Utilizing Feed Effectors and Passive Acoustic Monitoring for Semi-Intensive Pacific White Shrimp (*Litopenaeus vannamei*) Production

by

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Abstract

The efficiency and sustainability of shrimp farming have been improved through developments in farm management and technology such as passive acoustic monitoring (PAM), and fishmeal-free diets. To further improve the efficiency of shrimp aquaculture, the use of feed effectors has been suggested to increase the attractability and palatability of formulated diets. Our research trial aimed to expand upon previous Pacific white shrimp *Litopenaeus vannamei* research involving plant-based diets, feed effectors, and PAM in a laboratory setting, by conducting a feed trial in outdoor semi-intensive ponds stocked at 30 shrimp/m^2. A 13-week trial was conducted in sixteen 0.1-hectare ponds equipped with PAM-integrated feeders, which allowed for demand-style feeding. Four soy-optimized diets, consisting of an “all-plant” basal diet (AP) and three diets with an attractant (2% krill meal (KM), 2% squid meal (SM), 4% fish hydrolysate (FH)), were fed to the for a period of 74-75 days. Final production values were determined after fully harvesting each pond. Significantly more ($p=0.024$) of the FH diet was fed to the respective ponds than the AP diet, suggesting that the addition of FH to soy-optimized diets increases the intensity of the feed response in shrimp cultured in semi-intensive ponds. The same diets offered in predetermined amounts to shrimp in an outdoor, recirculating green water system also resulted in no significant differences ($p>0.05$) between treatment means for final weight or weekly growth rate. However, there was a significant difference ($p=0.021$) in survival between the AP treatment (85.83%) and the FH treatment (95.83%), which led to the final biomass ($p=0.004$) and FCR ($p<0.0001$) of the AP treatment being significantly different than all other treatments. The slightly poorer response observed in the AP treatment suggests that the addition of attractants may improve the performance of plant-based diets in RAS. However, further research is required to improve our understanding of the relationship between feed effectors and shrimp aquaculture.

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FM	Fish meal
AP	All plant
KM	Krill meal
SM	Squid meal
FH	Fish hydrolysate
PAM	Passive acoustic monitoring
PL	Post larvae
CPMC	Claude Peteet Mariculture Center
RAS	Recirculating aquaculture system
DO	Dissolved oxygen
TAN	Total ammonia nitrogen
SFP	Standard feeding protocol
ANOVA	Analysis of variance
ANPR	Apparent net protein retention

1 Introduction

The global population and demand for dietary protein are rapidly increasing, with overall protein consumption expected to double by 2050 (Steinfeld et al. 2006). The pressures placed on food security by population growth will be further compounded by the effects of environmental degradation on natural food sources (FAO 2011, Sundström et al. 2014, FAO 2020). Therefore, to maintain and protect natural food sources, food security objectives should also account for environmental objectives. Farming highly efficient aquatic organisms, such as shrimp, can provide a sustainable solution to food security. Aquaculture has proven to be one of the most efficient methods of producing protein and consequently, it is one of the fastest-growing food production sectors (Naylor et al. 2009, Béné et al. 2015). However, as aquaculture production continues to intensify, farming techniques and procedures must be refined and improved to accommodate the increasing demand for protein and environmental sustainability.

One of the largest and most scrutinized sectors of the aquaculture industry is shrimp production (Stonich and Bailey 2007, FAO 2020). Such scrutiny is the result of unsustainable farming practices that strained surrounding ecosystems and wild fisheries. Historically, ecosystems became degraded as shrimp were stocked and fed beyond the carrying capacity of a given location, commonly leading to eutrophication and silting (Dierberg and Kiattisimkul 1996, Páez-Osuna et al. 1998, Páez-Osuna et al. 1999, Páez-Osuna 2001). In addition, formulated diets for shrimp contained up to 50% of wild-caught protein (Tacon and Barg 1998), usually in the form of fishmeal (FM). The shrimp production industry has adopted new methods, technology, and techniques in feed and farm management to ensure the future of the industry is more sustainable than its past. Improvements in management techniques and technologies allow farmers to stock shrimp at high densities with excellent survival and yield, while also reducing environmental impact (Samocha et al. 2002). One new technology that is being implemented on shrimp farms is passive acoustic monitoring (PAM), which allows farmers to provide the exact amount of feed the shrimp demand, decreasing the risk of overfeeding and the resulting pollution while improving production (Bador et al. 2013, Ullman et al. 2019a, Ullman et al. 2019b, Reis et al. 2020). Furthermore, improvements in diet formulations allow for the reduction of wild caught ingredients without sacrificing production (Browdy et al. 2006, Reis *et al*. 2022b).

The two most common wild caught ingredients in aquaculture production diets are FM and fish oil (Tacon and Metian 2015, FAO 2020). FM is an ideal protein source due to its ease of digestibility and essential amino acid composition, and fish oil is a desirable source of fats due to its high omega-3 content (Olsen and Hasan 2012, Tacon and Metian 2015). No alternatives are currently available at a large-scale and a suitable price point to replace the fish oil-derived essential fatty acids found in fish oil within formulated diets (Turchini et al. 2010, Council 2011). Conversely, FM is already being replaced by many suitable alternative protein sources (Hardy 2010, Council 2011). One of the most common alternative protein sources in aquaculture feeds is soybean meal due to its high crude protein content and relatively balanced amino acid profile (Gatlin III et al. 2007). Recent studies suggest that shrimp can be fed diets formulated with only plant-based proteins (hereafter referred to as plant-based diets) without sacrificing production (Reis et al. 2022b). Reis et al. (2022b) observed no significant differences in growth between shrimp fed plant-based diets and diets containing FM or poultry by-product meal, when fed with demand style feeders. To further improve the efficiency and acceptability of plant-based diets, studies suggest the addition of feed effectors (chemoattractants, feeding incitants, and stimulants) for increased attractability and palatability (Nunes et al. 2006, Suresh and Nates 2011, Soares et al. 2021). By intensifying feeding response and rate of ingestion, feed effectors can potentially increase production while reducing the effects of nutrient leaching.

Reis et al. (2022b) demonstrated that FM can be completely replaced by soybean meal with no significant effect on shrimp production when using PAM-style feeders in semi-intensive pond culture. In a clear water lab trial conducted in aquaria equipped with PAM, Soares et al. (2021) further demonstrated that the addition of KM, SM, or FH to a soy protein diet, all led to higher attractability. Moreover, the addition of FH at 4%, led to significantly improved growth performance. The objective of the current work was to expand upon the preliminary work on plant-based diets, attractants, and PAM by:

i. Investigating the growth performance of *L. vannamei* fed soy protein diets with attractants using PAM in an outdoor semi-intensive pond setting.
ii. Examining the effects of the same diets and attractants offered to *L. vannamei* on specified rations in an outdoor green water recirculating aquaculture system (RAS) to verify data from the pond trial.

