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IDENTIFICATION AND HEALTH-PROMOTING PROPERTIES OF MILK CONTAINING A2 B-CASEIN – A REVIEW

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Abstract

Recent scientific findings on nutrition as well as new nutritional trends show the need for a deeper understanding of animal products. It has been increasingly recognized that consumption of dairy products, which contribute to increased release of opioid peptides, may have adverse health effects. This concerns mainly food allergy sufferers, people with digestive problems and autistic children. The milk's β-casein, the most frequent genetic variants of which are A1 and A2 casein, has recently aroused considerable attention for health reasons. Research shows that β-casomorphin-7 (BCM-7), which is formed in the process of digestion

of A1 casein, may contribute to some major human diseases such as atherosclerosis, sudden infant death syndrome, and cardiovascular diseases. Furthermore, numerous studies have demonstrated associations between BCM-7 and neurological problems such as autism and schizophrenia. Milk containing A2 β-casein does not have such properties. The presence of a specific β-casein variant in cow's milk are genetically determined. The role of A1 β-casein as an undesirable variant has resulted in an attempt to select dairy cows based on their polymorphism. The aim of this article is to present current research findings concerning the identification of cows for β-casein variant as well as its effects on human health.

Key words: A2 milk, β-casein variant, β-casomorphin-7, cows identification.

Dairy products, especially those made from cow's milk, form a major component of the human diet around the world, and their consumption is rising. Milk has beneficial nutritional properties; it is a source of proteins, lipids, vitamins, minerals and biologically active substances, which have multifaceted effects on the human organism (Kuczyńska et al., 2011). These bioactive milk components include immunoglobulins, hormones, cytokinins, polyamides, enzymes, nucleotides, mono- and polyunsaturated fatty acids, fat-soluble vitamins, carotenoids and phospholipids (Séverin and Wenshui, 2005), which stimulate immune function or act at the physiological level. Food products that contain "bioactive" compounds are known as functional foods. Unfortunately, many studies suggest that the growing consumption of milk products may increase the risk or aggravate the symptoms of some diseases, including gastrointestinal disorders (Barnett et al., 2014; Haq et al., 2014), immune disorders and inflammations (Holmer-Jensen et al., 2011; Haq et al., 2014). A special role in this regard is played by cow's milk proteins, which are a common source of bioactive

peptides released during digestion of casein and whey proteins. Biopeptides are defined as specific protein fragments which have positive effects on body function or condition and may ultimately influence health (Kitts and Weiler, 2003). In particular, caseins are a reserve of bioactive peptides, which are regulatory compounds acting like hormones, and may affect nutritive value of the milk (Lorenzini et al., 2007).

Cow's milk proteins

Cow's milk protein content ranges from 2.5 to 4.2%. The highest proportion is formed by caseins, which account for around 80% of the milk proteins. The caseins are of four types of polypeptides: αS1 (CSN1S1), αS2 (CSN1S2), β (CSN2) and κ (kappa-CSN3). They are encoded by four genes (CSN1S1, CSN1S2, CSN2 and CSN3, respectively) located on chromosome 6 (Rijnkels, 2002).

The whey proteins, which account for around 20% of all proteins, are β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and bovine serum albumin (BSA) (El-Agamy, 2007; Barłowska et al., 2011). Research has shown that the content of β-lactoglobulin, α-lactalbumin and lactoferrin in milk varies according to breed. The highest level was found in the milk of Polish Red cows compared to Jersey, Simmental, Polish Holstein-Friesian and White-backed breeds (Król et al., 2013). Whey proteins are biologically active compounds with antioxidant, immune-activating, antibacterial, antiviral and anti-cancerous properties. The bioactive effects of α-lactoglobulin and β-lactoglobulin whey proteins may be used in a wide range of functional food products. However, as a result of enzymatic hydrolysis (gastrointestinal digestive enzymes of plant or microbial origin) these proteins may form peptides, which may cause sensitivities or allergies (Szwajkowska et al., 2011). These active peptides (opioids) are chemical substances acting like morphine in the body. They bind to μ opioid receptors found in the central nervous system and in the digestive tract (Teschemacher, 2003) and thus may

alleviate pain among others. They are inactive in the parent protein sequence, but can be released during gastrointestinal digestion or processing of food (Kamiński et al., 2007).

