	0.18	0.70	0.54	1.9	0.17	< 0.1	< 0.2	7.96	114	0.25	33.2	3.24	2.07	< 4	7.80	0.43	45.3
45533	0.18	0.68	0.55	1.96	0.13	< 0.1	< 0.2	7.41	105	0.25	31.2	2.23	2.13	< 4	5.32	0.42	44.2
45534	0.18	0.70	0.55	1.96	0.15	< 0.1	< 0.2	7.65	111	0.25	31.3	2.38	2.08	< 4	5.32	0.43	44.5
45535	0.19	0.69	0.54	1.9	0.15	< 0.1	< 0.2	7.38	106	0.25	30.9	2.54	2.07	< 4	< 0.2	0.42	43.5
45536	0.25	0.68	0.53	1.87	0.15	2.41	< 0.2	11.3	90.3	0.28	44.9	9.93	2.30	< 4	4.64	0.42	41.3
45537	0.24	0.67	0.52	1.86	0.15	< 0.1	< 0.2	11.3	78.5	0.28	41.3	8.24	2.28	< 4	2.27	0.41	40.7
45538	0.26	0.69	0.50	1.77	0.19	< 0.1	< 0.2	11.4	130	0.30	42.5	7.90	2.30	< 4	117	0.41	43.0
45539	0.24	0.70	0.53	1.89	0.17	< 0.1	< 0.2	11.0	68.1	0.29	39.8	8.42	2.31	< 4	6.38	0.42	39.6
45540	0.29	0.63	0.45	1.58	0.18	< 0.1	< 0.2	11.7	105	0.29	38.1	6.64	2.25	< 4	11.6	0.40	45.4
45541	0.28	0.61	0.43	1.53	0.18	< 0.1	< 0.2	8.22	172	0.32	26.9	4.14	2.11	< 4	7.67	0.39	50.3
45542	0.32	0.59	0.40	1.42	0.19	< 0.1	< 0.2	10.5	256	0.30	34.8	2.42	2.08	< 4	43.8	0.42	50.9
45543	0.28	0.62	0.46	1.62	0.16	< 0.1	< 0.2	7.42	141	0.32	30.5	5.34	2.27	< 4	< 0.2	0.43	49.3
45544	0.30	0.64	0.46	1.64	0.17	< 0.1	< 0.2	9.49	79.5	0.31	27.8	4.15	2.20	< 4	19.5	0.42	49.0

45545	0.33	0.65	0.47	1.67	0.18	< 0.1	< 0.2	9.74	110	0.33	29.0	3.73	2.17	< 4	2.97	0.43	53.9
45546	0.32	0.64	0.47	1.67	0.17	< 0.1	< 0.2	10.6	82.8	0.27	31.5	2.76	2.20	< 4	3.55	0.43	41.0
45547	0.24	0.63	0.47	1.65	0.16	< 0.1	< 0.2	12.5	101	0.28	39.4	3.49	2.12	< 4	53.6	0.43	47.1
45548	0.26	0.61	0.43	1.54	0.17	< 0.1	< 0.2	11.6	109	0.26	26.7	11.5	2.15	< 4	8.66	0.44	48.1
45549	0.57	0.64	0.45	1.58	0.20	< 0.1	< 0.2	44.1	167	0.28	61.3	4.13	2.14	< 4	371	0.42	153
45550	0.48	0.81	0.51	1.80	0.30	< 0.1	< 0.2	14.8	331	0.42	71.2	2.96	2.17	< 4	1470	0.52	97.1
45551	0.53	0.61	0.44	1.57	0.17	< 0.1	< 0.2	14.1	1590	0.35	78.0	0.187	2.01	< 4	22.6	0.41	54.7
45552	0.43	0.59	0.43	1.54	0.16	< 0.1	< 0.2	15.0	713	0.34	48.2	2.03	2.00	< 4	12.2	0.40	56.3
45553	0.34	0.57	0.41	1.45	0.16	< 0.1	< 0.2	16.2	395	0.32	46.3	3.29	2.03	< 4	9.59	0.43	59.2
45554	0.35	0.60	0.43	1.52	0.18	< 0.1	< 0.2	17.0	695	0.34	53.7	1.88	2.07	< 4	11.1	0.43	58.9

TABLE 2A: COMPOSITION (% AS IS) OF THE BASAL DIETS USED IN THE DIGESTIBILITY AND GROWTH TRIALS.