2 Materials and Methods

Both the outdoor semi-intensive pond trial and the outdoor green water RAS trial were conducted at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center (CPMC) (Gulf Shores, AL, USA). Post larvae (PL) Pacific white shrimp were provided by American Penaeid (St. James City, FL, USA). The PL shrimp were transported to CPMC, where they were acclimated and nursed in an outdoor recirculating system. The nursery system consisted of 6 tanks, each with a volume of 6000L. For the first seven days, PLs were fed

PL Raceway Plus 1 produced by Zeigler Bros. Inc. (ZBI, Gardners, PA, USA) at 25% of body weight. For the following seven days, PLs were fed a mix (PL Raceway Plus 1 and 2) at 25%, 20%, and 15% of body weight. After 14 days in the nursery system, one group of juvenile shrimp (0.0125g ± 0.0009) were stocked into 16 outdoor, 0.1 ha ponds (46 x 20 x 1.0 m) at a density of 30 shrimp/m^2. After a longer nursery period, another group of juvenile shrimp (0.74g ± 0.024) were stocked into a green-water, recirculating system at 30 shrimp/tank (35 shrimp/m^2) in 24, 750L tanks.

2.1 Outdoor Pond Trial

The outdoor ponds were constructed with 1.52 mm high-density polyethylene, and each was lined with a 25 cm layer of sandy-loam soil substrate. Soil in the pond bottoms was tilled and then the ponds were filled with brackish water sourced from the intracoastal waterway in Gulf Shore, AL. Water was filtered with a 250-micron cloth mesh filter bag. To promote primary productivity prior to stocking, inorganic fertilizers (1687 mL of 32-0-0 and 303 mL 10-34-0 for 5.70 kg/ha of N and 1.03 kg/ha of P) were added to the ponds. Except for the week prior to harvest, systematic or planned water exchanges were not used. Water was drained from ponds in anticipation of major rain events to minimize overflow. The only water exchange occurred in response to possible harmful algal blooms when observed. Ponds with harmful algal blooms were also treated with Earthtec ® 20% copper sulfate pentahydrate (Earth Science Laboratories, Inc., Rogers, AR, USA) at 0.25ppm.

2.1.1 Feed Management

For the first 16 days, all ponds were hand-fed the same 1.5mm commercial diet (40% crude protein, 9% crude lipids) produced by Zeigler Bros. Inc. (ZBI, Gardners, PA, USA). After this period, shrimp in each pond were fed equal amounts hourly by a timer feeder system. The daily amount fed increased weekly from 1kg, 1.5kg, 3kg, 6kg, to a final rate of 8.2kg/day. On day 17, as the ponds were switched to the timer feeder system, the diets in each pond were changed to one of four 2.4mm custom, Ziegler-manufactured diets (~36-37% protein, ~6-8% lipids). Each diet was prepared around the same plant protein formulation (soybean meal and corn). One "all plant" (AP) diet served as the basal diet, and the three others were augmented with attractants: 2% KM, 2% SM, and 4% FH (Table 1). All diets were analyzed for amino acid profile and proximate composition by the University of Missouri Agricultural Experiment Station Chemical Laboratory (ESCL) (Tables 1 & 2). After 43 days of production, each pond was switched to an on-demand, PAM feeding system (AQ1 Feeder, AQ1 Systems Pty. Ltd., Tasmania, Australia). A hydrophone positioned in each pond collected acoustic signals produced by shrimp mandibular activity during feeding, thereby allowing the AQ1 system to supply feed to shrimp only when the acoustic signals were recognized. Each pond was limited to a maximum of 160 kg/ha/day due to aeration constraints. Aeration was provided to ponds with one 2-HP Aire-O2 (Aire-O2, Aeration Industries International, Inc., Minneapolis, MN, USA) as the primary source of mechanical aeration and one 1-HP Air-O-Lator (Kansas City, MO, USA) for supplemental aeration. Mechanical aeration was automatically initiated when dissolved oxygen (DO) levels were below 3mg/L and feeding was paused until DO was above 4.5mg/L.

2.1.2 Sampling and Harvest

Sampling of the shrimp was conducted once per week starting on day 15 of pond production. Cast nets (1.52m radius and 0.96cm mesh) were used to collect approximately 60 individuals in a maximum of ten throws from an area near the feeders. The shrimp sample was then group weighed, counted, and returned to the pond. In addition to providing weekly growth data, sampling allowed for general inspection of shrimp health. Water temperature and DO were

TABLE 1. Formulation (g/kg as is) of an all plant-based basal diet and three modifications including basal with krill meal (KM), basal with squid meal (SM), and basal with fish hydrolysate (FH) as attractants. Each diet was formulated to contain 36% protein and 8% lipid and was commercially-extruded (Zeigler Bros, Inc. Gardners, Pennsylvania, USA) as a 2.4mm sinking feed.

Diets	All Plant Basal	Basal +KM	Basal +SM	Basal +FH
Crude Protein	36.11	36.06	36.44	37.44
Moisture	9.77	9.17	9.10	9.54
Crude Fat	7.44	8.16	7.68	6.42
Crude Fiber	2.42	2.26	2.49	2.49
Ash	8.54	8.87	8.56	9.03
Soybean meal 47.5%[a]	56.0	54.0	54.0	56.0
Whole Wheat	19.1	19.1	19.1	15.1
Corn gluten meal 60%[a]	12.0	12.0	12.0	12.0
Fish hydrolysate	-	-	-	4.0
Krill meal	-	2.0	-	-
Squid meal	-	-	2.0	-
Fish oil	6.0	6.0	6.0	6.0
Lecithin	1.0	1.0	1.0	1.0
Vitamin Premix[b]	0.12	0.12	0.12	0.12
Mineral Premix[b]	0.12	0.12	0.12	0.12
Stabilized C[c]	0.02	0.02	0.02	0.02
Dicalcium phosphate	4.13	4.13	4.13	4.13
Bentonite	1.5	1.5	1.5	1.5
Copper Sulfate	0.01	0.01	0.01	0.01
Cost ($/kg)	1.28	1.41	1.40	1.33

[a] Protein content of ingredient
[b] Vitamin and mineral premixes are proprietary products and therefore the composition is not listed.
[c] Tiger C 35% activity

TABLE 2. Amino acid profile of an all plant-based basal diet (AP) and three modifications including basal with krill meal (KM), basal with squid meal (SM), and basal with fish hydrolysate (FH) as attractants. Analysis was performed by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed as g/100g as is.

