Development of a novel food dossier for water lentils

Duckweed, a new sustainable protein source for human nutrition

Ingrid van der Meer
Protein challenge

- Increase of both world population and meat consumption
  - World population will increase to 9 billion in 2050
  - Meat consumption will increase with 60% in 2030 compared to 2000
  - To produce 1 kg meat protein, up to 3-6 kg plant protein is needed
- Huge increase of protein demand world-wide

→ Need for new protein crops
Global need protein sources for animal production

- Global animal production is growing much faster than human population growth
- Current global feed need is 1 billion tons
- Animal production is reliant on soybeans
  - Soybean protein (meal) is a byproduct of soybean oil production
- Soybeans form the bulk of world commerce of feed protein
  - Other global feed protein supplies are canola meal, animal by-products and fishmeal (and other regional sources)
## Short list potential new protein sources

<table>
<thead>
<tr>
<th>category</th>
<th>Protein source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil seeds</td>
<td>Soybeans grown in Europe, rapeseed, sunflower seeds</td>
</tr>
<tr>
<td>Legumes</td>
<td>Peas, bean, lupines, chickpea, Lucerne (alfalfa)</td>
</tr>
<tr>
<td>Leaves</td>
<td>Grass, sugarbeet leaves</td>
</tr>
<tr>
<td>Aquatic plants</td>
<td>Algae, seaweeds, Duckweed (Lemna)</td>
</tr>
<tr>
<td>(Pseudo) cereals</td>
<td>Oats, quinoa</td>
</tr>
<tr>
<td>Insects</td>
<td>Mealworm, housefly, house cricket</td>
</tr>
</tbody>
</table>
Crop and protein yield per ha of various plant protein sources

<table>
<thead>
<tr>
<th>Plant Protein Source</th>
<th>Protein Content</th>
<th>Yield Biomass (tons dm/ha/y)</th>
<th>Potential Protein Yield (tons/ha/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil seeds – soybean</td>
<td>45%</td>
<td>1.5-3 tons</td>
<td>0.6-1.2 tons</td>
</tr>
<tr>
<td>Oil seeds – rapeseed</td>
<td>25%</td>
<td>3 tons</td>
<td>0.75 ton</td>
</tr>
<tr>
<td>Oil seeds – sunflower</td>
<td>23%</td>
<td>3 tons</td>
<td>0.7 ton</td>
</tr>
<tr>
<td>Legumes (pulses) – peas/beans/ lupine</td>
<td>17-35%</td>
<td>4-6 tons</td>
<td>1-2 tons</td>
</tr>
<tr>
<td>Legumes (forage) – lucerne</td>
<td>19%</td>
<td>13 tons</td>
<td>2.5 tons</td>
</tr>
<tr>
<td>Cereals – oat</td>
<td>12-15%</td>
<td>3-5 tons</td>
<td>0.4-0.75 ton</td>
</tr>
<tr>
<td>Pseudo cereals – quinoa</td>
<td>12-18%</td>
<td>3 tons</td>
<td>0.4-0.5 ton</td>
</tr>
<tr>
<td>Leaves – grass</td>
<td>12%</td>
<td>10-15 tons</td>
<td>1.2-2 tons</td>
</tr>
<tr>
<td>Leaves – (e.g. sugar beet leaves)</td>
<td>12%</td>
<td>4.5 tons</td>
<td>0.5 ton</td>
</tr>
<tr>
<td>Macro algae - seaweed</td>
<td>10-30%</td>
<td>25 tons</td>
<td>2.5-7.5 tons</td>
</tr>
<tr>
<td>Micro algae</td>
<td>25-50%</td>
<td>15-30 tons</td>
<td>4-15 tons</td>
</tr>
<tr>
<td>Duckweed</td>
<td>35-45%</td>
<td>30-40 tons</td>
<td>10-18 tons</td>
</tr>
</tbody>
</table>

Yield duckweed protein 10 x higher than soybean
Duckweed (Lemnoideae)

- Smallest flowering plant in the world
  - 1, 2 or 3 leaves with a single root hanging in the water
  - leaves can multiply vegetatively → exponential growth
  - 50% yield increase per day

- Protein rich plant with 35-45% protein
  - amino acid profile comparable to soy

- World-wide distribution
  - sub-tropical/ moderate climate
  - not many pathogens known
Nutritional components in duckweed

- **Other components:**
  - Proteins (35-45%); carbohydrates (15%); fibers (15%); fat (5%)
  - Vitamins: A, B1, B2, **B5**, B6, C, **E**
  - Beta-carotenoids
  - Polyphenols: flavonoids, tannins and anthocyanins

- **Easy to grow, easy to harvest, high protein yield per hectare**
  - Digestible by cattle, pigs, poultry, fish

→ **But, not yet used for human nutrition**
Main protein Rubisco

- Rubisco has good properties for food:
  - Nutritional value
    - Digestible
    - Balanced AA profile
    - Non-allergenic
  - Functionality
    - Good gelling
    - High foaming
    - Good emulsification properties
    - High solubility

But: Duckweed protein falls under Novel Food legislation
Hurdle duckweed for human consumption

- No legal authorisation to use duckweed as protein source for human consumption
  - Research needed to build novel food dossier
  - Composition, safety, digestibility, bio-availability and consumer acceptance

Safety aspects of novel protein sources, 2013 van der Spiegel et al
Data which the dossier should contain

Administrative data from the applicant.

