Report of the zero-fishmeal diets with Motiv

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PROJECT/TASK:
Evaluation of the impacts of Motiv on zero-fishmeal diets in a RAS system

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BACKGROUND

Motiv is a value-added feed ingredient developed specifically to enhance the productivity of shrimp. Based on laboratory and commercial trials, Motiv has demonstrated improvements in growth, survivability, as well as improvements in shrimp coloration with optimal inclusion of Motiv in replacing fishmeal has been observed at an inclusion of 7.5% to 10% Motiv in a shrimp diet. Replacing 100% fishmeal in a shrimp diet has been a big challenge for shrimp feed industry as some of the key nutrients in fishmeal such as cholesterol, choline chloride, phospholipids and fatty acids (EPA/DHA/ARA) and also the palatability and attractant characteristics are difficult to replace. Therefore, it is necessary to evaluate an opportunity of replacing 100% fishmeal in shrimp diets using Motiv and high-quality poultry meal together with the attractant and palatable ingredients/additives.

OBJECTIVES

To evaluate the impacts of Motiv diets in the zero-fishmeal diets on shrimp growth performances and health indicators as well as the approaches with attractant packages for zero-fishmeal diets.

MATERIALS AND METHODS

Trial location: RAS system at house in Hoc Mon District – Ho Chi Minh City – Vietnam.

Animals: *L. vannamei* weighing approximately 5g/pcs appearing in healthy conditions were selected for the trial. Shrimp sources were from a good nursery in Vietnam and to were reared on a common commercial nursery diet consisting of 42% protein until the beginning of the trial (to reach around 5g shrimp). 20 shrimps of approximate 5g were arranged for each styrofoam box (38 cm x 52 cm) and 12 styofoam boxes for 4 diets with 3 replicates for each diet.

Diets: Diets were approximately 42% protein and 7% Lipid. The fishmeal in the 25%-fishmeal control diet was replaced by 10% Motiv in combination with poultry meal and SBM and the attractant packages was added together with 10% Motiv in the treatment diets. The negative-control diet did not contain the attractant packages. All diets then will be sampled to sent to UpScience lab (in Vietnam) and Pacific Lab (in Singapore) for nutrient analysis and carotenoid analysis, respectively.

Treatment 1 (T1): Control diet is formulated with 25% fishmeal in the diet to be as the average fishmeal inclusion in shrimp commercial standard diet.

Treatment 2 (T2): The Negative- control diet with 10% Motiv plus SBM and high-quality poultry meal to have 0% fishmeal without attractant package.

Treatment 3 (T3): 10% Motiv plus SBM and high-quality poultry meal to have 0% fishmeal and added with attractant & palatability packages (Krill meal + a Cocktail mixture of Taurine, Glycine and Betaine).

Treatment 4 (T4): 10% Motiv plus SBM and high-quality poultry meal to have 0% fishmeal and added with attractant package (a Cocktail mixture of Taurine, Glycine and Betaine).

Formulated formula for all diets				
Ingredient	T1 (Control)	T2 (Negative control)	T3 (Krill + Cocktail)	T4 (Cocktail)
Fish Meal (Anchovy)	250.000	0.000	0.000	0.000
Motiv (Cargill US)	0.000	100.000	100.000	100.000
Soybean meal, 48% CP	320.000	308.000	278.000	278.000
Fish oil, Tuna oil	18.000	20.000	20.000	20.000
Wheat, flour	285.000	285.000	285.000	285.000
Krill meal	0.000	0.000	30.000	0.000
Wheat gluten meal, 78% CP	60.000	60.000	60.000	60.000
Dry yeast (AB Mauri)	30.000	30.000	30.000	30.000
HQ Poultry meal (65% protein)	0.000	125.000	112.00	140.000
Choline chloride, 60% choline	0.000	3.000	3.000	3.000
Soy lecithin	17.000	20.000	20.000	22.000
Cholesterol	0.000	0.885	0.760	0.885
MCP	4.875	6.990	8.515	7.390
Salt, NaCl	0.000	7.500	7.500	7.500
Stay-C 35	2.000	2.000	2.000	2.000
Shrimp Premix	7.500	7.500	7.500	7.500
DL-Methionine	2.000	3.700	3.400	3.700
L-Threonine	1.100	2.900	2.800	3.000
L-Lysine	0.000	5.600	5.200	5.600
Arginine	1.400	5.200	5.800	5.600
Histidine		2.300	2.400	2.500
Valine		2.400	2.500	2.700
Tryptophan		0.900	1.000	1.000
Glycine			3.500	3.500
Betaine			3.000	3.000
Taurine			5.000	5.000
Calcium propionate	1.000	1.000	1.000	1.000
BHT/BHA	0.125	0.125	0.125	0.125
Total	1000.000	1000.000	1000.000	1000.000
Formula cost (VND/kg)	30,413.838	27,549.260	29,792.297	28,619.090

Table 1.

