a2 Milk™
Overview of Technology

Dr Andrew J Clarke
Chief Scientific Officer

Outline

• What is a2 milk™?
• Production and Certification of a2 milk™?
• The a2 milk™ proposition
• a2 milk™ Intellectual property
• Review of dairy science and beta caseins.
• What is beta casein?
• The beta casein variants
• Milk protein bioactives
• Review of studies on beta casein
• a2 milk™ on the market
What is a2 milk™?

• a2 milk™ is a natural, high quality cow’s milk that
  – has been naturally obtained from specially selected animals
  – does not involve GE or GM.
  – Is produced to highly certified standards using proprietary technologies.
  – Is a value added premium product presenting benefits to the producers, retailers and consumers.
  – Complements existing lines of value added milks offering the consumer further choice.
  – Can be incorporated into a range of natural dairy products targeted at the informed and discerning consumer.

• Selected animals produce milk containing the A2 variant of the milk protein beta-casein at the exclusion of other major variants.

• A multitude of studies have been conducted into the beta casein variants, their digestion products and subsequent biological activity.

Production and Certification of a2 milk™

• Animals are tested using a hair or any other type of tissue sample from each cow.

• Following testing, cows having the trait for the production of the a2 type of beta-casein, not other major variants, are tagged and identified as a2 milk™ producers.

• Identified a2 milk™ producers are segregated from other dairy cows and their milk is gathered, stored and processed separately from other milk.

• A2 Milk Company implements quality control measures that are applied from the farm through to the packaged product.

• These measures include periodic testing of the milk at points of the supply chain to ensure that at least 99% of the beta-casein produced by these segregated cows is in the A2 type.
Consumer Proposition

- a2 milk™ is positioned as a line of premium low and no fat milks.
- A ‘new’ choice in protein content complimenting existing lines of value added milks. e.g. fat content, modified carbohydrate, fortified with vitamins or calcium, organic
- a2 milk™ is certified to have 2g of a2 beta-casein per serving.
- The natural and nutritional goodness of milk may be realised through a2 milk™

The a2 milk™ Proposition

a2 milk™ technology delivers incremental value throughout the chain.

- Farmer: Through patented test(s), identify and segregate higher value, pure a2 milk™ producing animals.
- Processor: a2 milk™ marketed using proprietary marks and rights licensed to limited number of partners
- Retailer: Consumer choice of a milk with a unique protein profile.
- Consumer: a2 milk™ is positioned as a line of premium, value added, low and no fat milks,
# a2 milk™ - US Patents and Patent Applications

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6,570,060</td>
<td>Food Product and Process / Milk Lacking beta casein A1</td>
</tr>
<tr>
<td>US 7,094,949</td>
<td>Breeding and Milking Cows For Milk Free of Beta Casein A1</td>
</tr>
<tr>
<td>US 10/405,358</td>
<td>Milk Lacking beta casein A1</td>
</tr>
<tr>
<td>US 6,451,368</td>
<td>Method of Selecting Non-Diabetogenic Milk or Milk Products and Milk or Milk Products So Selected.</td>
</tr>
<tr>
<td>US 10/207,709</td>
<td>Method of Selecting Non-Diabetogenic Milk or Milk Products and Milk or Milk Products So Selected.</td>
</tr>
<tr>
<td>US 10/515,940</td>
<td>Animal Genotyping Method</td>
</tr>
<tr>
<td>US 10/405,358</td>
<td>Milk Lacking beta casein A1</td>
</tr>
<tr>
<td>US 10/530,209</td>
<td>Therapeutic Uses of Beta-Casein A2 and Dietary Supplement Containing Beta-Casein A2</td>
</tr>
<tr>
<td>US 10/519,624</td>
<td>Method for Altering Fatty Acid Composition of Milk</td>
</tr>
</tbody>
</table>

## Review of dairy science and beta caseins

- **What is beta casein?**
  - Function and role

- **The beta casein variants**
  - The difference
  - Their digestion

- **Bioactives**
  - In milk
  - Yielded from beta casein

- **Review of studies on beta casein**
What is beta casein?

- About 30% of the protein in cows' milk is beta-casein.
- Beta-caseins play a key role in the transport of essential ions and nutrients such as calcium and phosphorous.
- It is known that the beta caseins in milk, when digested, can yield a plethora of bioactive protein fragments, a number of which have been well characterized with regard to potential biological activity.

Beta casein variants

- Research indicates that originally all cows carried the trait for only the A2 type of beta casein.
- At some point in history, owing to natural genetic mutation, a variant of the A2 gene and its associated protein appeared.
- Termed the A1 variant, it differed very slightly in composition from the original A2 beta-casein.
- Subsequently this newer variant has since given rise to a number of minor related sub variants, such as those termed B and C.
- The small differences between the beta casein variants may lead to the differential digestion following consumption, resulting in different yield of protein fragments, a number of which are biologically active or bioactive.

Evolution of beta casein variants

Figure from: MILK PROTEIN POLYMORPHISM: DETECTION AND DIFFUSION OF THE GENETIC VARIANTS IN BOS GENUS, Annali della Facoltà di Medicina Veterinaria, Vol. XIX, 1999. Università degli Studi di Parma

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

~10,000 yrs ago
Variant A1 evolves through natural mutation from beta casein A2 in bos taurus.

