An Evaluation of Feed Management, the Use of Automatic Feeders, and Feed Leaching in the Culture of Pacific White Shrimp *Litopenaeus vannamei*

by

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Abstract

Feed management is widely considered to be one of the most important factors of shrimp production, however it has been grossly under studied using standardized methods. New and improving technology is making the use of automatic feeders more realistic for many farmers and could reduce the feeding labor required for production. The objectives of the pond studies were to examine the impact of: 1) Number of daily feedings 2) daily feeding rate and 3) the use of acoustic feedback; on the growth and production performance of Pacific white shrimp *Litopenaeus vannamei* grown in ponds. Another related aspect of this works was the examination of the leaching of commercial feeds to help understand the mechanism of the benefit of increased daily feedings. One theory suggested by the pond trials was that the increased growth could be attributed to the shorter amount of time that the feed is in the water before being consumed. Shortening this time would result in less nutrients leaching from the feed. Hence, a tank based trial was conducted to evaluate the impact of leaching on the available nutrients in a feed and the growth of shrimp fed the leached feed. All experiments were conducted at the Alabama Department of Natural Resources in Claude Peteet Mariculture Center, Gulf Shores, AL, USA. The two pond trials were performed in 16, 0.1 ha ponds that were stocked at 17 shrimp/m^2 (trial 1) and 38 shrimp/m^2 (trial 2). Both trials examined the use of a standard feeding protocol (SFP)(1.3 g/wk, 1.2 FCR, and 1.5% weekly mortality), solar timer feeders, and AQ1 Systems acoustic feedback system. The feed leaching trial was performed in 24, round 800L tanks with a daily 5% water exchange from a shrimp production pond. Findings

of the pond trials showed that increasing the number of daily feedings from 2 to 6 resulted in improved growth and increased shrimp values. Increasing the daily feedings also allowed for a higher daily ration without significantly impacting the FCR. The AQ1 treatment also produced larger shrimp than the timer or SFP treatments in both trials. The larger shrimp resulted in a higher total yield and a higher shrimp value. An economic analysis performed on trial 1 demonstrated that the increased shrimp value of both the timer and AQ1 treatments can offset the costs of implementation in as little as one production cycle. The leaching trial confirmed a decreased biological value of leached feed as the long the feed was pre-leached the poorer the growth rate of the shrimp. These results confirm that the use of automated feeding systems reduced the time feed was in the water prior to consumption and increased the availability of feed over time. Both factors result in improved growth rates of the shrimp resulting in significant improvements in total biomass production under the same time period. These results, also confirm the efficacy of automatic feedback systems for the use in growout ponds.

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Introduction

Pacific white shrimp *Litopenaeus vannamei* are one of the most important aquaculture species grown with 3.9 million metric tons produced in 2015, which accounted for 53% of all farmed crustaceans (FAO 2015). Feed management is an integral part of the production of Pacific white shrimp, *Litopenaeus vannamei*, but has been neglected in the research to improve production as compared to diet studies (Carvalho and Nunes 2006).

Shrimp are naturally grazers, and therefore will tend to eat slowly and variably. Fox *et al.* (2001) takes this into consideration and states that feed should be applied frequently and only what will be consumed quickly to improve growth and minimize waste. This type of feed application is problemic because it is typically not cost effective to feed more than 2-4 times per day and monitoring feed consumption using feeding trays is labor intensive (Jory 1995, Ullman *et al.* 2017b). Programable feeders allow for feeding strategies that increase the number of feedings per day without increasing feeding labor (Bador *et al.* 2013). Acoustic technology, as seen in the AQ1 SF200 (AQ1 Systems, Tasmania, Australia), is capable of adjusting the feeding rate according to the feeding activity. This can help to reduce waste and increase growth because feed is only offered when the shrimp are actively feeding (Napaumpaiporn *et al.* 2013).

Feed is often one of the largest individual variable cost in an aquaculture enterprise. This indicates that significant savings can be made by properly adjusting the feeding rates and application to avoid waste (De Silva 1989). Reducing wasted feed not only has economic savings, but also reduces the waste products in a pond. Because feed is the original source of all pond waste products, proper feed management can significantly decrease the ammonia levels and biological oxygen demand (Boyd and Tucker 1998, Jory 2016).

Another problem related to mismanaged feeding in shrimp production is that of nutrients leaching out of the feed. When feed is not consumed quickly, it can lose nutrients into the water, where they are no longer available for the shrimp to consume (Carvalho and Nunes 2006). Substantial research has focused on improving diet formulations for shrimp (Sookying *et al.* 2011), but these formulations are irrelevant if the nutrients are not available for the shrimp to consume.

These series of studies were performed to improve the understanding of how feed management can be modified to improve the growth and performance of *L. vannamei* while reducing waste.

Chapter I: Effects of Four Different Feeding Techniques on the Pond Culture of Pacific White Shrimp *Litopenaeus vannamei*.

Abstract

The purpose of this study was to examine the effects of four feed management techniques on the production of Pacific white shrimp (*Litopenaeus vannamei*) using a 16-week trial performed in 16, 0.1 ha ponds stocked at 17 shrimp/m^2. Four treatments were used for this study; standard feeding protocol (SFP) fed twice daily, a 15% increase to the SFP fed twice daily, timer feeders programmed to feed 6 times daily following the SFP, and ad libitum using the AQ1 acoustic demand feeding system. Significant increases in final weights were seen using the timer and acoustic feeders (28.66 and 35.91 g respectively) when compared to the SFP and SFP + 15% (23.55 and 24.65 g respectively). The AQ1 treatment also resulted in significantly higher shrimp value ($21,198/ha) than the other treatments ($11,776 - $13,446/ha). No significant differences were seen in survival (72.2 ± 5.99%), FCR (1.03 ± 0.095), or water quality. Results demonstrate that increasing feed by 15% when feeding twice daily gives no advantage to production. Increasing feedings from 2 to 6 times per day did improve growth and economic

returns, and using acoustic feedback to feed based on feeding activity can further improve production and economic returns.

Introduction

Pacific white shrimp *Litopenaeus vannamei* is regarded as the primary cultured shrimp species worldwide and a high value commodity with around 3.8 million metric tons produced in 2015 (FAO 2015). Extensive research has focused on improving shrimp feed and reducing the animal protein needed in the diets (Sookying et al. 2011), but little has focused on improving the application and management of that feed (Carvalho and Nunes 2006). According to De Silva (1989), significant savings can be made on the feed costs with proper application.

Feed is the original source of all waste products in a pond system, which contributes to the degradation of water quality through increased ammonia and biological oxygen demand. According to Jory (1995) and Boyd and Tucker (1998) feed management can have a significant impact on water quality in pond systems. Therefore, over feeding, in addition to increasing feed costs, is likely to increase pollution in the pond, which results in increased cost of managing the water quality (Kaushik 2000).

One way of reducing wasted feed is by only applying feed that will be quickly consumed by the shrimp. Fox et al. (2001) states that feed should be applied frequently and only what will be consumed quickly in order to improve growth and reduce waste. Historically, feed was applied and consumption was monitored using manual labor to spread the feed and check feeding trays. Automatic feeders are gaining popularity because they can provide more feedings per day and require less labor than manual feeding (Jory 2016). Another development involves the use of hydrophones to monitor the feeding response of the shrimp in the pond to determine feeding activity and then feed based upon that response (Bador et al. 2013, Napaumpaiporn et al. 2013). All of these technology shifts can impact both production and water quality.

Improvements to feeding methods in shrimp production have been identified as an important part of improving shrimp production overall, but little work has been performed to demonstrate effective methods. Proper feed management facilitates efficient feed consumption, minimizes waste, and prevents overfeeding. Therefore, an understanding of feed management principles is critical for the efficient expansion of the industry. The objective of this study was to examine different feeding strategies to determine the impact on growth, production, and economic returns on the semi-intensive culture of Pacific white shrimp.

Materials and Methods

This trial was performed at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center, Gulf Shores, AL. Pacific white shrimp L. vannamei (2.06mg initial wt.) were obtained from Shrimp Improvement Systems(Islamorada, FL) and acclimated then nursed in a greenhouse according to the procedures described in Zelaya (2005) for 17 days prior to stocking in production ponds. The production cycle was performed in 16, 0.1 ha ponds and lasted for 16 weeks.

Juvenile L. vannamei (initial weight of 0.07 g) were collected from the nursery system and stocked at 17 shrimp/m^2 in the production ponds. The ponds used for the grow-out were approximately 0.1 ha in surface area (46 x 20 x 1.0m) and lined with 1.52 mm high-density polyethylene lining with a 25 cm layer of sandy-loam soil on the bottom. Ponds were filled using brackish water (11-13 g/L), filtered through a 250 µm sock, from the Intracoastal Canal between Mobile and Perdido Bay, Alabama and water exchange was minimized. Inorganic liquid fertilizers (1697 ml of 32-0-0 and 303 ml of 10-34-0) were added to the pond 14 days before stocking and reapplied at half the rate 10 days later later. Ponds that had a Secchi disk reading of more than 50 cm at the time of stocking received another fertilization treatment at half of the original strength. All ponds were aerated with a 1hp Airolator (Aquarian™, Air-O-Lator, Kansas City, Missouri) and used a 1 or 2-hp Aire-O_2 (Aire-O_2, Aeration Industries International, Inc. Minneapolis, Minnesota) as backup and/or supplemental aeration to try to maintain DO above 3 mg/L.

Feed and Treatments

The shrimp were fed a 1.5 mm commercial starter diet (40% CP and 9% CL) produced by Zeigler, Inc. (Gardners, Pennsylvania, USA) for the first four weeks and all treatments received equal amounts of feed twice per day by hand feeding. From weeks 4 – 16, a 2.4 mm high plant-based diet production diet (Table 1.) (36% CP and 8% CL) produced by Ziegler, Inc. was fed according to the treatments.

The four treatments used to evaluate the potential for automation were a Standard Feeding Protocol (SFP), SFP+15%, Timer feeding, and AQ1 feeding system (AQ1 Systems, Tasmania, Australia). The SFP was calculated based on an expected weight gain of 1.3g wk^{-1}, a feed conversion of 1.2, and a survival of 75% during the culture period. SFP+15% included a 15% increase in daily ration for the final 8 weeks of growth. The solar timer feeders (FIAP GmbH, Ursensollen Germany) were intended to feed using the same ration as the SFP, but fed at a rate similar to the SFP+15% due to feedings based on full seconds/feeding which rounded feed amount up. The AQ1 feeding system fed ad libitum using a hydrophone and computer software to monitor the feeding activity. The SFP and SFP+15 treatments were fed by hand

Table 1. Diet composition and analysis. Feed ingredients were sourced and diets manufactured by Zeigler, Inc. (Gardners, Pennsylvania, USA) using extrusion processing. Proximate analysis and ingredient composition of the high soy diet fed for weeks 4-16 in all treatments. Analysis was performed by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed as g/100g.

