Making a Claim: Factors impacting Protein Quality and a New Way for Measuring

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Overview

• Demand for high-protein foods
• Need for alternative protein sources
• Protein quality regulations around the world
• Requirements for protein claims in the U.S.
• PDCAAS protein quality test
• New animal safe PDCAAS test
• Future development in protein quality measurement
Protein Demand Continues through 2016

• Protein continues to be a hot trend in the food industry
  — Snacks, meals, beverages and more
  — Focus has shifted to protein from plant sources
  — FAO has declared 2016 the international year of the pulses
    o Low-cost source of protein and dietary fiber

Source – MINTEL 2013

High-protein, natural are trending in beverages
Source – Dairy Foods 2015
Animal protein may not be sustainable for supporting further growth of protein foods
Current Common Protein Sources

Traditional Protein Sources

- Cereals
- Legumes (pulses)
- Nuts & Seeds
- Dairy
- Meat & Seafood
- Egg

Lower Quality
Higher Quality
Some Potential Future Protein Sources

- Rice Bran
- Algae
- Krill
- Hemp
- Insects
- Wolffia
- RuBisCO
- Potato
- Palm Cake
- Fungi

* WSV = waste stream valorization
Alternative Protein Sources

• How can I compare these sources?

• How will they impact the final product?

• What concerns should I have?
Five regulatory authorities of the world for protein quality

- Canada (Health Canada)
- United States (FDA-USDA-FTC)
- European Union
- Codex Alimentarius
- Codex Alimentarius
- Codex Alimentarius
- Australia – New Zealand (FSANZ)

Protein quality testing around the world

Alternatives for Different Regulatory Regions
1. amino acid composition or reference amino acid profile;
2. protein digestibility-corrected amino acid score (PDCAAS);
3. protein efficiency ratio (PER);
4. protein rating;
5. reference protein without amino acid profile or method of determination.

Test generally required for protein content declaration
• United States – PDCAAS
• Canada – PER
• EU – PDCAAS depending on food
• FSANZ – PDCAAS depending on food
• CODEX – PDCAAS depending on food; Moving to DIAAS

U.S. Regulations for Protein Claims

• Food product must contain a minimum of 10% of the daily value of quality protein on both a per serving and per RACC (Reference Amount Customarily Consumed) basis

• The amount of quality protein must be determined by the PDCAAS method which includes both amino acid and digestibility measures

• The total protein claimed may be based on the total crude protein (Dumas combustion or Kjeldahl x 6.25)

(21CFR101.9(7))
Protein Claims - Overview

Threshold Requirement:
>10% DV of Protein (high quality) per RACC and per serving as determined by PDCAAS

Examples:

• **Nutrient Content**
  — Good Source of Protein, With Protein, Made With Xg of Protein, Contains Protein, Source of Protein

• **Statements of Fact**
  — Xg of protein (apart from NF panel)

• **Structure Function**
  — Protein helps build/maintain/repair muscles

• **Food Combination Claims**
  — Xg of protein when made with/eaten with milk, yogurt, chicken, etc.
    - Requires full context: A serving of product X when eaten with Y provides Xg of protein
Elements of PDCAAS Protein Quality Measurement

• Amino Acid Analysis
  — Acid hydrolysis for majority of amino acids
  — Performic acid oxidation for cysteine and methionine
    o Alternative approaches e.g. reduction/carboxymethylation
  — Base hydrolysis for tryptophan

• Determination of crude protein (nitrogen x 6.25)

• Calculation of limiting amino acid relative to ideal protein source

• Determination of protein digestibility

PDCAAS Value = Limiting Amino Acid Value x Digestibility
Ideal Protein – Contains all amino acids essential for human nutrition

<table>
<thead>
<tr>
<th>ESSENTIAL AMINO ACIDS for Human Nutrition</th>
<th>FAO Recommended Values (2011) – mg/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>20</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>32</td>
</tr>
<tr>
<td>Leucine</td>
<td>66</td>
</tr>
<tr>
<td>Lysine</td>
<td>57</td>
</tr>
<tr>
<td>Methionine + Cysteine</td>
<td>27</td>
</tr>
<tr>
<td>Phenylalanine + Tyrosine</td>
<td>52</td>
</tr>
<tr>
<td>Threonine</td>
<td>31</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>8.5</td>
</tr>
<tr>
<td>Valine</td>
<td>43</td>
</tr>
</tbody>
</table>
What molecular weight should be used for Amino Acids from Protein?