Ingredient (As basis g/kg feed)	Basal diet for digestibility	Basal diet for growth trial
Fishmeal	10.0	6.00
Soybean meal	32.5	51.70[1]
Corn protein concentrate	-	7.00
Menhaden fish oil	3.20	5.76[1]
Lecithin	1.0	1.00
Cholesterol	0.0	0.05
Whole wheat	47.6	23.0
Corn Starch	2.1	0.39[1]
Mineral premix	0.5	0.50
Vitamin premix	1.8	1.80
Choline chloride	0.2	0.20
Stay C 35% active	0.1	0.10
CaP-dibasic	0.0	2.50
Chromic oxide	1.0	0.00

[1]See Table 2b for adjustments for test diets.

TABLE 2B: BASAL DIET INGREDIENT MODIFICATION (G/100G AS IS) TO CREATE THE TEST DIETS. ALL OTHER INGREDIENTS ARE THE SAME AS THAT OF THE BASAL DIET (TABLE 1B)

Diet #	Soybean meal	Corn starch	Fish oil
2	49.30	2.87	5.68
3	48.70	3.59	5.56
4	49.80	2.44	5.61
5	49.40	2.69	5.76
6	49.30	2.78	5.77
7	47.60	4.36	5.89
8	48.50	3.73	5.62
9	47.30	4.9	5.65
10	47.90	4.31	5.64
11	47.70	5.5	4.65
12	47.80	4.44	5.61
13	47.10	5.45	5.30

Diet #	Soybean meal	Corn starch	Fish oil
14	44.30	7.62	5.93
15	45.00	7.69	5.16
16	44.30	7.94	5.61
17	47.40	4.88	5.57
18	47.30	5.14	5.41
19	45.30	7.15	5.40
20	48.10	4.14	5.61
21	49.80	2.5	5.55
22	47.10	5.14	5.61
23	46.10	6.29	5.46
24	44.40	7.54	5.91
25	44.40	7.44	6.01

TABLE 3A: RESPONSE OF JUVENILE SHRIMP (0.23 ± 0.02 G) FED WITH DIETS CONTAINED DIFFERENT SOURCES OF SOYBEAN MEAL OVER A 6-WEEKS EXPERIMENTAL PERIOD (TRIAL 1). VALUES REPRESENTED THE MEAN OF EIGHT REPLICATES FOR THE BASAL DIETS AND FOUR REPLICATES FOR THE REST.

Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	5.69	5.46	2302	1.73^{ab}	85.0	0.098
2	5.78	5.54	2283	1.70^{ab}	90.0	0.099
3	5.54	5.31	2269	1.73^{ab}	90.0	0.097
4	5.94	5.71	2458	1.60^{b}	87.5	0.101
5	5.71	5.50	2602	1.64^{b}	85.0	0.101
6	5.61	5.38	2365	1.68^{ab}	85.0	0.098
7	5.58	5.36	2466	1.69^{ab}	95.0	0.099
8	5.06	4.84	2210	1.97^{a}	80.0	0.094
9	5.28	5.05	2231	1.78^{ab}	82.5	0.095
10	5.34	5.10	2152	1.73^{ab}	92.5	0.095
11	5.62	5.39	2371	1.71^{ab}	80.0	0.099
12	5.18	4.96	2259	1.75^{ab}	97.5	0.095
13	5.42	5.19	2290	1.70^{ab}	90.0	0.097
14	5.23	4.99	2165	1.80^{ab}	85.0	0.095
PSE	0.39	0.38	217.65	0.13	7.94	0.003
P-value	0.07	0.07	0.23	0.06	0.07	0.067

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons

TABLE 3B: RESPONSE OF JUVENILE SHRIMP (0.67 ± 0.02 G) FED WITH DIETS CONTAINED DIFFERENT SOURCES OF SOYBEAN MEAL OVER A 5-WEEKS EXPERIMENTAL PERIOD (TRIAL 2). VALUES REPRESENTED THE MEAN OF FIVE REPLICATES.

Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	6.07^{ab}	5.40^{ab}	811^{ab}	1.86^{ab}	86^{ab}	0.092^{ab}
15	5.53^{b}	4.86^{b}	731^{ab}	1.93^{ab}	92^{ab}	0.087^{ab}
16	5.36^{b}	4.70^{b}	712.2^{b}	2.02^{a}	96^{a}	0.085^{b}
17	5.44^{b}	4.76^{b}	697^{b}	2.04^{a}	90^{ab}	0.085^{b}
18	5.52^{b}	4.85^{b}	717^{b}	1.97^{ab}	96^{a}	0.086^{b}
19	6.02^{ab}	5.36^{ab}	807^{ab}	1.81^{ab}	88^{ab}	0.092^{ab}
20	5.97^{ab}	5.31^{ab}	807^{ab}	1.79^{ab}	96^{a}	0.091^{ab}
21	6.33^{a}	5.66^{a}	851^{a}	1.67^{b}	92^{ab}	0.095^{a}
22	5.89^{ab}	5.20^{ab}	749^{ab}	1.84^{ab}	90^{ab}	0.089^{ab}
23	6.08^{ab}	5.39^{ab}	791^{ab}	1.77^{ab}	92^{ab}	0.091^{ab}
24	5.85^{ab}	5.17^{ab}	764^{ab}	1.84^{ab}	92^{ab}	0.089^{ab}
25	5.55^{ab}	4.86^{b}	707^{b}	1.99^{a}	80^{b}	0.086^{b}
PSE	0.37	0.37	60.08	0.14	7.19	0.004
P-value	0.001	0.001	0.001	0.002	0.041	0.001

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons

TABLE 3C: TOTAL GROWTH COEFFICIENTS (TGC) OF JUVENILE SHRIMP (AS A PERCENTAGE FROM TGC OF BASAL DIET) FED WITH DIETS CONTAINED DIFFERENT SOURCES OF SOYBEAN MEAL (TRIAL 1 & 2 COMBINED DATA).

Trt.	TGC	Trt.	TGC
2	100.42 abcd	14	96.08 abcd
3	98.885 abcd	15	94.08 abcd
4	102.57 ab	16	92.45 cd
5	102.16 abc	17	92.34 d
6	99.94 abcd	18	93.67 abcd
7	100.43 abcd	19	99.62 abcd
8	95.39 abcd	20	99.27 abcd
9	96.97 abcd	21	102.74 a
10	96.57 abcd	22	96.9 abcd
11	100.11 abcd	23	99.36 abcd
12	96.49 abcd	24	97.14 abcd
13	98.3 abcd	25	93.4 bcd
PSE	3.87		
P-value	0.00		

