Bovine Beta Casein Variants and Digestion

26 September 2017
Outline

• Research Timeline
• Beta Casein Variants
• Review of Studies on Beta Casein and Digestive Function
• Recent Research
• The Future
Research Timeline

1 Reflects calendar years
2 Beta casomorphin-7
Bovine Beta Casein ~2.5 grams per serve

Conventional A1 containing milk and the a2 Milk™ brand have the same general composition.

a2 Milk™ brand contains only the A2 type of the beta-casein protein.
A2 Beta Casein: The Original Variant

Ancient bos (Auroch)

>100,000 yrs ago
taurus and indicus species evolve from bos genus

~ 5 – 10 thousand years ago
Variant A1 evolves through natural mutation from beta casein A2 in bos taurus

- African bos indicus e.g. Africander (A2)
- Indian bos indicus e.g. Brahman (A2)
- African bos taurus e.g. Kuri (A2)
- European bos taurus e.g. Holstein, Jersey, Guernsey (A1 and A2 in all breeds)

Figure adapted from information reported in:
• Originally all domesticated cows produced milk containing only the A2 type of beta casein

• Owing to natural genetic mutation, a variant of the A2 protein appeared; termed A1 protein, differs very slightly in composition from the original A2 protein

• Both types have since given rise to a number of minor related “sub-variants”, such as those termed A3, B and C

• Beta casein variants can be divided into either “A1 type” or “A2 type” based on their digestion

• A1 and A2 variants are the primary types of beta-casein

• Variants A3, B and I are increasing in frequency
Digestion of Beta Casein Variants

- It is accepted that BCM-7 is produced from the incomplete digestion of the A1 but not A2 types of beta casein.

- BCM-7 is an established exorphin, or peptide that binds and ‘activates’ opiate receptors expressed by cells and tissues throughout the body. (EFSA 2009)

- BCM-7 is produced at physiological levels in healthy adult human small intestine (Boutrou et al, 2013)

- Searching scientific or medical databases will throw up hundreds of published studies on BCM-7 or beta casomorphin.
Barnett et al (2014)

A1 vs A2 protein consumption resulted in:
- Increased GI inflammation (MPO)
- Increased transit time (GITT)
- Increased DPPIV – enzyme that breaks down BCM-7 but also controls stomach emptying and metabolic hormones.
Ul Haq et al (2013)

- A1 protein induced inflammation is BCM-7 mediated through established immune/cell pathways.

• Boutrou et al (2013) provide time line and amount of BCM-7 production following the consumption of milk protein.
  – Milligram amounts, sufficient to elicit biological response from tissue in the gut
  – Peaks at 30 min and present for four hours following intake of milk.

• Ho et al (2014) Preliminary Human Clinical Trial reports the potential link between A1 beta casein, GI inflammation and symptoms of intolerance
  “a statistically significant positive association between abdominal pain and stool consistency was observed when participants consumed the A1 but not the A2 diet. Further studies of the role of A1 beta casein in milk intolerance are needed.” (Ho et al, 2014 and Pal et al, 2015)

JanssenDuijghuijsen et al (2016) Physiol Rep. 4 (20) reports detectable and increasing levels of BCM-7 in urine post exercise stress as a marker of intestinal permeability
A mechanism of BCM-7’s biological interactions with neuronal and gut epithelial cells was published in 2014.

BCM-7 binds the Mu Opiate receptor which is coupled to the EAAT3 receptor.

BCM-7 binding inhibits cysteine uptake by EAAT3 receptor, reducing the level of substrate for antioxidant production.

Consequently cellular glutathione (GSH) levels decrease putting the cell under oxidative stress.

Oxidative stress and subsequent inflammation triggers both changes in gene expression and epigenetic modification of DNA.
Neural stem cells were treated with BCM-7, BCM-9 (A2) and hBCM-7 (from human).

BCM-9 (A2) was comparable to hHBCM-7 in its effects where as BCM-7 was closer in effect to positive control morphine.

