Effects of Yeast Products on Immune Function and Disease Resistance of Hybrid Striped Bass

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The diagram illustrates various factors affecting the immune system. The central node labeled "Immune System" connects to multiple branches:

- **Temperature**
- **Photoperiod**
- **Protein Lipids**
- **Environmental factors**
- **Nutrients**
- **Micro-nutrients**
- **Stressors**
- **Immunomodulators other than micronutrients**

Subcategories around the central node include:

- **Management factors**:
  - Seasons (storm, typhoon, hurricane)
  - Stressors:
    - Glucans, adjuvants
  - Immuno-modulators other than micronutrients

- **Nutrients**:
  - Antioxidant Vitamins
  - Carotenoids
  - Heavy metals

The diagram is attributed to Verhac and Gabaudan 1997.
Non-specific immune response

**Natural barriers:** Skin and mucus

**When micro-organisms penetrate the body:**

**Cellular mechanisms:**
- Macrophage and neutrophil activities:
  - Chemotaxis
  - Phagocytosis
  - Pinocytosis
  - Killing: oxygen-dependent mechanisms
  - oxygen-independent mechanisms

**Extracellular killing:**
- Natural killer cells
- Eosinophils

**Soluble factors:**
- Complement
- Lysozyme
- C-reactive protein
- Transferrin
- Lactoferrin
- Ceruloplasmin
- Lectins, natural agglutinins
- Interferons

Verhac and Gabaudan 1997
Non-Nutritive Dietary Supplements Shown to Affect Fish Health

Immunostimulants
- heterogeneous group including
  - β-glucans
  - nucleotides
  - chitin

Probiotics
favorable microbial agents that alter intestinal flora, e.g., *Lactobacillus* and *Carnobacterium* sp.

Prebiotics
dietary constituents that alter the intestinal conditions to favor certain bacterial species
Three components in brewers yeast may have immunomodulating effects:

- **β-glucans** have been shown to enhance immune responses and disease resistance of several fish species (Gatlin 2002)

- **Nucleotides** recently have been reported to improve disease resistance of Atlantic salmon (Burrell et al. 2001) and common carp (Sakai et al. 2001)

- **Chitin** has been reported to have immunomodulatory effects in gilthead seabream (Esteban et al. 2001)
β-glucans

β-1,3- and β -1-6-linked polysaccharides

Sources
• yeast
• fungi

Mechanisms of action
• complement activation
• enhanced lysozyme production
• increased oxidative capacity of phagocytes

Injection has generally produced more favorable responses than oral administration (~0.2% of diet)
Nucleotides

Nitrogenous bases synthesized by various cells as precursors of DNA replication.

Their synthesis may become limited under conditions of disease or during wound repair.

Commercial products available for inclusion at 0.1 to 0.2% of diet.
Enhanced resistance to infection by bacterial, viral, rickettsial and ectoparasitic organisms has been reported in salmonids (Burrell et al. 2001)

Alterations in non-specific immune responses not consistently reported although potential mechanisms of action include:

- complement activation
- enhanced lysozyme production
- activation of macrophages
- increase natural killer cell activity
A mixture of partially autolyzed brewers yeast, dairy components and dried fermentation products
• Aquacultural production of hybrid striped bass is continuing to expand nationally and internationally.

• Infectious diseases have negatively impacted production of hybrid striped bass.

• Effective therapeutic agents are currently limited.
Disease outbreaks in hybrid striped bass are often caused by pathogenic bacteria such as *Streptococcus iniae*. At this time there are no efficacious chemotherapeutics that are approved to treat *Streptococcus* infection in hybrid striped bass.
Objectives

1. Determine the nutritional value of brewers yeast and Grobiotic AE™ as supplements in diets of hybrid striped bass.

2. Determine the potential of these supplements to enhance resistance of hybrid striped bass to *Streptococcus iniae*. 
Experimental Diets

*All diets formulated at 40% crude protein, 10% lipid, 3.5 kcal DE/g

<table>
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<tr>
<th>Ingredient (% dry weight)</th>
<th>Control</th>
<th>Brewtech 1%</th>
<th>Brewtech 2%</th>
<th>Grobiotic AE™ 1%</th>
<th>Grobiotic AE™ 2%</th>
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# Analyzed Composition of Experimental Diets

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<tr>
<th>% dry wt.</th>
<th>Basal Diet</th>
<th>Brewtech 1%</th>
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<th>Grobiotic 1%</th>
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<td>Dry matter</td>
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Feeding Trial 1

- Initial fish weight = 7.1 g.
- 13 fish stocked into each 110-L aquarium.
- Closed, recirculating system containing brackish water (1.5-2.0 ppt) at 25 ± 1 °C.
- Each diet was fed to fish in four replicate aquaria at the same fixed rate.
- Trial duration = 7 weeks.
Weight Gain

% of initial wt.

Control 1% 2% 1% 2%

Brewtech Brewtech Grobiotic Grobiotic Diet

7 wk
P = 0.45
Feed Efficiency

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<th>Diet</th>
<th>0.84</th>
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</table>

7 wk
P = 0.05
Survival

7 wk

P = 0.28
Feeding Trial 2

- Initial fish weight = 19.7 g
- 17 fish stocked into each 110-L aquarium
- Closed, recirculating system containing brackish water (1.5-2.0 ppt) at 25 ± 1 ºC
- Each diet fed to fish in three replicate aquaria at the same fixed rate
- Trial duration = 4 weeks
A virulent isolate of *Streptococcus iniae* was biochemically identified and provided by the Texas Veterinary Diagnostic Laboratory. The isolate was grown in BHI broth overnight at 27°C in a shaking water bath. Fish were exposed to $9.3 \times 10^5$ CFU/ml by immersion for 2 h, distributed into triplicate flow-through aquaria (10 fish each, 30 per treatment) and monitored 15 d.
• Dead or moribund fish removed twice daily for 19 d
• Brain and/or kidney tissue was streaked for bacterial isolation
• Death due to bacterial exposure confirmed via growth on selective agar
Immune Response Assays

The following non-specific immune responses were measured after 4 weeks of feeding:

- Serum lysozyme activity
- Blood neutrophil oxidative radical production
- Head kidney macrophage
  - Extracellular superoxide anion production
  - Intracellular superoxide anion production
Lysozyme Activity

4 wk

P = 0.7
Neutrophil Oxidative Radical Production

![Graph showing Neutrophil Oxidative Radical Production](chart.png)

- **X-axis**: Diet (Control, 1% Brewtech, 2% Brewtech, 1% Grobiotic, 2% Grobiotic)
- **Y-axis**: mg NBT diformazan/ml
- **Statistical Information**: 4 wk, P = 0.15
Extracellular Superoxide Anion Production (Trial 2)

4 wk

P < 0.01
Cumulative Survival During Bacterial Challenge (Trial 2)

Concentration of *S. iniae* suspension is $9.3 \times 10^5$ CFU/ml

Pr $< 0.01$
Conclusions

Brewers yeast and Grobiotic AE™ in the diet of hybrid striped bass:

• positively influenced growth performance
• significantly enhanced resistance to *Streptococcus iniae* infection
• increased macrophage respiratory burst activity
Future Research Plans

- Further investigate optimum dose and time of administering the two products
- Monitor changes in intestinal microflora
- Evaluate resistance of fish to other pathogenic organisms
Acknowledgements

• International Ingredient Corporation (St. Louis, MO) for providing Brewtech® dried brewers yeast and Grobiotic AE™.
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• Texas Veterinary Medicine Diagnostic Lab
• All the students at the Texas A&M Aquacultural Research and Teaching Facility