Ingredient	**g/100g**
Soybean meal 47.5%	50.00
Wheat	23.10
Poultry by-product meal	8.00
Corn gluten meal 60%	8.00
Dicalcium phosphate	3.13
Fish oil- Topdress	3.00
Fish oil- Mix	2.00
Bentonite	1.50
Lecithin	1.00
Vitamin Premix[b]	0.12
Mineral Premix[b]	0.12
Tiger C-35	0.02
Copper Sulfate	0.01
Component	**g/100g**
Phosphorus	1.20
Crude Protein[a]	37.22
Moisture	10.18
Crude Fat	7.01
Crude Fiber	2.56
Ash	8.55

[a] Percentage N x 6.25
[b] Vitamin and mineral premixes are proprietary products and therefore the composition is not listed.

twice daily (0800 and 1600 h). The timer treatment received feed 6 times daily (0800, 1000, 1200, 1400, 1600, and 1800 h). Timer and AQ1 treatments were non-randomly assigned to ponds due to constraints to the power supply and the SFP and SFP+15 treatments were randomly assigned to the remaining ponds. Feed was not offered at night due to the potential for water quality issues (Boyd 2015). Also, feeding was not possible at night in the AQ1 treatment due to sound interference with the aerators, an artifact of the small pond size.

Sampling and Water Quality

Shrimp were sampled weekly throughout the experimental period using a cast net (1.22 m radius and 0.95 cm mesh) to collect approximately 60 shrimp per pond. Weights were recorded and individual shrimp were visually inspected for general health. Ponds were monitored at least three times each day (dissolved oxygen, temperature, salinity, and pH), just before sunrise (0430 - 0500h), during the day (1400 – 1430 h), and at sunset (1900 – 2000 h) using a YSI ProPlus meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Secchi disk readings and total ammonia nitrogen (TAN) were monitored on a weekly basis. Water samples were taken in the morning at the bank and TAN was determined using an Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Water samples were also collected at weeks 0, 4, and 8 – 16 and shipped overnight, on ice, to Auburn, Alabama for more in-depth analysis. The process for that analysis is described in Jescovitch *et al.* (2017).

Harvest

The ponds were harvested over two days at the end of the 16-week culture period. The ponds were drained by about two thirds and aeration was provided using the Airolator the night before the pond was due to be harvested. The remaining water was drained and the shrimp were pumped out of the catch basin using a hydraulic fish pump equipped with a 25 cm diameter suction pipe (Aqualife-Life pump, Magic Valley Heli-arc and Manufacturing, Twin Falls, Idaho, USA). The pump was placed in the catch basin and shrimp pumped, de-watered, and collected into a hauling truck. Shrimp were rinsed, weighed in bulk and 150 were randomly selected to measure individual weights and size distribution.

Economic Analysis

The economic returns of each treatment was evaluated using an enterprise budget and treatments were compared using a partial budget following the formats in Engle (2010). The value of the shrimp was estimated using the harvest size distribution and prices reported for Latin American farmed white shrimp by Urner Barry using the three year average (2014-2016) (Urner Barry, Toms River, New Jersey, USA). Shrimp sizes and harvest quantities in the economic analysis were averaged between treatments for values that were not significantly different.

Statistical Analysis

Statistical analysis of the data was conducted with SAS 9.4 (SAS Institute, Cary, North Carolina, USA) to perform a one-way analysis of variance to determine significant difference (p-value < 0.05) among treatments. Student-Neuman-Keuls multiple range test was used to determine differences among treatments.

Results

Water quality parameters were maintained within typical ranges for shrimp production throughout the study (Table 2). Final mean individual weights at harvest were 23.55, 24.65, 28.66, and 37.32 g for the SFP, SFP + 15%, Timer, and AQ1 treatments respectively (Fig. 1). Survival was between 66.9% and 75.8%, and FCR ranged from 0.94 to 1.11, but no significant differences were seen between treatments (Table 3). Two ponds were excluded from the data. One pond from the Timer treatment was excluded due to high mortality in week 10 that resulted from a low DO incident. One pond was also excluded from the AQ1 treatment due to mechanical failure that resulted in significantly different feeding from the rest of the treatment.

Figure 2 shows the distribution of the harvested shrimp by size class as a percentage and the associated price per kg. The AQ1 treatment produced the majority of its shrimp (87%) in a larger size class (24-34 shrimp/kg, $9.24/kg) than the other treatments, which all produced the majority in the 35-45 shrimp/kg class ($8.12/kg). The feed input and feed cost was significantly higher in the AQ1 treatment (5407 kg/ha and $5289/ha) than all other treatments, with the lowest being the SFP (2586 kg/ha and $2841/ha) (Table 4). The value of the shrimp produced was significantly higher in the AQ1 treatment ($44,609/ha), but did not vary significantly in the SFP, SFP + 15%, or Timer treatments ($21,665/ha, $24,483/ha, and $26,761/ha) (Table 4).

Table 5 shows the partial budget that is used to assess the differences in costs and benefits for each of the treatments as shown in the enterprise budget (appendix 1). The partial budget shows that the AQ1 treatment had a net benefit of $10,153 per ha over the average of

Table 2. Summary of water quality parameters observed over the 16-week culture period for each of the four treatments. The values are shown as mean ± standard deviation. Minimum and maximum values are shown in parenthesis.

	SFP	SFP + 15%	Timer	AQ1
Morning DO[a] (mg/L)	4.54 ± 0.89 (1.48, 9.44)	4.60 ± 0.84 (1.52, 7.13)	4.53 ± 0.80 (1.92, 7.85)	4.33 ± 0.78 (1.55, 7.62)
Afternoon DO (mg/L)	9.84 ± 2.36 (3.65, 19.41)	9.10 ± 2.10 (3.34, 17.21)	8.90 ± 1.79 (2.70, 14.68)	8.95 ± 2.13 (3.12, 15.97)
Evening DO (mg/L)	8.99 ± 2.42 (3.28, 16.66)	8.36 ± 2.19 (2.61, 15.29)	7.67 ± 1.86 (1.16, 14.28)	7.88 ± 2.16 (2.09, 14.85)
Salinity (g/L)	11.89 ± 1.32 (9.52, 16.43)	11.70 ± 1.07 (9.92, 15.74)	11.89 ± 1.14 (9.82, 15.32)	11.93 ± 1.14 (9.95, 15.15)
pH	8.68 ± 0.64 (7.35, 10.17)	8.64 ± 0.59 (7.35, 10.17)	8.52 ± 0.57 (7.35, 9.77)	8.57 ± 0.58 (7.38, 9.87)
Temperature (°C)	31.4 ± 1.9 (22.5, 36.6)	31.4 ± 1.8 (25.7, 36.1)	31.4 ± 1.8 (26.3, 34.8)	31.6 ± 1.8 (26.3, 35.3)
TAN[b] (mg/L)	0.16 ± 0.51 (0.00, 3.00)	0.04 ± 0.11 (0.00, 0.50)	0.16 ± 0.62 (0.00, 4.00)	0.11 ± 0.24 (0.00, 1.00)
Secchi (cm)	52 ± 38 (10, 140)	61 ± 44 (15, 145)	55 ± 34 (15, 150)	60 ± 44 (15, 150)

[a] DO: Dissolved Oxygen

[b] TAN: Total ammonia-nitrogen

Figure 1. Final weight (g) and feed inputs (kg/ha) at the end of the 16 week trial.

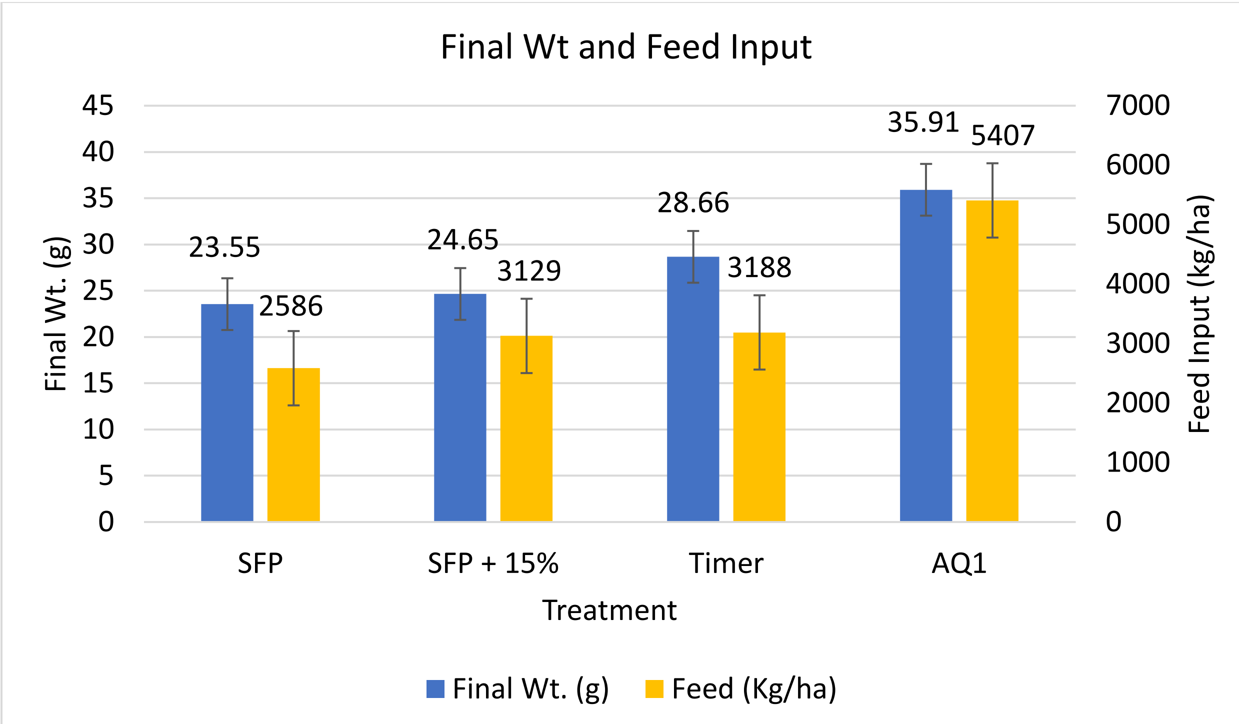


Table 3. Production results for Pacific white shrimp cultured in 0.1 ha earthen ponds over a 16-week culture period using varying feeding strategies including a standard feeding protocol (SFP), SFP with a 15% increase in feed inputs, automatic feeder with a timer, as well as an automatic feeder with acoustic feedback (AQ1)[1].

Treatment	Yield (kg/ha)	Final wt. (g)	Survival (%)	Feed conversion ratio	Weekly Growth Rate (g/wk)	Total Feed Input (kg/ha)
SFP	3068.5[a]	23.55[a]	75.8	0.94	1.47[a]	2586[a]
SFP + 15%	3032.5[a]	24.65[a]	72.2	1.04	1.54[a]	3129[a]
Timer[2]	3294.3[a]	28.66[b]	66.9	0.98	1.79[b]	3188[a]
AQ1[2]	4568.8[b]	35.91[c]	73.9	1.14	2.33[c]	5407[b]
P-Value	0.0016	<0.0001	0.3112	0.0598	<.0001	<0.0001
PSE[3]	226.6	1.1703	2.9779	0.0468	0.0593	169.84

[1] Mean values (n=4) in the same column with different superscripts are significantly different ($P < 0.05$) based on analysis of variance followed by Student Newman-Keuls multiple range test.

[2] n=3

[3] Pooled Standard Error

Table 4. Partial cost and value of shrimp cultured over a 16-week production period in ponds and maintained using four different feeding strategies.

