Selecting Beneficial Protein Components From all Dairy Animals for Manufacturing Next Generation Infant Formulas
What is the Problem?

• Breast milk is the perfect food for infants

• Infants who are fed breast milk alternatives:
  • Increased risk of infancy gastrointestinal and respiratory infections
  • Are at increased risk of obesity in later childhood and adulthood
    – Gain weight more quickly vs breast milk
  • Increased risk of type 2 diabetes
  • Have an altered gut microbiome
  • Possibility of lower IQ

Data from the UK Millenium Cohort of infants suggested that 53% of hospital admissions for diarrhoea and 27% for respiratory tract infections up to 8 months could have been prevented by exclusive breastfeeding.
What can we do to Increase the Nutritional Value of Infant Formula through the Protein Component?

<table>
<thead>
<tr>
<th>Water (%)</th>
<th>Fat (%)</th>
<th>Lactose (%)</th>
<th>Protein (%)</th>
<th>HMOs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>87</td>
<td>3.8</td>
<td>7</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Fat and lactose provide 50% and 40% of the total energy of the milk, respectively.

Figure 31.1. Composition of major components of human (left) and bovine (right) milk. Based on data from Coppa et al. (1993, 1999), Kunz et al. (2000) and Nasirpour et al. (2006).
The Role of Proteins In Human Milk

• A source of amino acids
• Mineral solubilisation and transport
• Source of oligosaccharides
• Cognitive development
• Bioactive
  • Peptides
  • Immunomodulatory functions
  • Proteases/lipases
  • Antibodies
  • Serum Albumin
• NON-SPECIFIC PROTECTIVE PROTEINS
  – Lysozyme
  – Lactoferrin
  – Lactoperoxidase

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### Major Human Milk Proteins

<table>
<thead>
<tr>
<th>Caseins (~40 %)</th>
<th>Whey Proteins (~60 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Casein</strong></td>
<td><strong>α-Lactalbumin</strong></td>
</tr>
<tr>
<td>0-5P</td>
<td>Ca &amp; Zn, lactose synthesis</td>
</tr>
<tr>
<td><strong>κ-Casein</strong></td>
<td><strong>Lactoferrin</strong></td>
</tr>
<tr>
<td>55 % weight is carbohydrate</td>
<td>Iron, antimicrobial</td>
</tr>
<tr>
<td><strong>αs1-Casein</strong></td>
<td><strong>Immunoglobulins</strong></td>
</tr>
<tr>
<td>Very small amount</td>
<td>Immunity</td>
</tr>
<tr>
<td></td>
<td><strong>Lysozyme</strong></td>
</tr>
<tr>
<td></td>
<td>Antimicrobial</td>
</tr>
<tr>
<td></td>
<td><strong>Serum Albumin</strong></td>
</tr>
<tr>
<td></td>
<td>Fatty acid transport</td>
</tr>
</tbody>
</table>

- Over 400 proteins identified in human milk
Human Milk is Not Static

**FIGURE 1.** Changes in whey protein (▲) and casein (■) concentrations during lactation in one mother. Data from reference 4.

**FIGURE 2.** Changes in casein content as a percentage of total protein (i.e., ratio of whey protein to casein) in 2 mothers during lactation. Data from reference 4.
Protein Differences

Protein (g/L Milk)

Proportions of Each Caseins

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# Gross Milk Compositions

<table>
<thead>
<tr>
<th></th>
<th>Buffalo</th>
<th>Camel</th>
<th>Cow</th>
<th>Goat</th>
<th>Sheep</th>
<th>Human</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>5.9</td>
<td>3</td>
<td>3.1</td>
<td>3.1</td>
<td>5.5</td>
<td><strong>1</strong></td>
<td>1.5</td>
</tr>
<tr>
<td>Fat</td>
<td>10.4</td>
<td>3.6</td>
<td>3.5</td>
<td>3.5</td>
<td>5.3</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.3</td>
<td>4.4</td>
<td>4.9</td>
<td>4.6</td>
<td>4.6</td>
<td><strong>7</strong></td>
<td>6.8</td>
</tr>
<tr>
<td>Micelle Diameter (nm)</td>
<td>188</td>
<td>300</td>
<td>180</td>
<td>260</td>
<td>193</td>
<td><strong>75</strong></td>
<td>N/A</td>
</tr>
</tbody>
</table>

Camel and human milks, with a low (3.5%, Kappeler et al., 1998) and a high (17%, Dev et al., 1994; Miranda et al., 2000) concentration of kappa casein.
Current State of Proteins in Formulas

Currently major function of proteins in formula is to provide amino acids

Standard 2.9.1 Infant formula products

2.9.1—9 Infant formula and follow-on formula—composition

(1) Infant formula must have:
   (a) an energy content of no less than 2500 kJ/L and no more than 3150 kJ/L; and
   (b) a protein content of no less than 0.45 g/100 kJ and no more than 0.7 g/100 kJ; and
   (c) a fat content of no less than 1.05 g/100 kJ and no more than 1.5 g/100 kJ.

2.9.1—10 Infant formula and follow-on formula—protein—further requirements

(1) The L-amino acids listed in the table to section S29—6 must be present in infant formula and follow-on formula at a level no less than the corresponding minimum level specified in the table.

