**A Summary Report prepared for Zeigler Bros.**
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# Comparison of two commercial feeds for the production of marketable Pacific White Shrimp, *Litopenaeus vannamei*, in a super-intensive biofloc dominated zero-exchange raceway system

Operating limited discharge recirculating aquaculture systems can reduce potential negative environmental impacts created by traditional pond culture practices using extensive water exchanges. In recent years studies at the Texas A&M AgriLife Research Mariculture Lab at Flour Bluff have focused on the use of a specially designed feed made by Zeigler Bros. (HI-35, Zeigler Bros., Gardners, PA) formulated for use in biofloc-dominated super-intensive zero-exchange systems. These experimental systems were operated at high densities (>300 shrimp/m$^3$), resulting in high biomass (>3-6 kg/m$^3$). A 2009 study at the lab showed that high yields (9.29 kg/m$^3$) and survival (88%) can be achieved with no water exchange and a density of 500 shrimp/m$^3$ while using foam fractionators or settling tanks to regulate levels of particulate matter in the culture medium. The study conducted last year showed that when the shrimp were fed two feeds, both containing 35% crude protein (HI-35 and SI-35), a significant improvement in shrimp performance was noticed when the shrimp were fed the HI-35 feed. Although no significant difference in survival was found between the two feeds, the shrimp fed the HI-35 feed had better yield (9.74 kg/m$^3$ vs. 8.71 kg/m$^3$), growth (2.03 g/wk vs. 1.76), FCR (1.25 vs. 1.43), and final weight (22.12 vs. 19.74 g).

The objectives of the current study were: 1. To evaluate the effect of 35% crude protein commercial feed used in the previous year study (HI-35) compared to an experimental feed with a higher protein level (40%) (EXP), both formulated for super-intensive biofloc-dominated shrimp production systems, on growth, survival, and FCR of *Litopenaeus vannamei*, 2. Study the effect of the two feeds on selected water quality indicators under zero water exchange, and 3. Evaluate the benefit of using continuous DO monitoring equipment in operating a super-intensive shrimp production system.

The study was conducted in six 40 m$^3$ (68.5 m$^2$) raceways (RWs). Each 25.4 m x 2.7 m RW was lined with EPDM liner (Firestone Specialty Products, Indianapolis, IN). RWs were equipped with a center longitudinal partition positioned over a 5.1 cm PVC pipe with sprayer nozzles. Each RW had six banks of three 5.1 cm airlift pumps positioned equidistance on both sides of the partition. In addition, each RW had six 0.91 cm long air diffusers (1.9 cm OD, Aero-Tube$^{TM}$, Tekni-plex Aeration, Austin, TX), a 2 HP centrifugal pump, and a Venturi injector capable of introducing atmospheric air or a mixture of oxygen and air. Raceways were filled with 35 m$^3$ of water used in a preceding 63-d nursery study and 5 $m^3$ of natural seawater with salinity adjusted to 30 ppt. Each RW was equipped with a small commercial foam fractionator (FF) (VL65, Aquatic Eco System, Apopka, FL) and a homemade settling tank (ST). Shrimp used in this study were produced from a cross between Taura resistant and fast-growth genetic lines received from KAVA Farms (Los Fresnos, TX). RW were stocked with juveniles (4.7 g) at a density of 324/$m^3$.

This study compared two feeds with three replicates each; a hyper-intensive feed (HI-35) and an experimental hyper-intensive feed (EXP), both produced by Zeigler Bros., Gardners, PA (Table 1).

Table 1. Proximate composition of the hyper-intensive feed (HI-35) and experimental hyper-intensive feed (EXP)

| Component | HI-35 | EXP |
|---|---|---|
| Crude Protein (%) | 35.8 | 39.5 |
| Lipid (%) | 8.7 | 9.2 |
| Fiber (%) | 1.9 | 3.0 |
| Ash (%) | 9.7 | 12.3 |
| Carbohydrate (%) | 37.2 | 31.0 |
| VPak® (%)[1] | 0.25 | 0 |

[1]A proprietary natural nutritional additive premix designed to enhance shrimp disease resistance and improve survivability

