The need for microalgae as a lipid rich resource in future aquafeed

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The use of fish meal and fish oil in fish feed

Annual global aquaculture production 2000-2010 (million tons) compared to volumes of fishmeal and fish oil used in aquaculture. Source: IFFO Positional Statement, Feb 2013.
Expected increased demand for fish feed and available supply of fish oil

- Production of salmon and rainbow trout in Norway in 2009 (FHL 2010)
  - 929 600 t fish produced
  - 1 150 500 t fish feed
- Expected increased production in the future

- The "Føre var" report:
  - Stable volumes of fish meal and fish oil
  - Increased need for aquaculture fish feed
  - Acute lack of fish meal and fish oil for aquafeed may arise in few years (<5 years)
  - Different feed resources may be important
  - Challenge for the salmon industry
## Possible sources to replace fish oil

<table>
<thead>
<tr>
<th>Novel sources of EPA and DHA fatty acids</th>
<th>Category</th>
<th>Source</th>
<th>Potential</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pelagic Fish</td>
<td>Trimmings and By-products</td>
<td>Represent 25% of global feed rawmaterial supply, and will continue to grow. Require industrial efforts on logistic</td>
<td>IFFO, 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less exploited fish species</td>
<td>Tuna, sardines, squid etc. Require industrial efforts on logistic and amended regulations</td>
<td>FAO, 2011</td>
</tr>
<tr>
<td></td>
<td>Zooplankton</td>
<td>Krill</td>
<td>Large biomass, ecological impact of harvest is disputed. High price - currently not feasible for feed applications. Estimated production in 2017 is 5000 t krill oil</td>
<td>Bostock, 2010 IFFO, 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calanus</td>
<td>Large biomass, challenging harvesting methods Uncertain potential and high price level - currently not feasible for feed applications</td>
<td>WO2010-077152A1</td>
</tr>
<tr>
<td></td>
<td>Microalgae</td>
<td>Photoautotrophic</td>
<td>Primary producers, sustainable production using renewables, biological and technological improvements can lead to competitive price level.</td>
<td>Norsker, 2011 Draisma, 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterotrophic</td>
<td>Microorganisms, mature technology, biological and technological improvements can lead to competitive price</td>
<td>US 7732170</td>
</tr>
<tr>
<td></td>
<td>Gene modified organisms</td>
<td>GM fungi</td>
<td>GM Yerrowia produces 55% EPA by fermentation (DuPont). Commercially used for salmon feed, approved by US FDA</td>
<td>US 89619A1</td>
</tr>
</tbody>
</table>
Cultivation of marine microalgae biomass

- Microalgae have a balanced chemical composition
  - 20-50 % protein
  - High lipid content (20-40%)
  - High content of n-3 poly unsaturated fatty acids
  - Contain immune stimulatory compounds
  - High content of pigments, vitamins and minerals

- High production yield
  - Production yield of 75 - 365 tons DW hectare\(^{-1}\) year\(^{-1}\)
  - Varies with cultivation conditions and technology
  - Highest yield in photo-bioreactors
  - Need only nutrients (N&P), CO\(_2\) and light energy

- Industrial applications of microalgae
  - Aquaculture
  - Human nutrition
  - Cosmetics
  - Biofuel – biodiesel?
Cultivation of microalgae in Norway - the light challenge

Harvesting the sun energy: Photosynthesis reactions

Photosynthetic active radiation in a greenhouse (60% transmission) throughout one year (1 h basis)

- The irradiation in South Norway is 1000 kWh year\(^{-1}\)
- 60% transmission in a greenhouse and 5% photosynthetic efficiency:
  - The potential biomass production is about 10 kg DW m\(^{-2}\) year\(^{-1}\), more realistic 5-7 kg DW m\(^{-2}\) year\(^{-1}\)
  - Corresponds to 9-12 kg CO\(_2\) m\(^{-2}\) year\(^{-1}\)

CO\(_2\) emission in Norway (2011): 52.7 mill tons year\(^{-1}\)

Calculations by Leiv Mortensen, UMB
Microalgae activities in Norway

- Laboratory experiments – Photo-autotrophic and Heterotrophic cultivation
  - Small volumes – typical 1-2 liter bioreactors
  - Growth and metabolic /chemical compound studies (temperature, nutrients, light responses, CO2)
  - Light source typical artificial light (50-200 μmol m⁻² s⁻¹)
  - R&D institutions: Bioforsk, Institute of Marine Research, Nofima, Norwegian Univ. of Science and Technology, SINTEF, Univ. of Bergen, Univ. of Life Sciences, Univ. of Oslo, Univ. of Stavanger, Univ. of Tromsø