(2) Despite subsection (1), L-amino acids listed in the table to section S29—6 may be added to infant formula or follow-on formula only in an amount necessary to improve protein quality.
Building the Next Generation Protein Component of Infant Formula

• 60/40 Whey to Casein Ratio
• Whey – Mixture of α-Lactalbumin, Lactoferrin, IgG, serum albumin
• Casein - β-Casein and κ-Casein

• Processing technologies using cow’s milk

• Alternative Milks? Is there a better starting protein source

• Altering the functionality of cow’s milk?

• Synthetic milk?
Processing Technologies using Cow’s Milk

• MFGM purification and addition
  • Modulates the neonatal gut microbiome and normalizes intestinal development (Bhinder et al. 2017 Scientific Reports).
  • MFGM supplementation to infant formula narrows the gap in cognitive development between breastfed and formula-fed infants (Timby et al. 2014 The American Journal of Clinical Nutrition).

• Lactoferrin purification and addition
  • Better iron uptake, transport, scavenging from bacteria
  • Resistant to digestion > bioactive in gastrointestinal tract
  • Lower incidence of diarrhea, lower respiratory tract illness, other infections, improvements of iron status

• β-Casein
  • Large scale processing now available
  • Source of many bioactive peptides
  • Commercial dephosphorylation > changes in clotting and digestibility
## Alternative Milks

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Camel</th>
<th>Cow</th>
<th>Buffalo</th>
<th>Sheep</th>
<th>Goat</th>
<th>Human</th>
<th>Donkey</th>
<th>Mare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids %</td>
<td>12.41</td>
<td>12.25</td>
<td>16.19</td>
<td>17.05</td>
<td>12.12</td>
<td>11.34</td>
<td>9.16</td>
<td>10.16</td>
</tr>
<tr>
<td>Fat</td>
<td>3.96</td>
<td>3.6</td>
<td>6.75</td>
<td>5.95</td>
<td>4.15</td>
<td>2.8</td>
<td>0.95</td>
<td>1.01</td>
</tr>
<tr>
<td>Milk solids non-fat %</td>
<td>8.45</td>
<td>8.65</td>
<td>9.44</td>
<td>11.1</td>
<td>7.97</td>
<td>8.54</td>
<td>8.21</td>
<td>9.15</td>
</tr>
<tr>
<td>Protein %</td>
<td>3.22</td>
<td>3.24</td>
<td>4.18</td>
<td>5.25</td>
<td>3.02</td>
<td>1.97</td>
<td>1.86</td>
<td>2.31</td>
</tr>
<tr>
<td>Fat:Casein</td>
<td>1.65</td>
<td>1.43</td>
<td>2.09</td>
<td>1.47</td>
<td>1.79</td>
<td>3.94</td>
<td>1.51</td>
<td>1.04</td>
</tr>
<tr>
<td>Casein %</td>
<td>2.4</td>
<td>2.51</td>
<td>3.22</td>
<td>4.06</td>
<td>2.32</td>
<td>0.71</td>
<td>0.63</td>
<td>0.97</td>
</tr>
<tr>
<td>Whey protein %</td>
<td>0.93</td>
<td>0.73</td>
<td>0.96</td>
<td>1.19</td>
<td>0.7</td>
<td>1.26</td>
<td>1.23</td>
<td>1.34</td>
</tr>
<tr>
<td>Whey protein:casein</td>
<td>0.36</td>
<td>0.24</td>
<td>0.27</td>
<td>0.29</td>
<td>0.28</td>
<td>1.77</td>
<td>1.94</td>
<td>1.38</td>
</tr>
<tr>
<td>Lactose %</td>
<td>4.56</td>
<td>4.65</td>
<td>4.45</td>
<td>4.91</td>
<td>4.21</td>
<td>6.3</td>
<td>5.95</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*EU only allowed to use proteins from Cow or Goats Milk*
Whey Proteins
Potential Routes to Alter Functionality

• **Stage of Lactation**
  - Total protein
  - Protein ratios e.g. Lactoferrin

• **Genetics**
  - Total Protein
  - Amount of glycosylation
  - Protein ratios
The Effects of Lactation Stage on Milk Components

![Graphs showing the effects of lactation stage on milk components: IgG, α-lactalbumin, albumin, and lactoferrin concentrations over milking number.](image-url)
An Example of Complexity – κ-Casein

• Bovine κ-casein
  • A, A1, B, C, D, E, F1, F2, G1, G2, H, I, and J variants
  • 1-3 phosphate groups
  • 1-6 O-linked glycans
  • Potential variants = 234
  • Actual found = >26
Higher ratio of glycosylated to non glycosylated κ-casein in small CM: leading to differences in CM assembly and growth.

Synthetic Milk

http://www.perfectdayfoods.com/
Conclusions

• Protein component extremely important

• Many different sources of protein, still many unknown
  • Camel milk may be promising – missing Beta lactoglobulin

• There are many potential new routes to improve infant formula
  • Natural variation
  • Processing
  • Alternative sources
  • Synthetic milk
Thank you

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