General description

Required information

I. Specification of the NF (origin and composition)
   - Origin of the NF (species, taxon)
   - Content of macronutrients, such as energy value, protein content etc.
   - Content of micronutrients and bioavailability of the micronutrients
   - Critical toxicants (potentially toxic inherent constituents, external contaminants)

II. Effect of the production process applied to the NF

III. History of the organism used as the source of the NF

IX. Anticipated intake/extent of use of the NF

X. Information from previous human exposure to the NF or its source

XI. Nutritional information on the NF

XII. Microbiological information on the NF

XIII. Toxicological information on the NF

Evaluation and conclusion by the applicant.

Summary by the applicant
Novel Food Applications

- Uptill now, 89 applications were approved and 3 applications were rejected

- Protein examples (approved):
  - Novel Food catalogue:
Stepping stones towards a dossier

- Production method
  - Only make use of defined substrate (like used for substrate grown greenhouse plants)
  - High productivity
  - High hygiene, high containment

- Strain/ecotype selection
  - Growth experiments
  - High yield; high protein content
  - No anti-nutritional factors (Ca oxalate)
High contained production possible
Product with nutritional value

- Protein
- Essential amino acids
- Vitamins
- Beta-carotenoids, flavonoids
- Fortification with Zinc, Selenium
Safety analyses

- Applied processing
  - Keep, minimal, simple and approved (like freeze or spray drying)

- Expected intake?

- Nutritional declaration

- Microbiological information
  - Certified lab: Salmonella, E. coli,

- Toxicological information
  - Metals: Cd, Pb, As, Hg, F
  - Aflatoxine: B1, B2, G1, G2
  - Pesticides: PCDD, PCDF, PCB, etc
  - Dioxins: TCDD, PeCDD, HxCDD, HpCDD, OCDD, etc
Results human trial

- How well is a single high dose tolerated?
- Is Duckweed a good bioavailable protein source?
- Will this be enough for the dossier?
  - Require long term /repeated exposures?
  - Require also to show tolerance to (food grade) isolated protein fraction?
Dossier can be further built by info on:

- Well tolerated by animals including piglets with similar digestive system
  - No change in growth/health with diet supplemented with 10% Duckweed

- Eaten by people in developing countries
  - India, Nepal, ...

- On the market in the USA
Will it support a dossier?

http://www.lentein.com/#process

Authorities in US that document/record adverse effects of users?
Goal and objectives of research

Analysis of safety aspects for human consumption and the nutritional value of duckweed for human food

1. Quality and safety: growth characteristics, biochemical composition, nutritional content, toxins, contaminants, anti-nutritional factors
2. Human digestibility (in vitro & in vivo)
3. Consumer acceptance
1. Analysis duckweed content and safety

- Duckweed lines grown under controlled conditions
- Biochemical analyses:
  - Protein content, protein/peptide profile (allergens), amino acid profile
    - Proteomics and amino acid analysis
  - Plant metabolite analysis: vitamins, carotenoids, polyphenols, flavonoids, calcium oxalate
    - Metabolomics
  - Safety analysis: toxins, micro-organisms, pesticides, contaminants, heavy metals
    - Diagnostic tests
2. Analysing digestibility *in vitro*

- **Homogenise (mouth)**
- **Peptic digestion (stomach) pH 2 1h/37°C**
- **Digestive enzymes (small intestine) pH 5.8**
  - Pancreatin, lipase, α-chymotrypsin
  - Bowel digestion pH 6.5 **bile salts (small intestine) 2h/37°C**
- **Centrifuge pH 7.5**

**Caco-2 cells; 21 days old resembling small intestinal cells**

**Supernatant that potentially passes mucus layer**

---

Food Analytical Methods
May 2014, Volume 7, Issue 5, pp 1047-1055

Date: 03 Dec 2013

**A Fast and Accurate UPLC Method for Analysis of Proteinogenic Amino Acids**

Esk J. Meussens, Albert N. T. van Zeeland, Marieke E. Bruins, Johan P. M. Sanders
2. Clinical study with duckweed protein

- Double-blind, randomized, cross-over design

- Analyse bio-availability, digestibility (post-prandial amino acid levels during time after intake)

- Monitoring possible adverse effects

N=20

Day 1 → Day 8
1 week
2. Analyse bioavailability in volunteers (*in vivo*)

- Start fasting overnight
- 'green smoothy'
- 4-5 hours
- Blood samples for amino acid analysis at t= -15, 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180
- Glucose, insulin, and standard clinical laboratory measurements at t= -15, 0, 180
- Aural temperature, heart rate, and blood pressure
- Interview with Physician/MD
- Monitoring of Adverse Effects
Consumer & industry survey

- **Consumer survey:** evaluate pros and cons for consumers
  - Send to contact list (>1000, age 18-70)
  - Results taken used for future research/proposals

- **Industry survey**
  - Use company oriented platforms to analyse potential interest of industry
  - Technical, nutritional application, sustainability
Conclusions

- **Problem**: Upcoming global shortage of vegetable protein
- **Solution**: Duckweed has high protein content, high yield/ha, good amino acid profile, no arable land needed, sustainable
- **Research goal**: Analyse safety aspects for human consumption and nutritional value as human food source
- **Methods**:
  - Quality, safety and nutritional composition
  - *In vitro* and *in vivo* digestibility and bio-availability
  - Consumer acceptance and interest of food industry
Thank you for listening

• Ingrid van der Meer
  Plant Metabolomics and Proteomics research

• Jurriaan Mes
  Addie van der Sluis
  Consumer science and Health research

• Adrie van der Werf
  Agrosystems research