Phylogenetic studies indicate that the A2 form was the progenitor or original form of beta casein gene in cattle

Figure adapted from information reported in:
Differential peptide yield from beta-casein variants

- Proline (not histidine) at residue 67 strongly favours the differential cleavage of A2 beta casein protein by proteolytic/digestive enzymes¹.

- Both in vitro¹ and in vivo²-³ studies have shown that a bioactive peptide, beta casomorphin-7 (BCM-7) is preferentially yielded upon digestion of β-casein A1 and related variants, but not A2.

- Production of BCM-7 from A1 may:
  - Preclude the production of other useful bioactives¹.

---


---

Difference between beta casein variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Amino Acid Position 67</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2 beta casein and related sub-variants</td>
<td>Tyr</td>
</tr>
<tr>
<td>A1 beta casein and related sub-variants</td>
<td>Tyr</td>
</tr>
</tbody>
</table>

Bioactives obtained from milk proteins

- Milk contains or may release bioactives shown to have beneficial effects, much of which are attributed to antibodies.

- Proteins found in the whey component of milk have been found to have antimicrobial and immunomodulatory properties as well as having important roles in iron absorption and calcium transport.

- As scientific research has progressed more bioactive proteins have been discovered in milk as well as a vast array of bioactive protein fragments or peptides that are encrypted in major milk proteins.

- These bioactives may be released by enzymatic digestion, either naturally, in the gastrointestinal tract, or by commercial processing.

- The structural difference between the beta casein variants may lead to a different yield of bioactives which in turn may impart a different range of effects.

Reviewed in:

Research on dairy & beta-caseins

- A significant accumulation of dairy science indicates . . .
  - “Small dietary changes, such as increasing dairy food intake, can improve the nutritional quality of the diet and play a beneficial role in health promotion and disease prevention.”

- Beta-casein science, conducted in a number of different countries over the past 15 years:
  - Shows that the major beta casein variants differ at a single amino acid, that may lead to a difference in the yield of bioactive peptides (protein fragments) following digestion.

- Research has linked this difference between beta-casein variants and subsequent bioactive yield to:
  - Heart health
  - Blood sugar
  - Neurological function

- Research on beta-caseins and associated hypotheses.
  - “There are seven strands of evidence relevant to the A1/A2 hypothesis: epidemiology, milk chemistry, pharmacology, animal experiments, human trials, clinical observations, and consumer experiences. It is the totality of these seven strands that makes the A2 hypothesis so intriguing.”

Studies on beta caseins and their digestion products

- The following studies debate the hypotheses relating to milk with high levels of the a2 beta casein variant.
- These hypotheses relate to:
  - Heart health
  - Blood sugar
  - Neurological function
  - Biological functions
- Further studies cited relate to a range of bioactives encoded in beta caseins, their potential yield, absorption and biological effects.

Reviews of published studies relating to beta-casein and health

Health implications of milk containing beta-casein with the A2 genetic variant.

“Populations that consume milk containing high levels of beta-casein A2 variant, have a lower incidence of cardiovascular disease and Type-1 Diabetes. Furthermore consumption of milk with the A2 variant may be associated with less severe symptoms of autism and schizophrenia.”


The A2 milk case: a critical review.

‘there is no convincing or even probable evidence that the A1 b-casein of cow milk has any adverse effects in humans’.


A critique of Truswell's A2 milk review.


Further research for consideration in 'the A2 milk case'.

**Epidemiological studies: beta-casein variants and heart health**

**Ischaemic heart disease, Type 1 diabetes, and cow milk A1 beta-casein.**

“The remarkable agreement between mortality and the consumption of this allele suggests that this factor is worthy of serious consideration as a potential source of cardiovascular disease when taken in conjunction with regional variations in the traditional risk factors.”


**beta-casein A1, ischaemic heart disease mortality, and other illnesses**

“A1 beta-casein per capita supply in milk and cream (A1/capita) was significantly and positively correlated with IHD in 20 affluent countries five years later over a 20-year period—providing an alternative hypothesis to explain the high IHD mortality rates in northern compared to southern Europe”


**Animal and Human studies: beta-casein variants and heart health**

**A casein variant in cow’s milk is atherogenic.**

“demonstrate for the first time that beta-casein A2 has a mildly atheroprotective effect while b-casein A1 is most definitely atherogenic….These results are consistent with epidemiological studies that suggest a strong relationship between mortality from cardiovascular disease and consumption of b-casein A1, even though other studies show no association between reported milk consumption per se and cardiovascular mortality”


**Effect of dietary supplementation with beta-casein A1 or A2 on markers of disease development in individuals at high risk of cardiovascular disease.**

“Total plasma cholesterol levels were significantly lower following 12 weeks of both casein A1 and A2 interventions but the decrease was not different between intervention. Plasma insulin, homocysteine, C-reactive protein, fibrinogen, protein C and S and von Willebrand factor levels were not different between the two casein supplements.”