Ruminant milk has been thoroughly studied for milk protein genes in cattle and goats, resulting in the identification and characterization of noticeable genetic variation. This genetic variation results in different milk protein variants, which influence milk composition and cheese-making properties (Martin et al., 2002). These effects are associated with functional modifications of the protein, mainly allele exchanges or deletions, which affect the biological properties of the coded protein. The identified different milk protein variants were used for breed characterization (Ceriotti et al., 2004), biodiversity investigations (Mahé et al., 1999) and evolution studies on both animal resources and milk protein genes (Ibeagha-Awemu et al., 2007).

β-casein

For health reasons, considerable interest in recent years has focused on β-casein, which has 209 amino acid residues in its protein chain (Farrel et al., 2003). There are 12 genetic variants of β-casein: A1, A2, A3, B, C, D, E, F, H1, H2, I, G, but only seven of these (A1, A2, A3, B, C, I and E) were detected in European cattle breeds (Massella et al., 2017). The most frequent genetic variants of β-casein (CSN2) are A1 and A2, variant B is less common, variants A3 and C are very rare (Farrell et al., 2004; Kamiński et al., 2007). Variant I comes from mutation of the A2 variant and has low frequency. However, in some breeds, such as Italian Holstein Friesian, Italian Red Pied and Dutch Holstein Friesian, frequency of this variant may reach 0.120-0.190 (Jann et al., 2002). Variant E was only identified in the Italian Piemontese breed (Voglino, 1972).

Because variant A2, found in cow's milk, structurally resembles β-casein in human breast milk, the milk may "imitate" breast milk and help the child to maintain optimal growth and

development (Sadler, 2013). It is also proposed that consumption of milk containing A2 β-casein may alleviate the symptoms of autism and schizophrenia (Ganguly et al., 2013).

Table 1. The change in the amino acid sequence of β-casein variants (Sebastiani et al., 2020)

β-casein variant	Change in amino acid sequence								
	36	37	67	72	88	93	106	122	138
A2	Glu	Glu	Pro	Gln	Leu	Met	His	Ser	Pro
A1	Glu	Glu	His	Gln	Leu	Met	His	Ser	Pro
A3	Glu	Glu	Pro	Gln	Leu	Met	Gln	Ser	Pro
B	Glu	Glu	His	Gln	Leu	Met	His	Arg	Pro
C	Glu	Lys	His	Gln	Leu	Met	His	Ser	Pro
D	Glu	Glu	Pro	Gln	Leu	Met	His	Ser	Pro
E	Lys	Glu	Pro	Gln	Leu	Met	His	Ser	Pro
F	Glu	Glu	His	Gln	Leu	Met	His	Ser	Leu
G	Glu	Glu	His	Gln	Leu	Met	His	Leu	Pro
H1	Glu	Glu	Pro	Gln	Ile	Met	His	Ser	Pro
H2	Glu	Glu	Pro	Glu	Leu	Leu	His	Ser	Glu
I	Glu	Glu	Pro	Gln	Leu	Leu	His	Ser	Pro

Arg: arginine; Gln: glutamine; Glu: glutamic acid; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Pro: proline; Ser: serine.

Depending on the genetic structure of cows, their milk may contain one or both types of β-casein. A1 and A2 caseins differ in protein structure, and the mutation that causes differences in the β-casein protein results from a single nucleotide polymorphism at codon 67 in exon 7 of the CCT gene (A2, proline), on CAT (A1, histidine); thus, A2 casein contains a proline residue, whereas A1 casein a histidine residue, which allows the previous seven amino acid residues to be removed and beta-casomorphine-7 (BCM-7) to be formed (Jinsmaa and Yoshikawa, 1999). (fig. 1).

