Evaluation of Biofuel Co-products as Ingredients for Aquafeeds

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Biofuel Co-products

Oil Seeds and Nuts
Algae, Coconut, Jatropha, Rapeseed, Camelina

Processing
Clean Crack Extrude Press

Oil

Biodiesel

By-products

Feeds
Outline

• Criteria and methodology for evaluation of an ingredient
• Substitution of fishmeal protein by biofuel co-products in aquafeeds (opportunity and concerns)
• Discussion on future researches
Essential Information for Evaluation of an Ingredient

- Nutrient requirement of a target species
- Sustainability for production of the ingredient
- Nutritional evaluation of the ingredient
Selection Criteria for an Ingredient
---Nutritional evaluation

- Chemical composition
- Pellet physical quality
- Effects on attractiveness and palatability
- Digestibility and utilization of nutrients
- Effect on product quality
Evaluation of an Ingredient

Chemical Composition

- Nutrient levels
- Nutrient balance (amino acid; fatty acid)
- Presence of anti-nutrients or contaminants
## Proximate Composition of Different Ingredients (%)

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Fishmeal</th>
<th>Soybean meal</th>
<th>DDGS</th>
<th>Camelina</th>
<th>Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>93</td>
<td>92</td>
<td>90</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td>Protein</td>
<td>68</td>
<td>46</td>
<td>28</td>
<td>29</td>
<td>32</td>
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<tr>
<td>Lipid</td>
<td>6.5</td>
<td>1.9</td>
<td>5.7</td>
<td>33.5</td>
<td>0.9</td>
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<tr>
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<td>18.5</td>
<td>5.1</td>
<td>5.2</td>
<td>3.5</td>
<td>17.9</td>
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<tr>
<td>Crude fiber</td>
<td>6.6</td>
<td>7.0</td>
<td></td>
<td></td>
<td>39.5</td>
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</tbody>
</table>
### Amino acid profiles of different ingredients (% of total amino acids)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Pollock meal</th>
<th>Menhaden</th>
<th>SBM</th>
<th>DDGS</th>
<th>Camelina</th>
<th>Algae meal</th>
</tr>
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<tbody>
<tr>
<td>Ala</td>
<td>6.2</td>
<td>7.8</td>
<td>5.1</td>
<td>7.5</td>
<td>5.3</td>
<td>10.6</td>
</tr>
<tr>
<td>Asp+ASN</td>
<td>8.8</td>
<td>9.6</td>
<td>8.9</td>
<td>6.6</td>
<td>7.8</td>
<td>6.6</td>
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<tr>
<td>Cys</td>
<td>0.8</td>
<td>0.6</td>
<td>1.0</td>
<td>2.2</td>
<td>1.4</td>
<td>0.8</td>
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<tr>
<td>Glu+Gln</td>
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<td>11.0</td>
<td>15.7</td>
<td>16.3</td>
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<td>12.1</td>
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<tr>
<td>Gly</td>
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<td>8.1</td>
<td>4.9</td>
<td>4.3</td>
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<td>9.9</td>
</tr>
<tr>
<td>Pro</td>
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<td>4.4</td>
<td>5.1</td>
<td>8.8</td>
<td>5.2</td>
<td>8.0</td>
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<tr>
<td>Ser</td>
<td>4.7</td>
<td>3.6</td>
<td>4.9</td>
<td>4.7</td>
<td>4.2</td>
<td>5.4</td>
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<tr>
<td>Tyr</td>
<td>3.8</td>
<td>3.5</td>
<td>3.5</td>
<td>5.6</td>
<td>3.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Taurine</td>
<td>2.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Arg</td>
<td>7.6</td>
<td>9.2</td>
<td>8.9</td>
<td>4.7</td>
<td>9.0</td>
<td>6.2</td>
</tr>
<tr>
<td>His</td>
<td>2.2</td>
<td>2.9</td>
<td>3.6</td>
<td>3.4</td>
<td>3.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Ile</td>
<td>5.6</td>
<td>4.7</td>
<td>5.3</td>
<td>4.1</td>
<td>5.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Leu</td>
<td>6.7</td>
<td>7.5</td>
<td>7.1</td>
<td>10.9</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Lys</td>
<td>8.5</td>
<td>9.6</td>
<td>6.8</td>
<td>3.3</td>
<td>6.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Met</td>
<td>2.7</td>
<td>2.6</td>
<td>1.5</td>
<td>2.2</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Phe</td>
<td>4.3</td>
<td>4.9</td>
<td>7.7</td>
<td>5.5</td>
<td>7.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Thr</td>
<td>4.8</td>
<td>4.7</td>
<td>4.6</td>
<td>3.7</td>
<td>4.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Val</td>
<td>5.5</td>
<td>5.8</td>
<td>5.5</td>
<td>5.3</td>
<td>6.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>
# Compositions of Traditional Ingredients & Microalgae