All diets were produced at ShrimpVet Lab. Micro-ingredients and macro-ingredients were mixed separately before final mixing. The cooking temperature and retention time were 93 0C and 30 minutes, respectively. Final pellets were dried at 55 0C for 75 minutes.

Analyzed nutrients of the diets (UpScience lab in Vietnam and Pacific Lab in Singapore)				
Nutrient	T1 (Control)	T2 (Negative control)	T3 (Krill + Cocktail)	T4 (Cocktail)
Moisture (%)	8.9	10.08	8.65	10.52
Crude Protein (%)	42.53	41.95	43	42.07
Crude Lipid	6.09	6.94	8.05	7.92
Ash (%)	8.03	6.53	6.64	6.43
Fiber (%)	1.38	1.78	1.57	1.50
Total Carotenoids (mg/kg)	6.90	36.97	35.42	35.86
Amino acid profile (g/100g)	38.43	37.95	39.09	38.47
Cystine (%)	0.54	0.58	0.58	0.57
Aspartic acid (%)	3.74	3.17	3.22	3.11
Methionine (%)	0.98	0.94	0.92	0.98
Threonine (%)	1.66	1.66	1.65	1.71
Serine (%)	1.91	1.89	1.91	1.91
Glutamic acid (%)	8.43	8.50	8.67	8.23
Glycine (%)	2.10	1.92	2.25	2.22
Alanine (%)	1.98	2.00	2.04	2.10
Valine (%)	1.91	1.90	1.99	1.95
Isoleucine (%)	1.73	1.51	1.57	1.55
Leucine (%)	3.04	3.31	3.39	3.31
Phenylalanine (%)	1.99	1.92	1.99	1.90
Histidine (%)	1.02	1.02	1.04	1.07
Lysine (%)	2.36	2.30	2.33	2.34
Arginine (%)	2.53	2.55	2.62	2.61
Proline (%)	2.50	2.81	2.92	2.94

Table 2.

CULTURE SYSTEM

A RAS system was used for the trial and consisted of a shrimp tank which was connected with a waste-settling and biofilter tank. Water from this tank is pumped to a probiotics tank and water from a probiotics tank then flows through the Styrofoam boxes before going back to the shrimp tank to be recirculated. Shrimp were stocked at 100 shrimp/m2.

Daily water exchange was done at 5% of the total tank to fill in the siphoning loss. Salinity at 10 ppt was produced by mixing the seawater and freshwater (tap-water) and water disinfected using Virkon (Bayer) at 1.2 ppm and/or H2O2 (50%) at 6 ppm and aerated for at least 48 hours before using disinfected water to fill in the loss water due to siphoning. Aeration is equipped in shrimp tank, probiotics tank, and air stones are added in the Styrofoam boxes to secure dissolved oxygen (DO) at least 5 mg/L. Salinity is fixed at ranges of 8 to 10 ppt during the trial. Mineral mixture and lime (CaCO3) were daily added at 50g to maintain alkalinity higher than 100 mg/L.

Figure 1. Experimental design with Styrofoam boxes served as replicates (3 boxes per each diet treatment).

MEASURED PARAMETERS

- pH was measured 2 times a day using pH meter (LAQUAtwin – made in Japan).
- Alkalinity was measured once week (using CP kit).
- Ammonia and Nitrite were measured daily (using Sera kit).
- Salinity was fixed at 10 ppt (using Hydrometer).
- DO was measured once week (using YSI – Pro20).

Feed consumption: daily feed observation and collection of uneaten feeds to estimate the uneaten feed amount by counting the uneaten pellets. 1g of each feed was counting three times for average number of pellets per g and using this average number of pellets per g to estimate the uneaten feed per day.

FCR: total feed consumption per live shrimps was the total feed consumption (both dead and live shrimp) – the feed eaten by dead shrimps. Feed eaten by dead shrimp was calculated as averaged feed consumption per shrimp until the date shrimp died. FCR is the average amount of feed per live shrimp divided by average weight growth per shrimp.

ADG is average weight growth per live shrimp divided by 32 days of feeding period.

Salinity-stress test: 10 shrimps from each treatment replicate were directly stocked in free-chlorine tap water (zero-salinity water) with aeration and recorded mortality after 12 hours.

Pigmentation (carotenoids): Samples of all diets were sent to UpScience Lab for nutrient analysis and samples of all diets were also sent to Pacific Lab in Singapore for total carotenoid measurement in all diets. Shrimp at the end of the trial were pulled from each treatment to have at least 150g per diet and sent to Pacific Lab in Singapore for total carotenoid measurement. 10-15 shrimp pulled from each diet was also boiled for 3 minutes and then directly added to ice water and wait for another 5 minutes to measure color scores using Salmon color pan.