Diets	AP	KM	SM	FH
Amino Acid				
Taurine §	0.14	0.15	0.16	0.16
Hydroxyproline	0.06	0.05	0.08	0.06
Aspartic Acid	3.54	3.57	3.54	3.73
Threonine	1.32	1.33	1.31	1.38
Serine	1.53	1.54	1.52	1.59
Glutamic Acid	6.88	6.89	6.77	7.12
Proline	2.17	2.16	2.14	2.23
Lanthionine §	0.05	0.05	0.05	0.05
Glycine	1.46	1.44	1.47	1.47
Alanine	1.88	1.89	1.86	1.94
Cysteine	0.52	0.50	0.51	0.54
Valine	1.80	1.80	1.78	1.87
Methionine	0.57	0.58	0.59	0.60
Isoleucine	1.71	1.72	1.69	1.78
Leucine	3.39	3.42	3.33	3.56
Tyrosine	1.40	1.42	1.39	1.45
Phenylalanine	1.96	1.96	1.93	2.05
Hydroxylysine	0.00	0.00	0.00	0.00
Ornithine §	0.03	0.04	0.03	0.03
Lysine	1.91	1.94	1.92	2.01
Histidine	0.88	0.89	0.88	0.92
Arginine	2.25	2.24	2.25	2.34
Tryptophan	0.41	0.37	0.44	0.42

measured continuously via optical probes connected to the feeding system in each pond. In addition, manual measurements were taken at sunrise (5:00-5:30 a.m.) and sunset (7:00-8:00 p.m.) for DO, temperature, salinity, and pH, using a YSI ProPlus Meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). Total ammonia nitrogen (TAN) was measured for each pond weekly using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA). Nitrite-N, nitrate-N, phosphate, calcium, magnesium, and alkalinity were measured for each pond every two weeks until week 8, then measurements were taken weekly using a photometer (WaterLink Spin Touch, LaMotte, Chestertown, MD).

The morning preceding the termination of the pond trial, a sample of shrimp was taken from each pond, dried, finely ground and sent to Midwest Laboratories (Omaha, NE, USA) for whole body proximate and mineral analysis. Another sample of 3-4 shrimp per pond was collected to obtain a pooled sample of hemolymph from the pericardial cavity. Samples were allowed to clot, then centrifuged and approximately 100uL of resulting supernatant was evaluated using a VetScan VS2 analyzer (Abaxis, Inc. Union City, CA) to measure levels of alkaline phosphatase, alanine aminotransferase, gamma glutamyl transferase, total bilirubin, blood urea nitrogen, and cholesterol.

After 93-97 days of production, all sixteen ponds were harvested. The harvesting process was delayed due to a hurricane, resulting in one pond being harvested 3 days prior to the rest. Ponds were gradually drained over the span of three days, until approximately one third of the water remained the evening prior to harvest. At the time of harvest, standpipes were lowered to drain the remaining water. The angle of the pond substrate directs shrimp to collect in a concrete catch basin at the lowest point. A hydraulic fish pump (Aqua-Life pump, Magic Valley Heli-arc and Manufacturing, Twin-Falls, Idaho, USA) was placed in the catch basin to accelerate draining and collect shrimp. The shrimp were pumped through a 25cm diameter suction pipe to a harvester, where they were dewatered and transferred to hauling tanks in the bed of a pickup truck. The truck was then unloaded under a pole barn where shrimp were dipped in an ice slurry containing EverFresh (Andenex-Chemie, Engelhard + Partner GmbH, Hamburg, Germany) solution as a melanosis inhibitor. Shrimp were then bulk-weighed to determine the biomass for the entire pond. An additional sample of 150 randomly selected shrimp were taken to measure individual weights. Based on the size distribution, the economic partial value of the shrimp was then calculated by subtracting feed costs from current market value. The market value used for the shrimp were based on the production values for South America, as reported by the Undercurrent News Portal for weeks 31 to 38 of 2019.

2.2 Green-water RAS Trial

Using the same four 2.4mm commercial diets as the pond trial, a 9-week growth trial was conducted in a 24-tank outdoor, green water, recirculating system. The research system consisted of a central reservoir (~1000L), treatment tanks (750L), and 1/3 horsepower circulation pump. Aeration was provided by a regenerative blower supplying multiple air stones in each tank. Water was sourced from an outdoor shrimp production pond using a sump pump. No filtration was used in this system and a 5% water exchange was conducted daily. Tanks were stocked with 0.74g shrimp at a density of 35 shrimp/m^2 (30/tank). Four treatments received equal, predetermined feed rations and two treatments received thirty percent more (+30%), which allowed for the comparison of near satiation and excessive feed regimens. Each treatment had four replicate tanks. The shrimp were provided with predetermined feed rations four times a day (07:00h, 11:00h, 15:00h, 19:00h). Feed inputs were increased throughout the trial based on growth predictions and a standardized feeding protocol (SFP) used by Davis et al. (2006). SFP was based on a weekly weight gain of

1.3g/week and an FCR of 1.2. Measurements were taken every morning (7:00-7:30 a.m.) and afternoon (3:00-3:30 p.m.) for DO, temperature, salinity, and pH, using a YSI ProPlus Meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). TAN was measured for the whole system once per week using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Statistical Analysis

All data from the pond trial and green water RAS trial were analyzed using Statistical Analysis System for Windows (V9.4, SAS Institute, Cary, NC, USA). A one-way analysis of variance (ANOVA) was used to determine statistically significant differences (p-value < 0.05) in growth and production parameters among treatments in both trials. The assumptions for ANOVA were met. When there was statistical significance in the ANOVA test, a Student-Newman-Keuls multiple range test was used to determine statistically significant differences between treatment means. Due to heavy storms, low salinity, and blue green algal blooms leading to low survival in the pond trial, one replicate from the KM treatment, three replicates from the SM treatment, and two replicates from the FH treatment were removed from the data set.