RWs 1, 3 and 5 were designated as the HI-35 treatment and RWs 2, 4 and 6 as the EXP treatment. Shrimp were fed continuously seven days a week using belt-feeders. For the first week daily rations were based on an assumed growth of 1.5 g/wk, FCR of 1.4, and mortality of 0.5%/wk. Rations were later adjusted based on observed feed consumption and results of the twice weekly shrimp sampling. Use of the FFs and the STs commenced at the study initiation. Foam fractionators and settling tanks were operated intermittently, targeting culture water TSS concentrations between 200 and 300 mg/L and settleable solids between 10 and 14 mL/L. Settling tank flow rates varied between 10 and 12 L/min. Raceways were maintained with zero water exchange throughout the trial and municipal freshwater was added to compensate for water losses due to evaporation and operation of the FFs and STs. Water temperature, salinity, dissolved oxygen, and pH were measured twice daily using a YSI 650 Series multi-probe (YSI Inc. Yellow Springs, OH). Settleable solids were measured every two or three days. Alkalinity and TSS were measured twice a week; while, VSS, TAN, $NO_2$, $NO_3$, and $PO_4$-P were measured weekly. Each RW was equipped with a YSI 5500D multi-parameter monitoring and alarm system with an optical DO probe (YSI Inc., Yellow Springs, OH). Data collected by the monitoring system was uploaded into a computer in the lab which could also be accessed from remote locations for real-time monitoring of the DO in the six RWs. Daily and weekly water quality data from the two treatments was analyzed by Linear Mixed Models, using Factor Analytic (First Order, Heterogeneous). Shrimp mean final weights; weekly growth rate, survival (arcsine transformed), FCR, and total yields were analyzed using one-way ANOVA. A significance level of $p<0.05$ was used for all statistical tests.

Oxygen supplementation was initiated on Day 8 and continued until the end of the 77 day study. From Day 8 until Day 57 supplementation was related to daily events (e.g., feeding, molasses addition). Beginning at Day 58, when shrimp biomass was estimated to be 7.15 $kg/m^3$, supplemental oxygen was used 24 hr/day as operating the Venturi with ambient air was not sufficient to maintain DO above the targeted 4 mg/L. The YSI 5500D monitors and their optical probes proved to be a valuable tool for the management of super-intensive shrimp culture, allowing quick adjustments to be made to minimize stress from low DO while setting upper and lower DO limits helped to optimize oxygen use. Tables 2 and 3 summarize the mean values of daily and weekly water quality indicators monitored in this study. Figures 3 through 8 show the weekly changes in selected water quality indicators. There were no statistically significant differences in DO, temperature, salinities, pH, TAN, $NO_3$-N, $PO_4$, and SS between treatments (Tables 2 & 3). Total ammonia nitrogen levels remained below 0.7 mg/L throughout the study, while $NO_2$-N level remained below 0.53 mg/L (Fig. 2) with significant differences between treatments. While solids were controlled by the use of the FFs and STs, TSS and VSS levels in the EXP treatment remained higher than the HI-35 treatment (Fig. 5, 7 & 9) however there were no statistically significant differences. Sodium bicarbonate and soda ash were added to RWs based on the targeted alkalinity of 180 mg/L $CaCO_3$ and pH of 7.4..

Table 2. Mean values of daily water quality indicators during grow-out study with *L vannamei* in 40 $m^3$ RWs.

| | | HI-35[1] | | EXP[2] | |
|---|---|---|---|---|---|
| | | Mean±SD | Min - Max | Mean±SD | Min - Max |
| Temperature (C) | a.m. | 29.1±1.1 | 25.2 – 30.9 | 29.0±1.1 | 25.2 – 30.8 |
| DO (mg/L) | a.m. | 5.1±0.5 | 4.2 – 6.5 | 4.9±0.5 | 3.7 – 6.1 |
| pH | a.m. | 7.4±0.1 | 7.1 – 7.9 | 7.3±0.2 | 7.0 – 7.8 |
| Salinity (ppt) | | 29.4±1.1 | 26.7 – 33.6 | 29.8±1.2 | 25.3 – 33.6 |

[1] RWs where shrimp were fed the HI-35 Zeigler Bros. feed.
[2] RWs where shrimp were fed the EXP Zeigler Bros. feed.