Shrimp at harvest were pulled from each treatment of each diet for total hemocyte count at ShrimpVet lab.

RESULTS

1. WATER QUALITY

Shrimp were reared in RAS system with the following water parameters:

- Ammonia (TAN): controlled bellows 0.5 mg/L using probiotics and molasses in a middle tank.
- Nitrite (NO2-N): controlled bellows 1 mg/L using probiotics and molasses in a middle tank.
- pH was measured and the ranges varied between 7.7 to 8.2 during the experiment.
- Alkalinity was measured and varied between 120 to 150 mg/L.
- Dissolved oxygen was measured and varied between 5.5 to 6.5 mg/L.
- Salinity was ranged at 8 to 10 ppt during the experiment.

2. DIETS & PIGMENTS

Diets with good Water Stability (around 2 hours) were produced. Although all the diets were formulated at approximately 42% protein and 7% lipid, the analyzed results (Table 2) indicate a little variation in proximate nutrients among the diets. However, when convert the analyzed nutrients into dry weight it seems not big differences in the proximate nutrients. Also, the amino acid profiles of all diets were similar among the diets, including total amino acid weight. Total carotenoids were 5 times higher in all Motiv diets (T2, T3, T4) than that of the control diet while total carotenoids measured in harvested shrimp showed similar pattern as that of the diets although concentration of carotenoids accumulated in shrimp is much lower than those in the diets (Table 3).

Carotenoids measured in diets and in shrimp muscle from the treatments				
Total carotenoids (mg/kg)	T1	T2	T3	T4
In diets	6.90	36.97	35.42	35.86
In shrimp muscle	0.98	1.92	1.80	1.89
Color scores (Salmon color fan)	21.0	21.5	22.0	21.5

Table 3.

Although carotenoids is similar values in shrimp muscle, the color score and color performances was the best in the shrimp fed on treatment T3 that consists of 10% Motiv and 3% Krill meal while color performances recorded better for all shrimp fed on Motiv diets than the shrimp fed on the control diet (Fig. 2).

Fig. 2. Color scores of shrimps for the 4 diet treatments (2 photos in different light-phone regimes).

3. SHRIMP PERFORMANCE

Raw data of harvested parameters						
Treatment	Reps	Initial mean weight (g)	Final mean weight (g)	ADG (g/day)	FCR	Final survival shrimp (%)
T1	1	4.67	13.10	0.263	1.59	85.00
	2	4.81	12.79	0.249	1.69	80.00
	3	4.60	12.98	0.262	1.55	85.00
Mean		4.69	12.99	0.26	1.61	83.33
SD		0.11	0.10	0.01	0.07	2.89
Mean ± SD		4,69 ± 0,11	12,69 ± 0,16	0,26 ± 0,01	1,61 ± 0,07	83,33 ± 2,89
T2	1	4.68	12.57	0.246	1.54	90.00
	2	4.71	12.72	0.250	1.51	90.00
	3	4.62	12.62	0.250	1.54	85.00
Mean		4.67	12.64	0.25	1.53	88.33
SD		0.05	0.08	0.00	0.02	2.89
Mean ± SD		4,67 ± 0,05	12,64 ± 0,08	0,25 ± 0,00	1,53 ± 0,02	88,33 ± 2,89
T3	1	4.73	12.39	0.239	1.61	85.00
	2	4.66	12.76	0.253	1.52	85.00
	3	4.59	12.81	0.257	1.42	90.00
Mean		4.66	12,65	0,25	1,52	86,67
SD		0.07	0,23	0,01	0,10	2,89
Mean ± SD		4,66 ± 0,07	12,65 ± 0,23	0,25 ± 0,01	1,52 ± 0,10	86,67 ± 2,89
T4	1	4.68	12.22	0.236	1.69	85.00
	2	4.47	12.28	0.244	1.71	80.00
	3	4.76	12.46	0.240	1.51	90.00
Mean		4,64	12,32	0,24	1,64	85.00
SD		0,15	0,12	0,00	0,11	5,00
Mean ± SD		4,64 ± 0,15	12,32 ± 0,12	0,24 ± 0,00	1,64 ± 0,11	85,00 ± 5,00

Table 4.

Stats, Duncan Test, 5% significant level, SPSS for the shrimp performances					
Treatment	Initial mean weight (g)	Final mean weight (g)	ADG (g/day)	FCR	Survival rates (%)
T1	4,69 ± 0,11a	12,69 ± 0,16a	0,26 ± 0,01a	1,61 ± 0,07a	83,33 ± 2,89a
T2	4,67 ± 0,05a	12,64 ± 0,08b	0,25 ± 0,00ab	1,53 ± 0,02a	88,33 ± 2,89a
T3	4,66 ± 0,07a	12,65 ± 0,23b	0,25 ± 0,01ab	1,52 ± 0,10a	86,67 ± 2,89a
T4	4,64 ± 0,15a	12,32 ± 0,12c	0,24 ± 0,00b	1,64 ± 0,11a	85,00 ± 5,00a

Table 5. Values are presented as Mean ± Stdev. The same letters on the same row are presenting not significant differences (P > 0.05).