3 Results

3.1 Outdoor Pond Trial

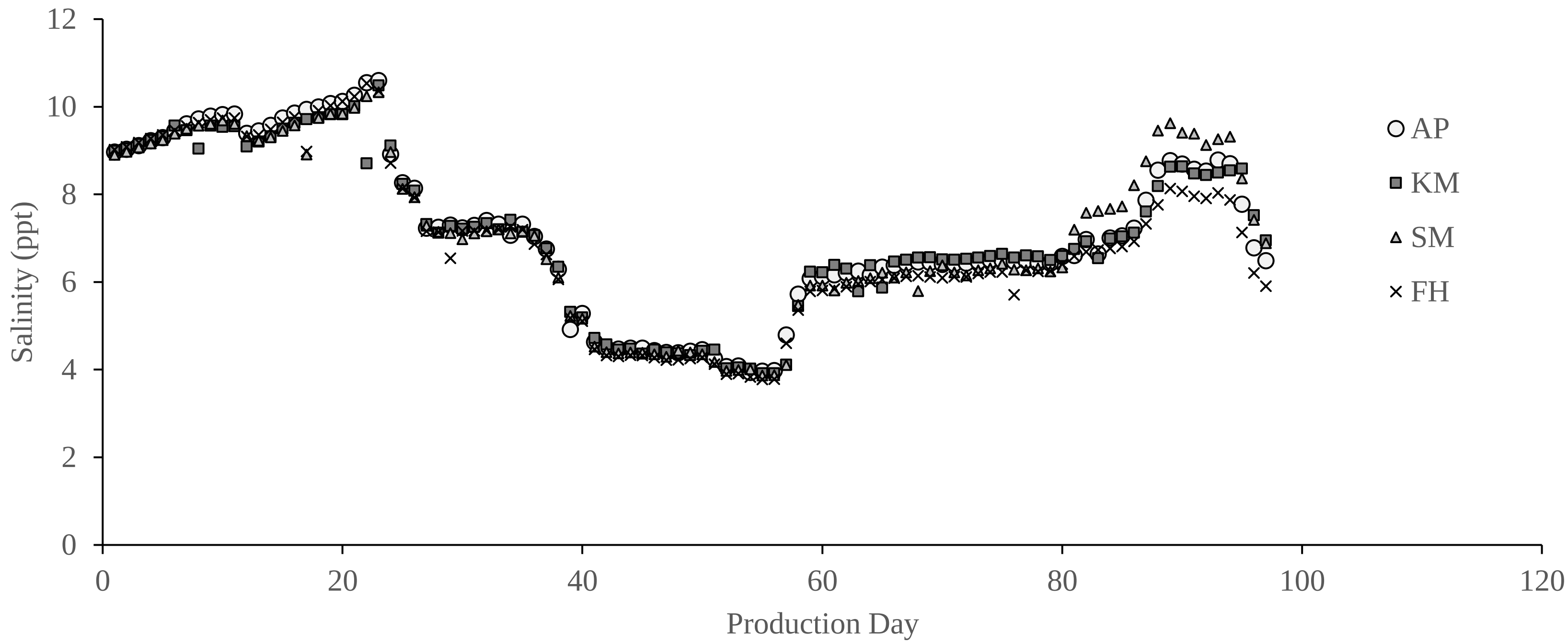
The mean daily water quality values for temperature, pH, DO (AM and PM), and salinity, as well as TAN, nitrite, nitrate, phosphate, calcium, magnesium, and alkalinity are summarized in Table 3. All water quality parameters were maintained at adequate levels for shrimp culture (Boyd and Tucker 1992). However, salinity did fluctuate substantially throughout the production period due to heavy rainfall (Figure 1). No statistically significant differences were found between

TABLE 3. Summary of water quality parameters observed over the 13-wk. growth trial in ponds and the 9-wk growth trial in a green-water, recirculating system. Values are presented as mean ± standard deviation with minimum and maximum values presented in parenthesis for dissolved oxygen (DO), salinity and total ammonia nitrogen (TAN).

	Pond Trial				Tank Trial
	AP	KM	SM	FH	
Morning DO[a] (mg/L)	3.96±1.03 (2.07, 10.82)	3.73±0.95 (1.53, 9.82)	3.79±0.96 (2.11, 9.45)	3.72±0.91 (1.15, 10.82)	7.40±0.94 (6.23, 8.31)
Night DO[a] (mg/L)	9.03±2.69 (3.49, 16.19)	8.62±2.62 (3.14, 16.97)	8.81±2.35 (2.87, 16.89)	8.35±2.79 (2.06, 18.67)	7.18±0.68 (6.23, 11.35)
Continuous DO[a] (mg/L)	6.61±3.13 (1.29, 36.99)	7.00±3.45 (0.77, 21.70)	6.88±3.19 (1.31, 27.05)	6.76±3.12 (0.85, 22.17)	-
Salinity (g/L)	7.16±1.92 (2.62, 11.85)	7.12±1.87 (2.38, 11.46)	7.16±2.00 (2.48, 11.10)	6.94±1.95 (2.48, 11.11)	7.25±1.45 (5.10, 9.15)
TAN[b] (mg/L)	0.61±1.33 (<0.001, 10.00)	0.50±1.35 (<0.001, 10.00)	0.39±0.87 (<0.001, 4.60)	0.97±1.74 (<0.000, 9.00)	0.09±0.11 (0.00, 0.30)
Temperature (°C)	30.45±1.87	30.59±1.82	30.50±1.83	30.64±1.84	27.96±1.56
pH	7.98±0.83	7.88±0.82	8.03±0.82	7.97±0.73	8.00±0.05
Nitrite	0.27±0.48	0.41±0.98	0.16±0.35	0.56±1.14	0.32±0.16
Nitrate	0.38±0.49	1.23±3.98	0.75±1.82	0.63±1.27	9.89±4.81
Phosphate	1.16±0.99	1.35±0.98	1.61±1.22	2.01±1.12	3.38±0.33
Calcium	73.61±29.01	71.11±28.66	75.29±31.25	75.00±26.01	78.33±32.68
Magnesium	219.38±61.96	211.45±66.35	226.13±75.85	214.90±59.90	220.50±36.40
Alkalinity	41.72±15.14	43.70±15.92	43.40±16.92	57.45±18.02	50.63±14.22

[a] DO - Dissolved Oxygen
[b] TAN -Total Ammonia Nitrogen

Figure 1. Average salinity recordings from daily measurements throughout the 93-97 day production cycle for *L. vannamei* reared in 0.1 ha semi-intensive ponds fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Juvenile shrimp (0.0125 g) were stocked at a density of 30 shrimp/m^2. (N=4)



treatments for any major production parameter apart from feed input (p=0.024) (Table 4) (Figures 2 & 3). Significantly more of the FH diet (9147.3 kg/ha) was fed to the respective ponds than the AP diet (6636.6 kg/ha) (Figure 4). Correspondingly the cost of feed was significantly higher (p=0.014) for the FH diet (12,333 $/ha) than the AP diet (8,732 $/ha). There were no other statistically significant differences (p>0.05) between treatments for economic parameters (Table 4).

Results from the proximate whole-body analysis of shrimp from the outdoor pond trial revealed a statistically significant difference (p=0.023) in iron content between treatments. The shrimp fed the AP diet (97.48ppm) and the shrimp fed the FH diet (98.50ppm) had significantly higher iron content than the shrimp fed the S diet (55.20ppm). There were no other statistically significant differences (p>0.05) between treatments for any other body composition parameters measured (Table 5). Based on the crude protein content of the shrimp (Table 5) and the crude protein content of the diets (Table 1), the apparent net protein retention (ANPR) was calculated. No statistically significant differences (p>0.05) were observed between treatment means for the calculated protein retention, which ranged from 28.35-33.83%. Analysis of the shrimp hemolymph using a VetScan VS2 analyzer (Abaxis, Inc. Union City, CA) found no significant differences between treatments for all measured parameters (Table 6).

3.2 Green-water RAS Trial

The mean water quality values for temperature, pH, DO, salinity, and TAN for the green-water RAS trial are summarized in Table 3. All water quality parameters were maintained within the typical range for shrimp production aquaculture (Boyd and Tucker 1992). The results of the growth parameters after the 9-week RAS trial are summarized in table 7. There were no statistically significant differences (p>0.05) between treatment means for final weight or weekly growth rate.

TABLE 4. Production results for *L. vannamei* reared in 0.1 ha semi-intensive ponds over a 13-wk culture period fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Juvenile shrimp (0.0125 g) were stocked at a density of 30 shrimp/m^2.