Table 3. Mean values of weekly water quality indicators during grow-out study with *L vannamei* in 40 $m^3$ RWs.

| | HI-35[1] | | EXP[2] | |
|---|---|---|---|---|
| | Mean±SD | Min - Max | Mean±SD | Min - Max |
| TAN (mg/L) | 0.52±0.24 | 0.05 – 1.19 | 0.70±0.56 | 0.11 – 3.35 |
| $NO_2$-N (mg/L) | 0.35±0.90[a] | 0.00 – 5.19 | 0.52±0.82[b] | 0.00 – 3.56 |
| $NO_3$-N (mg/L) | 184.58±78.26 | 83.73 – 319.60 | 202.55±101.2 | 61.18 – 401.02 |
| ALK (mg/L) | 147.28±30.98 | 86 – 219 | 126.69±26.79 | 78 – 172 |
| TSS (mg/L) | 381.42±114.10 | 142 - 617 | 428.30±123.85 | 250 – 692 |
| VSS (mg/L) | 258.89±68.81 | 67 – 392 | 289.94±76.60 | 133 – 508 |
| $PO_4$-P (mg/L) | 57.42±36.84 | 15.31 – 175.41 | 65.61±41.32 | 10.07 – 217.87 |
| SS (mL/L) | 14.87±8.39 | 0.5 – 30 | 12.28±9.53 | 0 – 40 |

[1] RWs where shrimp were fed the HI-35 Zeigler Bros. feed.
[2] RWs where shrimp were fed the EXP Zeigler Bros. feed.
Different letters in the same row represent statistical differences.

Analyses of shrimp performance based on harvest data (Table 4) showed no significant differences in mean final weights (27.22 vs. 28.8 g), yields (8.21 vs. 7.79 kg/$m^3$), weekly growth (2.05 vs. 2.16 g/wk), and FCR (1.59 vs. 1.72) of the shrimp fed the HI-35 and the EXP feeds, respectively. Significantly higher survival was found for the shrimp fed the HI-35 (93.14% vs. 83.35%). Although there was a little difference in cost between the two feeds ($1.94/kg vs. $1.92/kg, for the EXP and HI-35, respectively) a preliminary economic analysis of profitability indicates that the HI-35 and EXP feeds would be commercially viable when shrimp are sold at $6.00/lb.

Table 4. Summary of mean final weight, weekly growth, yield, survival, FCR, and water usage from a 77 day grow-out study of *Litopenaeus vannamei* in greenhouse-enclosed raceways operated with no water exchange.

| Feed | Yield (kg/$m^3$) | Survival (%) | Av. Wt. (g) | Growth (g/wk) | FCR |
|---|---|---|---|---|---|
| HI-35[1] | 8.21 | 93.14% | 27.22 | 2.05 | 1.59 |
| EXP[2] | 7.79 | 83.35% | 28.80 | 2.16 | 1.72 |
| Diff | 0.42 | 9.79 | (1.58) | (0.11) | 0.13 |

[1] RWs where shrimp were fed the HI-35 Zeigler Bros. feed.
[2] RWs where shrimp were fed the EXP Zeigler Bros. feed.

Weekly changes in shrimp average weights during the 77 day trial are presented in Figure 1. When looking at the changes in weekly growth and FCR by treatment during this period (Figure 2), more information emerges regarding the feed effect on shrimp performance. The data showed that the shrimp fed the EXP had a growth rate between 4.4 and 3.4 g/wk with FCR values between 0.45 and 1.1 for the first 31 days of the trial.

Figure 1. Weekly changes in shrimp average weights in a 77-d study with *Litopenaeus vannamei* when fed two feeds under biofloc dominated and no exchange conditions.