Results indicates the control diet with 25% fishmeal performed the best in final mean weight with significant differences (P< 0.05) than all the shrimp feed on the other three diets, consisting of Motiv or both Motiv & krill diet while ADG is not significant between the control diet vs the negative-control diet (T2) and the Motiv diet consisting of both mixture of attractant and krill meal but the control diet has significant difference than the Motiv diet consisting of only the mixture of attractant packages (the T4: treatment with the Cocktail). It was expected to have better shrimp performances in the treatment T4 with the mixture of the attractant packages compared to the negative-control diet (T2) without the mixture of the attractant packages; however, results show the final mean weight was not as good for shrimp fed on T4-treatment diet while it was good for both the treatment T2 (without attractant) and T3 (with both attractant and krill meal). It seems that palatability is more important than the attractant for Penaeus vannamei shrimp. FCR and survival rates were not significant differences among shrimp fed on the control and all the treatment diets.

Considering feed cost production, the negative-control treatment (T2) becomes the lowest feed production cost (feed cost per kg of shrimp) and significant difference than the other diet treatments, including the control diet while the control diet is not significant difference to the T3 & T4 diet treatments (Table 6).

Feed production cost	
Diet treatment	Feed production cost (VND/kg of shrimp
T1	48,964 ± 2,193a
T2	41,690 ± 471.97b
T3	45,184 ± 2.813ab
T4	47,125 ± 2,659a

Table 6. Values are presented as Mean ± Stdev. The same letters on the same column are presenting not significant differences (P > 0.05).

In conclusion, although final weight was the best performances in the control diet, the ADG, FCR and survival rates were not significant differences among the control diet (T1), the negative-control diet (T2) and the treatment diet consisting of both krill meal and the mixture of the attractants (T3) while the treatment diet consisting of only the attractant package (T4) showed the lowest final mean weight and ADG. The future study should focus on testing palatability for Penaeus vannamei to have better investigations for successful with the zero-fishmeal shrimp diet.

4. HEALTH PERFORMANCE VIA STRESS TEST AND HEMOCYTE COUNTS

Mortality recorded from the stress test directly at 0-salinity water and hemocyte count		
Treatment shrimp	Mortality at 12 hours (%)	Hemocyte count (x10^6 cell/ml)
T1	30.33 ± 5.57a	17.12 ± 1.21b
T2	30.00 ± 17.32a	20.07 ± 2.72bc
T3	23.33 ± 5.57a	23.58 ± 3.56c
T4	36.67 ± 5.57a	10.34 ± 1.72a

Table 7. (Stats, Duncan, 5% Significant level, SPSS) – the same letters on the same column present no significant differences.

Results indicates no significant differences for shrimp mortality at 0-salinity test after 12 hours among the shrimp fed on the control and the Motiv diets while total hemocyte counts shows higher numbers in the negative-control treatment (T2) and the Treatment T3 consisting of both Krill meal and the mixture of the attractants and significant differences than the treatment T4 (consisting of only the mixture of the attractants). Hemocyte count was the best in shrimp fed on the treatment consisting of both krill meal and attractant packages (T3) and significant differences to the shrimp fed on the control diet & the diet T4. A little higher in FCR and lower in both final mean weight and ADG for shrimp fed on the treatment T4 seems to notice something not good when stimulating Penaeus vannamei shrimp eating with supplemental attractants like glycine & betaine.

CONCLUSION

1. Final mean weight was the best in the control fishmeal-base diet.
2. ADG was not significant differences among the control diet, the negative-control diet and the Motiv diet consisting of both krill meal and the mixture of the attractants.
3. FCR, final survival rates and mortality at 12 hours of the 0-salinity test were not significant different among the control diet and the Motiv diets with or without the mixture of the attractants.
4. Total carotenoids were higher in all shrimp fed on the Motiv diets vs the shrimp fed on the control diet and that resulted in better color performances in shrimp fed on Motiv diet vs the shrimp fed on the control diet, especially the shrimp fed on the Motiv diet consisting of krill meal performed the best color enhancement and color scores.
5. Hemocyte count also showed the significant higher cell number in shrimp fed on the Motiv diet also consisting of krill meal.

SUGGESTION

Further investigation should focus on palatability ingredients/additives to be successful on the zero-fishmeal shrimp diets for *Penaeus vannamei*.