The Impacts of Probiotics and Prebiotics in RAS Pompano Aquaculture

Susan Laramore
Fish Health

Stress and Disease
- Effects Production and Profitability

Disease can be reduced by good management
- But not eliminated

Diseases are often combated by
- Chemicals and antibiotics, but...
  - Potential risks to consumers and the environment

Alternatives
- Probiotics, prebiotics, immunostimulants
Fish Gastrointestinal Tract

- Gut microbiome consists of trillions of microbes
- Develops throughout a fishes lifetime
- Impacted by bacteria from the water and feed
- Balanced GIT – 85% good guys
  - Produce antimicrobial agents that promote an immune response
Probiotics, Prebiotics, Synbionts

- **Probiotics** = live beneficial bacteria that improve intestinal microbial balance
  - LABs and *Bacillus sp.*

- **Prebiotics** = non-digestible feed ingredients that selectively stimulate growth or activity of probiotics
  - fructooligosaccharides (FOS), mannanooligosaccharides, inulin and β-glucan

- **Synbionts** = Probiotics + Prebiotics

- **Modulate the non-specific immune system**
  - First line of defense
Why Project was Initiated

Unaware of investigations concerning the effect of synbionts during the entire culture cycle of pompano or any other fish species (egg to harvest)

“One Increase nutrient availability, health status, and survival of Florida Pompano utilizing synbiotic feed additives”

One of three project objectives
Hematological Indices

- Red blood cells (RBCs, Erythrocytes)
- White blood cells (WBCs, Leukocytes)
- Total counts
- Differential leukocyte counts
  - Lymphocytes, thrombocytes, heterophils, monocytes, eosinophils
  - Lymphocytes – produce B (antibodies) and T (killer cells)
  - Granulocytes – produce enzymes
    - eosinophils, heterophils
  - Phagocytes – heterophils, monocytes
Immune System Function

- **Phagocytosis**
  - Engulfing, destroying, discharging
  - Macrophages, monocytes, heterophils

- **Superoxide dismutase (SOD)**
  - Breaks down harmful oxygen molecules
  - Speeds up chemical reactions

- **Lysozyme Activity**
  - Anti-microbial activity
  - Gram positive bacteria
Digestive Enzymes

- **Amylase**
  - converts starch into simple sugars
- **Lipase**
  - breaks down fats
- **Protease**
  - breaks proteins into peptides and amino acids
- **Alkaline Phosphatase**
  - breaks down proteins, protects GIT from bacteria and aids in digestion
- **Proteins**
Experiment 1 – Synbioint Screening

Ten treatments

Control, Probiotic, Probiotic + Prebiotic 1 (4 levels), Probiotic + Prebiotic 2 (4 levels)

- *Bacillus* sp blend + *Pedicoccus acidilactici* ($10^6$ CFU g$^{-1}$)
- FOS or β-glucan (0.5, 1.0, 2.0 and 4.0 g kg$^{-1}$)

Three months

5 to 50 g larval pompano

8 - 16 Fish/Treatment
Hematological Indices

Proportion of RBC’s or WBC’s

- (P<0.001)

Differential Leukocyte Counts

- Lymphocytes (P=0.1733)
- Thrombocytes (P=0.3341)
- Monocytes (P=0.0142)
  - β-Glu 2.0, FOS 4.0
- Granulocytes
  - Heterophils (P=0.0511)
    - β-Glu 1.0 & 2.0, FOS 4.0
  - Eosinophils (P=0.2912)
Immune Function

- **Phagocytosis**
  - NS (P=0.857)
  - $\beta$-Gluc 1.0/Bac, FOS 2.0/Bac

- **SOD**
  - NS (P=0.872)
  - $\beta$-Gluc 1.0/Bac, FOS 4.0/Bac

- **Lysozyme**
  - NS (P=0.344)
  - $\beta$-Gluc 2.0/Bac, FOS 1.0/Bac
Digestive Enzymes

- **Protein**
  - NS (P=0.69)

- **Alkaline Phosphatase**
  - NS (P_pro=0.72, P_tiss=0.76)

- **Amylase**
  - NS (P_pro=0.42, P_tiss=0.47)

- **Lipase**
  - Sig (P_pro=0.011, P_tiss=0.013)
    - Highest in FOS 4.0/Bac

- **Protease**
  - NS (P_pro=0.260, P_tiss=0.241)
Take Away

• No clear-cut winner
  • 0.5% prebiotic addition didn’t perform well
  • 4% prebiotic addition did not increase benefit in β-Gluc
    • Cost prohibitive for FOS
• Best performance in...
  • β-Gluc 1.0 and 2.0/Bacillus sp + P. acidilactici
  • FOS 2.0 and 4.0/Bacillus sp + P. acidilactici
Experiment 2 – Pellet Type ± Synbiont

Four treatments

- Hard pellet ± Synbiont
- Soft pellet ± Synbiont
  - *Pedicoccus acidilactici* (10^6 CFU g\(^{-1}\))
  - β-glucan (1.0 g kg\(^{-1}\))

Three months

- 250 g pompano
- 12 Fish/Treatment
Hematological Indices

Proportion of RBC’s or WBC’s
- NS (P=0.277)