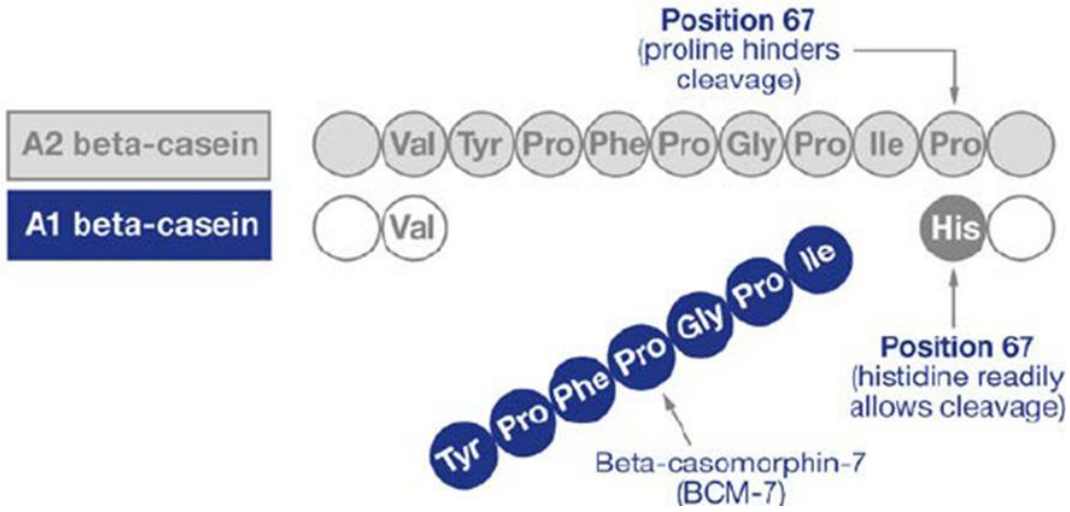

Fig. 1. Release of beta-casomorphine-7 from β-casein A1 variant in the human digestive system (Woodford, 2009)

Research has shown that BCM-7, which is formed from digestion of A1 casein and is a major bioactive peptide with strong opioid activity, may contribute to the development of some major diseases in humans. Importantly, this undesirable effect was not observed for A2 casein. This may be due to the fact that in the milk with A1 casein, BCM-7 level is 4-fold higher than in A2 milk (Kamiński et al., 2007). BCM-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) as a peptide with morphine-like activity was first identified in 1979 (Brantl et al., 1979).

Effect of β-casomorphine 7 (BCM-7) on human health

Research has proved that BCM-7 may play a considerable role in the etiology of many human diseases. It was demonstrated that BCM-7 may increase the risk of ischemic heart disease, coronary artery atherosclerosis, type 1 diabetes and sudden infant death syndrome (Thorsdottir et al., 2000; McLachlan, 2001; Laugesen and Elliott, 2003; Sun et al., 2003; Tailford et al., 2003, Kamiński et al., 2007). BCM-7 may act as an immunosuppressant and

impair tolerance to dietary antigens in the gut immune system, which, in turn, may contribute to the onset of type 1 diabetes (Clemens, 2011). Populations that consume milk containing high levels of β-casein A2 have a lower incidence of cardiovascular disease and type 1 diabetes. *In vitro* studies confirmed that high levels of BCM-7 are associated with increased levels of lymphocytes and other inflammatory markers (Pal et al., 2015). In turn, Jianqin et al. (2016) demonstrated that consumption of milk containing A1 casein manifests in symptoms similar to those of lactose intolerance, leads to gastrointestinal inflammation and longer transit time, and slows cognitive processing speed and accuracy. Furthermore, because elimination of A1 β-casein attenuated these effects, some symptoms of lactose intolerance may stem from inflammation it triggers, and can be avoided by consuming milk containing only the A2 type of casein. It is now thought that BCM-7 may also increase the risk of diseases such as atherosclerosis, sudden infant death syndrome, and cardiovascular diseases (Ganguly et al., 2013). A number of studies have also pointed to the associations between A1 β-casein/BCM-7 and neurological problems such as autism (Reichelt and Knivsberg, 2003; Kawashti et al., 2006) and schizophrenia (Niebuhr et al., 2011; Severance et al., 2011). It has also been reported that elevated BCM-7 immunoreactivity is associated with delayed psychomotor development in infants (Kost et al., 2009). Some results imply that A1 β-casein and its peptide derivatives also affect information processing in the brain. It has also been demonstrated that food-derived opioid peptides have a variety of direct effects on neural cells, including the expression of genes involved in redox and methylation processes, and epigenetic regulation. It was suggested that milk-derived peptides may induce inflammation and systemic oxidation, including in the central nervous system (Trivedi et al., 2014), and these effects might impact on development or information processing.