Treatment	Total Feed Input (kg/ha)	Feed Cost ($/ha)	Shrimp Value ($/ha)	Partial Income[3] ($/ha)
SFP	2586[a]	2841[a]	21,665[a]	18,824[a]
SFP + 15%	3129[a]	3102[a]	24,483[a]	21,381[a]
Timer[2]	3188[a]	3159[a]	26,761[a]	23,602[a]
AQ1[2]	5407[b]	5289[b]	44,609[b]	39,320[b]
P-Value	<0.0001	<0.0001	<0.0001	0.0004
PSE	169.84	163.05	837.33	769.34

[1] Mean values (n=4) in the same column with different superscripts are significantly different ($P < 0.05$) based on analysis of variance followed by Student Newman-Keuls multiple range test.

[2] n=3

[3] Partial income= shrimp value-feed cost

[4] Pooled Standard Error

Table 5. Partial budget describing the change in benefits and costs for the Timer and AQ1 treatments compared to the SFP in the enterprise budget (appendix 1).

		Timer	AQ1
Category	**Description**	**Value or Cost**	**Value or Cost**
Benefits			
Additional Revenue			
	Change in Shrimp Size and/or Yield	$2,160	$20,478
Reduced Costs			
	Feeding Labor	$570	$570
	Interest on Operating Costs	$34	0
Total Additional Benefits		**$2,764**	**$21,048**
Costs			
Additional Costs			
	Change in Shrimp Feed Fed, 2.5 mm	$0	$2,383
	Feed freight, 2.5 mm	$0	$590
	Labor - checking DO	$0	$570
	AQ1 battery	$10	$0
	Software license	$0	$3,500
	Internet	$0	$1,200
	Interest on Operating Costs	$0	$460
	Depreciation (capital items, equipment and machinery)	$440	$1,152
	Interest on Loans (capital items, equipment and machinery	$88	$227
	Repairs & Maintenance (capital items, equipment and machinery)	$9	$813
Reduced Revenue		--	--
Total Additional Costs		**$547**	**$10,895**
Net Benefit per ha		**$2,217**	**$10,153**

the SFP and SFP + 15% and the timer treatment also out produced the SFP and SFP + 15% average by $2,261 per ha.

Discussion

Traditional feeding tables have been reported as typically underfeeding during the first 40 days of production and over feeding during the second half (Zeigler 2014). Site specific improvements to traditional feeding tables has the potential to improve shrimp production by reducing the cost of feed and improving water quality through reduced waste. Davis *et al.* (2006) reported reductions in FCR and increases in growth and final weight when comparing the SFP to traditional feed management techniques at this site. Work conducted by Van *et al.* (2017) indicated that adjusting the SFP at two feedings per day could make some improvements but they were minimal.

One reason for two feedings per day having limits is the fact that the feed is in the water for an extended period of time prior to consumption. It is well established that the nutrient profile of a feed will shift as it is immersed in water. For example, studies by Carvalho and Nunes (2006) as well as Watson *et al.* (2015) indicate a direct relationship between the amount of time that feed is in the water and the amount of nutrients that leach out of it. In our research, shrimp offered feed using the timed feeder at 6 feedings per day were larger than those maintained on the SFP or SFP+15%, which received feed only twice per day. Given that feed intake of shrimp is relatively slow, one can infer that when offering two feedings per day feed is consumed over a longer period of time (or not consumed at all) and thus has lower nutritive values. Therefore, when comparing 2 vs 6 feedings per day one of the improvements is likely due to the reduced amount of time the feed is in the water.

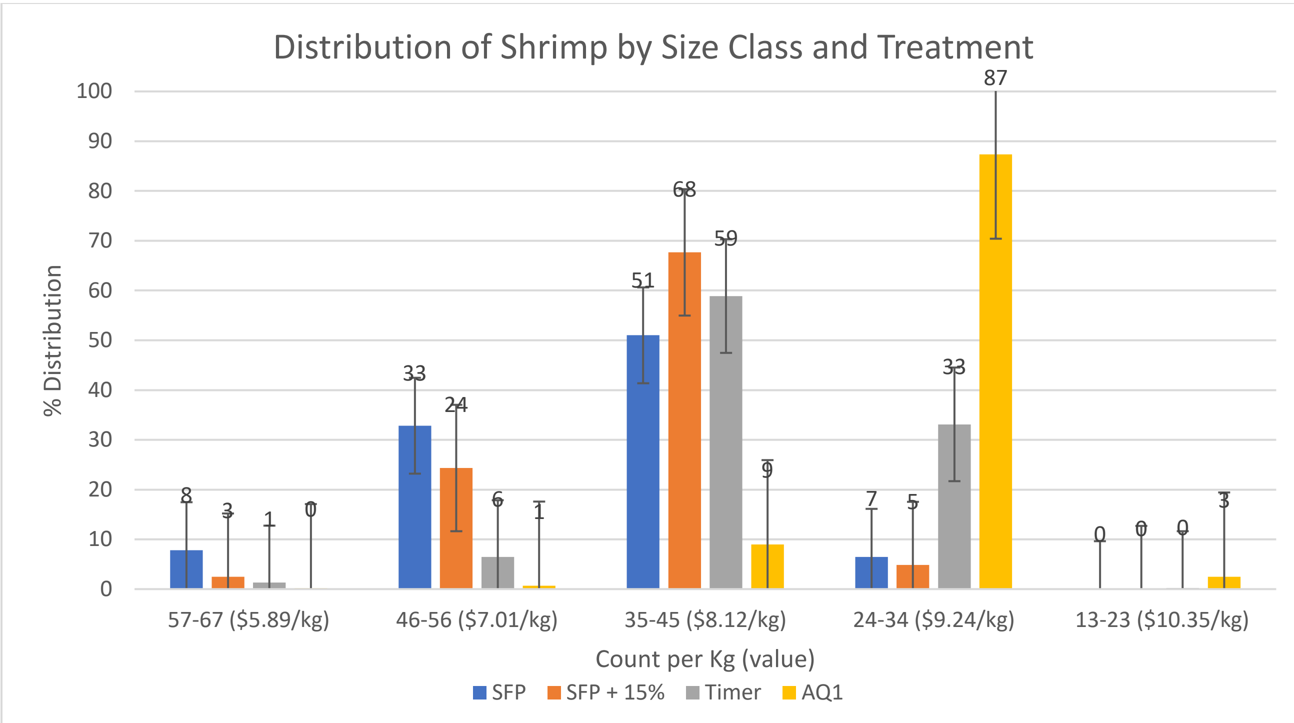
The results from this study indicate that increasing the amount of feed without increasing the number of feedings does not have a significant impact on the growth between the SFP and SFP+15% treatments (table 3 and Fig 1). These results are supported by the results from Van et al. (2017), which used the same standard feeding protocol and did not find improvements when increasing the feed by 10% in green-water tanks. Comparing the Timer treatment to the SFP+15% in the present study, it is seen that the 15% increase in feed is utilized when the number of feedings is increased, resulting in a significantly higher mean weight.

Robertson *et al.* (1993) indicated improved production when moving from 1 to 4 feedings per day, but Velasco *et al.* (1999) did not record any improvements when increasing the feeding beyond 1 time per day. Some potential reasons that this trial did see differences when moving from 2 to 6 feedings are differences in the experimental protocols. This would include difference in culture habitat (ponds vs. tanks), feed formulation (36% vs. 19.5% protein), and length of study (112 days vs 20 and 28 days). While the present study did result in a higher final weight when feeding 6 times per day as compared to 2 feedings per day (Fig. 1), the final shrimp biomass was not significantly different than the SFP or SFP+15%, indicating that the lower survival could have contributed to the increased final weight. While the 6 feedings per day did not result in significantly higher production, the AQ1 treatment did while feeding more feed and many more times per day. This may be due to the fact that the SFP was designed using 2 feedings per day and is optimized at that level, therefore the feeding protocol will need to be modified when feeding more frequently.

The final FCR and growth rates were better than what was anticipated and survival was similar to what was expected according to the SFP. The SFP fed with an anticipated FCR of 1.2, growth of 1.3 g/wk and 75% survival, but the results were 0.94, 1.47 g/wk and 75.8% for FCR, weekly growth and survival, respectively (Table 3). Similar SFP has been used in other reports from this group. Sookying et al. (2011), which grew shrimp at the same density and an equivalent SFP, reported an FCR of 1.17, growth at 1.6 g/wk, and a survival of 61%. Venero *et al.* (2007) reported an FCR of 1.0, growth at 1.38 g/wk, and final survival of 88% for shrimp grown at the same location. The consistently low FCR indicate that the SFP used is conservative and that natural productivity in the pond plays a significant role at this location. Roy *et al.* (2012) used a similar feeding protocol (FCR and growth rate: 1.25 and 1.5 g/wk) fed twice daily that showed no significant difference in final weight for juvenile shrimp (initial weight 0.772 g) grown in greenwater tanks when the feeding ration was reduced by 40%. Roy et al. (2012) also grew shrimp in greenwater tanks without any feed input to 1.90 g (initial weight 0.25g) with 98% survival. All of this supports restricting the feed inputs when feeding twice daily to increase the use of natural productivity for growth.

Producing the highest value of shrimp per unit of input is the goal of most shrimp production operations. Income for shrimp sales are driven not only by the total weight of the shrimp harvested, but also by the individual weights, with larger shrimp commanding a higher price. Figure 2 shows the distribution of harvested shrimp by size class as a percentage. The AQ1 and timer treatments produced a higher proportion of their total production as shrimp in

Figure 2. Percent of each treatment that falls into each size class in count per kg.



larger size classes than the SFP or SFP+15%. The AQ1 treatments also produced a higher proportion of its shrimp within a single size class than the SFP or SFP+15%. Ly *et al.* (2005) observed a similar decrease in size variation with increased feedings in juvenile orange-spotted grouper Epinephelus coioides, which may indicate less competition for feed and more even feeding between individuals. Table 4 shows that the value of the shrimp produced by the AQ1 treatment ($44,609/ha) was 60% more than that produced by the Timer treatment ($26,761/ha), 55% more than the SFP + 15% ($24,483/ha), and 49% more than the SFP ($21,665/ha).

Table 5 shows the partial budget that compares the Timer and AQ1 treatments to the average of the SFP and SFP + 15% as described in the enterprise budget (appendix 1). Some equipment for the AQ1 treatment can be shared between multiple ponds (SF200 Base Station- 2 ponds, Software License- up to 10 ponds, computer- up to 10 ponds), therefore the equipment costs do not scale linearly. It should also be noted that since this is a research facility, the labor required is an estimate based on the working schedule and equipment available. While statistical analysis could not be performed on the budget, the results here are consistent with the observations of Bador et al. (2013) in that the AQ1 treatment grossly out produced all other treatments.

On site water quality analyzed in this study showed no significant differences between treatments for all parameters that were analyzed. Jescovitch et al. (2017) performed further analysis on the water samples and found TAN to be significantly higher in the SFP + 15% treatment for week 10 and in the AQ1 treatment in week 14. Nitrite-nitrogen was also found to be significantly higher in the SFP treatment in week 11 and in the AQ1 treatment in week 15.

These levels were maintained within the suitable levels for shrimp production and did not cause any apparent mortalities. The ponds were clearly capable of processing the nutrient load presented under the reported conditions. However, it should be emphasized that the automated systems increased the feed inputs, which if employed at higher densities may exceed the processing capacity of the system. Consequently, the implementation of automated systems at higher densities must consider the increased load placed on the system.

