Alternative protein sources for monogastrics: composition and functional assessment

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Aim of project

- To characterize the protein component of new/alternative protein sources using proteomics

- To predict functionality of protein sources using bioinformatics

- To assess functional properties of new/alternative protein sources using animal models (mice and pigs)

- To elucidate underlying mechanisms
Topics of today’s presentation

- To characterize the protein component of new/alternative protein sources using proteomics
- To predict functionality of protein sources using bioinformatics
- To assess functional properties of new/alternative protein sources using animal models (mice and pigs)
- To elucidate underlying mechanisms
**In vivo digestion process**

Digestion of proteins in gastro-intestinal tract

- **Proteomics:** MS
- **Genomics:** NGS
- **Transcriptomics:** Microarray

- **Cytokines**: Systemic effects
- **Metabolites**: MS/GC

**Digestion of proteins in gastro-intestinal tract**

- **Commensal bacteria**
  - SCFA
  - LPS
  - Sphingolipids

- **Immune responses**
  - IL-18
  - IL-22
  - IL-23

- **Antigen presentation**
  - DC
  - B cell
  - T cell

- **Immune cell activation**
  - M cell
  - Plasma cell
  - Treg

**Trends in Immunology**

**References:**

- ELISA
- Transcriptomics
- Proteomics
- Genomics
Proteomic analysis of feed ingredients

**Feed Ingredients**

- **CAS**: Casein (feed grade)
- **DWP**: Delactosed Whey Powder
- **SDPP**: Spray Dried Plasma Protein
- **SBM**: Soybean Meal
- **WGM**: Wheat Gluten Meal
- **YMW**: Yellow Meal Worm

**Proteomics analysis MS**

1. **Protein Sample** (Filter Aided Sample Preparation)
2. Fragmented using enzyme
3. Spectrum of fragment generated
4. Identified peptides and proteins
5. Results
6. Match
7. Genomics driven database

**Bioinformatic analysis**
Bioinformatic analysis of feed ingredients

- List of identified peptides and proteins
- Selected top 90% of the total calculated protein content
- Amino acid composition (ACC) prediction

- Prediction of bioactivity

- In silico digestion with pepsin, trypsin and chymotrypsin

- Compare ACC values obtained with conventional analytical method
Results: proteomic analysis

<table>
<thead>
<tr>
<th>CAS</th>
<th>DWP</th>
<th>SDPP</th>
<th>SBM</th>
<th>WGM</th>
<th>YMW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

Number of identified proteins

<table>
<thead>
<tr>
<th>70</th>
<th>130</th>
<th>210</th>
<th>748</th>
<th>586</th>
<th>43</th>
</tr>
</thead>
</table>

Number of proteins forming 90% of total protein fraction

| 3   | 3   | 25  | 68  | 24  | 19  |
Results bio-informatic analysis: amino acid composition

--- MS-based predicted ACC
--- Chemically defined ACC

r: 0.94
Results bio-informatic analysis: amino acid composition

- **CAS**: $r: 0.94$
- **DWP**: $r: 0.94$
- **SDPP**: $r: 0.86$
- **SBM**: $r: 0.92$
- **WGM**: $r: 0.94$
- **YMW**: $r: 0.87$

--- MS-based predicted ACC
--- Chemically defined ACC
Results: bioactivity

Proportion of bio-functional properties of proteins
• MS-based analysis provides more detailed information on the composition of complex protein sources compared to conventional (nutritional) analytical approaches.

• MS-based analysis allows the detection of individual proteins in complex matrices at very high resolution.

• MS-based approach was effective in predicting the amino acid composition of protein sources.

• MS-based analysis allows the prediction of bio-functional properties of protein.
## Chemical composition of the experimental diet

was replaced by the “protein derived from new sources”. The diets were identical with respect to all other nutrients compared to AIN 93-G

