



# **a2 Milk™**

## **Overview of Technology**

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### **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**

A background image showing a splash of white milk against a light cream color. The splash is centered and creates a sense of motion and freshness.

## 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.

A background image showing a splash of white milk against a light cream color. The splash is centered and creates a sense of motion and freshness.

## 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**

Consumer choice of a milk with a unique protein profile.

a2 milk™ is positioned as a line of premium, value added, low and no fat milks,

## **a2 milk™ - US Patents and Patent Applications**

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

## **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<sup>1</sup>
- At some point in history, owing to natural genetic mutation, a variant of the A2 gene and its associated protein appeared<sup>1</sup>.
- Termed the A1 variant, it differed very slightly in composition from the original A2 beta-casein<sup>2</sup>
- Subsequently this newer variant has since given rise to a number of minor related sub variants, such as those termed B and C<sup>1</sup>.
- The small differences between the beta casein variants may lead to the differential digestion following consumption, resulting in different yield of protein fragments<sup>1</sup>, a number of which are biologically active or bioactive<sup>3</sup>.

1. [Advanced Dairy Chemistry: Volume 1 - Proteins, Second Edition, edited by P.F. Fox and P. L. H. McSweeney pp 424 & 430  
2. Jinsmaa, Y & Yoshikawa, M. 1999. Enzymatic release of neocasomorphin and beta-casomorphin from bovine beta-casein. *Peptides* V20(8), pp 957-962.  
3. Shah, N. 2000. Effects of Milk-derived bioactives: an overview. *British Journal of Nutrition*. 84. Suppl. 1. S3-310.

## Evolution of beta casein variants

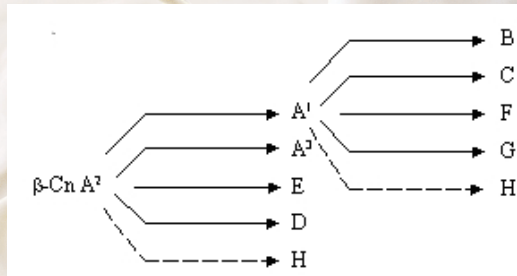


Figure from: MILK PROTEIN POLYMORPHISM: DETECTION AND DIFFUSION OF THE GENETIC VARIANTS IN BOS GENUS, Annali della Facoltà di Medicina Veterinaria, Vol. XIX, 1999. Università degli Studi di Parma ]

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

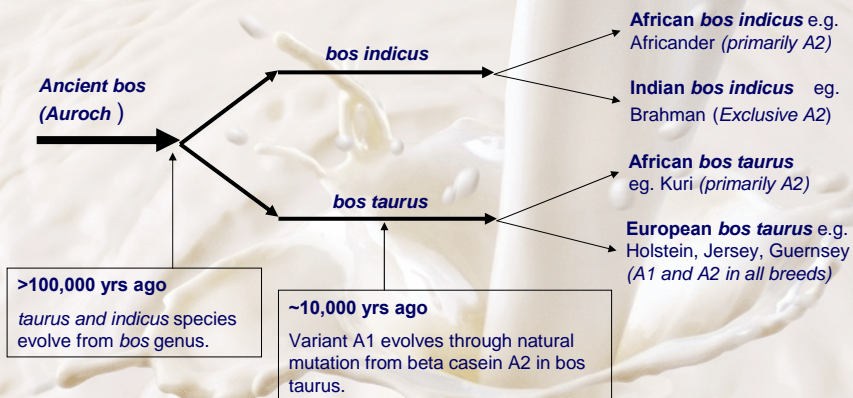


Figure adapted from information reported in:

1. Advanced Dairy Chemistry: Volume 1 - Proteins, Second Edition, edited by P.F. Fox and P. L. H. McSweeney pp 424 & 430
2. 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
3. Daniel G. Bradley, Ronan T. Loftus, Patrick Cunningham, David E. MacHugh (1998) 'Genetics and domestic cattle Origins' Evolutionary Anthropology V6:3 pp79-86

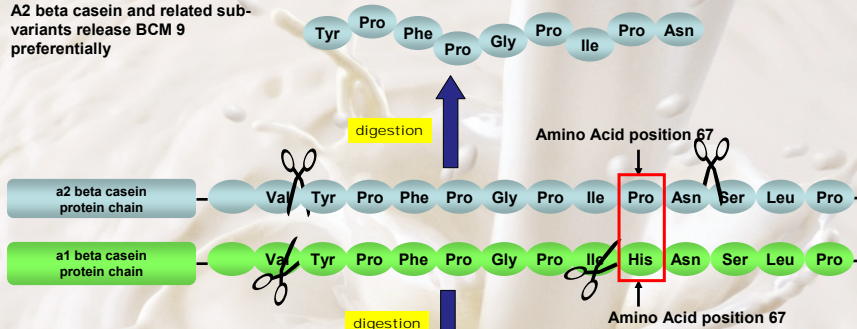
## Differential peptide yield from beta-casein variants