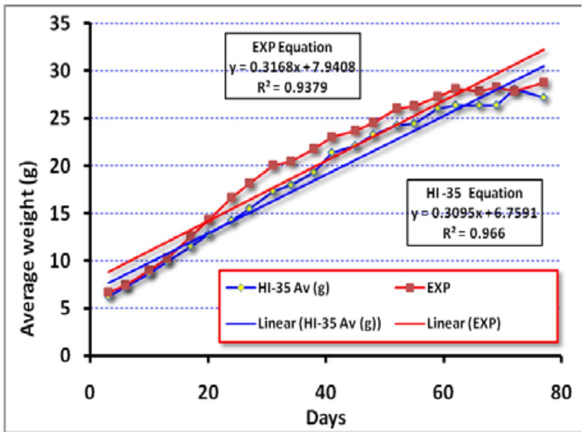
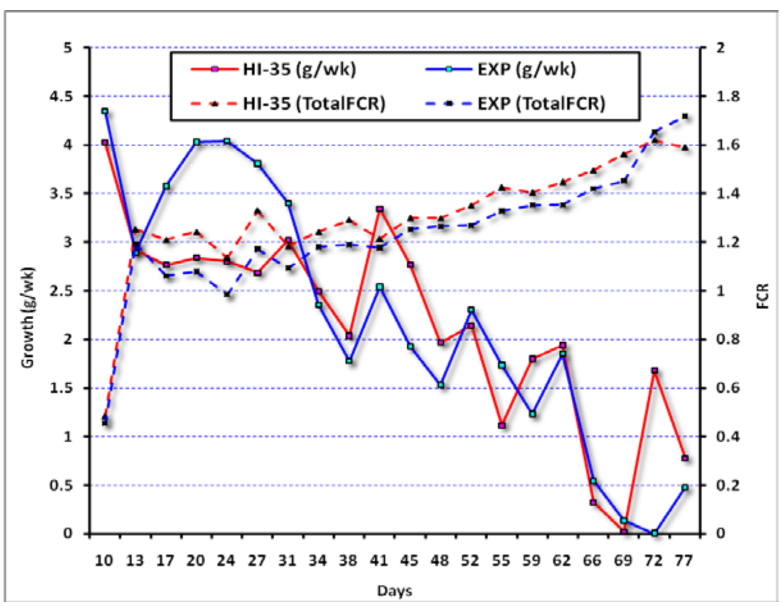



Figure 2. Weekly changes in shrimp growth and FCR values in a 77-d study with *Litopenaeus vannamei* when fed two feeds under biofloc dominated and no exchange conditions
Leandro please change total FCR to Intermittent FCR



On the other hand, the shrimp fed the HI-35 had a growth rate between 4.0 and 3.0 g/wk with FCR values between 0.45 and 1.2 for the same period. Shrimp mortality was first noticed on Day 22 in one of the raceways which was fed the EXP feed. This mortality continued to spread into the other RWs and finally came into a complete stop on Day 52 of the trial. It is interesting to note that our records showed a larger number of dead shrimp in the RWs offered the EXP feed than the HI-35. Although no mortality was noticed from Day 52 on, shrimp growth until the termination of the study was significantly reduced resulting in poor FCR for both treatments.

In order to identify the cause for the observed mortality, preserved and live shrimp samples were sent to a disease diagnostic lab. Reports by the lab suggested shrimp infection by vibriosis. Isolation and identification of the bacteria using 16s rRNA sequencing tool suggested the presence of several species of *Vibrio* to include: *V. parahaemolyticus*, *V. owensii*, *V. communis*, and *V. alginolyticus*. Furthermore, RT-PCR tests showed no infections by TSV, YHV, IMNV or PvNV.

**Discussion**

Although shrimp growth and FCR during the first few weeks of the trial were extremely good, especially for the shrimp fed the EXP feed, once the infection by *Vibrio* was noticed, shrimp performance started to decline. It is interesting to note that the shrimp fed the HI-35 had significantly higher survival than those fed the EXP. We assume that the difference in shrimp survival rate between the two feeds is related to the diet composition. Preliminary information suggests that supplementation of the HI-35 feed by VPak™ may have contributed to the improved survival. Although significant reduction in shrimp growth was noticed in both treatments once the shrimp were infected by the *Vibrio*, the overall growth rate was similar to the average rate observed in last year study when the shrimp were fed the HI-35. Nevertheless, unlike the low FCR observed last year, the FCR values from this year study were much higher. We assume that this poor FCR was the result of the negative impact of the bacterial infection on feed utilization by the shrimp.

**Acknowledgements**

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Picture 1: View of a raceway full of water

Picture 2: A general view of an empty raceway

Picture 3: RW drain and filter pipe.