	AP	KM	SM	FH	*P*-value	PSE[3]
g/week[1]	1.41	1.45	1.28	1.47	0.2838	0.0723
Weight (g)[1]	19.40	19.93	17.60	20.13	0.3332	1.0280
Feed Input (Kg/ha)[2]	6636.3[b]	7556.1[ab]	8145.0[ab]	9147.3[a]	0.0237	329.142
Survival (%)[2]	69.90	77.38	76.56	83.17	0.5155	4.9987
Yield (kg/ha)[2]	4044.6	4778.3	4831.5	5155.5	0.2139	286.971
FCR [2]	1.65	1.63	1.69	1.79	0.9492	0.1552
ANPR [2,4]	31.50	33.83	31.55	28.35	0.8067	3.0315
Electric Use (kwh/ha)[1]	13,110	13,558	11,275	12,818	0.4559	1027.57
Production Cost ($/kg)[2]	2.17	2.34	2.40	2.41	0.9002	0.218
Feed Cost ($/ha)[2]	8,732[b]	10,845[ab]	11,597[a]	12,333[a]	0.0143	451.64
Shrimp value ($/ha)[2]	30,039	36,299	37,136	39,311	0.2162	2440.02
Partial Income ($/ha)[2,5]	21,307	25,454	25,539	26,976	0.6186	2692.2

[1]n=4 for all treatments
[2]n=4 for AP, n=3 for KM, n=1 for SM, n=2 for FH
[3]PSE: Pooled Standard Error
[4]Apparent net protein retention
[5]Shrimp value minus feed cost

Figure 2. Average weight for individual *L. vannamei* reared in 0.1 ha semi-intensive ponds over a 13-wk culture period fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Juvenile shrimp (0.0125 g) were stocked at a density of 30 shrimp/m^2. (N=4)

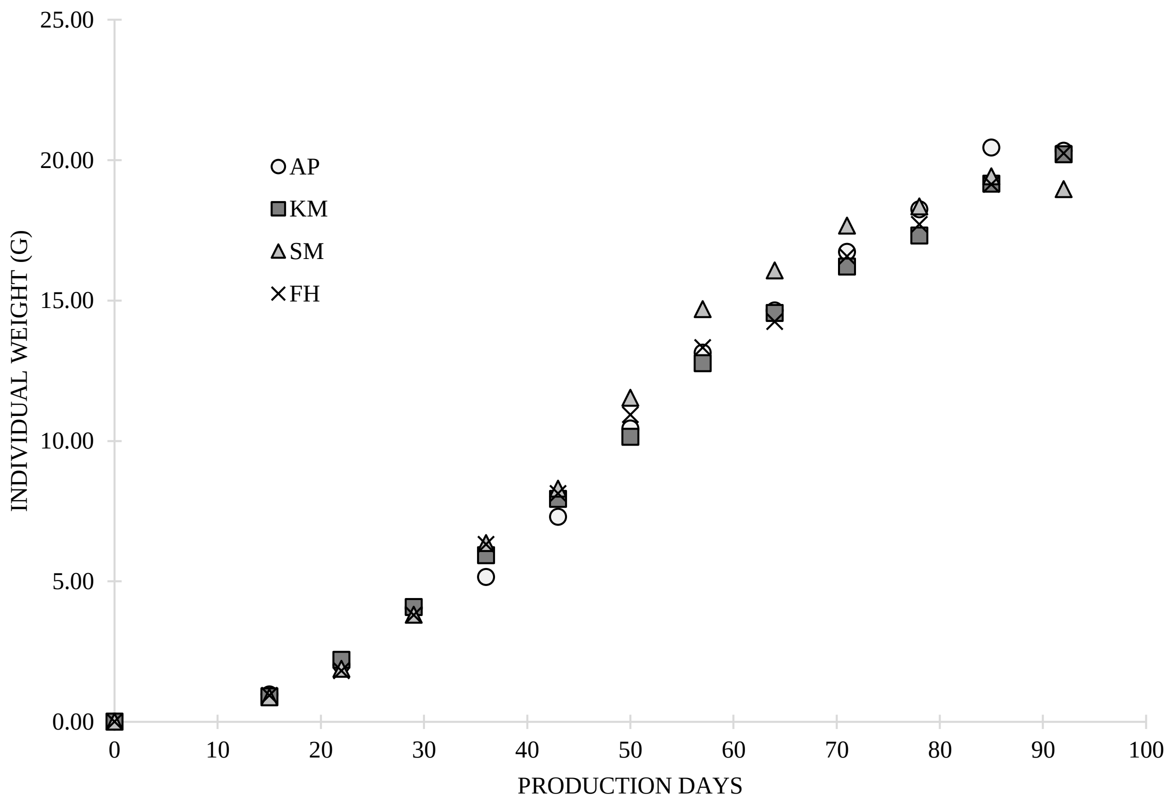


Figure 3. Average final yield and total feed inputs for *L. vannamei* reared in 0.1 ha semi-intensive ponds over a 13-wk culture period fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Juvenile shrimp (0.0125 g) were stocked at a density of 30 shrimp/m^2. Feed input for FH was significantly higher (p=0.0237) than the AP treatment. (N=4 for AP, N=3 for KM, N=1 for SM, N=2 for FH)