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons

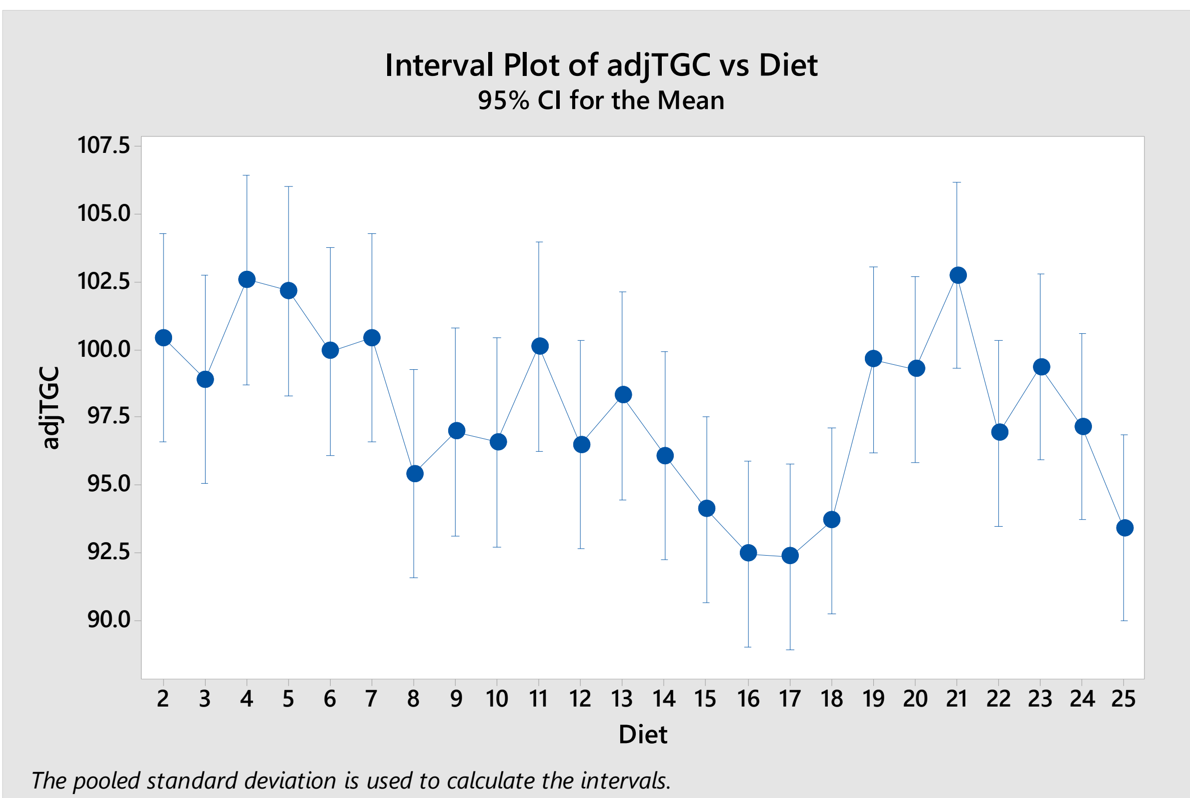
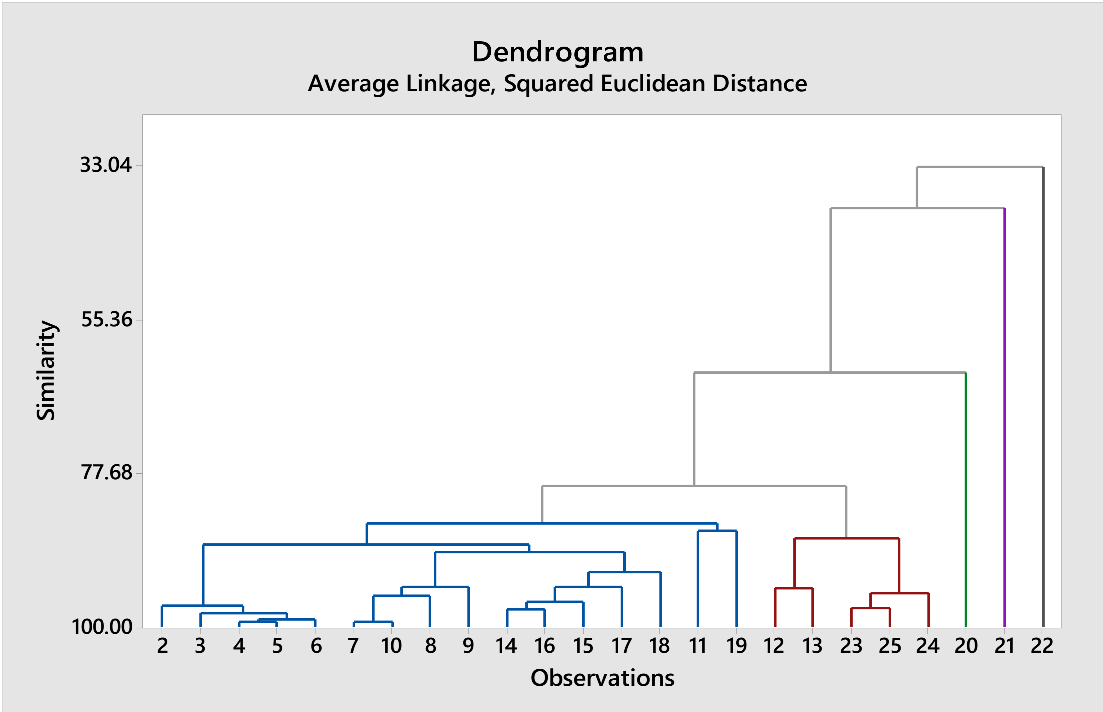


TABLE 4: PRINCIPLE COMPONENT ANALYSIS OF CHEMICAL CHARACTERISTICS OF SBM SOURCES.