- **Proline (not histidine) at residue 67 strongly favours the differential cleavage of A2 beta casein protein by proteolytic/digestive enzymes<sup>1</sup>.**
- **Both in vitro<sup>1</sup> and in vivo<sup>2,3</sup> studies have shown that a bioactive peptide, beta casomorphin-7 (BCM-7) is preferentially yielded upon digestion of  $\beta$ -casein A1 and related variants, but not A2.**
- **Production of BCM-7 from A1 may:**
  - **Preclude the production of other useful bioactives<sup>1</sup>**

1. Jinsmaa, Y & Yoshikawa, M. 1999. Enzymatic release of neocasomorphin and beta-casomorphin from bovine beta-casein. *Peptides* V20(8), pp 957-962.  
 2. Svedberg, J., et al. 1985. Demonstration of  $\beta$ -casomorphin immunoreactive materials in *In vitro* digests of bovine milk and in small intestine contents after bovine milk ingestion in adult humans. *Peptides* V6, pp 825-830.  
 3. Chabance, B et al. 1998. Casein peptide release and passage to the blood in humans during digestion of milk or yoghurt. *Biochimie* 80(2), pp 155-165.

## Difference between beta casein variants

A2 beta casein and related sub-variants release BCM 9 preferentially



A1 beta casein and related sub-variants release BCM 7 preferentially

Release of BCM-7 may preclude yield of other important bioactives from beta casein

Adapted from: Gobetti M, Stepaniak L, De Angelis M, Corsetti A, Di Cagno R. *Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing*. *Crit Rev Food Sci Nutr*. 2002;42(3):223-39. & Jinsmaa, Y & Yoshikawa, M. 1999. *Enzymatic release of neocasomorphin and beta-casomorphin from bovine beta-casein*. *Peptides* V20(8), pp 957-962.

## 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:*

- Shah, N. 2000. *Effects of Milk-derived bioactives: an overview. British Journal of Nutrition. 84. Suppl. 1. S3-310.*
- Meisel H. (2004) *Multifunctional peptides encrypted in milk proteins. Biofactors 21(1-4):55-61*
- German JB, Dillard C.J, Ward RE. (2002) *Bioactive components in milk. Curr Opin Clin Nutr Metab Care. 5(6):653-8*

## 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.”<sup>1</sup>*
- **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.”<sup>2</sup>*

1. National Dairy Council, Dairy Council Digest 75 (3), June 2004

2. Woodford KB. (2006) A critique of Truswell's A2 milk review. *Eur J Clin Nutr. 60(3):437-9*

## 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.”*

Bell SJ, Grochoski GT, Clarke AJ. (2006) Crit Rev Food Sci Nutr 46(1):93-100

### 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’.*

Truswell AS. (2005) Eur J Clin Nutr. 59(5):623-31

### A critique of Truswell's A2 milk review.

Woodford KB. (2006) Eur J Clin Nutr. 60(3):437-9

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

Allison AJ, Clarke AJ. (2006) Eur J Clin Nutr. 60(7):921-4; 924-5

## **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."*

Laugesen M & Elliott R, 2003. New Zealand Medical Journal 116(1168) U295.

### **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"*

McLachlan, C. N. S. 2001. Medical Hypotheses. 56(2), 262-272.

## **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".*

Taillford KA, et al. 2003 Atherosclerosis. 170(1):13-9.

### **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."*

Chin-Dusting J, et al (2006) Br J Nutr. 95(1):136- 44 &  
Venn BJ et al. (2006) Atherosclerosis. 188(1):175-8

## Human studies: beta-casein variants and heart health

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

*“Significant increase in antibodies against oxidized LDL particles (IgoxLDL) 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 IgoxLDL”*

Steinerová A, et al 2004 Ltr to the Editor. Atherosclerosis 173(1) pp 147-148

### **Antibodies against oxidised LDL in infants**

Steinerova A, et al/2001.. Clin. Chem. 47(6) pp 1137-8

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

Torreilles J & Guerin MC. 1995. C R Seances Soc Biol Fil 189(5):933-42

## 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.”*

Birgisdottir BE, et al. (2006) Ann Nutr Metab. 50(3):177-83.

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

Birgisdottir BE, et al.I. 2002. Diabetes Nutr. Metab. 15 (4) 240-245

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

Laugesen M & Elliott R, 2003. New Zealand Medical Journal 116(1168) U295.

## 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 I diabetes, but could enhance the outcome of diabetes in some cases. Other diet components such as wheat could be more important promoters of Type I diabetes.”*

Beales PE, et al (2002). Diabetologia 45 (9) 1240-1246.

### **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.”*

Padberg S, et al. (1999) Dtsch Med Wochenschr. 124 (50) 1518-21.