- Commercial cultivation of microalgae
  - Microalgae cultivation as feed in aquaculture hatcheries (marine fishes, bivalves)
  - Cultivation of selected microalgae for feeding live prey and for “green water”

- Large scale production of microalgae biomass
  - Challenge to be started up in Norway
  - Several companies / groups aims on producing microalgae for different purposes:
    - Omega 3 oil, β-glucan, functional food, feed ingredients
    - Bioenergy
    - Cultivation units
Some microalgae project activities in Norway

- University of Life Science (UMB), Ås
  - Microalgae to oyster larvae (Interreg project):
    »Cultivation conditions of microalgae and content of EPA and DHA
  - Use microalgae to remove N & P from wastewater (IPN project from RCN)
  - Use of solar energy for CO₂ capture, algae cultivation and hydrogen production (RCN project, cooperation with Bioforsk)

- University of Tromsø
  - Mass production of microalgae the last 25 years for fish and shellfish.
  - Partner of CRI MabCent project (lipids from big diatoms for human health and fish feed)

- CO₂ to Bio - project
  - Proposed industrial production of algal biomass at Mongstad based on captured CO₂
  - Algae biomass rich in omega 3 fatty acids will be used in aquafeed
  - Main partners: UniResearch, Univ. Bergen, Nofima, Hordaland Industry Cluster, EWOS

- SINTEF and NTNU
  - Cultivation facilities (1-2 L units - 300 L cylinders – 250 L tubular photobioreactor)
  - ALGAFEED (Potential of replacing fish meal and fish oil with microalgae in aquafeed)
  - Projects on bioenergy (Optimized Solar Cell, Lipido, Local biogas facilities)
  - Use of microalgae in fish fry cultivation
Cultivation of microalgae for aquaculture

- Feed for mussels (sallops, oysters)
- Feed for shrimp production
- Juvenile production of marine fish
  - Green water (“Magic Green Water”)
  - Cultivation feed for live feed
  - Adjustment feed for live feed
- Use in fish feed
  - Replacement of fish meal and fish oil
  - Feed ingredients
    - Lipid, EPA and DHA
    - Immunostimulants
    - Pigments (Carotenoids)
### Reported EPA or DHA concentrations and phototrophic productivities.

Values are based on cultivation conditions used for each individual study.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cell density [g DW/l]</th>
<th>EPA/DHA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[% of DW]</td>
<td>[mg DW/l]</td>
</tr>
<tr>
<td>Nannochloropsis sp.</td>
<td>7-8</td>
<td>5-6</td>
<td>[ ]</td>
</tr>
<tr>
<td>N. oculata</td>
<td>0.4-1</td>
<td>4-5</td>
<td>20-50</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>2.6-3.1</td>
<td>0.148</td>
<td>Sánchez-Mirón et al. (2003)</td>
</tr>
<tr>
<td>Isochrysis galbana</td>
<td>3-10</td>
<td>6-7</td>
<td>Fradique (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zhang 2003</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td>3-10</td>
<td>15-30*</td>
<td>0.29/0.14</td>
</tr>
</tbody>
</table>

* of total fatty acids, TFA.

### Reported DHA concentrations and heterotrophic productivities.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cell density [g/l]</th>
<th>DHA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[% of TFA]</td>
<td>[g/l]</td>
</tr>
<tr>
<td>Thraustochytrid strain 12B</td>
<td>21</td>
<td>50-55</td>
<td>5.6</td>
</tr>
<tr>
<td>S. limacinum SR21</td>
<td>59</td>
<td>~65</td>
<td>15.5</td>
</tr>
<tr>
<td>Aurantiochytrium sp.</td>
<td>90-100</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>Schizochytrium sp.</td>
<td>160-180</td>
<td>40</td>
<td>40-45</td>
</tr>
</tbody>
</table>
Three possible pathways to achieve higher biomass productivity and/or higher content of EPA and DHA in microalgae

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Resource</th>
<th>Principle</th>
<th>Potential increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploit the physiological potential</td>
<td>High productivity strain</td>
<td>Metabolic stress by growth conditions</td>
<td>→ Lipid yield: 2-4 fold</td>
</tr>
<tr>
<td>Improve strains by selection &amp; breeding</td>
<td>Diverse collection of strains</td>
<td>Selection pressure for phenotype</td>
<td>→ Productivity: 2-4 fold and/or</td>
</tr>
<tr>
<td>Improve strains by genetic modification</td>
<td>Appropriate strain Molecular tools</td>
<td>Mutagenesis or metabolic engineering</td>
<td>→ Lipid yield: 2-4 fold</td>
</tr>
</tbody>
</table>

