# Use of Tilapia Effluent as a Nutritional Source for the Pacific White Legged Shrimp in Recirculating Systems

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## Introduction

Previous studies have demonstrated that Pacific white shrimp (*Litopenaeus vannamei*) are able to utilize natural biota and bacterial supplements, such as microbial flocs, as nutrients (McIntosh et al., 2000; Burford et al., 2004). Tilapia wastewater therefore, potentially represents a valuable source of nutrition for shrimp, since it contains organic and suspended matter (Jiménez-Montealegre et al., 2002). Both of these materials can be readily converted into microbial flocs using aerobic bioreactors (Grady et al., 1999; Metcalf and Eddy, 2003).

The purpose of the present study was to investigate whether effluent, derived from a commercial tilapia operation, could be employed as a suitable form of nutrition for Pacific white legged shrimp. Untreated settled solids from the tilapia farm and microbial flocs were generated using custom-made bioreactors. These treated effluent sources were examined against each other, and commercial shrimp feed for their potential nutritional value using growth performance as the prime indicator.

## Materials and Methods

*System and husbandry*

Six Aquatic Habitats™ (AHAB) benchtop systems (Aquatic Ecosystems, Apopka, FL, USA) were employed. Each system consisted of of four 10-L aquaria, 38 L/min magnetic driven pump, 35 L aerated sump with Siporex™ Biomedia, 10-inch, 50-micron canister filter, 10 inch canister carbon filter, 25-watt UV sterilizer, and 250-watt submersible heater. All treatments were conducted in 2.0 g/L salinity derived from synthetic sea salt (Crystal Sea, Marineland, Baltimore, MD, USA)

Certified, specific pathogen-free (SPF) postlarval shrimp were supplied by The Oceanic Institute (Kailua-Kona, Hawaii, USA). Salinity of the shipped samples was lowered from 18 g/L to approximately 2.0 g/L using the acclimation procedure described by Van Wyk et al. (1999). The shrimp were maintained at this salinity for an additional 10 days prior to the start of the experiment and were conditioned on a 35% crude protein (CP), ground shrimp feed (Melick Aqua Feeds, Catawissa, PA, USA).

*Diets and experimental design*

This study was conducted over a 40 day period with four diets that included the aforementioned commercial shrimp feed as components. They included: (diet one) 100% shrimp feed, (diet two) 50% shrimp feed with 50% microbial flocs, (diet three) 50% shrimp feed with 50% untreated solids, and (diet four) 50% shrimp feed. All diets were fed *ad libitum*, but a surplus of feed was available for consumption during a 24 hour period. Every 24 hours, all feed was completely removed from the shrimp aquaria by siphoning with a 60 mL syringe.

Microbial flocs, for diet two, were generated from tilapia effluent that was treated in 12 aerobic batch bioreactors (38-L) until the soluble chemical oxygen demand (sCOD, between 86 and 97 mg/L) was reduced by greater than 80% (in most cases greater than 90%). The tilapia effluent was collected from a local commercial recirculating aquaculture system (RAS) tilapia farm. The effluent was diverted and collected while a settling basin was being drained as part of the normal operations at the farm to discharge settled solids. The untreated solids for diet three were collected from the tilapia effluent and fed directly to the shrimp without treatment. The microbial flocs and untreated solids were concentrated by allowing solids to settle for approximately 30 to 45 minutes. As part of diets two and three, the microbial flocs and settled solids were fed on alternating days, making up 50% of the diet. For diet four, shrimp were fed shrimp feed on alternating days, thereby making up 50% of the shrimp feed available as compared to diet one.

The four dietary treatments were randomly distributed amongst the six AHAB benchtop systems. The treatments were conducted in sextuplicate for all diets. Each aquarium was initially stocked with five juvenile *L. vannamei* with a mean weight of 26.3 ± 1.3 mg. Survival was monitored every 48 hours, and final weights were measured for all surviving shrimp in each aquarium at the end of the feeding trial. Shrimp were patted dry with Kim Tech Wipes and weighed to the nearest 0.0001 g. Specific growth rates (SGR) were determined using the following formula (Ricker, 1975).

$$\mathrm{SGR}\left(\frac{\%}{\mathrm{d}}\right) = \frac{100 * [\log_e \text{shrimp final mass (g)} - \log_e \text{shrimp initial mass (g)}]}{\text{time (d)}} \qquad \text{(Eq. 1)}$$

*Water quality*

Water quality was monitored for nitrite, nitrate, and total ammonia nitrogen (TAN) by spectrophotometry (HACH DR/2400 Spectrophotometer, HACH Co., Loveland, CO, USA). Salinity, temperature, and dissolved oxygen (DO) were measured using a YSI model 85 probe (Yellow Springs Inc., Yellow Springs, OH, USA). A HI 9024 pH meter (HANNA Instruments, Woonsocket, RI, USA) was used to measure pH. COD was determined using Method 5220D (APHA, 1998) for the generation of microbial flocs used in diet two.

