THE EFFECT OF TWO COMMERCIAL FEEDS AND DIFFERENT C:N RATIOS ON SELECTED WATER QUALITY INDICATORS AND PERFORMANCE OF Litopenaeus vannamei JUVENILES CULTURED AT HIGH DENSITY IN A BIOFLOC-DOMINATED ZERO-EXCHANGE OUTDOOR TANK SYSTEM

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Introduction

Operating zero-exchange biofloc-dominated systems can potentially:

- Increase bio-security
- Increase production
- Reduce effluent discharge
- Reduce water usage

The driving force of these systems is the microbial biofloc which can:

- Help maintain optimal water quality
- Serve as a supplemental food source
Introduction

Choosing a appropriate feed is important:

- Maximize shrimp growth
- Reduce feed cost
- Minimize negative impact on WQ

Developing microbial flocs is necessary to:

- Control nitrogen
- Recycle feed

Inoculation and adding carbohydrates are practical and effective means of enhancing the development of microbial flocs
Objectives & Experimental Design

Evaluate the effect of two commercial shrimp feeds and different C:N ratios on selected water quality indicators and shrimp performance in a biofloc-dominated zero-exchange tank system.

2 × 5 Factorial Experiment Design

- **Semi-intensive feed**
  - C/N=9
  - C/N-Adjust
  - C/N=12
  - C/N=15
  - C/N=18

- **Hyper-intensive feed**
Materials & Methods

Juvenile *L. vannamei*
- Average weight: $2.21 \pm 0.11$ g
- Stocking density: 300 shrimp/m$^3$

Experimental system
- Forty 800-L HDPE tanks
- Equipped with 2 airstones for aeration
- Filled with biofloc-rich water (500 L)

Tank management
- No water exchange
- Freshwater was added to compensate for evaporative losses
- $\text{NaHCO}_3$ was added to maintain pH above 7.2
Materials & Methods

Feeds and Feeding

- Two 35% CP commercial feeds: Semi-intensive & Hyper-intensive feeds (SI-35 & HI-35)
- Feed offered in two equal portions during the day: 08:30 and 18:30
- Rations were adjusted based on feed tray observations and weekly shrimp growth sampling

<table>
<thead>
<tr>
<th>Proximate composition of the two experimental feeds (% dry weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
</tr>
<tr>
<td>----------------</td>
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<tr>
<td>SI-35</td>
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<tr>
<td>HI-35</td>
</tr>
</tbody>
</table>
Materials & Methods

Carbohydrate supplementation
- C/N: 9, 12, 15, 18 based on the carbon and nitrogen contents of the feeds and molasses, respectively
- The C/N: Adjust treatment was based on the actual TAN in the culture water (6 g of C was added for each 1 g of TAN)
- 35% CP commercial feed had C/N=9

Water quality monitoring
- Temperature, salinity, dissolved oxygen, and pH were recorded twice daily
- SS, TSS, VSS, NH$_4$-N, NO$_2$-N, NO$_3$-N, alkalinity, cBOD$_5$, and turbidity were measured weekly
Materials & Methods

Shrimp performance

- Survival (%) = 100 × (final shrimp count / initial shrimp count)
- Weekly growth rate (g/week) = (final average weight − initial average weight) / culture weeks
- Biomass = total harvest shrimp weight / water volume
- FCR = total dry weight of feed offered / total shrimp wet weight gained
## Results

A Two-way Linear Mixed Model showing the effects of feed and C/N ratio on selected water quality parameters during 6-week study with *L. vannamei*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Significant† ($P$ value)</th>
<th>Diet</th>
<th>C/N</th>
<th>Diet $\times$ C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>NS (0.382)</td>
<td></td>
<td>*** (0.000)</td>
<td>NS (0.942)</td>
</tr>
<tr>
<td>TSS</td>
<td>*** (0.000)</td>
<td></td>
<td>*** (0.000)</td>
<td>NS (0.686)</td>
</tr>
<tr>
<td>VSS</td>
<td>** (0.002)</td>
<td></td>
<td>*** (0.000)</td>
<td>NS (0.797)</td>
</tr>
<tr>
<td>TAN</td>
<td>NS (0.977)</td>
<td></td>
<td>NS (0.514)</td>
<td>NS (0.988)</td>
</tr>
<tr>
<td>NO$_2$-N</td>
<td>NS (0.914)</td>
<td></td>
<td>* (0.014)</td>
<td>NS (0.842)</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>NS (0.972)</td>
<td></td>
<td>*** (0.000)</td>
<td>NS (0.686)</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>* (0.043)</td>
<td></td>
<td>*** (0.000)</td>
<td>NS (0.984)</td>
</tr>
<tr>
<td>cBOD$_5$</td>
<td>* (0.046)</td>
<td></td>
<td>** (0.003)</td>
<td>NS (0.389)</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NS (0.985)</td>
<td></td>
<td>*** (0.000)</td>
<td>NS (0.143)</td>
</tr>
</tbody>
</table>