Conclusion

The results of this study clearly indicate an advantage in production for acoustic feeding systems over traditional hand feeding systems using two feedings per day. It also implies that improvements can be made by increasing the number of feedings per day using less sophisticated automated feeders. Based on this and previous research, increasing the amount of feed without concurrent increases in feeding does not result in improved production. Presumably, because intake is already optimized for two feedings per day. The Timer and AQ1 treatments represent a significant investment, but also show that the increased returns can pay off those investments in as little as one production cycle. Future research on this subject should focus on refining feeding and production protocols when using automated feeding systems.

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Chapter II: Feed Management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*

Abstract

The purpose of this study was to examine the effects of using automatic feeding and acoustic feedback systems on the pond culture of Pacific white shrimp *Litopenaeus vannamei*. This trial was performed using 16, 0.1 ha ponds stocked at 38 shrimp/m^2 and grown for 13 weeks. The treatments used were a standard feeding protocol (SFP) fed twice daily, a Timer treatment fed a 15% increase to the SFP offering feed 6 times per day (Timer 15), a timer treatment fed a 30% increase to the SFP offering feed 6 times per day (Timer 30), and the AQ1 acoustic feedback system that fed based on feeding activity (AQ1). At the conclusion of the pond production trial, final individual weights of the shrimp were significantly different for all treatments 19.74 g, 25.15 g, 27.52 g, and 32.04 g for the SFP, Timer 15, Timer 30, and AQ1 treatments respectively. The shrimp yield for the AQ1 treatment (7430 kg/ha) was also significantly higher than the SFP (4843 kg/ha) or Timer 15 (5629 kg/ha) treatments. The AQ1 treatment also produced a significantly higher value of shrimp ($65,587/ha) than the SFP ($32,982/ha) or the Timer 15 ($44,279/ha). No significant differences were seen in FCR (1.07 – 1.24) or survival (58.5 – 63.9%). The results demonstrated that increasing the number of daily feedings from 2 to 6 can allow for a higher daily feeding rate, which translated into higher

growth and production. Improvements of feed inputs using feedback technologies improved the production as the number of feedings and quantity of feed was increased, resulting in further improvements in production. The results of this study indicate that increasing the number of daily feedings in the pond production of shrimp has the potential to significantly increase growth and can reduce the labor requirements for feeding.

Introduction

Feed management is an integral part of all aquaculture practices and becomes particularly difficult in shrimp production due to the difficulty of monitoring consumption. It has been reported that when traditional feeding tables are used in shrimp production, the shrimp are often under fed in the first 40 days of production and over fed during the second half of the cycle (Zeigler 2014). Despite the problem of feed management, little research has focused on improving the feeding techniques in shrimp production (Carvalho and Nunes 2006). Ullman *et al.* (2017a) and Bador et al. (2013) have shown that the use of modern equipment and technology to spread out the feeding and feed according to activity can have significant improvements on the size, yield, and returns in semi-intensive shrimp culture.

Shrimp are typically slow, continuous feeders and it is beneficial to growth and production to feed small meals frequently to provide a constant source of fresh feeds. Ullman et al. (2017a) demonstrated that increasing the number of feeding daily from 2 to 6 resulted in a significant increase in final weight. This could be a result of less physical breakdown of feed before consumption as it is not in the water as long (Obaldo *et al.* 2002). Lim and Cuzon (1994), suggest that feed stability requirements could be reduced if diets are attractive and consumed quickly. This could also be relevant to feed management as feeds with lower stability may be formulated with lower cost ingredients.

Another benefit of dynamic feed management is the potential for reduced waste from feed that is fed, but not consumed. Over feeding has also been shown to increase pollution in a pond and increase the cost of managing the water quality (Kaushik 2000). Without a way to monitor feed intake, there is no way to adjust the amount of feed to prevent over feeding the shrimp.

The use of feeding trays have been widely implemented to adjust the feeding rates according to consumption (Vicava 1995) and is probably the most common feed management technique. Casillas-Hernandez *et al.* (2007) used a standard feeding table and feeding trays to examine the effects of adjusting the feed based on demand and showed a significant increase in individual growth and overall yield of *Litopenaeus vannamei* with no change in FCR in a 29 week pond study. This supports the use of feeding trays, but the increased labor that is required to manually monitor the consumption may outweigh the production benefits (Jory 2016, Ullman et al. 2017b). The high labor requirements can also limit the number of feedings

Ullman et al. (2017a) showed that feeding 6 times per day, and the use of the acoustic feedback system in the AQ1 (AQ1 Systems, Tasmania, Australia) are capable of increasing the feeding rate and the related production results. Presently there is limited information of feed management and in particular automated feeding systems. Hence the objective of this study was to examine the effects of increasing the daily feeding rate when feeding 6 times per day and to evaluate the efficacy of the AQ1 system when growing *L. vannamei* stocked at 38 shrimp/m^2.

Materials and Methods

Pacific white shrimp *L. vannamei* post larvae (2.05 mg initial wt.) were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and were nursed for 13 days following the procedures outlined in (Zelaya 2005) prior to stocking. The trial was performed over 13 weeks in 16, 0.1 ha ponds located at the Claude Peteet Mariculture Center, Alabama Department of Conservation and Natural Resources (Gulf Shores, AL, USA).

Juvenile *L. vannamei* (initial weight 0.04g) were stocked from the nursery into the ponds at a density of 38 shrimp/m^2. The ponds used for the trial were approximately 0.1 ha in surface area (46 x 20 x 1.0m), lined with 1.52mm high-density polyethylene lining, and had a 25 cm layer of sandy-loam soil as a substrate. Ponds were filled using brackish water (8.5 – 9.8 g/L), filtered through a 250 µm sock, from the Intracoastal Canal between Mobile Bay and Perdido Bay, Alabama and water exchange was minimized. Inorganic liquid fertilizers (1697 ml of 32-0-0 and 303 ml of 10-34-0) were added to the pond eleven days before stocking and reapplied at half the rate three days before stocking to ponds that had a Secchi disk reading of more than 50 cm. Some ponds required a treatment of agricultural lime at day 25 and were treated with 7.2 kg/ha if alkalinity was below 20 ppm and 5.4 kg/ha if alkalinity was 20 – 30 ppm. All ponds were aerated using a 1 hp aerator (Aquarian™, Air-O-Lator, Kansas City, MO, USA) and used a 1 or 2 hp aerator (Aire-O_2, Aeration Industries International, Inc. Minneapolis, MN, USA) as backup and/or supplemental aeration to maintain DO above 3 mg/L

Feed and Treatments

The four treatments used in this trial were a Standard Feeding Protocol (SFP), a timer feeding treatment that fed a 15% increase to the SFP (Timer 15), a timer feeding treatment that fed a 30% increase to the SFP (Timer 30), and the AQ1 shrimp feeding system. The SFP was calculated based on an expected weight gain of 1.3 g/wk, a FCR of 1.2, and a weekly mortality of 1.5% during the 13 week culture period. The SFP treatment was fed twice per day (0800 and 1600 h) by hand spreading the feed. Both of the timer treatments used solar battery powered feeders (Solarfütterer, FIAP GmbH, Ursensollen, Germany) that are programed to feed the daily ration over 6 feedings (0800, 1000, 1200, 1400, 1600, and 1800 h). The AQ1 system fed ad libitum using a hydrophone and computer software to monitor the feeding activity and adjust the feed input accordingly. The AQ1 treatment was not randomly assigned to ponds due to the constraints of the power supply with adjacent ponds sharing a controller. The SFP, Timer 15, and Timer 30 treatments were randomly assigned to the remaining ponds.

A 1.5mm commercial starter diet (40% CP and 9% CL) produced by Zeigler, Inc. (Gardners, PA, USA) was fed for the first 15 days and all treatments received equal amounts of feed twice per day by hand feeding. Starting on day 16, a commercial semi-intensive grower diet (CP 35% and CL 7%) (Shrimp Grower SI-35) produced by Zeigler, Inc. was used for the remainder of the trial. Timer feeding was started on day 22 with equal feedings six times per day for the Timer 15, Timer 30, and AQ1 treatments. The AQ1 system was started on day 35 and was set to a 15kg per day limit.

Sampling and Water Quality

Shrimp were sampled weekly throughout the experiment using a cast net (1.22m radius and 0.95 cm mesh) to collect approximately 60 shrimp per pond. Group weights were recorded and individual shrimp were examined for general health. Ponds were monitored for dissolved oxygen (DO), temperature, salinity, and pH at least three times per day using a YSI ProPlus meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA); just before sunrise (0500 - 0600h), during the day (1400 – 1430h), and at sunset (1900 – 2000h). The aerators were turned on for 10-15min prior to the afternoon DO reading to reduce effects of stratification. Secchi disk readings and total ammonia nitrogen (TAN) were monitored on a weekly basis. Water samples were taken in the morning at the bank and TAN was determined using an Orion ion selective electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Harvest

The ponds were harvested over three days at the end of the 13-week culture period. Ponds were partially drained then the night before harvest the level was reduced to about one third and aeration was provided using the Airolator. On the day of harvest, the remaining water was drained and the shrimp were pumped out of the catch basin using a hydraulic fish pump equipped with a 25 cm diameter suction pipe (Aqua-Life pump, Magic Valley Heli-arc and Manufacturing, Twin-Falls, Idaho, USA). The pump was placed in the catch basin and shrimp were pumped, de-watered, and collected into a hauling truck. Shrimp were then rinsed, weighed in bulk, and 150 were randomly selected to measure individual weights and size distribution. A subsample of these shrimp were collected and frozen for subsequent analysis. Whole body proximate with minerals analysis of the shrimp was performed by Midwest Laboratories (Omaha, NE, USA)

Shrimp values

Shrimp prices used were the three year average (2014-2016) as reported by Urner Barry (Urner Barry, Toms River, NJ, USA) for Latin American Farmed white shrimp, whole. The partial value was calculated by subtracting the feed costs from the production value. The feed prices were \$1.69/kg for the starter diet and \$0.96/kg for the grower diet.

Statistical Analysis

Statistical analysis of the data was conducted with SAS 9.4 (SAS Institute, Cary, NC, USA) to perform a one-way analysis of variance to determine significant difference (p-value < 0.05) among treatments. Student-Newman-Keuls multiple range test was used to determine differences among treatments.

Results

In this trial, water quality parameters were maintained within the typical ranges for shrimp production throughout the study (table 1), except for one pond that was excluded from the AQ1 treatment due to a high mortality event in week 11 resulting from an aerator failure that caused low DO.

The average number of morning low DO (< 3.0 mg/L) events per pond for each treatment is shown in figure 1. The low morning DO readings were summed for each treatment and averaged across the ponds. The average low DO counts were graphed with the total feed inputs to show how the feed input level affects the biological oxygen demand (BOD). Figure 2 shows the low morning DO counts for each pond plotted with the total feed inputs for the pond to show the relationship between the feed inputs and BOD for each pond.

Production data is shown in table 2. The final mean individual shrimp weights were significantly different between all treatments with values of 19.74, 25.15, 27.52, and 32.04 g for shrimp reared using the SFP, Timer 15, Timer 30, and AQ1 treatments respectively. The yield for the AQ1 treatment (7430 kg/ha) was significantly higher than the SFP (4843 kg/ha) and Timer 15 treatment (5629 kg/ha) but was not significantly different from the Timer 30 treatment (6416 kg/ha). FCR ranged between 1.07 and 1.24 and survival was between 58.5% and 63.9% but no significant differences were seen between the treatments.

Table 1. Summary of water quality parameters observed over the 16-week culture period for each of the four treatments. The values are shown as mean ± standard deviation. Minimum and maximum values are shown in parenthesis.