*Statistical analysis*

Statistical analyses was performed using SAS v13.0 for windows (SAS Institute Inc., Cary, NC, USA). Differences in water quality were considered significant when $P < 0.05$. A one-way ANOVA was employed and, when appropriate, a Duncan's Multiple Range Test was used to test significant differences ($P < 0.05$) between treatments for 40 day survival and growth.

## Results and Discussion

Water quality results are presented in Table 1. No differences in DO, nitrate, nitrite, pH, salinity, TAN, and temperature were observed between any of the AHAB systems. Levels of nitrite and TAN never peaked higher than 0.169 and 0.07 mg/L, respectively. The minimum DO concentration observed during this study was 4.70 mg/L. The salinity during this study was maintained at 2.0 g/L and did not deviate by more than 10%.

Table 1. Water quality results for the 6 AHAB systems, mean values (95% confidence interval). Number of sampling events denoted by n.

| AHAB system | Dissolved oxygen [mg/l] | Nitrate-N [mg/L] | Nitrite-N [mg/L] |
|---|---|---|---|
| | n = 13 | n = 9 | n = 9 |
| 1 | 5.21 (4.87-5.55) | 15 (5.5-25) | 0.020 (0-0.072) |
| 2 | 5.18 (4.76-5.61) | 16 (5.9-26) | 0.022 (0-0.077) |
| 3 | 5.20 (4.82-5.57) | 18 (5.5-31) | 0.029 (0-0.11) |
| 4 | 5.06 (4.73-5.39) | 20 (6.7-33) | 0.032 (0-0.14) |
| 5 | 5.16 (4.85-5.47) | 12 (5.9-18) | 0.017 (0-0.049) |
| 6 | 5.10 (4.71-5.50) | 18 (5.9-31) | 0.012 (0-0.024) |

| AHAB system | pH | Total ammonia-N [mg/L] | Temperature [$^{o}$C] |
|---|---|---|---|
| | n = 11 | n = 11 | n = 28 |
| 1 | 8.45 (8.37-8.53) | 0.018 (0-0.045) | 29.8 (28.5-31.1) |
| 2 | 8.48 (8.35-8.62) | 0.020 (0-0.040) | 29.4 (27.7-31.1) |
| 3 | 8.50 (8.37-8.62) | 0.022 (0.002-0.041) | 29.1 (27.9-30.4) |
| 4 | 8.53 (8.37-8.68) | 0.021 (0-0.048) | 30.0 (28.3-31.6) |
| 5 | 8.45 (8.24-8.65) | 0.025 (0-0.065) | 29.4 (28.0-30.7) |
| 6 | 8.49 (8.34-8.65) | 0.029 (0-0.070) | 29.5 (27.7-31.3) |

Water quality in this study was considered optimal for juvenile *L. vannamei* in terms of DO (Van Wyk et al., 1999), nitrogen constituents (Wickins, 1976; Lin and Chen, 2001; Sowers et al., 2004) and temperature (Ponce-Palafox et al., 1997). Statistical analysis of the variations in diets was strengthened because there were no differences in water quality between the AHAB systems.

Final mass and survival rates varied significantly ($P < 0.05$) for the shrimp fed the different diets (Table 2). In terms of final mass, diet one out-performed all other diets, and diet two was significantly better than diet four. It should be noted that shrimp fed diet four were 53% smaller than the shrimp fed diet one. This was expected because shrimp fed diet one received twice the protein level. This justified the use of diet four as a means of directly comparing diets two and three, in which shrimp were fed 50% of the feed, but were supplemented with microbial flocs and untreated solids, respectively. The microbial flocs used in diet two contributed significantly to growth, whereas the untreated solids used in diet three did not. Shrimp fed diet two and diet three at the end of the 40 day trial were, respectively, 44% and 25% larger than diet four. With respect to survival rates, rates for diets two and four were higher than that of diet one. However, diet three survival rates were not statistically different from any of the other feeding regimes.

Table 2. Final mass, SGR, and survival of shrimp as determined on day 40, alphas denote significant differences.

| Diet | SGR [1/d] | Final mass [mg] | Survival [%] |
|---|---|---|---|
| Diet one: 100% shrimp feed | 6.68 | $385.0^{a}$ | $73^{b}$ |
| Diet two: 50% shrimp feed, 50% microbial flocs | 5.71 | $259.1^{b}$ | $93^{a}$ |
| Diet three: 50% shrimp feed, 50% untreated solids | 5.35 | $225.7^{b,c}$ | $87^{a,b}$ |
| Diet four: 50% shrimp feed | 4.80 | $180.2^{c}$ | $93^{a}$ |
| Pooled error | | 153400 | 226.7 |
| P > F | | <0.0001 | 0.1027 |

## Conclusions

This study demonstrated that the microbial flocs generated in bioreactors using tilapia effluent contributed significantly to shrimp growth. This alternative source of nutrition could potentially be used to reduce feed costs if dual culture of fish and shrimp was employed. McIntosh et al. (2000) and Burford et al. (2004) demonstrated the importance of microbial floc systems for the culture of shrimp. However, unlike previous studies, the microbial flocs generated herein were from a waste stream that would have otherwise been discharged. The recycling of nutrients in fish effluents to feed shrimp can potentially save the shrimp farming industry money, making a positive step towards sustainability.

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