† $P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant.
Results

Water quality

**SI-35**

**HI-35**

**SS (mL L⁻¹)**

**Sampling time (week)**

<table>
<thead>
<tr>
<th>CN-9</th>
<th>CN-Adjust</th>
<th>CN-12</th>
<th>CN-15</th>
<th>CN-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>1</td>
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<td>6</td>
<td>6</td>
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<td>6</td>
</tr>
</tbody>
</table>
Results

Water quality

![Graphs showing TSS (mg L⁻¹) vs. Sampling time (week) for SI-35 and HI-35.](image-url)
Results

Water quality

![Graph showing changes in VSS over time for different sampling sites.](image)

- **SI-35**
  - VSS (mg L⁻¹)
  - Sampling time (week)
  - CN-9, CN-Adjust, CN-12, CN-15, CN-18

- **HI-35**
  - VSS (mg L⁻¹)
  - Sampling time (week)
  - CN-9, CN-Adjust, CN-12, CN-15, CN-18
Results

Water quality

![Graph showing water quality data over time for different sampling locations and water quality parameters.]

- **SI-35**
  - TAN (mg L⁻¹) values increase over time for all locations, with CN-18 showing the highest values.

- **HI-35**
  - Similar trend observed, with CN-18 again showing the highest values.

*Note: The graph includes bars indicating the mean and error bars for each sample point. The X-axis represents sampling time (week), and the Y-axis represents TAN concentration (mg L⁻¹). The different colors represent different locations (CN-9, CN-Adjust, CN-12, CN-15, CN-18).*
Results

Water quality

![Graph showing NO2-N levels over time for different sites.](image-url)
**Results**

**Water quality**

![Graph showing water quality results over time](image_url)

**NO$_3$-N (mg L$^{-1}$)**

- **SI-35**
- **HI-35**

Sampling time (week)

[Graphs showing changes in NO$_3$-N levels over time for different conditions (CN-9, CN-Adjust, CN-12, CN-15, CN-18)].
Results

Water quality

**Results**

**Water quality**

- **CN-9**
- **CN-Adjust**
- **CN-12**
- **CN-15**
- **CN-18**

**Alkalinity (mg L$^{-1}$)**

**Sampling time (week)**

**SI-35**

**HI-35**
Results

Water quality

![Graphs showing water quality results for SI-35 and HI-35 over sampling time (weeks)]
Results

Water quality

**Turbidity (NTU)**

**Sampling time (week)**

SI-35

HI-35
Results

C/N < 12

Water quality

C/N ≥ 12
# Results

Two-way Repeated Measures ANOVA showing the effects of feed and C/N ratio on shrimp performance at the end of 6-week study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Significant† (P value)</th>
<th>Diet</th>
<th>C/N</th>
<th>Diet × C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight</td>
<td>*** (0.000)</td>
<td>***</td>
<td>***</td>
<td>NS (0.591)</td>
</tr>
<tr>
<td>Growth rate</td>
<td>*** (0.000)</td>
<td>***</td>
<td>***</td>
<td>NS (0.575)</td>
</tr>
<tr>
<td>Survival</td>
<td>* (0.041)</td>
<td>**</td>
<td>**</td>
<td>NS (0.556)</td>
</tr>
<tr>
<td>Yield</td>
<td>*** (0.000)</td>
<td>***</td>
<td>***</td>
<td>NS (0.619)</td>
</tr>
<tr>
<td>FCR</td>
<td>*** (0.000)</td>
<td>***</td>
<td>***</td>
<td>NS (0.956)</td>
</tr>
</tbody>
</table>