Variable	PC1	PC2	PC3	PC4	PC5
Trypsin Inhibitor	0.2138	-0.0050	0.0739	-0.3508	-0.1167
Fructose	0.1786	-0.0550	0.3096	0.3782	0.1568
Glucose	0.2267	-0.0594	0.2669	0.3216	0.1670
Sucrose	-0.3112	0.1642	0.0682	-0.1364	0.2045
Raffinose	0.1874	0.0245	-0.3020	-0.1670	-0.1315
Stachyose	-0.1868	0.2506	-0.1062	-0.2097	0.2323
ADF	0.2267	-0.1123	0.2917	-0.1822	-0.0175
NDF	0.1904	-0.0955	0.3059	-0.1194	-0.0022
Lignin	0.0105	-0.1362	-0.1243	0.2744	-0.1507
Ca	0.2973	0.2157	-0.1229	0.1022	0.1329
P	-0.1727	0.2926	0.2966	0.0502	-0.1050
P in PA	-0.2487	0.0980	0.3494	-0.0185	0.0911
Total PA	-0.2468	0.0900	0.3534	-0.0176	0.0833
Non-phytate P	0.1013	0.3683	-0.0315	0.0939	-0.2835
Cu	0.1345	0.2130	-0.2365	0.0576	0.5020
Fe	0.3297	-0.0279	0.1597	0.0647	0.0656
Mg	0.2447	0.2164	-0.0286	0.0541	-0.4240
Mn	0.2428	0.2542	0.0932	0.1746	0.1493
Mo	-0.2113	0.0006	-0.1997	0.4188	-0.0452
K	-0.2281	0.0951	-0.0520	0.4057	-0.1609
Na	0.0538	0.4160	0.0612	0.0040	-0.1989
S	-0.0456	0.3789	0.1278	-0.1142	-0.1657
Zn	0.1336	0.3068	-0.1470	-0.0292	0.3467
Eigen value	7.0844	5.0033	3.2787	2.0938	1.5463
% variance	30.8	21.8	14.3	9.1	6.7
Cumulative %	30.8	52.6	66.8	75.9	82.6

FIGURE 1: DENDROGRAM OF CLUSTER ANALYSIS AND SCORE PLOT OF PCA



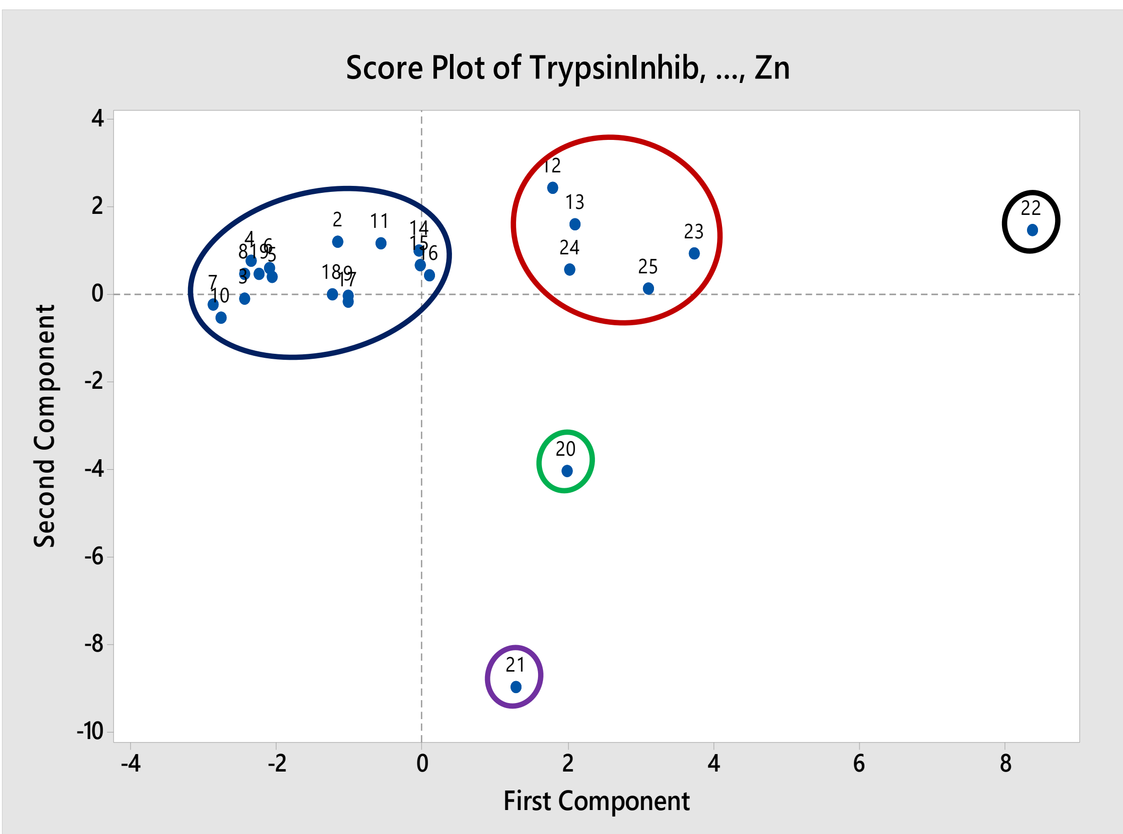


TABLE 5: MULTIPLE LINEAR REGRESSION OF THERMAL GROWTH COEFFICIENT (TGC) WITH PRINCIPLE COMPONENTS (PC1, PC2, PC3, PC4, PC5)