Based on Meireles et al. (2003) & Courchesne et al. (2009).
Potential of using microalgae to partially replace fish oil and fish meal in aquaculture fish feeds

1. Content of lipids and omega 3 fatty acids in selected microalgae

2. Digestibility of microalgae based fish feed given to
   • Mink (model species)
   • Atlantic salmon
   • Atlantic cod

3. Effect of microalgae based fish feed on growth of
   • Atlantic salmon
   • Atlantic cod

Prymnesiophyceae:
   • Isochrysis T-Iso (Isochrysis T-Iso)
Bacillariophyceae:
   • Phaeodactylum tricornutum
Estigmatophyceae:
   • Nannochloropsis sp.
Protein Lipid

80%

Digestibility of algae-based feed by mink

Nutrient digestibility in fish fed diets:
Increased addition of microalgae (% on DW)

Microalgae content of the feed:
0.3%, 6% and 12%
P. tricornutum (DW)

Nutrient digestibilities (%)

Atlantic salmon

Cod

Reitan, KI, Eriksen, T, Berge, GM, Ruyter, B, Sørensen, M, Galloway; TF, Kjørvik, E,
Nutrient digestibility and effect on gut morphology of diets with increasing content of
dried microalgae Phaeodactylum tricornutum in Atlantic cod and Atlantic salmon.
Growth of salmon and cod
Microalgae (*P. tricornutum*) content:
0.3%, 6% and 12% (DW)

**Atlantic salmon:**
- Feeding period: 82 days
- 31 ind. pr. tank
- Similar feed uptake
- No difference in feed digestion
- No difference in growth up to 6% microalgae content
- No difference in gut morphology

**Atlantic cod:**
- Feeding period: 95 days
- 20 ind. pr. tank
- Forsøksperiode
- Similar feed uptake
- No difference in feed digestion
- Tendency to increased growth with algae inclusion
- Significant difference in skin pigmentation
- No difference in gut morphology
How can fish meal and fish oil be replaced by microalgae?

- Different species have different fatty acid composition and production yield
  - Heterotrophic production of DHA?
  - Phototrophic production of EPA?

- Possible co-products from microorganisms for value added production

- Protein will be interesting co-product for use in fish feed

- EHS (Environment, health and safety) issues

- Possible anti-nutrients from microorganisms

- Need to obtain stable large volume production to come into the market
  - Some feed producers want to get batches of 30,000 tons microalgae

- What will the production price be?
Processing of the microalgae before use in aqua feeds

Flow scheme showing the processing of microalgae following various routes.
Production cost (from ProAlge Results, 2013)

- Need to develop cost efficient production technology for the biomass

- Price competitive with fish oil (EPA + DHA equivalents)

- Heterotrophic production of DHA (and EPA)

- Phototrophic production of microalgae for EPA (and DHA)

Base case estimates of production costs in USD per kg EPA/DHA equivalents
USD per kg EPA/DHA

- Flat Panels: $39.06
- Tubular: $55.31
- Open Pond Raceway: $135.19

Blue bars represent Spain, and red bars represent the Netherlands.
## Comparison of production costs per unit EPA and DHA based on phototrophic and heterotrophic production

Production cost estimates based on techno-economic analysis and cost projections (chapter 8).

<table>
<thead>
<tr>
<th>Production principle</th>
<th>Estimated production cost (USD per kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA+DHA</td>
</tr>
<tr>
<td><strong>Phototrophic production</strong></td>
<td></td>
</tr>
<tr>
<td>Current production cost</td>
<td>39.1</td>
</tr>
<tr>
<td>Production cost after optimization</td>
<td>11.9</td>
</tr>
<tr>
<td><strong>Heterotrophic production</strong></td>
<td></td>
</tr>
<tr>
<td>Current production cost</td>
<td>19.0</td>
</tr>
<tr>
<td>Production cost after optimization</td>
<td>11.5</td>
</tr>
</tbody>
</table>
Future challenges and recommendations

- Screen the biodiversity to find novel productive strains (EPA and DHA)
- Model systems that allow genetic modified organisms giving higher productivity and EPA-DHA content
- Identify novel strains with optimal production characteristics
- Develop low energy cultivation systems
- Develop low cost harvesting and dewatering systems
- Develop efficient feed processing technology with use of algae as raw materials
- LCA analysis for using microalgae in fish feed
- Develop cost efficient production line
- Novel value chains

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SINTEF

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