Project Number:	1830-352-0501-C
Project Title:	Increase the Inclusion Rates of Soybean Meal in Shrimp Diets through Trait-enhanced Soybeans
Organization:	Virginia Polytechnic Institute and State University
Principal Investigator Name:	Dr. Bo Zhang

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.

The project has been completed as expected.

Results

- *Treatment diets nutrient composition*
  The nutrient profile of all seven treatment diets after formulation is presented in Table 1.
- *1.2. Water quality*
  During the 6–week feeding trial, water conditions were as follows (mean ± SD): temperature 29.6 ± 0.65 ºC, salinity 28.4 ± 2.60 g/L, dissolved oxygen 5.81 ± 0.25 mg/L, total ammonia nitrogen 0.14 ± 0.09 mg/L, nitrite 0.03 ± 0.02 mg/L, nitrate 23.7 ± 6.99 mg/L, alkalinity 168 ± 20.5 mg/L, and pH 8.62 ± 0.10.
- *Shrimp growth performance*
  Shrimp survival, animal weight gains, and FCRs during the six-weeks are presented in figures 1, 2 and 3, respectively. There were no significant differences among treatments in either study after statistical analyses ($p>0.05$).

Discussion

With the commercial and environmental un-sustainabilities of fishmeal utilization for fish and shellfish culture, soy byproducts have gained attention due to their more stable production and lower cost. Despite soybean meal being a major protein source for aqua feed ingredients, it presents the disadvantage of having anti-nutritional factors that can lead to poor growth of animals (Tangendjaja, 2015). In efforts to help the aquaculture industry overcome this problematic in soy products, a novel soy variety V12 (low in trypsin inhibitors and oligosaccharides) was selected for production of SBM. This study aimed to delineate the effects of various inclusion rates of V12 SBM in shrimp growth.

In this work, the quality of the water was adequate for shrimp growth (Kuhn et al., 2010; Van Wyk et al., 1999). There were no differences between the water culture conditions between the six A-habb systems utilized (data not shown). These adequate water quality levels made it possible to effectively assess the influence of the soy dietary regimens in the survival, weight gains and FCR of the shrimp.

The inclusion of the V12 SBM in the diets of shrimp did not impair the survival, weight gains or FCR of the animals compared to the control fishmeal group. Nevertheless, it is worth mentioning that there is a trend suggesting that increasing the inclusion rates of the V12 SBM results in the detriment of the previously mentioned growth parameters. This pattern can be observed in Fig. 1A, where survival seems to decrease as inclusion rates go from 25% to 65%, relative to the control diet, with up to the 45% V12 SBM being comparable with mean values for the non-treatment control diet. The net shrimp weight gains (Fig 2A) and FCR (Fig. 3A) also follow this trend. It seems reasonable that this pattern is occurring as diets higher in V12 SBM have lower levels in methionine and lysine (Table 3), two growth limiting essential amino acids in shrimp. Yet, all these findings were not significant ($p > 0.05$).

Soybean meal derived from the novel V12, does not improve the growth of Pacific shrimp compared to the commercially available soy protein concentrate. There is a pattern showing that an 80% inclusion rate of the V12 SBM appeared to harm the mean survival, weight gains, and FCR of the crustacean (Fig 1B, 2B, and 3B, respectively), relative to the SPC. But these findings were not significant ($p>0.05$). Despite the low occurrence of trypsin inhibitors and oligosaccharides in the diet of interest, the growth performance of the shrimp appears to be harmed by an unknown factor.

Conclusion

The present study evaluated various inclusion rates of SBM from the novel V12 soy variety to replace fishmeal as an aquafeed source of protein. To our knowledge, this is the first study investigating the effects of utilizing SBM from a soy variety being low in trypsin inhibitors and oligosaccharides in shrimp growth**. Fishmeal substitution with V12 SBM, up to 45%, shows potential as an alternative vegetable protein source, without compromising the growth performance of the shrimp.** Given the high sustainability of soy, the use of SBM from the V12 soy variety can help decrease the pressure on fishmeal usage for aquafeeds. Further investigation is needed to confirm possible animal physiology benefits of the use in the diet, for example trypsin activity in the digestive tract.

Table 1. Analysis of nutrients, minerals and essential amino acids of different treatment diets.