	SFP	Timer 15	Timer 30	AQ1
Morning DO† (mg/L)	4.52 ± 1.11	4.56 ± 1.07	4.41 ± 1.14	4.15 ± 1.40
	(1.37, 9.02)	(1.87, 9.13)	(1.47, 10.36)	(1.14, 10.69)
Afternoon DO (mg/L)	8.62 ± 2.17	8.78 ± 2.82	9.15 ± 2.48	8.98 ± 2.48
	(2.94, 15.40)	(1.47, 18.23)	(2.60, 18.59)	(1.21, 18.72)
Evening DO (mg/L)	7.70 ± 2.19	7.72 ± 2.31	8.33 ± 2.40	7.81 ± 2.53
	(1.02, 14.05)	(2.56, 14.64)	(1.86, 18.23)	(1.07, 15.99)
Salinity (g/L)	6.66 ± 1.76	6.72 ± 1.59	6.74 ± 1.52	6.75 ± 1.55
	(3.92, 10.93)	(3.97, 10.61)	(4.47, 10.35)	(4.50, 10.45)
pH	7.54 ± 0.54	7.49 ± 0.43	8.44 ± 0.61	7.56 ± 0.50
	(6.71, 9.11)	(6.81, 9.21)	(7.05, 9.94)	(6.81, 9.24)
Temperature (°C)	29.3 ± 1.7	29.4 ± 1.74	29.2 ± 1.73	29.4 ± 1.7
	(24.6, 37.7)	(24.6, 33.5)	(24.6, 33.4)	(26.3, 35.3)
TAN‡ (mg/L)	0.17 ± 0.47	0.09 ± 0.15	0..29 ± 1.15	0.12 ± 0.23
	(0.00, 2.00)	(0.00, 0.50)	(0.00, 7.00)	(0.00, 0.90)
Secchi (cm)	79 ± 55	88 ± 58	77 ± 54	82 ± 55
	(15, 170)	(10, 170)	(15, 170)	(15, 170)

† DO: Dissolved Oxygen

‡ TAN: Total ammonia-nitrogen

Figure 1. Average number of morning dissolved oxygen (DO) readings below 3.0 mg/L for the last 60 days of culture and the average total feed input for each treatment.

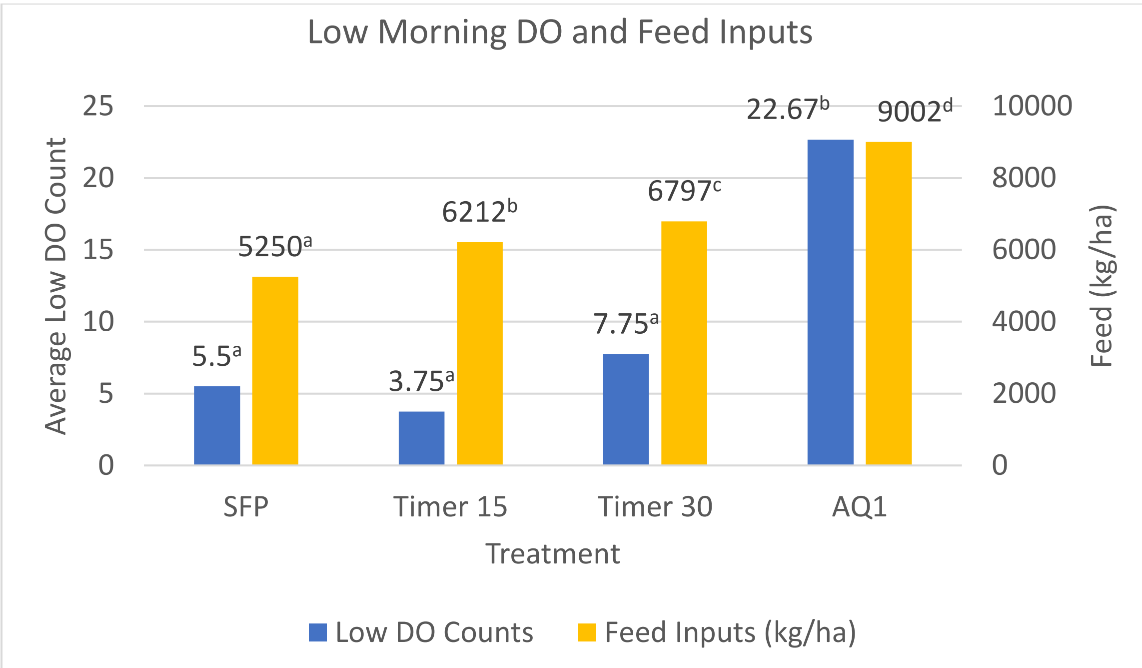


Figure 2. The relationship between total feed inputs and the number of low DO (< 3.0 mg/L) events for morning DO readings for each pond.

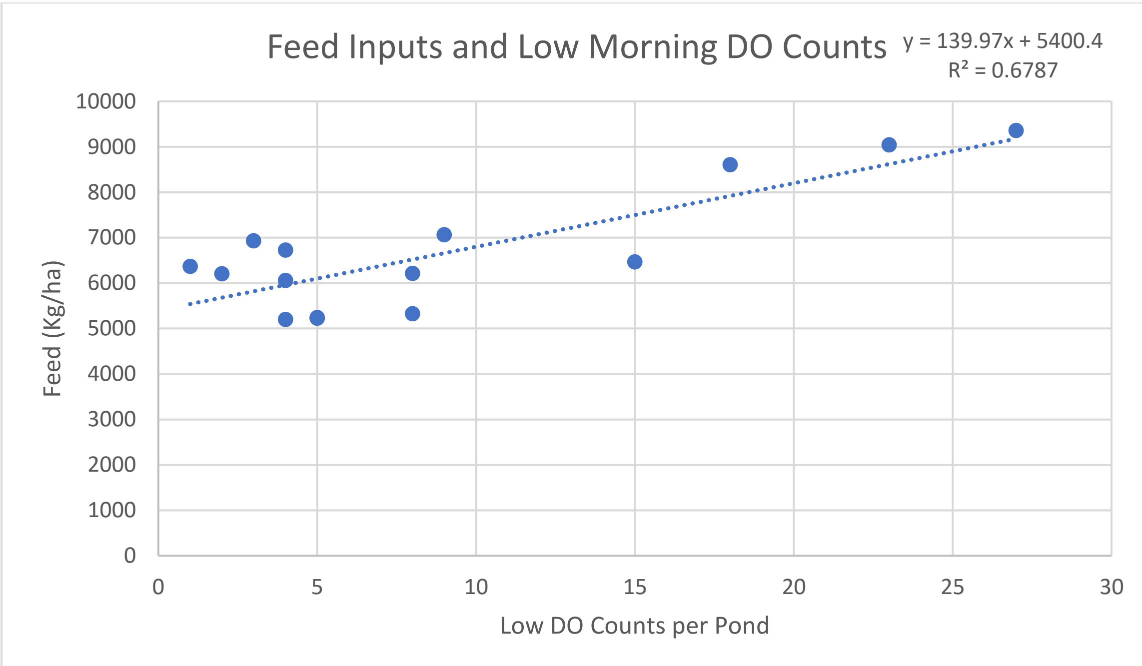


Table 2. Mean production results for Pacific white shrimp *Litopenaeus vannamei* grow in 0.1 ha ponds using four different feeding strategies. [†]

Treatment	Final Weight (g)	Yield (kg/ha)	Survival (%)	FCR	Feed (kg/ha)	Weekly Growth (g/wk)
SFP	19.74[a]	4843[a]	63.9	1.13	5250[a]	1.49[a]
Timer 15	25.15[b]	5629[a]	58.5	1.12	6212[b]	1.89[b]
Timer 30	27.52[c]	6416[ab]	61.2	1.07	6797[c]	2.07[c]
AQ1[‡]	32.04[d]	7430[b]	60.9	1.24	9002[d]	2.41[d]
P- Value	<.0001	0.008	0.7486	0.4099	<.0001	<.0001
PSE[§]	0.6059	399.4	3.4735	0.0628	11.12408	0.045603

[†]Mean values (n=4) in the same column with different superscripts are significantly different ($P < 0.05$) based on analysis of variance followed by Student Newman-Keuls multiple range test.
[‡] n=3
[§] Pooled Standard Error

Table 3 summarizes the shrimp values, costs, and value over selected costs associated with this trial. Significant differences are present between all treatments for the total feed inputs with 5250, 6212, 6797, and 9002 kg/ha for the SFP, Timer 15, Timer 30, and AQ1 treatments respectively. Subsequently, the feed cost per ha was significantly different for all treatments with $5138, $6063, $6623, and $8741 per ha for the SFP, Timer 15, Timer 30 and AQ1 treatments respectively. Shrimp value and partial value for the AQ1 treatment ($65,587/ha and $57,485/ha) was significantly higher than the SFP ($32,982/ha and $28,257/ha) and Timer 15 treatments ($44,279/ha and $38,688/ha), but not significantly different than the Timer 30 treatment ($52,687/ha and $52,687/ha).

Feed inputs per day over the course of the culture period are shown in figure 3. The average is plotted for the SFP, Timer 15, and Timer 30 treatments because daily inputs were similar between ponds. The AQ1 treatment is plotted as individual ponds because there is a large variation in feeding rate between pond and each day.

The whole body analysis of the shrimp is displayed in table 4. The AQ1 treatment produced shrimp with a significantly higher fat percentage (7.78%) than all other treatments. The Timer 30 (6.25%) treatment was significantly higher than the SFP (4.63%), but the Timer 15 (5.77%)treatment was not significantly different than either the SFP or Timer 30.

Discussion

Previous research using automatic feeders has shown the potential for significant improvements to growth, yield, and value in the pond culture of shrimp. Ullman et al. (2017a) performed a similar trial at lower densities and reported similar results supporting the use of automatic feeders at a stocking density of 17 shrimp/m^2. That trial used the same SFP, Timer

Table 3. Costs and values of Pacific white shrimp *Litopenaeus* vannamei shrimp grown in 0.1 ha ponds over 13 weeks using four different feeding strategies.

Trt	Total Feed Input (kg/ha)	Feed Cost ($/ha)	Shrimp Value ($/ha)	Partial Income ($/ha)
SFP	5250[a]	5138[a]	32,982[a]	28,257[a]
Timer 15	6212[b]	6063[b]	44,279[ab]	38,688[ab]
Timer 30	6797[c]	6623[c]	52,687[bc]	49,190[bc]
AQ1	9002[d]	8741[d]	65,587[c]	57,485[c]
P- Value	<.0001	<.0001	0.0007	0.0007
PSE[†]	11.12	10.67912	358.4	334.7141

[†] Pooled Standard Error

Figure 3. Daily feed inputs as means for the SFP, Timer 15, and Timer 30 treatments and as individual pond data for the AQ1 treatment.