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>DM</th>
<th>Ash</th>
<th>CP</th>
<th>EE</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menhaden meal</td>
<td>91.9</td>
<td>20.7</td>
<td>62.8</td>
<td>9.3</td>
<td>739</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>92.5</td>
<td>6.8</td>
<td>47.8</td>
<td>1.9</td>
<td>53</td>
</tr>
<tr>
<td>Chlorella A</td>
<td>95.9</td>
<td>19.4</td>
<td>38.2</td>
<td>4.0</td>
<td>5882</td>
</tr>
<tr>
<td>Chlorella B</td>
<td>97.3</td>
<td>22.4</td>
<td>31.5</td>
<td>3.6</td>
<td>1592</td>
</tr>
<tr>
<td>Haematococcus pluvialis</td>
<td>91.6</td>
<td>10.8</td>
<td>40.3</td>
<td>5.0</td>
<td>75</td>
</tr>
</tbody>
</table>
Evaluation of an Ingredient
Pellet Physical Quality

- Ingredient particle sizes
- Pellet durability
- Expansion of pellets after extrusion
- Oil absorption capacity of pellets
- Pellet water stability
Particle Size of Ingredients

- Physical parameters of pellets
  homogeneity, density and water stability
- Nutrient utilization

Particle size < 200µm
Effect of Different Particle Size on Floating and Water Stability of Pellets

![Bar chart showing the effect of different particle sizes on floating and water stability of pellets. The chart compares 200 µm and 400 µm particle sizes.](chart.png)
Pellet Durability

Tumble pellets & 5-½” hex nuts @10 minutes at 50 rpm

↓

Sieve and remove the fine particles

↓

PD (%)=100*pellet after tumbling (g) /pellet before tumbling (g)
Pellet Expansion Measurement

Base diet

70% base diet + 30% test ingredient

Expansion: % change in the diameter of the test pellet

$$=\left(\frac{\text{pellet width} - \text{die diameter}}{\text{die diameter}}\right) \times 100$$
## Pellet Characteristic & Density

<table>
<thead>
<tr>
<th>Pellet characteristic</th>
<th>Sea water @ 20°C</th>
<th>Fresh water @ 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Density (g/L)</td>
<td></td>
</tr>
<tr>
<td>Fast sinking</td>
<td>&gt;640</td>
<td>&gt;600</td>
</tr>
<tr>
<td>Slow sinking</td>
<td>580-600</td>
<td>540-560</td>
</tr>
<tr>
<td>Neutral</td>
<td>520-540</td>
<td>480-520</td>
</tr>
<tr>
<td>Floating</td>
<td>&lt;480</td>
<td>&lt;440</td>
</tr>
</tbody>
</table>
Oil Absorption Capacity

Weigh pellet sample (W1)

Fat coated pellets

Drain pellets for 10 mins

Weigh pellet sample (W2)

Oil absorption capacity = \( 100 \times \frac{(W2 - W1)}{W1} \)
Water stability% = \frac{100 \times \text{dry matter of retention pellets (g)}}{\text{dry weight of original pellets (g)}}
Pellet Water Stability Affects the Dry Matter and Lipid levels of Stomach Contents

Baeverfjord et al., Aquaculture (2006), 1335
Water Stability of Pellets Affects Apparent Digestibility of a Diet

High WS

Low WS

%
Summary

- Different ingredients can significantly affect both physical and chemical quality of feeds!
- Different ingredient may require different feed processing method!
- Processing method is important for quality of an ingredient as well as a feed!
Evaluation of an Ingredient
Attractability/Palatability Test
Measurement of Food Intake
Evaluation of an Ingredient
Digestibility