Free Amino Acids

Glycine

Water released

Alanine

Protein

Hydrolysis by Amino Acid Analysis splits the amino acids in the protein apart by adding water back to the peptide bond so they can be measured.

H₂O

(Water 18 g/mol)
Guidance of FAO/WHO on Amino Acid Molecular weight to be used for Protein

FAO/WHO 2001 Rome Working Group Consultation recommended that protein should be measured as the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water).
Effect of including water in Amino Acid Calculations

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Minus Water</th>
<th>Plus Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dehydrated</td>
<td>Hydrated</td>
</tr>
<tr>
<td></td>
<td>Amino Acid</td>
<td>Amino Acid</td>
</tr>
<tr>
<td></td>
<td>Content</td>
<td>Content</td>
</tr>
<tr>
<td></td>
<td>(g/100g)</td>
<td>(g/100g)</td>
</tr>
<tr>
<td></td>
<td>Ratio to FAO</td>
<td>Ratio to FAO</td>
</tr>
<tr>
<td></td>
<td>2011 Nutrition</td>
<td>2011 Nutrition</td>
</tr>
<tr>
<td>L-Cysteine + L-Methionine*</td>
<td>2.72</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td>1.254</td>
<td>1.446</td>
</tr>
<tr>
<td>L-Tryptophan*</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>1.551</td>
<td>1.551</td>
</tr>
<tr>
<td>L-HydroxyProline</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>6.27</td>
<td>7.26</td>
</tr>
<tr>
<td>L-Threonine*</td>
<td>2.31</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>0.926</td>
<td>1.091</td>
</tr>
<tr>
<td>L-Serine</td>
<td>2.79</td>
<td>3.37</td>
</tr>
<tr>
<td>L-Glutamic Acid</td>
<td>11.68</td>
<td>13.31</td>
</tr>
<tr>
<td>L-Proline</td>
<td>2.78</td>
<td>3.30</td>
</tr>
<tr>
<td>L-Glycine</td>
<td>2.38</td>
<td>3.14</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>3.19</td>
<td>4.00</td>
</tr>
<tr>
<td>L-Valine*</td>
<td>3.96</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td>1.144</td>
<td>1.352</td>
</tr>
<tr>
<td>L-Isoleucine*</td>
<td>3.08</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>1.198</td>
<td>1.388</td>
</tr>
<tr>
<td>L-Leucine*</td>
<td>5.52</td>
<td>6.40</td>
</tr>
<tr>
<td></td>
<td>1.041</td>
<td>1.207</td>
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<tr>
<td>L-Tyrosine + L-Phenylalanine*</td>
<td>7.31</td>
<td>8.16</td>
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<tr>
<td></td>
<td>1.748</td>
<td>1.953</td>
</tr>
<tr>
<td>L-Lysine*</td>
<td>3.06</td>
<td>3.49</td>
</tr>
<tr>
<td></td>
<td>0.667</td>
<td>0.761</td>
</tr>
<tr>
<td>L-Histidine*</td>
<td>1.62</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>1.006</td>
<td>1.138</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>5.54</td>
<td>6.18</td>
</tr>
</tbody>
</table>

Total Protein by Amino Acid Analysis = 65.27 (Minus Water) 75.59 (Plus Water)

16% higher result

Minus Water            Plus Water
Crude Protein (%) = 80.4 80.4
Animal Safe Digestibility = 0.88 0.88
First Limiting Amino Acid = L-Lysine L-Lysine
Amino Acid Score = 0.667 0.761
PDCAAS Value = 0.59 0.67