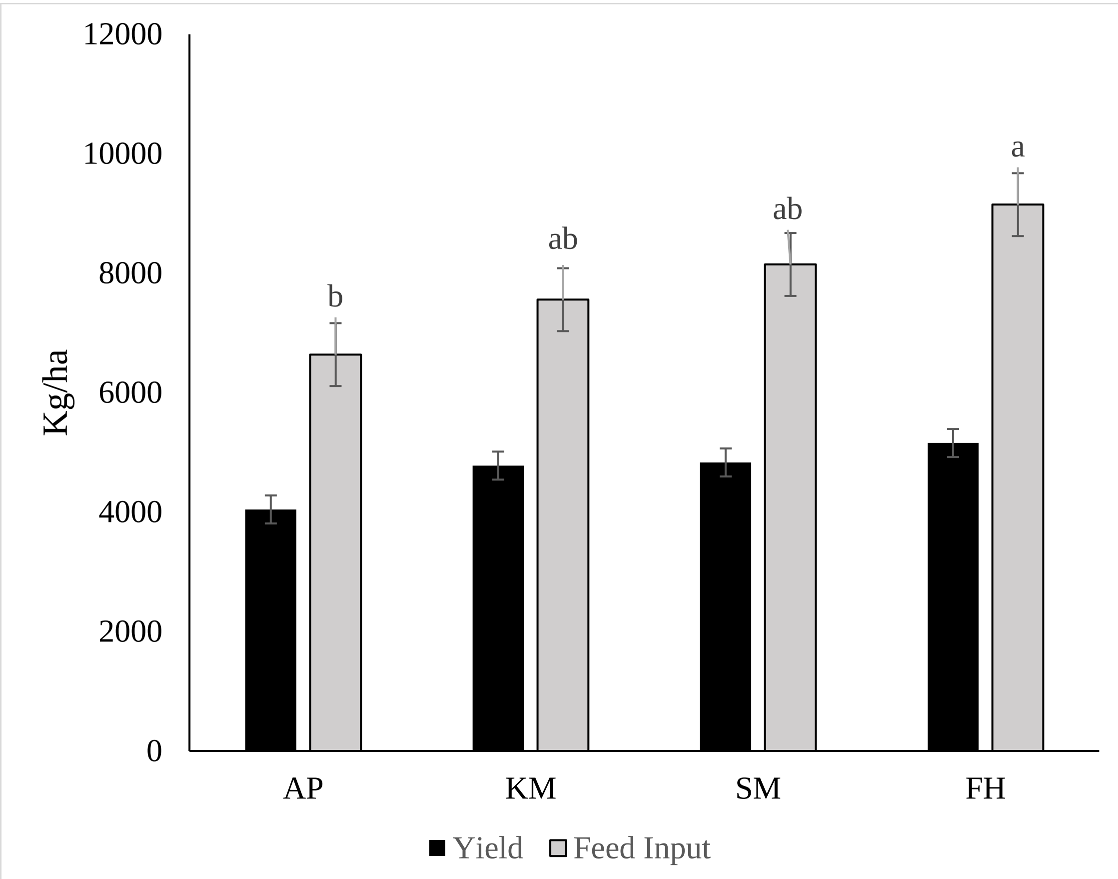


Figure 4. Average daily feed inputs in kg/day for *L. vannamei* reared in 0.1 ha semi-intensive ponds over a 13-wk culture period fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Juvenile shrimp (0.0125 g) were stocked at a density of 30 shrimp/m^2.

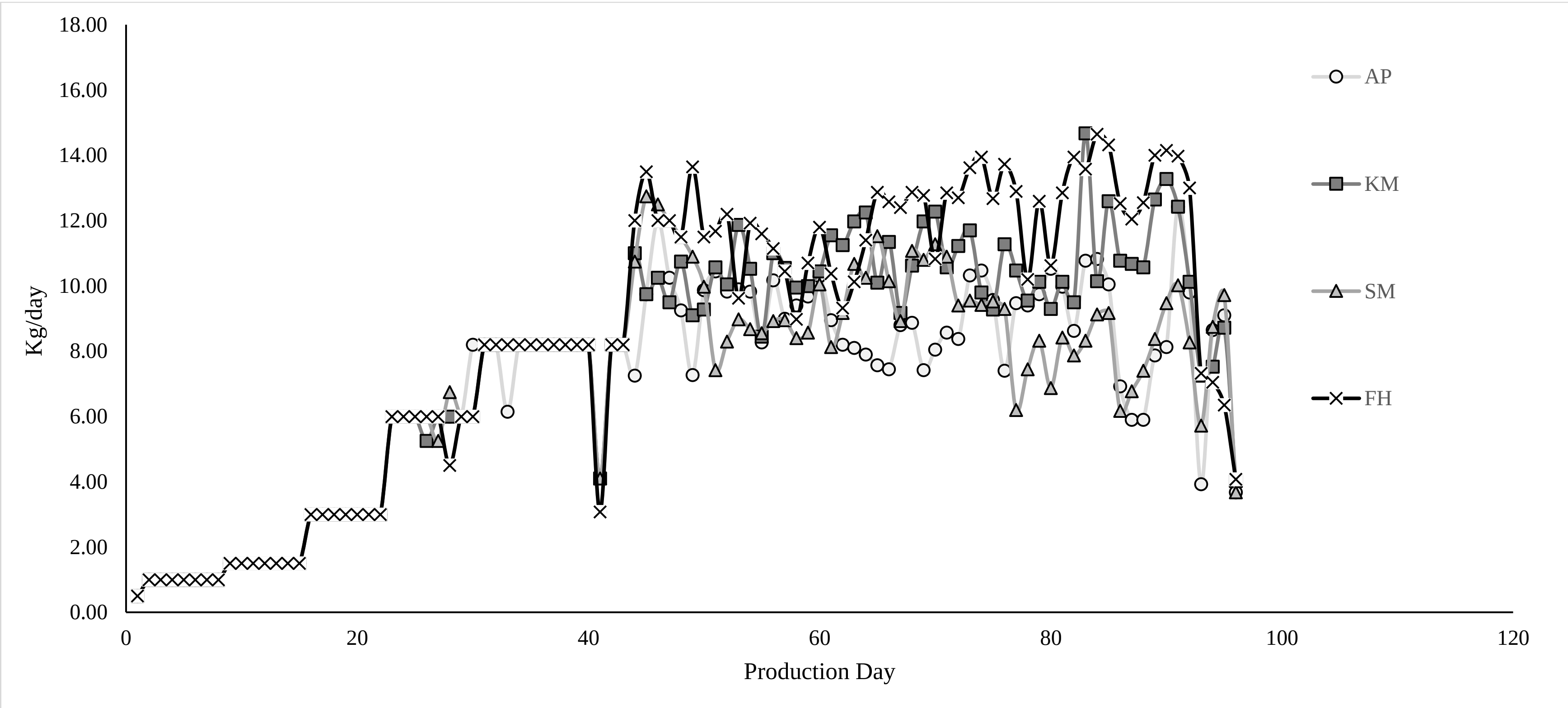


TABLE 5. Mean whole-body composition of *L. vannamei* reared in 0.1 ha semi-intensive ponds over a 13-week culture period fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Juvenile shrimp (0.0125 g) were stocked at a density of 30 shrimp/m^2 and analysed by Midwest Laboratories (Omaha, NE, USA). All analysis other than moisture are on a dry weight basis.

Pond Trial	AP	KM	SM	FH	*P*-value	PSE
Moisture %	75.25	75.90	76.55	76.50	0.6636	0.8079
Protein %	76.30	77.93	77.28	76.87	0.8252	1.2450
Fat %	5.84	5.56	5.48	6.03	0.9445	0.6752
Ash %	12.08	11.80	13.73	12.43	0.4167	0.8588
Sulfur %	0.74	0.73	0.72	0.74	0.8391	0.0134
Phosphorus %	1.22	1.26	1.28	1.16	0.1662	0.0341
Potassium %	1.23	1.25	1.31	1.23	0.3621	0.0344
Magnesium %	0.28	0.29	0.29	0.26	0.5565	0.0173
Calcium %	2.53	2.83	2.89	2.43	0.4297	0.2127
Sodium %	0.79	0.80	0.92	0.84	0.3668	0.0564
Iron (ppm)	97.48[a]	74.43[ab]	55.20[b]	98.50[a]	0.0226	9.1635
Copper (ppm)	117.25	118.50	111.75	119	0.5513	3.7914
Zinc (ppm)	62.63	63.33	62.93	62.67	0.9447	0.8991