	Control	25% V12	45% V12	65% V12	80% V12	Soy protein concentrate
Proximate and mineral levels (g/100g dry matter)						
Total protein‡	39.8	39.5	38.0	37.8	41.7*	47.8
Carbohydrate[a]	30.8	34.9	38.9	43.8	43.9	34.8
Total Ash	13.1	11.3	8.96	7.29	5.83	4.66
Crude fat	9.98	9.45	9.35	8.38	8.55	9.15
Acid detergent fiber	2.3	2.7	5.4	3.6	4.9	1.8
Calcium	3.69	2.63	1.81	1.21	0.95	0.73
Phosphorus	2.42	1.95	1.52	1.25	1.12	1.07
Potassium	0.9	1.06	1.21	1.4	1.49	0.43
Magnesium	0.23	0.23	0.22	0.24	0.23	0.14
Sodium	0.46	0.35	0.25	0.16	0.1	0.69
Sulfur	0.66	0.59	0.49	0.46	0.41	0.45
Essential amino acid levels (g/100g dry matter)						
Arginine	2.56	2.78	2.71	2.86	ND	3.34
Histidine	1.03	1.08	0.98	0.93	ND	1.18
Isoleucine	1.65	1.68	1.66	1.68	ND	2.19
Leucine	3.07	3.05	2.94	2.95	ND	3.7
Lysine	2.91	2.89	2.62	2.4	ND	2.91
Methionine	1.36	1.16	0.82	0.74	ND	0.8
Phenylalanine	1.7	1.76	1.83	1.84	ND	2.42
Threonine	1.27	1.25	1.29	1.3	ND	1.76
Tryptophan	0.45	0.36	0.42	0.47	ND	0.58
Valine	1.96	1.96	1.88	1.87	ND	2.31
Trace element levels (mg/kg)						
Copper	29.9	33.4	38.6	47.9	48.2	36.4
Iron	522	428	349	289	198	215
Manganese	107	94.6	91.3	87.8	81.2	71.6
Zinc	113	99.6	86.4	85.4	76.8	83.8

‡ Total protein was calculated by the addition of the percentages of nonessential and essential amino acids present
* Denotes total protein being calculated as crude protein
[a] Calculated according to Merrill and Watt (1973). Carbohydrate = 100 – (total ash + crude fiber + moisture + crude lipid + crude protein)

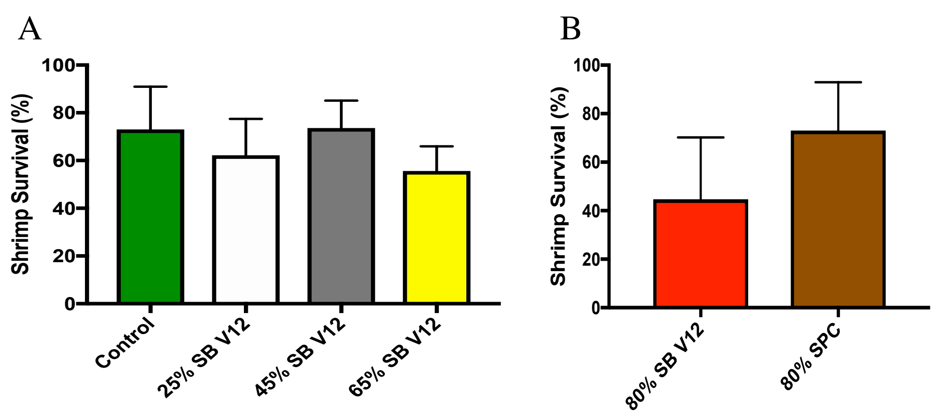

Figure 1. Shrimp survival per treatment diet. Graph (A) compares the survival between the control treatment diet to the survival of the soybean (SB) V12 treatment groups replacing fishmeal at 25%, 45% and 65%. Graph (B) shows the survival between groups when fishmeal was replaced at 80% with either SB V12, or soy protein concentrate (SPC). For (A), n=5; and for B, n=3. Data graphed are mean ± SD. No significant differences were recorded.