## 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 exorphins formed in the intestine from incomplete digestion of gluten and casein.”*

Cade R et al. (2000) Nutritional Neuroscience 3: 57-72.

### **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.”*

Reichelt KL, Knivsberg AM. (2003) Nutr Neurosci 6(1):19-28

### **Reports on dietary intervention in autistic disorders**

Knivsber AM, Reichelt KL, Nodland M. (2001). Nutr Neurosci. 4(1):25-37

## 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."*

Jinsmaa, Y & Yoshikawa, M. (1999). Peptides V20(8), pp 957-962.

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

Pfeuffer M, Schrezenmeir J. (2000) Br J Nutr 84 Suppl 1:S155-9.

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

Chabance B, *et al.* (1998) Biochimie 80(2), pp 155-165.

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

Pasi A, *et al* (1993) Res Commun Chem Pathol Pharmacol. 80(3):305-22.

## Studies on bioactivity of digestion products of beta-casein variants

### Rotational behaviour produced by intranigral injections of bovine and human beta-casomorphins in rats. Herrera-Marschitz M, *et al* 1989.

Psychopharmacology (Berl).99(3):357-61

### beta-Casomorphins induce apnea and irregular breathing in adult rats and newborn rabbits. Hedner J, Hedner T. 1987 Life Sci 41(20):2303-12.

### Delayed behavioral effects of beta-casomorphin-7 depend on age and gender of albino rat pups Dubynin VA, *et al* 2000.. Bull. Exp. Biol. Med. 130(11), pp1031-1034.

### Effect of opioid active therapeutics on the ascending reflex pathway in the rat ileum. Allescher HD, *et al* 2000. Neuropeptides. 34(3-4):181-6.

### Effects of beta-casomorphin derivatives on gastrointestinal transit in mice. Becker A, *et al* 1990. Biomed Biochim Acta. 49(11):1203-7.

## Studies on bioactivity of digestion products of beta-casein variants

**Intragastric beta-casomorphin (1-7) attenuates the suppression of fat intake by enterostatin.** White CL, *et al.* 2000. *Peptides* 2000 21(9):1377-81

**Effects of peptides derived from dietary proteins on mucus secretion in rat jejunum.** Claustre J, *et al.* 2002 *Am J Physiol Gastrointest Liver Physiol* Sep;283(3):G521-8.

**Beta-casomorphin (BCM) and human colonic lamina propria lymphocyte proliferation** Elitsur Y, Luk GD. (1991) *Clin Exp Immunol.* 85(3):493-7.

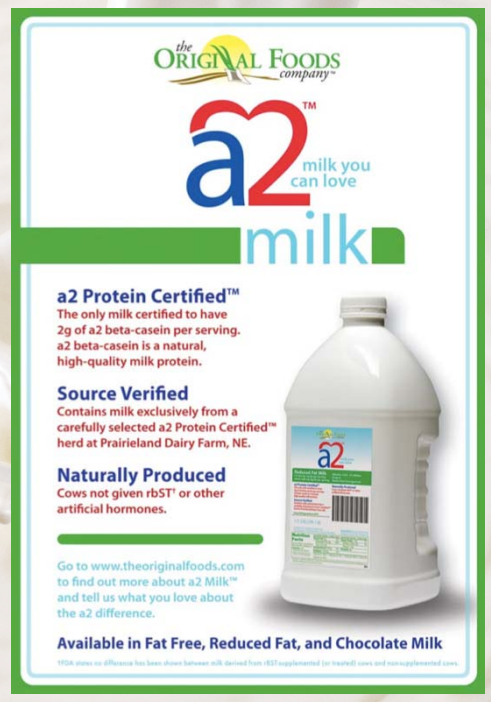
**Pseudoallergic skin reactions to opiate sequences of bovine casein in healthy children.** Kurek M, *et al* 1995. *Rocz Akad Med Bialymst.* 40(3):480-5.

**A naturally occurring opioid peptide from cow's milk, beta-casomorphine-7, is a direct histamine releaser in man.** Kurek M, *et al.* 1992. *Int Arch Allergy Immunol.* 97(2):115-20.

## 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.



**a2 Protein Certified™**  
The only milk certified to have 2g of a2 beta-casein per serving. a2 beta-casein is a natural, high-quality milk protein.

**Source Verified**  
Contains milk exclusively from a carefully selected a2 Protein Certified™ herd at PrairieLand Dairy Farm, NE.

**Naturally Produced**  
Cows not given rbST<sup>1</sup> or other artificial hormones.

Go to [www.theoriginalfoods.com](http://www.theoriginalfoods.com) to find out more about a2 Milk™ and tell us what you love about the a2 difference.

**Available in Fat Free, Reduced Fat, and Chocolate Milk**

100% cows or influence has been shown between milk derived from (a2) supplemented (or treated) cows and non-supplemented cows.

**For further information . . .**

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