Cosajaba oil
A bioactive feed ingredient for aquaculture


Cosajaba Development Team

Prof. Mark E. Cook, Ph.D.
Co-inventor. Founded 4 companies based on WARF technologies.
Professor of Animal Sciences since 1983.

Terence P. Barry, Ph.D.
Co-inventor. Founded 2 companies: Willow Creek Aquaculture & AquaMost
Sr. Scientist and Director of UW Aquaculture Research Lab.

Jake M. Olson, Ph.D.
Co-inventor. Ph.D. Molecular/Environmental Toxicology.
Post-doc since 2016. Research focus: lipids and immune function.

Teams of undergraduate and post-graduate researchers
UW-Aquaculture Research Lab: 3 students
Cook lab in Animal Sciences: 5 students

Confidential (April 2017)
Aquaculture on the rise

- World population predicted to be 9.6 Billion by 2050
- A 40% increase in demand for dietary protein expected
- Aquaculture expected to fill supply-demand gap

FAO. 2016
Aquaculture vs terrestrial animal production efficiency

<table>
<thead>
<tr>
<th></th>
<th>Fish</th>
<th>Chicken</th>
<th>Pig</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Retention</td>
<td>31 %</td>
<td>21 %</td>
<td>18 %</td>
<td>15 %</td>
</tr>
<tr>
<td>Energy Retention</td>
<td>23 %</td>
<td>10 %</td>
<td>14 %</td>
<td>27 %</td>
</tr>
<tr>
<td>Edible Yield</td>
<td>68 %</td>
<td>46 %</td>
<td>52 %</td>
<td>41 %</td>
</tr>
<tr>
<td>Feed Conversion Ratio (FCR)</td>
<td>1.1</td>
<td>2.2</td>
<td>3.0</td>
<td>4.10</td>
</tr>
<tr>
<td>Edible Meat pr 100 kg fed</td>
<td>61 kg</td>
<td>21 kg</td>
<td>17 kg</td>
<td>4.10 kg</td>
</tr>
</tbody>
</table>

Marine Harvest. 2016

Atlantic salmon

- Salmonidae, same family as rainbow trout
- Popular for its high protein content, vitamins, minerals, and omega 3s
- Roughly 70% of production is farmed
Primary Issue: Seawater mortality of Atlantic salmon

- Substantial growth at sea
  
  **Seawater transfer**

(5-25% mortality, ave. is 10%)
- Opportunistic infection is substantial

**Smoltification**
After ~1 year post-hatch

- In Norway (#1), mortality losses equals $250M/year
Fish encounter a variety of stressors

Environmental Stress
- Noises and vibrations
- Incorrect decor or tank mates
- Incorrect water conditions

Pathogen Excess
- Introduction of ill fish
- Pathogens unknown to fish

Physical Damage
- From other fish & bullying
- From objects
- Incorrect substrate

Bad Water Quality
- Ammonia, nitrates & nitrites
- Fluctuating conditions
- Toxin build-up

Other Causes
- Low genetic immunity
- Incorrect diet
- Bad luck (sods law)

Initial period after seawater transfer is critical for survival

<table>
<thead>
<tr>
<th>Number of fish with viraemia</th>
<th>IM</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase-A (2 weeks post-transfer)</td>
<td>16/18</td>
<td>7/15</td>
</tr>
<tr>
<td>(89 %)</td>
<td>(47 %)</td>
<td></td>
</tr>
<tr>
<td>Phase-B (9 weeks post-transfer)</td>
<td>12/15</td>
<td>2/15</td>
</tr>
<tr>
<td>(80 %)</td>
<td>(13 %)</td>
<td></td>
</tr>
</tbody>
</table>

Plasma (Phase-A) or serum (Phase-B) samples of IM and BI treatments at 7 dpi analyzed. The values represent the number of positive samples/number of analyzed samples, and percentage of positive samples (in brackets)

Jarungsriapisit et al., 2016
Effect of seawater transfer on immune function

- Is immune suppression contributing to salmon mortality?

Johansson et al., 2016

Increased transport stress = increased mortality

- Transport stress likely contributed to mortality

- During smoltification, salmon are fragile
- Seawater transfer is often abrupt, maladaptation can occur

Table 2
Transport time and meteorological conditions during five different commercial smolt transports in Norway and Scotland

<table>
<thead>
<tr>
<th>Transport</th>
<th>Time (days)</th>
<th>Transport time (h)</th>
<th>Average wind direction</th>
<th>Average wind speed (m/s)</th>
<th>Gust of wind (m/s)</th>
<th>Average wave height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>03.06.00</td>
<td>6.5</td>
<td>NW</td>
<td>12</td>
<td>18</td>
<td>3.0-3.5</td>
</tr>
<tr>
<td>II</td>
<td>04.06.02</td>
<td>40</td>
<td>SE to NW</td>
<td>5</td>
<td>7</td>
<td>0.4-1.0</td>
</tr>
<tr>
<td>III</td>
<td>14.05.00</td>
<td>4.0</td>
<td>W</td>
<td>8</td>
<td>11</td>
<td>2.0</td>
</tr>
<tr>
<td>IV</td>
<td>14.05.00</td>
<td>4.0</td>
<td>No wind</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>30.05.00</td>
<td>4.0</td>
<td>No wind</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Confidential (April 2017)
Hypoxia stress is common at sea