It is worth adding that over the last 40 years, there has been a significant increase around the world in the number of children diagnosed with autism-related developmental abnormalities.

Although intensive research is ongoing to find the reasons for this phenomenon, etiology of the disease has not been adequately studied (Skonieczna-Żydecka et al., 2017). Among the environmental factors that aggravate autism symptoms, increasing mention is made of inadequate diet, including the use of milk products (Lange et al., 2015). A recent study by Jarmołowska et al. (2019) conducted with a group of healthy and autistic children consuming commercially available pasteurized milk showed that autistic children had significantly higher BCM-7 level in the blood and urine. Other studies confirmed that avoidance of opioid peptides found in milk products by autistic children improves their emotional condition, increases their capacity to build social relationships, and reduces the symptoms of autoimmune diseases (Pennesi and Klein, 2012; Pedersen et al., 2014).

Because the clinical implications of A1 β-casein milk on human health are still under discussion, particular attention should be drawn to protein polymorphism, and more in-depth studies are needed to verify the extent and nature of its interaction with the human digestive tract and human body (Kamiński et al., 2007). A report published by the European Food Safety Authority, due to contradictory results for the potential effect of β-casomorphine and related peptides on health as well as the inability to establish a cause-effect relationship between oral ingestion of β-casomorphine or related peptides and the etiology of any suggested diseases, does not recommend a formal risk of these peptides (EFSA, 2009). However, according to Caroli et al. (2009) this does not preclude that the milk from cows of certain genotypes could be recommended as more appropriate for humans with some health problems.

Identification and occurrence of β-casein gene variants in different breeds of cattle

The presence of type 1 β-casein in cow's milk is mainly determined by genetic factors. The role of A1 β-casein as an undesirable variant has led researchers to select dairy cows based on

their β-casein polymorphism. Breeding for A2 milk involves no risk because there is no negative correlation between the presence of A2A2 gene and other traits. Research has shown that genotyping bulls for β-casein and giving preference to the A2 allele may bring two benefits: increase breeding value in terms of protein content, and reduce the incidence of the A1 protein variant, which is considered a risk factor for human health (Oleński et al., 2010). This means that milk producers may safely include this criterion in their breeding without any risk. The significance of research into human health and determination of β-casein polymorphism in dairy cattle has led to the development of many research methods based on microarray or DNA amplification (PCR-restriction fragment length polymorphism; bidirectional allele-specific PCR; multiplex PCR) and sequencing, focusing on nucleotide chain mutations (Massella et al., 2017).

Many studies are conducted worldwide to identify cows and bulls for A2 β-casein (Table 2). Sebastiani et al. (2020), who investigated Italian Holstein-Friesian cows from 17 farms located in central Italy, found that the most frequent alleles were A2 (60.65%), followed by A1 (30.39%), B (5.68%), I (3.10%), A3 (0.15%), and allele C (0.03%). Massella et al. (2017) obtained similar results for 1226 Holstein Friesian cows and 4 Braunvieh cows raised in Italy – the desirable A2 allele was more frequent (0.546) that the A1 allele (0.371). However, heterozygous genotype A1A2 was more frequent (0.403) than homozygous genotype A2A2 (0.301).