Potential of over-declaring protein content!
Digestibility Measurement: Rat PDCAAS Method

**Sample**
Food or Ingredient

**Measure**
- Total Nitrogen, Amino Acid Profile, Fat, Ash, Moisture, Sugars, and Fiber for feed calculation

**Feed**
Live Rats
Tested in parallel with casein standard (min. 10 rats)

**Measure**
Food intake, body weights, Fecal N₂

**Sacrifice**
Rats must be of appropriate age for studies

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Drawbacks to rat method for digestibility measurement

Timing
  - 2-3 month turnaround

Cost
  - $5,000+/sample

Method
  - Use of animals for product testing
Additional drawbacks to rat PDCAAS method

• Large amount of sample required (1 to 1.5 kg)

• Cost of full proximate analysis
  ○ Formulation of balanced rat feed

• Over-fortification with protein
  ○ Processing effects on score unpredictable
  ○ Significant over-use of expensive ingredients
Development of ASAP-Quality Score Method (Animal-Safe Accurate Protein Quality Score)

1. Determine Amino Acid Composition
   - Measure using AOAC method 994.12

2. Simulate Human Digestion
   - Digest proteins into amino acids
   - React amino acids with Ninhydrin and measure
   - Correct measurement with Amino Acid profile from Step 1

3. Report Results
   - % Quality Protein
   - Amino Acid Profile
   - Digestibility Score

Method Reference – US Pat Appl No. 14/599,050: IN VITRO METHOD FOR ESTIMATING IN VIVO PROTEIN DIGESTIBILITY, Plank, DW.
ASAP-Quality score digestion overview

Pepsin Digest, pH 2

Trypsin / Chymotrypsin Digest, pH 7.5

Digestion generally similar to stomach and small intestine

TCA Precipitate, Centrifuge

React supernatant with Ninhydrin

Quantify Absorbance

Adjust result by Amino Acid Composition

Quantification of reactive amines created by digest
Correlation of ASAP-Digestibility Score to Rat Digestibility Score

<table>
<thead>
<tr>
<th>Matrix</th>
<th>ASAP-Quality Digestibility Score</th>
<th>Rat Digestibility Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>100.2</td>
<td>100.0</td>
</tr>
<tr>
<td>Rolled Oats</td>
<td>82.7</td>
<td>83.0</td>
</tr>
<tr>
<td>Lentils</td>
<td>85.4</td>
<td>86.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>89.5</td>
<td>91.0</td>
</tr>
<tr>
<td>Split Pea</td>
<td>85.2</td>
<td>84.0</td>
</tr>
<tr>
<td>Sunflowers Seeds</td>
<td>86.0</td>
<td>94.0</td>
</tr>
<tr>
<td>Black-eyed peas</td>
<td>83.7</td>
<td>84.0</td>
</tr>
<tr>
<td>Kidney Beans</td>
<td>81.5</td>
<td>81.0</td>
</tr>
<tr>
<td>Peanuts Roasted High Oleic</td>
<td>71.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Sunflower Kernels Roasted No Salt SL80</td>
<td>77.0</td>
<td>79.0</td>
</tr>
<tr>
<td>Peanut Butter #7</td>
<td>92.0</td>
<td>93.0</td>
</tr>
<tr>
<td>Pea Protein Bar Fruit and Nut</td>
<td>90.0</td>
<td>89.0</td>
</tr>
<tr>
<td>Oats Rolled #15</td>
<td>93.0</td>
<td>95.0</td>
</tr>
<tr>
<td>High Pro Nutty Granola Cluster</td>
<td>97.0</td>
<td>97.0</td>
</tr>
<tr>
<td>Granola Base #7 Sucrose/Canola Natural</td>
<td>98.0</td>
<td>99.0</td>
</tr>
<tr>
<td>Granola Bar #1</td>
<td>78.0</td>
<td>78.0</td>
</tr>
<tr>
<td>Granola Bar #2</td>
<td>84.0</td>
<td>84.5</td>
</tr>
<tr>
<td>Chicken Stock Concentrate Salt</td>
<td>86.0</td>
<td>85.5</td>
</tr>
<tr>
<td>Granola base #5</td>
<td>94.0</td>
<td>93.0</td>
</tr>
</tbody>
</table>