In vitro

In vivo
• Manual stripping; Rectal suction
• Siphoning; Settlement
• Dissection

Fecal collection
Evaluation of an Ingredient Growth Trial

Green water system

Clean water system
Integrated Researches
Nutritional studies
Biochemistry
Feed processing technology
Substitution of Fishmeal Protein by an Algae Meal in Feed for Pacific Threadfin (Moi)

Commercial Feed
48-50% protein
14% lipid

Ol Feed
33-40% protein
10-12% lipid
## Formulation of Test Diets for Juvenile Moi

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Replacement of fishmeal protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Pollock meal</td>
<td>30</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>20</td>
</tr>
<tr>
<td>Algae meal (29.5% CP)</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>50</td>
</tr>
</tbody>
</table>

Crude protein: 37% Crude lipid: 13%: Gross energy: 19.3 kJ/kg
Effect of Algae Meal on Palatability of Fish

![Bar chart showing the effect of algae meal on food intake percentage of fish.](chart)

- **Y-axis**: Food intake (% of feed fed)
- **X-axis**: Replacement of fishmeal protein (%)
- **Data Points**:
  - 0% replacement: A
  - 7.5% replacement: AB
  - 15% replacement: AB
  - 30% replacement: BC
  - 45% replacement: C
  - Commercial: AB

The chart indicates that there is a significant effect of algae meal on the palatability of fish diets, with lower food intake percentages as the replacement of fishmeal protein increases.
Effect of Algae Meal on Digestibility of Dietary Nutrients for Fish

![Bar chart showing the effect of Algae Meal on Digestibility of Dietary Nutrients for Fish. The x-axis represents the replacement of fishmeal protein (%), and the y-axis represents ADC (%). The chart compares dry matter and crude protein.]
Effect of Algae Meal on Growth of Fish
Effect of Algae Meal on Feed Utilization

![Graph showing the effect of algae meal replacement on fishmeal protein replacement (%). The graph compares FCR (feed conversion ratio) and PER (protein efficiency ratio) at different replacement levels: 0%, 7.5%, 15%, 30%, 45%, and Commercial. The data indicates a decrease in FCR and an increase in PER as the replacement level increases.](image-url)
Effect of Different Diets on Nutritional Composition of Fish Fillet

![Graph showing the effect of different diets on nutritional composition of fish fillet. The x-axis represents the replacement of fishmeal protein (%), and the y-axis represents the % of wet weight. The graph indicates that the nutritional composition changes with different levels of fishmeal protein replacement.](image-url)
Optimal Replacement Level of Fish Meal Protein by the Algae Meal

\[ y = -0.0002x^2 - 0.0073x + 1.7089 \]

\[ R^2 = 0.9603 \]

Replacement of fishmeal protein (%)

SGR (%)

X=18.3%
## Amino Acids Contents of Test Diets and Ingredients

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Commercial</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Fishmeal</th>
<th>Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g diet as fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dispensable AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>0.46</td>
<td>0.50</td>
<td>0.46</td>
<td>0.41</td>
<td>0.33</td>
<td>0.28</td>
<td>2.74</td>
<td>0.02</td>
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<tr>
<td><strong>Indispensable AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>1.08</td>
<td>0.81</td>
<td>0.79</td>
<td>0.77</td>
<td>0.71</td>
<td>0.70</td>
<td>4.94</td>
<td>0.34</td>
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<tr>
<td>Phenylalanine</td>
<td>2.37</td>
<td>1.67</td>
<td>1.65</td>
<td>1.56</td>
<td>1.52</td>
<td>1.46</td>
<td>2.09</td>
<td>1.66</td>
</tr>
</tbody>
</table>
Summary

- The algae meal can replace 18% fishmeal protein based on performance of fish;
- The substitution level can be up to 30% based on feed utilization and nutritional composition of fish;
- Deficiency of amino acids in the algae meal may be one of the reasons for the poor performance of fish fed the high algae diet.
## Substitution of Fishmeal Protein by an Algae Meal in Shrimp Feeds

### Formulation of Test Diets for Shrimp

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Replacement of fish meal protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Menhaden meal</td>
<td>15</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>25</td>
</tr>
<tr>
<td>Algae meal (29.5% CP)</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>60</td>
</tr>
</tbody>
</table>