Model p-value= 0.016 $R^2 = 0.127$	Parameter estimates	p- value for each variable
PC1	-0.1643	0.3108
PC2	0.4516	0.0195
PC3	0.5929	0.0142
PC4	-0.1286	0.6726
PC5	0.4413	0.2052

TABLE 6: PEARSON CORRELATION COEFFICIENTS OF TGC WITH RAFFINOSE, ADF, NDF, PHOSPHORUS, PHOSPHORUS IN PHYTIC ACID, TOTAL PHYTIC ACID, NON-PHYTATE PHOSPHORUS, SODIUM, SULFUR AND ZINC.

Variable	r-value	p-value	Variable	r-value	p- value
Raffinose	-0.358	0.086	Total phytic acid	0.426	0.038
ADF	0.256	0.228	Non-phytate phosphorus	0.140	0.514
NDF	0.298	0.157	Sodium	0.353	0.091
Phosphorus	0.469	0.021	Sulfur	0.327	0.119
Phosphorus in phytic acid	0.429	0.037	Zinc	0.199	0.351

Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.

KPI included obtaining the soy samples, completing the digestibility trial and growth trials. All of these tasks were completed. However, data resulting from the digestibility trail was deemed inappropriate (see below).

Expected Outputs/Deliverables - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why.

The proposed research explored the possible relationships between chemical composition of soybean meals and digestibility coefficients and the biological response of the shrimp to various sources. It was hoped that a descriptive matric of composition will correspond to nutrient profiles and digestible nutrients to allow for a better evaluation of the quality of soybean meals for the shrimp feed industry. Albeit this research did not produce as robust of result as previous trials it does contribute to our understanding of variation on soybean meal and corresponding effects on growth.

Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.)

The 25 soy samples were included in a digestibility trial. We have completed Cr, Energy, Amino acids and a portion of the Kjeldahl (protein) analyses. Once the Cr, Energy and AA results were completed we started calculating apparent nutrient (Dry Matter, Energy, Protein and AA) digestibility (ADMD, AED, APD, AAAD) based on Cr, Energy and protein (sum of AA and the initial Kjeldahl analysis). Unfortunately, digestibility coefficients for the diets were extremely low which resulted in corresponding low ingredient digestibility values. In previous trials we have found that the sum of AA and Kjeldahal Nitrogen resulted in similar digestibility values for protein. Hence, these results were calculated confirming the poor response is not an analytical error in protein determinations. We also repeated a number of the diet fecal samples for Cr to confirm that it was not a Cr marker analysis issue. No analytical error could be identified that resulted in the low values.

The same basal diet was used in another digestibility trial for a different project. These samples were collected at a different time with a different set of shrimp. These values returned typical digestibility values for the basal diet and test ingredients. We have also discussed, the potential of diet contaminating the fecal samples but the analytical trends do not support this theory. Hence, the results are not due to either analysis or diet and are not likely due to the collection process (contamination of fecal samples with feed). My though is that the shrimp had a low grade infection that did not result in mortality but impaired the digestive process. Irrespective of the reasons, the data is not reasonable and is consequently unusable.

What, if any, follow-up steps are required to capture benefits for all US soybean farmers? Describe in a few sentences how the results of this project will be or should be used.

We have invested considerable resource into this project and feel that we can improve some of the results to provide a better product. We had enough digestibility diet to support a second collection of fecal samples which has just now been completed. We are working to analyze the chromium levels of the fecal samples as a first step to developing a new set of digestibility calculations. If these turn out reasonable then it is suggested that additional fund be spent to analyze these sample. This is a large data set for which the soy samples have been characterize hence it would prudent to work to re-analyze the data to get as much information as we can. I have invested in the collection of second set of fecal samples however, if the analyses are to be completed we will need financial assistance.