Picture 4: The YSI 5500 monitoring system

Picture 5: Optical DO probe

Picture 6: Foam fractionator

Picture 7: Imhoff cones with bacterial flocs

Fig. 3: Weekly changes in TAN

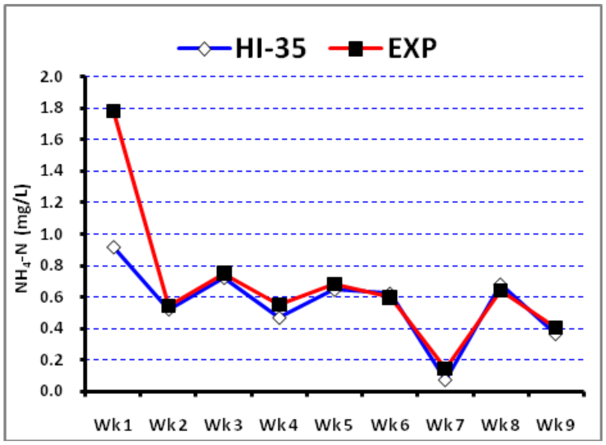


Fig. 4: Weekly changes in $NO_2$-N

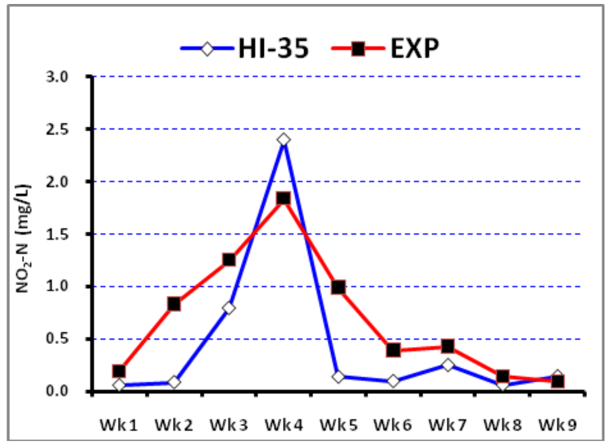


Fig. 5: Weekly changes in $NO_3$-N

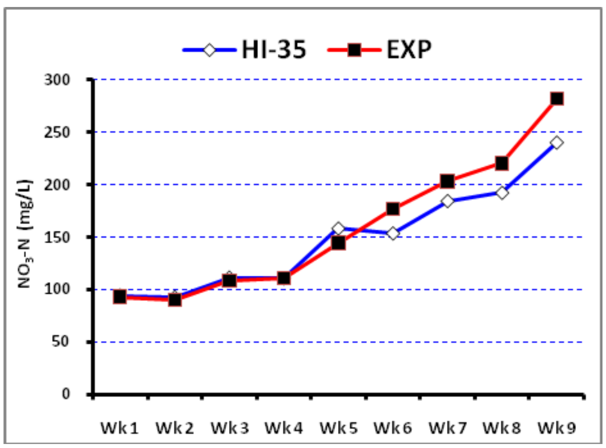


Fig. 6: Weekly changes in $PO_4$-P

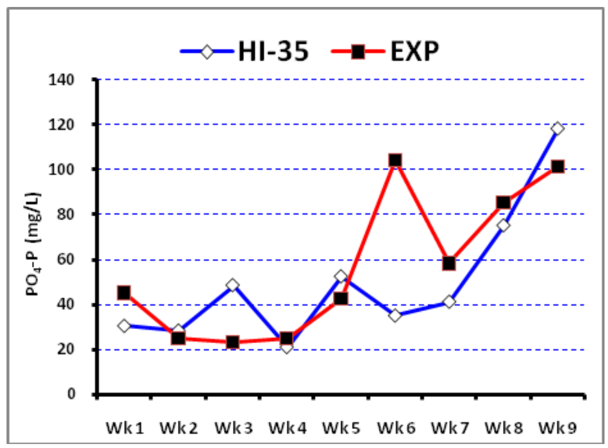


Fig. 7: Weekly changes in TSS

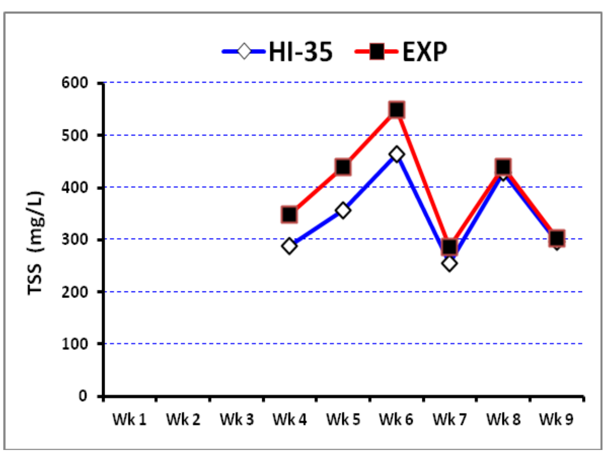


Fig. 8: Weekly changes in Alkalinity