Tank Trial	AP	KM	SM	FH	AP +30%	KM +30%	*P*-value	PSE
Protein %	78.05	74.30	76.45	77.28	75.70	75.63	0.2925	1.1477
Fat %	6.38[a]	7.63[a]	6.48[a]	7.13[a]	7.77[a]	7.96[a]	0.0230	0.3626
Ash %	10.75	10.92	10.39	11.23	9.65	9.69	0.1809	0.4979
Sulfur %	0.76	0.73	0.71	0.73	0.75	0.70	0.1230	0.0153
Phosphorus %	1.17[ab]	1.19[ab]	1.17[ab]	1.28[a]	1.06[b]	1.16[ab]	0.0422	0.0416
Potassium %	1.23	1.23	1.19	1.23	1.15	1.17	0.3318	0.0311
Magnesium %	0.26	0.24	0.25	0.26	0.23	0.27	0.4770	0.0135
Calcium %	2.85	2.76	2.66	2.95	2.55	3.01	0.6884	0.2227
Sodium %	0.80	0.81	0.77	0.78	0.76	0.76	0.1735	0.0156
Copper (ppm)	115.75	122.75	128.25	130.50	111.68	122.75	0.0990	4.8397
Zinc (ppm)	63.00	62.83	62.43	62.98	63.03	62.65	0.9996	1.4629

TABLE 6. Mean levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), total bilirubin (TBIL), blood urea nitrogen (BUN), and cholesterol (CHOL) in hemolymph collected from *L. vannamei* after a 13-wk culture period in ponds (N=4). The shrimp received four different diets produced by Zeigler Bros Inc. (Garners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). Analysis was conducted using a VetScan VS2 analyzer (Abaxis, Inc. Union City, CA). (N=4)

	AP	KM	SM	FH	*P*-value	PSE[1]
ALP (U/L)[2]	57.58	74.90	63.98	48.84	0.4849	11.7881
ALT (U/L)	189.49	146.34	99.12	122.35	0.2912	32.6507
GGT (U/L)	9.51	8.01	4.44	7.39	0.0732	1.2288
TBIL (mg/dL)	0.88	0.73	0.52	0.64	0.2168	0.1130
BUN (mg/dL)	3.17	3.26	2.89	4.58	0.6360	0.9821
CHOL (mg/dL)	21.07	23.99	23.24	24.20	0.9613	4.6451

[1]PSE: Pooled Standard Error

[2]U/L: units per liter

TABLE 7. Production results for *L. vannamei* reared in 750l tanks (green water system) over a 9-wk culture period fed four different diets produced by Zeigler Bros Inc. (Gardners, PA, USA). The diets were an all plant (AP) basal diet, basal diet with krill (KM), basal diet with squid (SM), and basal diet with fish hydrolysate (FH). To compare results of feed inputs near satiation and in excess, two treatments received 30% more (SFP+30%) feed. Nursed shrimp (0.74 g) were stocked at a density of 35 shrimp/m^2. (N=4)

Treatment	Final wt	g/week	Biomass (g)	Survival (%)	FCR	ANPR[2]
AP	11.20	1.16	288.15^{b}	85.83^{b}	1.39^{c}	35.88^{ab}
KM	11.46	1.19	320.70^{a}	93.33^{ab}	1.25^{d}	37.42^{ab}
SM	11.63	1.21	322.45^{a}	92.50^{ab}	1.24^{d}	39.35^{a}
FH	11.30	1.17	324.65^{a}	95.83^{a}	1.23^{d}	37.07^{ab}
AP (SFP+30%)	11.76	1.22	310.90^{a}	88.33^{ab}	1.67^{a}	28.10^{c}
KM (SFP+30%)	11.96	1.25	335.05^{a}	93.33^{ab}	1.55^{b}	32.75^{b}
P-Value	0.3228	0.3207	0.004	0.0214	<0.0001	0.0005
PSE[1]	0.2549	0.0281	7.0689	1.9740	0.0321	1.4684

[1]PSE. Pooled Standard Error
[2]Apparent Net Protein Retention

However, the mean final biomass of shrimp fed the AP diet (288.2g) was significantly less (P=0.004) than the mean biomass of all other treatments (310.9-335.1g). Mean survival of shrimp fed the AP diet (85.83%) was significantly lower (p=0.021) than mean survival of shrimp fed the FH diet (95.83%). No statistically significant differences were observed between mean FCR values from the K (1.25), S (1.24), or FH (1.23) treatments, yet they were all significantly lower (p<0.0001) than the other treatments. The FCR of the shrimp fed the AP+30% diet (1.67) was significantly higher than that of shrimp fed the KM+30% diet (1.55) and both were significantly higher than that of shrimp fed the AP diet (1.39). Shrimp from both the AP+30% treatment and KM+30% treatment had significantly lower ANPR than all other treatments and the AP+30% treatment (28.10) had significantly lower ANPR than the KM+30% treatment (32.75) (Table 7).

The results from the proximate whole-body analysis of the shrimp from the RAS trial are summarized in table 5. The mean fat content as a percentage of dry weight was determined to be statistically different (p=0.023) among treatments. However, a post hoc Student-Newman-Keuls multiple range test did not detect any differences between treatment means. The phosphorus content of the shrimp fed the FH diet (1.28%) was significantly higher (p=0.042) than that of the shrimp fed the AP+30% diet (1.06%). Apart from fat and phosphorus content, there were no statistically significant differences (p>0.05) between treatments for any other body composition parameters examined.

4 Discussion

Overfeeding farm raised shrimp produces an economic and environmental problem. Uneaten feed and leached nutrients are major contributors to diminished water quality in the culture system, as well as in effluent waters (Boyd and Musig 1992, Boyd and Tucker 1998). Since commercial feeds typically account for the largest variable cost of semi-intensive and intensive shrimp production, wasted feed is also a major contributor to diminished profits (Shang et al. 1998, Phuong et al. 2007). Therefore, improvements in technologies, culture techniques, and diet formulations that lead to more efficient consumption can greatly improve the economic and environmental sustainability of a shrimp farming operation.

The use of a PAM integrated feeding system to monitor the acoustic signals of shrimp and feed based on real time demand has resulted in improved growth performance in shrimp when compared to traditional feeding protocols (Ullman et al. 2019a, Ullman et al. 2019b, Reis et al. 2020). Reis et al (2022b) demonstrated that under pond conditions, plant-based diets can produce the same growth performance as diets that contain FM or poultry by-product meal when fed with PAM feeders. Although these pond-based results indicate that feeding based on real-time demand can result in similar performance, plant-based diets are often considered to have lower attractability and/or palatability than those with fishmeal. Given the reluctance of many groups to accept plant-based diets, there is a need to not only validate but to continually improve formulations.

To further improve the performance of plant-based diets, the use of attractant supplements such as KM, SM, and FH have been tested with varying degrees of success (Nunes et al. 2006, Grey et al. 2009, Nunes et al. 2019, Leonardi et al. 2021, Soares et al. 2021). Nunes et al. (2019) determined that a KM diet supplement is a powerful feed effector and growth enhancer and Leonardi et al. (2021) showed that diets supplemented with KM resulted in significantly higher yields with significantly lower feed cost per kg shrimp. A study by Yuan et al. (2021) found that FH and SM supplements to a "bland diet" significantly increased the feed intake of shrimp. Additionally, it was observed that the SM diet was consumed at a significantly higher rate than diets containing casein and FM. In a laboratory feed trial, Soares et al. (2021) observed significantly improved growth performance in shrimp fed a KM enriched plant-based diet with no significant difference between 2% and 4% inclusion. Furthermore, a FH treatment at 4% inclusion resulted in significantly increased consumption over the basal diet.