Human studies: beta-casein variants and heart health

Significant increase in antibodies against oxidized LDL particles (Ig ox LDL) in three-month old infants who received milk formulae.

“Significant increase in antibodies against oxidized LDL particles (Ig ox LDL) in three-month old infants who received milk formula.”

“...release of beta casomorphin-7 during A1 variant of beta casein metabolism. This substance is known to be involved in the oxidation of LDL.”

“As human milk does not contain beta casein A1 and infant formulas are based on bovine milk, we can express a hypothesis that beta casein A1 is the substance, which caused increased production of Ig ox LDL.”


Antibodies against oxidised LDL in infants

Casein-derived peptides can promote human LDL (oxidation by a peroxidase-dependent & metal-independent process.

Epidemiological studies relating to beta-casein variants and blood sugar

Lower consumption of cow milk protein A1 beta-casein at 2 years of age, rather than consumption among 11- to 14-year-old adolescents, may explain the lower rate of type 1 diabetes in Iceland than in Scandinavia.

“This study supports that lower consumption of A1 beta-casein might be related to the lower incidence of type 1 diabetes in Iceland than in Scandinavia. Additionally it indicates that consumption in young childhood might be of more importance for the development of the disease incidence than consumption in adolescence.”


Variation in consumption of cow milk proteins and lower rate of Type 1 diabetes in Iceland versus the other 4 Nordic countries.

Ischaemic heart disease, Type 1 diabetes, and cow milk A1 beta-casein.
Studies relating to beta-casein variants and blood sugar

A multi-centre, blinded international trial of the effect of A (1) and A (2) beta-casein variants on diabetes incidence in two rodent models of spontaneous type 1 diabetes.

“Milk caseins are unlikely to be exclusive promoters of Type 1 diabetes, but could enhance the outcome of diabetes in some cases. Other diet components such as wheat could be more important promoters of Type 1 diabetes.”


The significance of A1 and A2 antibodies against beta-casein in type-1 diabetes mellitus

“Because the A1 variant of beta-casein correlates with the onset of IDDM, but can also occur in normal controls, this may confirm the hypothesis of a defective oral immunotolerance to cow’s milk antigens in IDDM.”


Research on peptides derived from beta-casein variants and brain function

Autism and Schizophrenia: Intestinal Disorders

“Our data provide support for the proposal that many patients with schizophrenia or autism suffer due to absorption of exomorphins formed in the intestine from incomplete digestion of gluten and casein.”


Can the pathophysiology of autism be explained by the nature of the discovered urine peptides?

“the data presented form the basis of an autism model, where we suggest that exorphins and serotonin uptake modulators are key mediators for the development of autism.”


Reports on dietary intervention in autistic disorders

Studies on digestion products of beta-casein variants and beta casomorphins

Enzymatic release of neocasomorphin and beta-casomorphin from bovine beta-casein
“beta-Casomorphin-7 was released only from a genetic variant of beta-casein containing a His residue at the 67th position of the peptide chain.”


Bioactive substances in milk with properties decreasing risk of cardiovascular diseases.

Casein peptide release and passage to the blood in humans during digestion of milk or yoghurt.

beta-Casomorphin-immunoreactivity in the brain stem of the human infant.


Studies on bioactivity of digestion products of beta-casein variants


Studies on bioactivity of digestion products of beta-casein variants


a2 Milk™ on the market

• Milk launched in 2003 in Australasia
• Now available in over 1000 major supermarkets, health food outlets and other retail channels.
• Endorsed by consumers who report enjoyment and benefits of milk through a2 milk™
  – My 3 and 1 year old sons love this milk - they would never have a full bottle of the ordinary milk instead they have two bottles in a row at night. They also sleep better. My boys love the a2 milk. I recommend any parent that truly cares about their children’s health and well being should be giving them the real thing milk, its like having your own cow in the backyard!!
    • Sandra Taouk, Picton, Australia
  – I just wanted to say thanks! I can drink milk again after 10 years on soy! I was finding it difficult to restrict all dairy and soy, gluten and stay low GI. My Dr suggested I try a2 milk . . . boy was I skeptical! But, I tried it and now I can have my low GI muesli in the morning!
    • T. Bentz, Campbelltown, Australia
  – I tried your milk and the sticky throat thing stopped immediately and my stomach feels normal again (rather than bordering on seedy all the time). I am a big fan of your milk and will recommend it to all mums, to everyone.
    • L. Oehlert, Gold Coast, Australia
• Consumer response to a2 milk™ leads to further hypotheses relating beta caseins, their derived bioactives and realizing their potential benefits.
• a2 Milk™ in the U.S.

• Commercialized in North America by A2 Milk Company LLC
  – Joint venture between A2 Corporation Ltd of New Zealand and IdeaSphere Incorporated.

• Produced under license by The Original Foods Company, Nebraska.
  – Presently available in Hy-Vee Supermarkets throughout the Midwest.

For further information . . .

Please contact . . .

Mr Grant Prentice  grant@a2milk.com
President, A2MCo LLC

Dr Andrew Clarke  andrew@a2corporation.com
CSO, A2 Corporation Ltd.