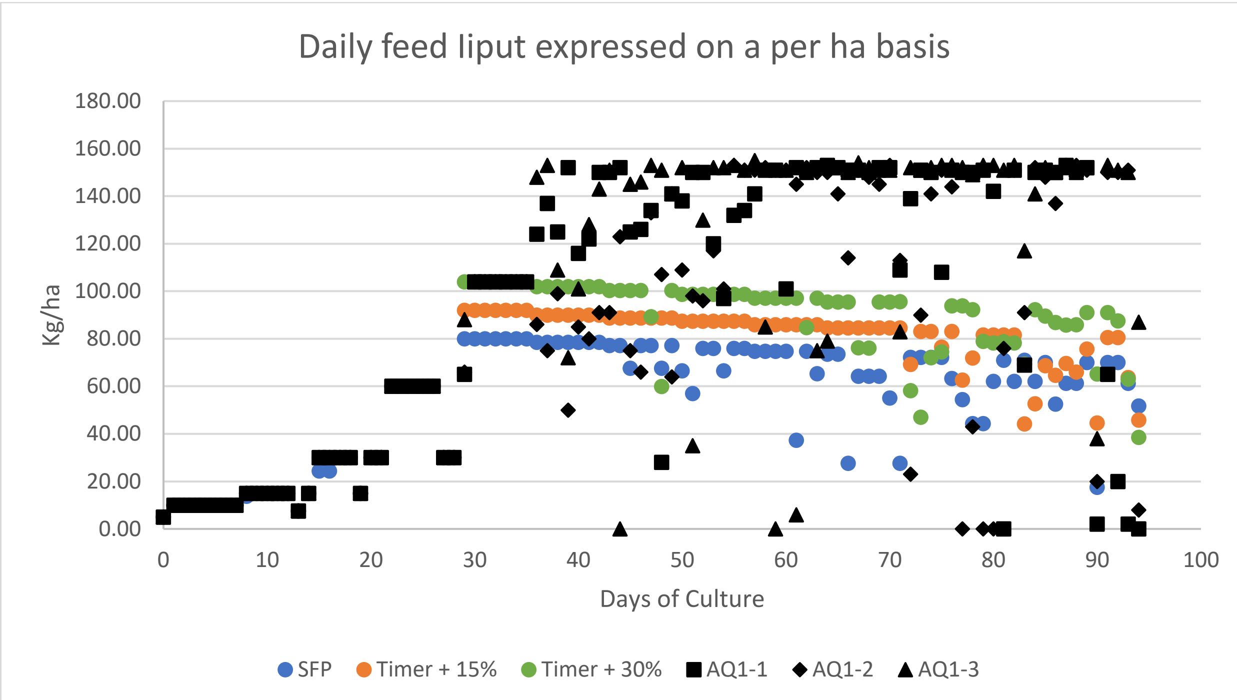


Table 4. Means of whole body composition analysis for each treatment. Analysis performed by Midwest Laboratories (Omaha, Nebraska, USA).

Treatment	SFP	Timer 15	Timer 30	AQ1[2]	P-Value	PSE
Moisture (%)	7.07[a]	7.76[ab]	8.38[b]	8.60[b]	0.0296	0.3144
Dry Matter (%)	92.93[a]	92.24[ab]	91.63[b]	91.40[b]	0.0296	0.3144
Protein (%)	72.65	73.15	72.75	71.27	0.4742	0.7824
Fat (%)	4.63[a]	5.77[ab]	6.25[b]	7.78[c]	0.0021	0.3907
Ash (%)	12.73[a]	11.18[ab]	10.40[b]	10.35[b]	0.0316	0.5238
Sulfur (%)	0.78	0.79	0.78	0.76	0.5117	0.0140
Phosphate (%)	1.26	1.24	1.26	1.21	0.7363	0.0303
Potassium (%)	1.22	1.21	1.22	1.15	0.3826	0.6038
Magnessium (%)	0.31[a]	0.30[ab]	0.27[b]	0.27[b]	0.0235	0.0095
Calcium (%)	2.78	2.73	2.25	2.44	0.0806	0.1434
Sodium (%)	0.81[a]	0.75[ab]	0.69[b]	0.69[b]	0.0110	0.0217
Iron (ppm)	296.25	133.45	154.50	149.67	0.1107	47.162
Manganese (ppm)	5.68	5.10	4.20	4.73	0.1107	0.3883
Copper (ppm)	121.25	125.00	113.50	121.67	0.5429	5.5890
Zinc (ppm)	69.08	70.93	68.70	68.20	0.4720	1.2109

[1]Mean values (n=4) in the same row with different superscripts are significantly different ($P < 0.05$) based on analysis of variance followed by Student Newman-Keuls multiple range test.
[2] n=3
[3] Pooled Standard Error

15, and AQ1 equipment (12 kg/day max) as in this trial over a 16 week production period. The final weights of the two trials are similar in that the AQ1 produced shrimp that were 37% and 38% larger than the SFP treatment and 23% and 22% larger than the Timer 15 treatments in the 17 shrimp/m^2 and the 38 shrimp/m^2 trials respectively.

The AQ1 treatment produced a higher yield and larger shrimp than the SFP or Timer 15 treatments, which translated into a higher shrimp value. Ullman et al. (2017a) evaluated the economic costs and benefits of the timer and AQ1 systems using enterprise and partial budgets that showed a net benefit of $2,216/ha and $10,153/ha for the timer and AQ1 treatments respectively when stocked at 17 shrimp/m^2. Upon further investigation, the authors decided that gross assumptions were used to evaluate the economic returns of a research facility as a production facility. According to Engle (2010) “An enterprise budget must be developed for a specific type and size production unit,” therefore the budget of a 1.6 ha research facility is unlikely to correlate to that of a much larger production farm. Hence, the implementation and labor costs and benefits are not evaluated in this study due to a lack of information, but the cost of the systems used in this trial are described in table 5. Some units of the AQ1 system can be used for multiple ponds, therefore the costs of implementation do not scale linearly. This information can be used along with the production results to evaluate the efficacy of the systems, but future endeavors will require an individualized evaluation to determine the costs and benefits unique to that operation.

The proximate analysis showed an increase in percent fats for the shrimp that was correlated to the increasing feed amounts, which shows an increase in stored energy with increased availability.

Table 5. The feeder equipment costs of the Timer 15, Timer 30, and AQ1 treatments as used setup for 4 ponds in each treatment at Claude Peteet Mariculture Center, Gulf Shores, AL, USA. Utility description is given to the AQ1 equipment to describe the maximum utility that was not necessarily used here.

Timer 15 and Timer 30				AQ1				
Item	Units	Price	Cost	Item	Units	Price	Cost	Utility
Feeder	4	$589	$2,356	Feeder	4	$650	$2,600	-
Batteries	4	$10	$40	Hydrophone	4	$880	$3,520	Runs up to 2 feeders
				Control Box	2	$2,450	$4,900	Runs up to 2 hydrophones
Total			**$2,396**	Base station	1	$400	$400	Runs up to 50 control boxes
				Computer	1	$1,000	$1,000	Runs up to 50 control boxes
				Software License	1	$3,500	$3,500	Price dependent upon number of units.
				Total Fixed Costs			**$15,920**	
				Internet	1	$1,200	$1,200	
				Software Renewal	1	$525	$525	15% Software License
				Total Annual Cost			**$1,725**	

On-site water quality parameters in this trial were not significantly different for any treatment. Casillas-Hernandez et al. (2007) found significantly lower TAN was measured in treatments that were fed using feeding trays (feed adjusted for consumption) when compared to those fed using a feeding table. While it is widely accepted that nutrients leach out of feed if it sits in the water unconsumed, more research is needed to determine the full effect of uneaten feed on water quality.

The average DO was not significantly different for any treatments, but the data was affected by the supplemental aeration that was necessary to maintain DO above 3.0 mg/L at night and during algae crashes. The average number of low DO (< 3.0 mg/L) occurrences for each treatment in the last 60 days of culture and the average total feed per ha is displayed in figure 2 ($y = 5.551x – 3.96$). The number of low DO events is significantly higher for the AQ1 treatment as compared to the others as is the feed amount. The low DO events for the SFP, Timer 15, and Timer 30 treatments are not significantly different whereas the amount of feed increases among those treatments. This indicates that there is a lower BOD in the pond when the feed is spread out over more feedings (Timer 15 and Timer 30), and that the quantity of feed delivered by the AQ1 was very close to the processing capacity of the pond with our level or aeration and therefore caused a higher occurrence of lower nighttime DOs.

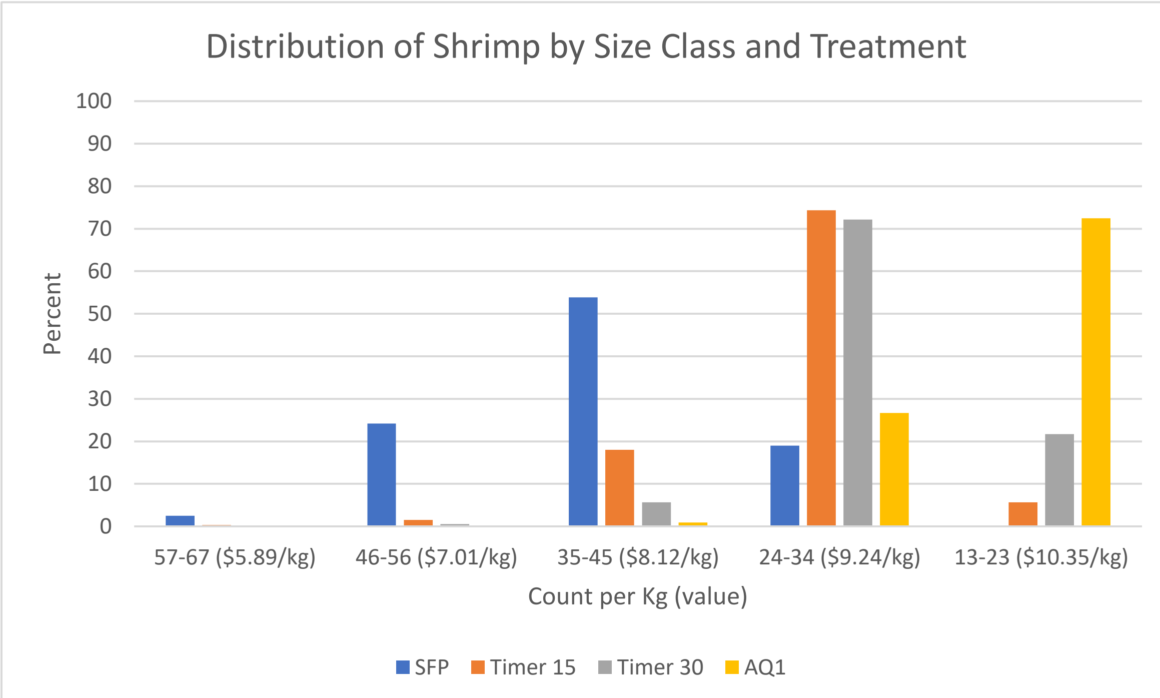
Figure 2 presents the daily feed input means for each of the SFP, Timer 15, and Timer 30 treatments are shown along with the individual pond AQ1 feed inputs . The daily variation in the AQ1 treatment indicates that the feeding rate is adjusted according to the demand from the shrimp. This also supports the use of a dynamic feeding program to maximize consumption and avoid waste.

Shrimp are sold and marketed according to size classes, which are displayed in figure 4 along with the distribution for each treatment and the value for each class. The Timer 30 and AQ1 treatments resulted in shrimp that were more clustered within a size class than the SFP or Timer 15 treatments. This was also observed in Ullman et al. (2017a) and was suggested to be the result of lower competition as seen in Ly et al. (2005) with juvenile orange-spotted grouper *Epinephelus coioides*.