Most previous attractant studies have been conducted in a laboratory as opposed to a production-style pond environment. Consequently, there is a disconnect between laboratory and field-based results. Under laboratory conditions, Soares et al. (2021) observed that some feed modifiers resulted in the potential improvement of shrimp production. Observing similar diets in the field would be necessary before any conclusions could be drawn on the commercial implications of these feed modifiers. Thus, our outdoor, semi-intensive pond trial utilizing PAM feeding was warranted. With the implementation of PAM feeders, shrimp can be fed based on demand, thereby allowing for the evaluation of feed effectors under field conditions. Allowing shrimp the ability to self-regulate feed inputs permitted them to feed to satiation and therefore consume different amounts of feed. Each AQ1-equipped feeder was programmed with similar parameters with an upper feed limit of 160kg/ha/day to prevent poor water quality. In the final weeks of production, feeders are more likely to hit the upper feed limit, however the AP, KM, and SM treatments each reached the upper feed limit less than 1.5% of the production cycle on average. The FH treatment reached the upper feed limit approximately 13% of the production cycle on average. This indicates that feed inputs were not restricted for the majority of the production cycle and that FH resulted in higher feeding activity.

Further evidence of FH increasing the intensity of feeding activity was observed in the feed input and feed cost results of the outdoor pond trial. Significantly more of the FH diet was fed to respective ponds than the AP diet, which correspondingly led to a higher feed cost of the FH diet. These results indicate that the acoustic response was substantially higher in the FH ponds which suggests that FH is a powerful attractant. Increased attractability of diets supplemented with FH corresponds with the results of Soares et al. (2021), albeit they also observed significantly increased attractability and growth performance in shrimp fed diets with KM. No other significant differences were observed for major production parameters in the pond trial; however, a proximate whole-body analysis of the shrimp revealed a statistically significant difference in iron content between the AP and FH treatments. This difference is unlikely to be the result of feed formulation and may be the result of variation between ponds. Although the data likely doesn't apply to feed formulations, whole-body analysis results are crucial for creating a record of shrimp characteristics that can be compared to many different production cycles in the future.

To further develop a record and to evaluate general health, an analysis of the shrimp hemolymph was conducted with a VetScan VS2 analyzer. Hemolymph enzymes such as alkaline phosphatase and alanine transferase are crucial for the proper function of the shrimp metabolism and immune system, therefore they can be used as indicators for hepatopancreatic disorders (Abdollahi-Arpanahi 2018, Hussain 2021). However, no observable response was observed between treatments for any hemolymph chemistry measured, indicating that the addition of attractant supplements does not have a negative effect on health.

To verify data collected from the outdoor pond trial, the same four diets were tested in a green water, recirculating system. The tank-based system allowed for a more controlled culture environment than the ponds while still using the same water. PAM was not used in the RAS trial since aeration, pumps, and water flow cause too much noise for the hydrophone to accurately identify shrimp feeding activity (Bart et al. 2001, Peixoto et al. 2020, Reis et al. 2022a). Therefore, shrimp received a specified ration based on growth predictions and SFP used by Davis et al. (2006). At the end of the 9-week trial, we observed that the AP treatment had significantly lower survival than the FH treatment. Correspondingly, final biomass of the AP treatment was also significantly lower than the other treatments. Since the shrimp were being fed on a specified ration, and no sampling was conducted, the feed ration was not adjusted for mortality, thus, the FCR of the AP treatment was also significantly different than the other treatments. A proximate whole-body analysis of the shrimp revealed a statistically significant difference in phosphorus content and fat content between treatments. Although, no mean separation was observed for fat content between treatments. The slightly higher values are likely due to variation in the data and overfeeding in the in the case of the excess (+30%) treatments. The higher phosphorus content of the FH treatment may be the result of the acidity of the FH supplement decreasing the pH of the feed and thereby increasing the availability of phosphorus (Sugiura et al. 1998, Baruah et al. 2007, Romano et al. 2015). However, the FH diet in this study only had a marginally lower pH than the other diets, suggesting that diet pH is unlikely to be the sole reason for the variation in phosphorus content. The excess treatments had significantly reduced ANPR, which is likely due to the fact that shrimp were fed more protein than required. Reduced survival also reduces ANPR as feed inputs were fixed. This may explain why the AP treatment is lower than the KM, SM, and FH treatments, albeit not significantly so. The slightly poorer overall response observed in the AP and AP+30% treatments suggests that the addition of feed effectors may improve the performance of plant-based diets in RAS. Although the tank-based trial was not carried out with demand style feeding, the significant results are likely not due to restricted feeding since the excess treatments did not perform better.

Multiple replicates from the pond trial had to be removed from the data set due to low survival unrelated to treatment. Unusually intense precipitation diluted the ponds with freshwater, bringing the average salinity to 2-3ppt. While this low salinity is still within reported parameters for shrimp production(Menz and Blake 1980, Bray et al. 1994, Roy et al. 2010), it also provides better conditions for the development of harmful blue-green algae blooms (Paerl and Tucker 1995, Wangwibulkit et al. 2008). Low survival due to blue-green algae blooms (*Microcystis spp*.) resulted in the removal of three replicates (1 KM, 1 SM, 1 FH). An additional three replicates were removed due to low survival because of harvest delays and salinity changes caused by Hurricane Ida (2 SM, 1 FH). Due to the proximity of the mortality events to the final harvest, growth parameters such as weekly weight and average weekly growth rate could still be used to determine significance with at least three replicates for each treatment. However, no significant differences were observed, reinforcing the observation that the addition of attractants to plant-based diets did not significantly improve production performance in semi-intensive ponds under these culture conditions.

Semi-intensive ponds provide shrimp with natural productivity and organic matter to feed on, but formulated diets are also required for optimal growth performance (De Silva 1995, Moss and Pruder 1995, Soares et al. 2005, Roy et al. 2012). A benefit of this culture method for production aquaculture, especially in combination with PAM, is that the presence of natural productivity can contribute to general nutrition (Moriarty 1997), and animals are more likely to feed to satiation (Diana et al. 1994, Correia et al. 2003, Carvajal-Valdés et al. 2012). For research purposes, the ability to feed shrimp to satiation and model commercial production styles can provide invaluable data that is not possible in a lab. However, the primary productivity and other external factors in outdoor ponds present a source of noise, which may require more data collection to observe an effect of treatment. Such noise in the data may explain why no improvements in production were observed in our pond trial. In tank-based systems, without many external factors or abundant primary productivity, data will have better resolution. Such clarity may explain why the green water RAS trial in this study showed significant improvements in production in shrimp fed diets with an attractant. To improve the economic and environmental sustainability of the shrimp farming industry, it is critical to further research into the complex relationship between feed effectors, plant-based diets, and production-style pond culture.

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