1 based on published literature values
2 based on direct analysis of the same sample by both methods

- \[ y = 0.9286x + 7.1024 \]
- \[ R^2 = 0.9205 \]
Correlation of ASAP-Quality Score to Final Rat PDCAAS Value

\[ y = 1.0113x - 0.0233 \]

\[ R^2 = 0.9784 \]

\( N = 20 \)
Advantages of ASAP-Quality Score

• Does not use animals for testing

• High correlation to animal test

• Significantly reduces testing time
  o 15-days versus 2 to 3 months

• Significantly reduces costs
  o $1,500 per sample versus $5,000+ per sample

• Eliminates ingredient waste
  o Reduces need for over-fortification by food developers
How to improve a PDCAAS result

![Bar chart showing PDCAAS scores for different types of protein. Whole Wheat and Peanuts are low quality proteins, while Whey Protein, Soy Protein Isolate, Egg White, and Beef are high quality proteins.](image)
# Protein Complementation

2 individually inferior proteins combined in the right proportion to result in delivery of a complete protein.

<table>
<thead>
<tr>
<th>Food</th>
<th>Limited Amino Acid (LAA)</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beans</td>
<td>Methionine</td>
<td>Grains, nuts, seeds</td>
</tr>
<tr>
<td>Grains</td>
<td>Lysine, Threonine</td>
<td>Legumes</td>
</tr>
<tr>
<td>Nuts/seeds</td>
<td>Lysine</td>
<td>Legumes</td>
</tr>
<tr>
<td>Legumes</td>
<td>Tryptophan, Methionine</td>
<td>Grains, nuts, seeds</td>
</tr>
<tr>
<td>Corn</td>
<td>Tryptophan, lysine</td>
<td>Legumes</td>
</tr>
</tbody>
</table>
Combining complementary protein compositions

Pea protein concentrate
LAA = Met & Cys

Brown rice protein concentrate
LAA = Lys

60% pea + 40% rice = 1.053
Medallion Complementation Database

• Contains amino acid analysis data and digestibility data from a wide range of ingredients
  o Source of data is primarily General Mills
  o Customer data never added to database unless specific permission granted

• Database can be used to model best ingredient matches for highest protein quality

• Targeting Q3 2016 for general availability to Medallion customers
ASAP-Quality Score Next Steps

• Conduct International Collaborative Study
  o AOAC Validation
  o Official acceptance as alternative for animal testing

• Develop correlation to DIAAS Protein Quality Method
  o New FAO international standard for protein quality
DIAAS Protein Quality Method

- **Digestible Indispensable Amino Acid Score (DIAAS)**

- Based on true ileal digestibility of each amino acid
  - Measured at end of small intestine
  - Excludes large intestine fermentation

- Preferably determined in humans

- Alternatively:
  - Growing pigs (FAO recommended)
  - Growing rats
Drawbacks of DIAAS Method

- **High cost per sample**
  - $15,000 to $20,000 USD per sample
  - 10-growing pigs per sample
- **Longer time for results**
  - 4 to 6 months
- **Low capacity**
  - No commercial labs currently available
  - Private arrangements with Universities
Adapting ASAP-Quality to fit the DIAAS Method

• Collaborate with Ingredient Manufacturers
  • Reduced development cost

• Run samples in parallel
  • Homogenously split samples
  • Analyze by DIAAS
  • Analyze by ASAP-Quality

• Develop new protocol/equation to fit ASAP to DIAAS
Summary

• Alternative protein sources are needed for the food supply

• Protein quality measurement is required for understanding the value of a protein source

• Current PDCAAS quality measurement has drawbacks including use of animals for testing

• ASAP Quality Score offers –
  o Animal free
  o Good correlation to rat digestibility
  o Much less expensive
  o Faster turnaround time for results

• DIAAS method may become next protein quality tool