Conclusion

This study indicates a clear advantage in growth and production to the automated feed back control of feed inputs over a hand feeding twice a day or the use of simple automatic feeding systems with increased feed inputs. It is also shown that the daily feeding rate can be increased without significantly affecting water quality or FCR by increasing the number of feedings from 2 to 6. The increased production was also shown to translate into higher shrimp values, which could offset the costs of the feeders. Further research is needed as a full economic analysis of a production facility to analyze the costs and benefits of feed automation.

Figure 4. Percent of each treatment that falls into each size class in count per kg.



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Chapter III: The effects of feed leaching on the growth of Pacific white shrimp *Litopenaeus vannamei* in a green-water tank system

Abstract

The purpose of this study was to examine the effects of leaching on the nutritional qualities of commercial shrimp feed and the growth of Pacific white shrimp *Litopenaeus vannamei*. This trial was performed in 24 green-water tanks with 5% daily water exchange using water from a shrimp production pond. Six treatments were used to examine the effects of leaching at 0, 0,5, 1, 2, 4, and 6 hrs. With regards to nutrient profiles, proximate composition, amino acids and select minerals were evaluated. In general, proteins and lipids increased as the feed was leached, but did not follow a well defined trend. Of the amino acids analyzed, taurine showed a negative trend relative to the leaching period in the amino acid analysis. Potassium, sodium, and sulfur also showed negative trends in the mineral analysis. The 0 and 0.5 hr treatments produced shrimp that were similar in size and significantly bigger than all other treatments In general, the size of shrimp linearly decrease with increase leaching time in excess of 0.5 hrs. Based on these results, feed that is exposed to water for more than 0.5 hrs can have an impact on the nutritional profile of the feed and a significant impact on the growth of the shrimp.

Introduction

Traditional feeding methods for shrimp production have typically fed 2-4 times per day due to labor constraints (Carvalho and Nunes 2006). The adaptation of automatic feeders has allowed farmers to increase the number of feedings without increasing the labor required (Bador et al. 2013). Ullman et al. (2017a) showed that growth was improved in *Litopenaeus vannamei* that were fed 6 times per day when compared to shrimp that were fed twice daily, but speculation was left open as to the cause of the increased performance. One speculation is that the feed had a higher nutritional value when fed 6 times per day because a smaller amount was fed each feeding and it may have been consumed more quickly and thus leached out less nutrients. The purpose of this study is to determine the effect that leaching has on the nutritional value of a commercial shrimp feed and the effect on the growth of *L. vannamei* in a green-water tank system.

Materials and methods

Culture System

The trial was performed at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center, Gulf Shores, AL. Pacific white shrimp *L. vannamei* (2.1 mg initial weight) were obtained from Shrimp Improvement Systems, Islamorada, FL and acclimated and nursed in a greenhouse for 30 days prior to stocking.

Shrimp were grown in an outdoor, semi-recirculating green-water system for 8 weeks. Water was pumped from a production pond for 2 hrs per day at a rate of 10 L/min to provide a daily water exchange of 5% and a source of natural productivity from the pond. Each tank was stocked with 30 shrimp (35 shrimp/m^2) that were hand sorted to a uniform size (0.30 g). The system consisted of 24, 800 L culture tanks and an 800 L sump. A 1-hp regenerative blower provided air via air stones to each of the tanks and a 0.33 hp pump circulated the water through the system.

At the end of the 8 week trial, the shrimp were counted and group weighed. The biomass, final weight, weight gain, weekly weight gain, Feed Conversion Ratio (FCR) (*Feed Fed (g) ÷ Weight Gained (g)*), and survival were then calculated (table 4).

Diets

A commercial extruded shrimp diet (35% CP and 7% L) produced by Zeigler, Inc. (Gardners, Pennsylvania, USA) was used as the base for all diets in this study. Six treatments were used to evaluate the effect of leaching feed on the growth of shrimp. Each treatment used feed leached for an amount of time as follows; 0 hrs (not leached), 0.5 hrs, 1 hr, 2 hrs, 4 hrs, and 6 hrs. The leaching was performed on 70cm x 53cm drying racks with window screen on the bottom. 1 kg of feed was spread evenly across each tray and the trays were lowered into a 15 cm deep pool of fresh water (Auburn, Alabama, USA) so that the tray floated without touching the bottom. After the prescribed amount of time, the trays were removed from the water and dried in two stages. The first stage had the trays placed outdoors in the sun with a fan blowing air across the feed for two hours to allow drainage of the bulk of the water. The second stage had the feed trays placed in a fan-ventilated oven for 24 hours to complete the drying process (~10% moisture). Dried pellet clumps were broken up by hand. Diets were analyzed for proximate and mineral composition by Midwest Laboratories (Omaha, Nebraska, USA) and for amino acids by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, Missouri, USA). The mineral and amino acid contents of each diet are displayed in table 1 as the percent change when compared to the unleached feed (0h). The true numbers are not shown to protect the feed formulation as it is proprietary information of the manufacturer.

Feeding

The standard feeding protocol (SFP) was calculated using an expected growth of 1.3 g/wk, an FCR of 1.2, and 100% survival and was fed twice per day (0800 h and 1600 h). Feeding for each treatment was adjusted based on the dry matter retention after leaching. The dry matter was calculated by leaching a known weight of feed (7.00g with 3 replicates) and subtracting the amount left after drying and comparing that to the unleached feed. The percent of the SFP that was fed is listed in table 2.

Water Quality

Tanks were monitored twice daily (0700 h and 1500 h) for dissolve oxygen, temperature, salility, and pH using a YSI ProPlus meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Total ammonia Nitrogen (TAN) was monitored twice weekly using an Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Nitrite and Nitrate were monitored weekly using test kits 3352-01 and 3354-01 respectively (LaMotte Company, Chestertown, Maryland, USA). Water quality results are shown in table 3.

Table 1. Feeds were analyzed on a dry matter basis and then presented as percent change from the unleached feed (0h).

Treatment	0.5h	1h	2h	4h	6h
Proximate					
Protein (crude)	2.7	6.1	6.4	1.5	5.6
Lipid (crude)	31.6	70.2	43.4	46.4	61.1
Fiber (acid detergent)	11.7	68.3	30.0	25.0	-15.0
Ash	-4.5	-5.4	-5.4	-8.0	-4.5
Minerals					
Sulfur	-8.9	-8.9	-8.9	-19.6	-10.7
Phosphorus	6.4	10.7	2.1	-3.6	7.1
Potassium	-50.0	-66.7	-80.2	-83.3	-84.1
Magnesium	50.8	55.7	60.7	41.0	54.1
Calcium	3.7	10.6	10.3	4.8	18.3
Sodium	-18.8	-31.3	-50.0	-62.5	-62.5
Iron	18.0	26.2	27.7	23.5	39.0
Manganese	31.5	37.8	32.7	30.3	45.8
Copper	34.5	28.7	23.6	8.9	21.5
Zinc	0.4	13.5	-0.4	8.7	18.8
Amino Acids					
Taurine	-18.18	-40.91	-40.91	-36.36	-50.00
Hydroxyproline	29.17	37.50	87.50	25.00	8.33
Aspartic Acid	6.29	9.43	11.01	10.69	11.64
Threonine	14.18	17.16	20.90	20.15	20.15
Serine	17.20	14.01	35.67	26.11	21.66
Glutamic Acid	5.61	7.78	11.75	11.39	8.50
Proline	14.68	16.51	18.81	18.81	18.81
Lanthionine	0.00	8.33	-25.00	-4.17	12.50
Glycine	16.27	16.27	19.14	18.66	18.18
Alanine	13.81	17.68	18.78	16.02	19.89
Cysteine	31.75	34.92	36.51	36.51	41.27
Valine	17.03	21.43	21.98	21.43	25.82
Methionine	-16.67	-15.48	-14.29	-14.29	-14.29
Isoleucine	10.69	14.47	12.58	13.21	15.72
Leucine	10.74	14.07	13.70	16.67	16.30
Tyrosine	5.83	2.50	5.00	5.83	6.67
Phenylalanine	9.52	13.10	11.31	14.29	14.88
Hydroxylysine	0.00	0.00	-11.11	-11.11	-11.11
Ornithine	0.00	16.67	-16.67	0.00	16.67
Lysine	0.84	0.84	2.11	-0.42	-0.84
Histidine	2.35	4.71	3.53	4.71	5.88
Arginine	8.30	9.17	10.92	13.54	10.48
Tryptophan	-6.38	-8.51	-8.51	-4.26	2.13

Table 2. Percent of the standard feeding protocol fed to each treatment based on the dry matter retained after leaching.

Treatment (Leaching Hours)	% Fed
0h	100
0.5h	92.8
1h	90.3
2h	88.7
4h	87.1
6h	86.2

Table 3. Summary of water quality over the 8-week culture period of Litopenaeus vannamei fed a leached diet in green-water tanks. Values shown are means ± one standard deviation with minimum and maximum values shown in parenthesis.

Parameter	Mean
Temperature (°C)	27.25 ± 1.76
	(23.2, 31.9)
Dissolved Oxygen (mg/L)	6.92 ± 0.70
	(3.31, 11.86)
pH	7.82 ± 0.27
	(7.12, 9.64)
Salinity (mg/L)	7.72 ± 0.83
	(6.21, 8.95)
Total Ammonia Nitrogen	0.19 ± 0.20
	(0.00, 0.60)
Nitrite	0.47 ± 0.31
	(0.17, 0.99)
Nitrate	6.29 ± 2.35
	(4.40, 8.80)

Statistical Analysis

The statistical analysis was conducted using SAS 9.4 (SAS Institute, Cary, NC, USA) to perform a one-way analysis of variance to determine significant differences among treatments. A Student-Newman-Keuls multiple range test was used to determine the differences between treatment means. Significant differences among treatments were determined at a p-level < 0.05.

Results

The experiment was terminated and growth performance was evaluated on day 52. The growth parameters are presented in table 4. No significant differences were observed between treatments for percent survival (94.2 – 100%) and FCR (1.10 – 1.35) with both values being typical for this systems. The final weight of shrimp was highest in treatment 0.5h (7.53 g) and 0h (7.50 g), which were not significantly different from each other but were significantly higher than all other treatments. The 6h (5.61 g) treatment produced shrimp that were significantly smaller than the 0h, 0.5h, and 1h (6.63 g) treatments, but not significantly different than the 2h(6.30 g) and 4h(6.49 g) treatments. As compared to all other treatments the final biomass was significantly higher for treatments 0h (221.2 g) and 0.5h (216.7 g). The biomass of shrimp on the 6h treatment (168.4 g), was significantly lower than those maintained on the 1 hr treatment. In general, there was a linear decrease in biomass and final weight of shrimp offered feeds leached for more than 0.5 hrs.

Proximate, mineral and Amino acid data for each diet is presented in Table 1. as a percent difference as compared to treatment 0h. The diet analysis should be considered

Table 4. Responses of juvenile (0.30 g) *Litopenaeus vannamei* grown in outdoor green-water tanks for 8-weeks and fed leached diets.