Plots of reported intolerance symptoms of participants consuming a2 Milk™ vs conventional milk in double blinded crossover clinical trial.
(a) Participants confirmed as lactase deficient
(b) Participants without lactase deficiency, and
(Figure 3) is combined data

Gastrointestinal (GI) transit time also plotted for feeding groups and phases, showing increased (6hrs) transit time in conventional (A1/A2) fed groups compared to a2 Milk™ fed
Conventional milk reported to increase inflammatory and immune markers

- Reported in three animal studies in 2014
- Confirmed in clinical trials in participants reported and confirmed as milk protein or lactose intolerance
- Images taken with a pill endoscope (camera) demonstrate increased gut inflammation with A1 protein containing milk

a2 Milk™ associated with improved levels of SCFA; markers of healthy gut bacteria

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sequence 1</th>
<th>Sequence 2</th>
<th>Mixed-effects ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PI</td>
<td>Estimate^2</td>
</tr>
<tr>
<td>hs-CRP (mg/dL)</td>
<td>1.08 ± 1.03</td>
<td>1.07 ± 1.11</td>
<td>0.0722</td>
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<tr>
<td>Hb (g/L)</td>
<td>142.8 ± 20.1</td>
<td>145.5 ± 17.7</td>
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<td>L-4 (ng/L)</td>
<td>119 ± 4.3</td>
<td>120 ± 3.7</td>
<td>&lt;0.0001</td>
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<td>IgA (g/L)</td>
<td>10.6 ± 2.1</td>
<td>11.1 ± 1.9</td>
<td>0.1426</td>
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<td>IgE (IU/mL)</td>
<td>58.0 ± 31.2</td>
<td>60.7 ± 33.3</td>
<td>5.9688</td>
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<td>IgG1 (µg/mL)</td>
<td>33.0 ± 26.3</td>
<td>28.5 ± 28.6</td>
<td>0.2424</td>
</tr>
</tbody>
</table>

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Clinical data reports a2 Milk™ vs. conventional milk associated with higher levels of ‘healthy’ gut bacteria.
HVN – aMiGo Trial (a2 Milk™ for gut comfort)

Assessed for eligibility (n=101)
- Excluded (n=42)
  - Appointment issues
  - Criteria not met
  - No longer required to screen (8)

Lactose Challenge Enrolment (n=59)
- Lactose Intolerant (DI) (n=26)
- Lactose Intolerant (DI) (n=10)
- Tolerant (n=10)

Dairy Intolerant (DI) (n=23)

Milk Challenge Enrolment (40)

Randomized
- Conventional
- a2 Milk™
- Lactose Free
Ethnic Distribution

- No non-Caucasians in Dairy Intolerant group
- Fewer non-Caucasian absorbers
Dairy avoidance in dairy avoiders?

How much milk do you drink each day?

- 3 glasses (750ml)
- 2 glasses (500ml)
- 1 glass (250ml)
- None, I don’t use milk

**Count**

- Dairy Intolerant
- Lactose Intolerant
- Tolerant
Lactose intolerant subjects experience greater symptoms with conventional milk.
Malabsorption reduced with a₂ Milk™

• Breath hydrogen decreased after a₂ Milk™ compared to conventional
• Urinary galactose not different
Summary: The aMiGo Trial

- In lactose intolerant individuals, a2 Milk™
  - Reduced nausea and faecal urgency than conventional milk, similar to lactose-free
  - Breath hydrogen increased later and less after a2 Milk™ than conventional in lactose intolerant subjects
The Future

- Confirm and extend acute study observations around A1 protein free milk benefits to lactose intolerance.
- Examine and characterise the mechanism by which the effects are imparted.
- Identify breathalomic or metabolomic markers to identify respondent individuals.
- Determine the proportion of respondents of selected population groups or market.
- Study medium to long term influence on metabolic and inflammatory markers and outcomes.
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