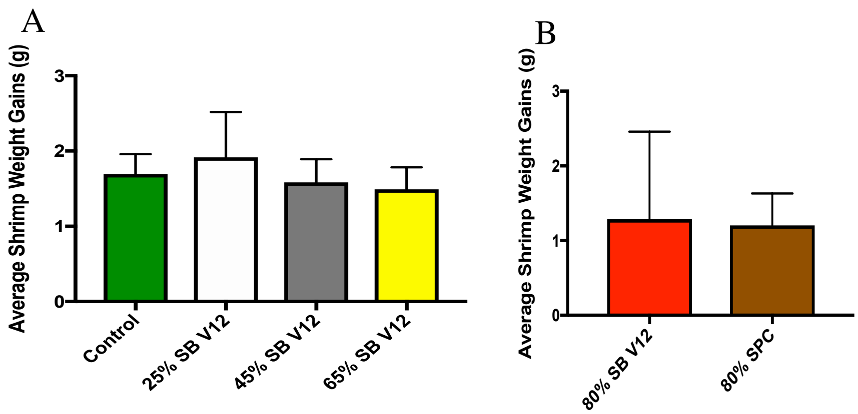

Figure 2. Average shrimp weight gains per treatment diet. Graph (A) represents the weight gains (g) of shrimp per either the control diet group, or the other groups where soybean (SB) V12 was used to replace fishmeal at 25%, 45% and 65%. Graph (B) show the shrimp weight gain data of the groups when fishmeal was replaced at 80% with either SB V12, or soy protein concentrate (SPC). For (A), n=5; and for B, n=3. Data graphed are mean ± SD. No significant differences were recorded.

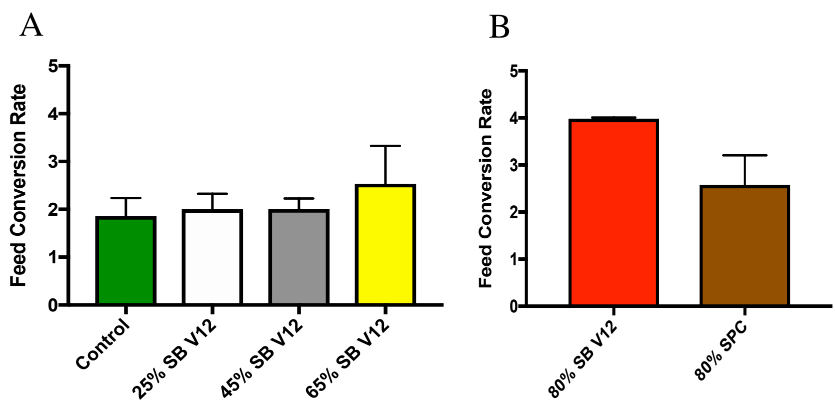

Figure 3. Feed conversion rate (FCR) per treatment diet. Graph (A) represents the FCR of shrimp of either the control diet group, or the other groups where soybean (SB) V12 was used to replace fishmeal at 25%, 45% and 65%. Graph (B) shows the FCR data of the treatment groups when fishmeal was replaced at 80% with either SB V12, or soy protein concentrate (SPC). For (A), n=5; and for B, n=3. Data graphed are mean ± SD. No significant differences were recorded.

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.

KPI1: VSBM has at least 20% more inclusion rate than commercial SBM in shrimp diets.
We decided to remove commercial SBM in the study because SBM has unknown supplements.

KPI2: The maximum inclusion rate of VSBM is not significantly different from LSPC in shrimp diets.
VSBM doesn't have significant difference from LSPC on all three parameters, and 45% inclusion rate of VSBM showed similar shrimp survival rate, better average shrimp gains and lower feed conversion rate (good) than LSPC.

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.

1) A soybean variety with enhanced seed compositions that can increase SBM inclusion rate in shrimp diets.

V12-4590 will be released in March 2019 for either commercial production or germplam with enhanced traits. We are looking for seed companies to license this value added variety.

2) Aquaculture producers' awareness of the benefits of using VSMB

Because we just completed all the expts, we haven't had a chance to present the benefits of VSMB to aquaculture producers. We will make effort to do it in Mid-Atlantic region (pls see below).

3) Publication(s) and presentation(s)

Because we just completed all the expts, we didn't present the study in 2018. However, Zhang will present the results at annual Virginia Crop Improvement Association conference on 2/21/19 and annual Virginia Soybean Board meeting on 3/7/19. Kuhn will also present the results at regional aquaculture meetings in 2019. A manuscript is underwritten now, and will be submitted to J of Aquaculture Nutrition by March, 2019.

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

We had hard time to purchase baby shrimps in 2018, which delayed our expts.

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.

The follow-up steps include disseminating research results to soybean growers and aquaculture farmers, supplying VSBM to aquaculture farmers through seed companies, soybean farmers, meal processors, and end users, and the more importantly, conducting a fish feeding trial to see the benefits of VSBM on fish.

List any relevant performance metrics not captured in KPI's.

This is the first study investigating the effects of utilizing SBM from a soy variety being low in trypsin inhibitors and oligosaccharides in shrimp growth.

Fishmeal substitution with V12 SBM, up to 45%, shows potential as an alternative vegetable protein source, without compromising the growth performance of the shrimp.