Differential Leukocyte Counts
- Thrombocytes (P=0.378)
- Lymphocytes (P=0.056)
  - Hard vs Hard β-glucan (P=0.0188)
- Monocytes (P=0.806)
- Granulocytes
  - Heterophils (P=0.549)
  - Eosinophils (P=0.749)
Immune Function

- **Phagocytosis**
  - Sig (P=0.023)
- **SOD**
  - NS (P=0.743)
- **Lysozyme**
  - NS (P=0.590)

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<thead>
<tr>
<th>OBJ 2 Phagocytosis Activity</th>
<th>Macrophage Engulfing (% Positive)</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>Mean</td>
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<tr>
<td>Hard Pellet</td>
<td>20</td>
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<tr>
<td>Hard Pellet (β-glucan)</td>
<td>29.8</td>
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<tr>
<td>Soft Pellet</td>
<td>36.2</td>
</tr>
<tr>
<td>Soft Pellet (β-glucan)</td>
<td>36.7</td>
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<tr>
<td>ANOVA Omnibus p =</td>
<td>0.023*</td>
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<thead>
<tr>
<th>A Priori Orthogonal Contrast</th>
<th>Macrophage Engulfing (% Positive)</th>
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<tbody>
<tr>
<td>Hard vs Soft</td>
<td>0.0076*</td>
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<tr>
<td>Hard vs Hard β-glucan</td>
<td>0.0998</td>
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<tr>
<td>Soft vs Soft β-glucan</td>
<td>0.9471</td>
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<tr>
<td>No β-glucan vs -glucan</td>
<td>0.2195</td>
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Digestive Enzymes

- **Protein (mg/L)**
  - NS (P=0.199)
- **Alkaline Phosphatase (U/mg)**
  - NS (Ppro=0.862)
- **Amylase (U/mg)**
  - Sig (Ppro=0.030)
  - ↑ Hard vs ↓ Soft (P=0.005)
- **Lipase (U/mg)**
  - NS (Ppro=0.308)
- **Protease (mg/L)**
  - NS (Ppro=0.259)
More Questions Than Answers!

- The synbiontic provided no apparent health benefits
  - Differences were only seen between pellet type

Was the sample size too small to detect differences?

Did we choose the right levels and synbiont combination?

Was addition at 250 g too late in life cycle for any benefit?

Were the fish too healthy to detect any benefit?
Experiment 4 – Early Synbiont Application

**Phase 1**
- 1 month
- 2 Treat
- 16 fish/Trt

**Phase 2**
- 1 month
- 4 Treat
- 8 fish/Trt

**Phase 3**
- 8 months
- + Pellet type
- 16 Treat
- 8 fish/Trt
- Stats for 4:
  - Hard, Soft, Non, Syn
  - 24 fish/Trt

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**Figure 1.** Dietary treatments during experiment 4 with Florida pompano from first feeding through mean weight of 100g.
Hematological Indices

- Only significant differences were seen in Phase 1:
  - Proportion of RBC’s or WBC’s
    - RBCs in Non Synbiont (P=0.019)
  - Differential Leukocyte Counts
    - Heterophils (P=0.0167)
    - Non Synbiont
    - Thrombocytes (P=0.058)
    - Synbiont
Immune Function

**Phagocytosis (Phase 3 only)**
- Sig (P=<0.001)
  - Non Synbiont
  - Synbiont*pellet interaction; Soft

**SOD**
- Sig Phase 3 (P=0.033)
  - Pellet type (P=0.0226), Syn (P=0.0659)
  - Soft Non vs Hard Syn

**Lysozyme**
- NS all phases (P=0.940, 0.344, 0.086)
Digestive Enzymes

• Phase 1 – NS
• Phase 2 – NS
• Phase 3 - Sig
  • Alkaline Phosphatase (P=0.005 – 0.05)
  • Pellet*synbiont interaction (P=0.002-0.004)
  • Hard non = Soft syn > Soft non, Hard syn
• Amylase (P=0.0339 – 0.05)
  • Pellet*synbiont interaction (P=0.004-0.007)
  • Hard syn = Soft non > Hard non, Soft syn
More to Tease out!

• Phase 1 - early response (larvae – 50 g) to synbionts
  • Hematological only
    • Syn = deceased RBCs, decreased heterophils, increased platelets

• Phase 2 – addition or removal of synbionts (50 – 100 g)
  • No difference was seen in tested health parameters

• Phase 3 – Pellet type + addition or removal of synbionts (100 g to harvest)
  • No Hematological responses
  • Immune function responses
    • Phagocytosis, SOD
  • Digestive enzyme responses
    • Alkaline phosphatase, Amylase

*Still to compare phase 3
  – Tease out Hard pellets only
  - Affects of the phase 2 “swap”
Are Synbiotics beneficial in RAS Pompano Culture?

- Inconclusive
  - Impacts of Stress, Disease Challenge should be evaluated
  - Right combinations, optimal levels
  - Statistical power analysis

Usefulness of Health Indices

- Early life stages - Hematological Assays
  - RBCs vs WBCs, heterophils, lymphocytes, thrombocytes
- Later life stages – Immune system Function, Digestive Enzymes
  - Immune = phagocytosis, SOD
  - Digestive = lipase, alkaline phosphatase, amylase
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