† *P < 0.05; **P < 0.01; *** P < 0.001; NS, not significant.
### Shrimp performance

Means ± S.D. of final weight, growth, survival, yield, and FCR of *L. vannamei* at the end of 6-week study

<table>
<thead>
<tr>
<th></th>
<th>Final Wt. (g)</th>
<th>Growth (g /wk)</th>
<th>Survival (%)</th>
<th>Yield (kg m⁻³)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SI-35</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN-9</td>
<td>8.52 ± 0.26 a</td>
<td>1.05 ± 0.04 ab</td>
<td>98.50 ± 2.62 ab</td>
<td>2.46 ± 0.06 b</td>
<td>1.55 ± 0.02 ab</td>
</tr>
<tr>
<td>CN-Adjust</td>
<td>8.51 ± 0.14 ab</td>
<td>1.05 ± 0.06 ab</td>
<td>99.33 ± 1.46 b</td>
<td>2.48 ± 0.04 b</td>
<td>1.54 ± 0.02 a</td>
</tr>
<tr>
<td>CN-12</td>
<td>8.75 ± 0.18 b</td>
<td>1.09 ± 0.04 b</td>
<td>96.67 ± 0.36 a</td>
<td>2.48 ± 0.02 b</td>
<td>1.52 ± 0.01 a</td>
</tr>
<tr>
<td>CN-15</td>
<td>8.24 ± 0.36 ab</td>
<td>1.01 ± 0.02 ab</td>
<td>98.83 ± 0.26 ab</td>
<td>2.39 ± 0.10 ab</td>
<td>1.62 ± 0.08 ab</td>
</tr>
<tr>
<td>CN-18</td>
<td>8.09 ± 0.46 a</td>
<td>0.98 ± 0.06 a</td>
<td>96.00 ± 2.18 a</td>
<td>2.27 ± 0.08 a</td>
<td>1.71 ± 0.06 b</td>
</tr>
<tr>
<td><strong>HI-35</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN-9</td>
<td>9.84 ± 0.28 bc</td>
<td>1.27 ± 0.04 c</td>
<td>97.33 ± 0.36 a</td>
<td>2.81 ± 0.08 b</td>
<td>1.29 ± 0.08 b</td>
</tr>
<tr>
<td>CN-Adjust</td>
<td>9.75 ± 0.36 bc</td>
<td>1.26 ± 0.02 bc</td>
<td>97.50 ± 0.76 a</td>
<td>2.79 ± 0.10 b</td>
<td>1.30 ± 0.10 b</td>
</tr>
<tr>
<td>CN-12</td>
<td>9.99 ± 0.22 c</td>
<td>1.30 ± 0.02 c</td>
<td>96.50 ± 2.12 a</td>
<td>2.83 ± 0.02 b</td>
<td>1.27 ± 0.02 b</td>
</tr>
<tr>
<td>CN-15</td>
<td>9.20 ± 0.16 ab</td>
<td>1.17 ± 0.06 ab</td>
<td>97.83 ± 0.30 a</td>
<td>2.64 ± 0.04 a</td>
<td>1.40 ± 0.04 a</td>
</tr>
<tr>
<td>CN-18</td>
<td>9.03 ± 0.36 a</td>
<td>1.14 ± 0.08 a</td>
<td>95.67 ± 1.64 a</td>
<td>2.53 ± 0.12 a</td>
<td>1.47 ± 0.12 a</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D. (n = 4).
For each feed, values in the same row with different superscripts are significantly different (P < 0.05) based on Tukey HSD test.
Summary

- No significant differences in inorganic N species (TAN, NO₂-N, NO₃-N) between the two feeds
- Growth & FCR of shrimp fed the HI-35 was significantly better than with the SI-35
- C/N ratio affected microbial communities
  - C/N ratio <12 → dominated by algae (*green-water*)
  - C/N ratio >12 → dominated by heterotrophic bacteria (*brown-water*)
  - C/N ratio >12 → increase in biofloc volume with the increase in C/N ratio
- Higher C/N ratio resulted in lower NO₃-N concentrations and greater biofloc volume
- C/N ratio of 12 showed best shrimp performance in both feeds
Acknowledgements

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