Treatment (Leaching Hours)	Biomass (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Weekly Weight Gain (g/wk)	FCR[1]	Survival (%)
0h	221.2[a]	7.50[a]	7.20[a]	2403[a]	0.90[a]	1.15	98.3
0.5h	216.7[a]	7.53[a]	7.23[a]	2420[a]	0.91[a]	1.10	95.8
1h	187.2[b]	6.63[b]	6.33[b]	2104[ab]	0.80[ab]	1.24	94.2
2h	184.2[bc]	6.30[bc]	5.99[bc]	1995[bc]	0.75[bc]	1.25	97.5
4h	186.2[bc]	6.49[bc]	6.18[bc]	2046[bc]	0.77[bc]	1.20	95.8
6h	168.4[c]	5.61[c]	5.31[c]	1787[c]	0.67[c]	1.35	100.0
P-Value	0.0050	0.0002	0.0002	<.0001	0.0002	0.0951	0.5748
PSE[2]	7.54	0.20	0.20	61.94	0.03	0.05	1.92

Mean values (n=4) in the same column with different superscripts are significantly different (p>0.05) based on analysis of variance followed by Student Newman-Keuls multiple range test.

[1]Feed Conversion Ratio

[2]Pooled Standard Error

preliminary as no replicates were performed and hence, there was no statistical analysis. In general protein, lipids, ash and fiber increased with leaching. In the mineral analysis, potassium (50.00%, 66.67%, 80.16%, 83.33% and 84.13%), sodium (18.75%, 31.25%, 50.00%, 62.50%, and 62.50%), and sulfur (8.93%, 8.93%, 8.93%, 19.64%, 10.71%) showed negative trends as compared to the 0h treatment for all other treatments (0.5h, 1h, 2h, 4h, and 6h) respectively. In the amino acid analysis, methionine showed a 16% loss from the 0h to 0.5h diet and stabilized from there in the longer leaching periods. Taurine displayed a negative trend with the leaching period with 18.18%, 40.91%, 40.91%, 36.6%, and 50.00% loss for the 0.5h, 1h, 2h, 4h, and 6h treatments respectively.

Discussion

Previous research has demonstrated that increasing the number of feeding per day improved shrimp performance. It has been hypothesized that part of this is due to a reduction of time that the feed is in the water. Hence, we evaluated the biological response of juvenile shrimp to feeds that were previously leached and then offered at a rate equivalent to what would be available at that that time period. Thus providing an estimate of growth potential of the feed after it was leached at each time period. .

In general there was no difference in growth or feed conversion for shrimp feed feed leached for up to 0.5hrs. There after there was a general decrease in performance. Based on mean separation, significantly reduced growth was seen in this trial for the feed that was leached for 6 hrs as compared to the feed that was leached for 0, 0.5, and 1 hr. Regression analysis of both final weights and biomass results in significant decreases over time (R-square 0.5493 and 0.4061, respectively)

In this experimental design, we chose to feed an equivalent ration that would be available should the feed be in the water for the given amount of time and then consumed. Hence, the ration was slightly reduced to mimic what would happen under production conditions. Hence, part of the reduced growth is simply due to a reduced quantity of feed being available. However, it also appears that this is due to the quality of that feed. This trial was run in a green water system for which a number for feed management trials have been conducted(Venero et al. 2007, Silva 2013, Van 2016). In other trials as feed is reduce FCR has increased as the shrimp consume more natural foods and probably are more likely to consume all of the feed. The increased FCR of shrimp offered the leached feed and reduced ration would be a clear indication of reduced nutritional value of the feed.

As would be expected, components of the feed with low water solubility (e.g. protein, lipids, fiber and some minerals) increased in concentration primary due to water soluble components such as soluble carbohydrates, mineral and free amino acids (e.g. taurine) leached from the feed This occurred very quickly primarily in the first 0.5 to 1 hr of leaching. It would be expected that this loss of nutrients would then lead to reduced performance of the shrimp,

Carvalho and Nunes (2006) observed decreases of 5.51% and 1.37% for crude protein and crude lipid respectively when the feed was leached for 8 hrs. No notable trend was seen for crude protein or crude lipid in this study, but it should be noted that the feed used for the current study was an extruded feed, while Carvalho and Nunes (2006) used a pelleted feed. The difference in processing may have had an effect on the leaching properties of the feeds. Potassium, sodium and sulfur showed negative trends relative to the leaching period. It is unlikely that potassium had any effect on the shrimp because most marine species are capable of obtaining sufficient potassium from the water as long as sodium and chloride ions are balanced (Davis *et al.* 1992). Sodium was also shown to have no effect on rainbow trout when included at 1% and 2% of the dry matter(Salman and Eddy 1988). The decrease in sulfur may be related to the decrease in methionine and taurine as those are sulfur containing amino acids.

Methionine levels showed a steep decrease after a short time in the water but did not decrease further after 0.5h. This could indicate a portion of the methionine was in a free form and dissolved easily into the water. Methionine is one of the first limiting amino acids in plant based diets, therefore its levels should be closely monitored (Nunes *et al.* 2014). Taurine was the only amino acid in this study to show a notable trend, decreasing by 50% in the 6h treatment. This is likely due to taurine not being incorporated into proteins and is found primarily in the free form. Leaching of taurine has been evaluated by (Watson et al. 2015)and found to easily leach from the feed (59.5 ± 16.5% over 40 min.)

Fox *et al.* (2006) showed a significantly higher leaching rates in 0ppt water than in 15ppt or 30ppt. While leaching in this study was lower than other studies for some factors, the mineral balance could have affected the leaching rate. Another factor that this study did not take into consideration is the pellet stability and how that may have been affected by the leaching and/or re-drying the pellet. The stability of the pellet is particularly important in shrimp because they handle the feed with their chela prior to consuming it (Obaldo et al. 2002).

Conclusion

The leaching of a commercial shrimp feed for more than 0.5 hrs prior to feeding to Pacific white shrimp *L. vannamei* in a green-water tank system significantly reduce the growth and increase the FCR of the shrimp. Hence, this study supports the theory that part of the effects of multiple feedings is the reduced time that feed is in the water prior to consumptions This data supports increasing the number of daily feedings with smaller rations to prevent leaching of nutrients. Future research is needed to examine the nutrients lost and how the feed formulation and processing affects the leaching rate and subsequent growth and performance of shrimp.

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Conclusion

The findings of the pond studies indicate that increasing the number of daily feedings from 2 to 6 can significantly improve the growth and performance of Pacific white shrimp *Litopenaeus vannamei*. Increasing the daily feedings also allows more feed to be fed without affecting the FCR, which can contribute additionally to the growth. It was also found that the use of acoustic feedback systems are capable of adjusting the feeding rate to feed only when the shrimp are actively feeding. This resulted in improved growth, as seen historically with the use of feeding trays, but with much less labor and with real time adjustments based on feeding response. The economic analysis of these systems indicated that the equipment costs of the timer or AQ1 feeders can be made up within one growing cycle, which makes automation a sound investment for shrimp producers.

The leaching of a commercial shrimp feed for more than 0.5 hrs prior to feeding to Pacific white shrimp *L. vannamei* in a green-water tank system significantly reduced the growth and increased the FCR of the shrimp. Hence, this study supports the theory that part of the effects of multiple feedings is the reduced time that feed is in the water prior to consumptions This data supports increasing the number of daily feedings with smaller rations to prevent leaching of nutrients, which can improve growth and efficiency in the production of *L. vannamei*.

The conclusion of this thesis indicates a need for increasing the number of daily feedings in order to improve growth and reduce feeding waste. Individual evaluation is needed for each farm to determine what level of automation is appropriate according to available capital, infrastructure, and production goals. The use of automatic feeders has been shown to be a viable option for increasing the daily feedings without increasing the feeding labor and is highly recommended to improve performance of the shrimp under commercial conditions.

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Appendices

Appendix 1. Enterprise budget per ha for the production of Pacific white shrimp Litopenaeus vannamei at Claude Peteet Mariculture center in Gulf Shores, AL, USA. All values are averaged for the SFP and SFP + 15% treatments to account for no significant difference. Total production and feed amounts are averaged between the SFP, SFP + 15%, and Timer treatments to account for no significant difference.

			SFP and SFP + 15%		Timer		AQ1	
	Unit	$/unit	Quantity	Value/Cost ($)	Quantity	Value/Cost ($)	Quantity	Value/Cost ($)
1. Gross Receipts								
13-23 count/kg	kg	$10.35	-	$0	7	$72	161	$1,669
24-34 count/kg	kg	$9.24	177	$1,639	1037	$9,578	4505	$41,614
35-45 count/kg	kg	$8.12	1,858	$15,093	1844	$14,980	140	$1,135
46-56 count/kg	kg	$7.01	895	$6,274	202	$1,414	11	$75
57-67 count/kg	kg	$5.89	162	$954	42	$246	11	$63
68-78 count/kg	kg	$4.78	29	$137	-	$0	11	$51
79-89 count/kg	kg	$3.67	8	$29	-	$0	-	$0
90-110 count/kg	kg	$2.11	3	$5	-	$0	-	$0
Total Receipts			**3,132**	**$24,131**	**3,132**	**$26,291**	**4,839**	**$44,609**
2. Variable Costs								
PL & nursery phase								
PL cost	$/1000	$8.00	60	$480	60	$480	60	$480

Artemia and PL feeds	total	$750	25%	$188	25%	$188	25%	$188
PL pick up cost	total	$800	25%	$200	25%	$200	25%	$200
Feed								
1.5 mm Raceway 40-9	kg	$1.70	128	$218	128	$218	128	$218
2.5 mm CS Experimental feed	kg	$0.97	2,840	$2,755	2,840	$2,755	5,297	$5,138
Freight								
Feed freight 1.5 mm Raceway 40-9	kg	$0.75	128	$96	128	$96	128	$96
Feed freight 2.5 mm CS Exp feed	kg	$0.24	2,840	$682	2,840	$682	5,297	$1,271
Labor								
Stocking	hr	$10.00	4	$40	4	$40	4	$40
Feeding	hr	$10.00	114	$1,140	57	$570	57	$570
Checking DO	hr	$10.00	57	$570	57	$570	114	$1,140
Harvesting	hr	$10.00	36	$360	36	$360	36	$360
Electricity								
Aeration	Kwhr	$0.14	4,032	$565	4,032	$565	4,032	$565
Battery for Timer	ea	$10.00	-	$0	1	$10	-	$0
Software license	Annual	$3,500	-	$0	-	$0	1.00	$3,500
Internet	Annual	$1,200	-	$0	-	$0	1.00	$1,200
Fuel								
Golf cart gas	liter	$0.53	75	$40	75	$40	75	$40
Chemicals								
32-0-0 & 10-34-0 fertilizers	liter	$3.24	12	$39	12	$39	12	$39
Harvest Costs								
Ice	135 lb blk	$10.50	16.25	$171	16.25	$171	16.25	$171
"Spot Away" shirmp rinse	case	$220.00	0.25	$55	0.25	$55	0.25	$55

Tractor rental	$/week	$500.00	0.25	$125	0.25	$125	0.25	$125
Harvester rental	$/week	$1,133.00	0.25	$283	0.25	$283	0.25	$283
Interest on Operating Costs	percent	6.00%	$28,388	$1,703	$27,828	$1,670	$36,061	$2,164
Total Variable Costs				$8,486		$7,892		$16,619
3. Income Above Variable Costs				$15,646		$18,399		$27,990
4. Fixed Costs								
Depreciation on capital items, machinery & equipment				$2,800		$3,240		$3,952
Interest on Loans (capital items and M&E items)				$342		$430		$569
Repairs & Maintenance (capital and M&E items)				$615		$624		$1,428
Total Fixed Costs				$3,757		$4,294		$5,948
5. Total Costs (VC + FC)				$12,243		$12,186		$22,567
6. Net Return to Management				$11,889		$14,105		$22,042