- Salmon experience dramatic fluctuation in dissolved oxygen in seawater
- O$_2$ levels may drop down to 30% air saturation (late summer and early fall, temperature, stocking density)
- Chronic hypoxia reduces immune function (Kvamme et al., 2013)

Burt et al., 2011; Oppedal et al., 2011

- Salmon mortality = Contribution of several non-lethal stressors?
- Effective strategies to overcome stress would be valuable to salmon industry

Animal Sciences Department Animal BioProducts Initiative

- 37-49% of animal is inedible
- >50 billion pounds of by-products produced each year

Bioproducts

Materials and chemicals from animal by-products
- Pharmaceuticals
- Biomedical devices
- Bioactive molecules
- Manufacturing chemicals

Rendering Products
- soaps, solvents, adhesives, crayons, cosmetics, antifreeze, feed additives, etc

Edible Products

Confidential (April 2017)
Preening

- In avian species, function is not completely understood
- Variety of wax ester compounds and other unique lipids

Cosajaba oil: Abundantly uncommon

- Crude extract of avian preen gland (low value-byproduct)
- 9 billion broiler chickens in US
- Hypothesized anti-inflammatory and anti-proliferative properties
- What are the benefits of an anti-inflammatory in fish diets?
Crude gland extraction process

- Scaled procedure based on lab experiments

Cosajaba oil as a dietary fish feed ingredient

- High product stability versus fish and vegetable oils
- Safe for use in animal feeds (meets AAFCO definition)
- Pre-pellet application
- Good Palatability
- No adverse effects on fat deposition or body condition
Dietary Cosajaba oil effects on growth and mortality

Effects of dietary cosajaba oil on arthritic inflammation

- Cosajaba oil fed to mice with collagen induced arthritis
- Arthritic severity and pro-inflammatory markers decreased

![Graph showing change in arthritic severity over 9wk period]

Pro-inflammatory cytokines in arthritic paws decreased over 9 wks feeding
Can Cosajaba oil increase growth/feed efficiency of fish?

Cosajaba increases growth and survival in pilot experiments: Fathead Minnows

**Increased growth**

- **Control**
- **2% Cosajaba**

**Increased survival**

- **Control**
- **2% Cosajaba**
Cosajaba increases temperature-stress tolerance: Yellow cichlid

![Yellow cichlid image]

**Increased growth**

Increased survival

Cosajaba increases growth and survival: Walleye

![Walleye image]

**Increased growth**

**Increased survival**

Confidential (April 2017)
Cosajaba increases walleye tolerance to hypoxia

- 25% increase in hypoxia tolerance vs controls

Cosajaba as a growth and stress-tolerance promoter in Rainbow trout
Cosajaba increases tolerance to hypoxia

- 20% increase in tolerance compared to fish oil

Cosajaba increases osmoregulation during seawater challenge

- 12% increase in osmoregulation compared to fish oil
Cosajaba oil as a dietary anti-stress promoter

Atlantic salmon

Why Cosajaba fits with Atlantic salmon

<table>
<thead>
<tr>
<th>Contributors of salmon mortality</th>
<th>Established effects of Cosajaba oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater stress</td>
<td>Increases osmoregulatory function (10%)</td>
</tr>
<tr>
<td>Hypoxia stress</td>
<td>Increases hypoxia tolerance (25% walleye, 20% Rainbow trout)</td>
</tr>
<tr>
<td>Temperature stress</td>
<td>Increases temperature stress tolerance (60% vs 0% survival in control)</td>
</tr>
<tr>
<td>Transport stress</td>
<td>Rainbow trout and Atlantic salmon experiments in progress</td>
</tr>
<tr>
<td>Pathogen stress</td>
<td>Atlantic salmon experiment in progress</td>
</tr>
</tbody>
</table>
Financial analysis for Atlantic salmon

- Mortality opportunity in Norwegian Atlantic salmon: **27M fish/yr**
- Value of saved fish due to Cosajaba (30% saved): **$73M/yr**
- Based on US supply of poultry glands, CO can meet 100% of Norwegian AS market need at current dietary level (2%)
- Dose-response experiments in progress
- Length-of-feeding requirements need refinement

Next steps

- Refine Cosajaba oil extraction/processing (COGS) and scale up production
- Determine dose-response effects of CO
- Further investigate CO’s mechanism of action
- Evaluate other markets where CO may provide benefits (within aquaculture, animal agriculture, humans, other)
- Perform efficacy field trials in Atlantic salmon
- Continue to refine economic viability of CO
Thank you!

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References