Crude protein: 33%; Crude lipid: 9%
Water Stability and Durability of Pellets

Replaces 64% fishmeal protein
Algae Meal Enhances Palatability of Shrimp

Algae meal supplementation (% diet)

Food intake (% of feed fed)

Bar chart showing the effect of algae meal supplementation on food intake of shrimp. The supplementation levels are 0, 5, 10, 20, 30, and commercial feed. The data shows that algae meal supplementation enhanced the food intake of shrimp, with significant differences observed at certain levels.
Growth of Shrimp Fed Diets with Different Levels of Algae Meal

![Bar chart showing final body weight of shrimp fed diets with different levels of algae meal supplementation. The x-axis represents the percentage of algae meal supplementation (0%, 5%, 10%, 20%, 30%, and commercial), and the y-axis represents final body weight (g). The chart indicates that the highest final body weight was achieved with 5% algae meal supplementation, followed by 30% and commercial diets, while 0% supplementation resulted in the lowest final body weight.}
Digestibility of Dietary Nutrients in Shrimp

Test diet for digestibility: 30% ingredient +70% reference diet
Summary

Algae meal tested

• Enhances shrimp palatability;
• Does not affect pellet durability but decreases water stability of pellet;
• Tends to decrease growth of shrimp;
• Has lower digestibility than fish meal.
Sources of Lipid/Long Chain PUFA

- Dinoflagellate alga (Cryptothecodinium Cohnii)
- Thraustochytrids (schizochytrium)
- Isochrysis galbana
- Pavlova lutheru
- Nannochloropsis occulata

http://phytoonline.mdamirpp.net/
### Fatty Acids Profiles of Different Ingredients

(% of Total Fatty Acids)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>18:2n-6</th>
<th>18:3n-3</th>
<th>20:4n-6</th>
<th>20:5n-3</th>
<th>22:6n-3</th>
<th>Chol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menhaden oil</td>
<td>1.3</td>
<td>0.3</td>
<td>0.2</td>
<td>11</td>
<td>9.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>1.4</td>
<td>0.6</td>
<td>1.6</td>
<td>11.2</td>
<td>12.6</td>
<td>0.57</td>
</tr>
<tr>
<td>Tallow oil</td>
<td>3.1</td>
<td>0.6</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.1</td>
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<tr>
<td>Soybean oil</td>
<td>51</td>
<td>6.8</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Corn oil</td>
<td>58</td>
<td>0.7</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Chaetoceros sp</td>
<td>1</td>
<td>0.4</td>
<td>3</td>
<td>16.7</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td>2.1</td>
<td>2.1</td>
<td>0.5</td>
<td>28.3</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>Isochrysis galbana</td>
<td>8.6</td>
<td>4.5</td>
<td>---</td>
<td>0.9</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>Cryptomonas sp</td>
<td>0.6</td>
<td>25.1</td>
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<td>12</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Rhodomonas sp</td>
<td>1.9</td>
<td>25.2</td>
<td>---</td>
<td>8.7</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Schizochytrium sp</td>
<td>0.7</td>
<td>0.11</td>
<td>2.9</td>
<td>0.6</td>
<td>31.4</td>
<td></td>
</tr>
</tbody>
</table>

Chol, cholesterol, % of diet

Nutritional Pigments

Dunaliella Salina produces β-carotene

Chlorella produces lutin and astaxanthin

Haematococcus produces mixed carotenoids

http://www.themagicisbac.com/
Astromanthin Improves Pigmentation in Shrimp

Ju et al. 2011
Concerns

- Nutrient levels and balance
- Anti-nutritional factors or contaminants
- Availability of nutrient (digestibility, palatability)
- Processing methods for ingredients and feeds
- Quality of end product (flavor, texture and nutritional values)
- Production and cost of a by-product
Future Research

• More research to update nutrient requirements and thus formulation for different farmed fish
  --- Culture system/condition
  --- New species or family of animal
Future Research

• Multidisciplinary collaborative researches
  ---ingredient selection
  ---selective breeding for optimal family
  ---processing technology development
Future Research

• Standard research diet development
  Ingredients
  Formulation
  Feed processing protocol
  Evaluation methods
Acknowledgements

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ALOHA!