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>CAS</th>
<th>DWP</th>
<th>SDPP</th>
<th>WGM</th>
<th>YMW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, g/kg</td>
<td>928 (914)</td>
<td>931 (957)</td>
<td>944 (930)</td>
<td>933 (924)</td>
<td>936 (917)</td>
<td>942 (929)</td>
</tr>
<tr>
<td>Crude protein, g/kg</td>
<td>150 (152.9)</td>
<td>263 (267.8)</td>
<td>79 (79.6)</td>
<td>240 (177.6)</td>
<td>140 (148.3)</td>
<td></td>
</tr>
<tr>
<td>Ash, g/kg</td>
<td>50 (43)</td>
<td>40 (29)</td>
<td>83 (77)</td>
<td>42 (36)</td>
<td>65 (65)</td>
<td></td>
</tr>
<tr>
<td>Crude fibre, g/kg</td>
<td>55</td>
<td>44</td>
<td>44</td>
<td>46</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Crude fat, g/kg</td>
<td>78 (75.5)</td>
<td>73 (65.3)</td>
<td>86 (73.7)</td>
<td>77 (66.6)</td>
<td>87 (70.3)</td>
<td>174 (160.1)</td>
</tr>
<tr>
<td>Starch, g/kg</td>
<td>251</td>
<td>249</td>
<td>249</td>
<td>249</td>
<td>268</td>
<td>261</td>
</tr>
<tr>
<td>Sugar, g/kg</td>
<td>295</td>
<td>263</td>
<td>403</td>
<td>263</td>
<td>271</td>
<td>263</td>
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<tr>
<td>NSP, g/kg</td>
<td>71</td>
<td>8</td>
<td>17</td>
<td>14.9</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Gross energy, KJ/g</td>
<td>171 (17.1)</td>
<td>166 (16.3)</td>
<td>184 (18.4)</td>
<td>18 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca, g/kg</td>
<td>5.9</td>
<td>5.5</td>
<td>10.0</td>
<td>5.2</td>
<td>5.2</td>
<td>6.1</td>
</tr>
<tr>
<td>P, g/kg</td>
<td>3.7</td>
<td>3.3</td>
<td>6.2</td>
<td>1.9</td>
<td>2.3</td>
<td>4.0</td>
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<tr>
<td>K, g/kg</td>
<td>1.0</td>
<td>1.0</td>
<td>16.5</td>
<td>4.5</td>
<td>4.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Na, g/kg</td>
<td>1.1</td>
<td>1.2</td>
<td>5.9</td>
<td>8.2</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Cl, g/kg</td>
<td>1.7</td>
<td>1.7</td>
<td>4.4</td>
<td>12.7</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Linoleic acid, g/kg</td>
<td>37.7</td>
<td>35.8</td>
<td>35.8</td>
<td>35.8</td>
<td>35.8</td>
<td>35.8</td>
</tr>
<tr>
<td>Electrolyte balance, Meq</td>
<td>266</td>
<td>94</td>
<td>388</td>
<td>115.2</td>
<td>106</td>
<td>92</td>
</tr>
</tbody>
</table>

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**Timeline**

- Daily feed intake measurement
- Weekly body weight measurement
- Sampling of ileal tissue and content
- Sampling of blood and urine

**Experimental diet**

<table>
<thead>
<tr>
<th>Age of mice (days)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>35</td>
<td>35</td>
<td>42</td>
<td>49</td>
<td>56</td>
</tr>
</tbody>
</table>

**Animals:** C57BL/6

**Sex of Animal:** Male
### Performance parameters

**Feed Intake**

- **Average feed intake (g/day):**
  - Day 36-42: SBM, CAS, DWP, SDPP, WGM, YMW
  - Day 43-49: SBM, CAS, DWP, SDPP, WGM, YMW
  - Day 50-56: SBM, CAS, DWP, SDPP, WGM, YMW

**Body weight gain**

- **Body weight (g):**
  - Day 35: SBM, CAS, DWP, SDPP, WGM, YMW
  - Day 42: SBM, CAS, DWP, SDPP, WGM, YMW
  - Day 49: SBM, CAS, DWP, SDPP, WGM, YMW
  - Day 56: SBM, CAS, DWP, SDPP, WGM, YMW

Bars and whiskers represent means ± SD (n = 6).
- *P < 0.05
- **P < 0.01
- ***P < 0.001
Local response: Ileal gene expression profile (microarray)

<table>
<thead>
<tr>
<th>Comparison of experimental diet vs SBM</th>
<th>Number upregulated gene-sets</th>
<th>Number down regulated gene-sets</th>
<th>Number of significantly enriched gene-sets (FDR &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS</td>
<td>559</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>DWP</td>
<td>561</td>
<td>0</td>
<td>124</td>
</tr>
<tr>
<td>SDPP</td>
<td>591</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>WGM</td>
<td>516</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>YMW</td>
<td>559</td>
<td>0</td>
<td>98</td>
</tr>
</tbody>
</table>
Local response: Functional analysis of gene expression data at ileal tissue
Local response: ileal microbiota
Systemic response: Cytokines and Chemokines in blood

- Panel of 23 biomarkers were analysed
- Increase of granulocyte colony stimulating factor (G-CSF) in SBM fed mice
- Increase of Eotaxin for DWP- and YMW-fed mice
- Increase of IL-12p70 in DWP-fed mice
- Decrease of G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5, IL-6, IL-13 and monocyte chemotactic protein (MCP) in WGM-fed mice compared to SBM-fed mice
Systemic response: Metabolites

- Metabolites
  - Amines (Urine)
    - Number of Analytes: 53
  - Acyl carnitines (Urine)
    - Number of Analytes: 16
  - Amines (Serum)
    - Number of Analytes: 41
Systemic response: Amines, urine
Mice experiment: conclusions and discussion

- Diets based on different protein sources affect host responses:
  - local (ileal microbiota, expression of (immune-related) genes)
  - systemic (serum cytokines/chemokines and urine metabolites)
- SBM differs clearly from the other experimental diets
- Diet specific effects identified (not shown here)
- DWP and YMW responses more similar to each other
- Knowledge may help to formulate monogastric diets
Acknowledgement

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