Miluchová et al. (2014), who studied β-casein polymorphism in three cattle breeds, found that homozygous genotype A1A1, heterozygous genotype A1A2 and homozygous genotype A2A2 had a frequency of 0.1261, 0.3333 and 0.5405 in the Simmental breed; 0.1379, 0.4598 and 0.4023 in the Holstein breed; and 0.3034, 0.5168 and 0.1798 respectively in the Pinzgau breed. A2 allele was more frequent in the Simmental and Holstein population (0.7072 and 0.6322), and A1 allele in the Pinzgau population (0.5618).

Many studies also involved local breeds of cattle, in which the frequency of A2A2 genotype is much higher than in high-producing breeds. Frequency of the A1 allele was found to range from 0.01-0.06 in Guernsey, 0.12 in Jersey, 0.31-0.66 in Holstein-Friesian to 0.720 in Ayrshire (Kamiński et al., 2007).

A study performed with Dangi (Indian Zebu) and Holstein-Friesian (HF) crossbreds showed that in 75% HF crosses, allele frequency was 0.13 (A1) and 0.86 (A2), and genotype frequency was 0.06 (A1A1), 0.13 (A1A2) and 0.81 (A2A2). For 62.5% HF crosses, these values were 0.03 (A1) and 0.97 (A2); and 0.00 (A1A1), 0.06 (A1A2), 0.94 (A2A2). In Zebu breed of cattle, only the homozygous A2A2 genotype was observed. Therefore, the gene frequency of A1 allele was zero, which shows the presence of the allele (A2) in naturally evolved native breed of cattle (Jawane et al., 2018). Similar studies with cows of the local Gir breed and Guzerá cows showed allele A2 had a frequency of 0.98 and 0.97, and genotype A2A2 a frequency of 0.96 and 0.93, respectively (Rangel et al., 2017).

In Poland, this type of research was conducted with Holstein-Friesians (Kamiński et al., 2006) and the Polish Red conservation breed (Cieślińska et al., 2019). In a study with Polish HF bulls, Kamiński et al. (2006) identified three genotypes (A1/A1, A2/A2, A1/A2) and the frequency of A1 and A2 was 0.402 and 0.598, respectively. Oleński et al. (2012), who studied 650 Polish HF bulls born between 1997 and 2003, observed the following frequencies: allele A1 – 0.33, allele A2 – 0.67, genotype A1A1 – 0.122, genotype A2A2 – 0.452. Cieślińska et al. (2019), who investigated 24 bulls and 177 cows of the Polish Red breed, found the frequency of A2 β-casein in the Polish Red population to be 0.47. A2 β-casein had a frequency of 0.58 and 0.37 in bulls and cows, respectively. Due to the health-promoting properties of milk from conservation breeds, it is justified to investigate breeding bulls of these breeds for the frequency of these two variants, and the high frequency of A2 β-casein

allele (0.58) among the bulls of this breed may increase its frequency in the entire population (Cieślińska et al., 2019).

Table 2. Occurrence of β-casein A1 and A2 gene variants in various cattle breeds (adapted from Kamiński et al., 2007; Şahin et al., 2018; Cieślińska et al., 2019)

Breed	**Country**	***N***	**Allele frequency**		**References**
			A1	**A2**	
HF	Denmark	415	0.266	0.614	Gustavsson et al., 2014
	The Netherlands	1929	0.28	0.50	Visker et al., 2010
	The Netherlands	1629	0.029	0.69	Heck et al., 2009
	Poland	177	0.32	0.68	Cieślińska et al., 2012
	Poland	650	0.35	0.65	Oleński et al., 2012
	Italy	1226	0.371	0.546	Massella et al., 2017
	Poland	143	0.40	0.60	Kamiński et al., 2006
	China	133	0.432	0.459	Dai et al., 2016
	Iran	119	0.50	0.50	Gholami et al., 2016
Simmental	Slovakia	111	0.292	0.707	Miluchová et al. (2014)
	Croatia	621	0.190	0.630	Curik et al., 1997
	Germany	229	0.343	0.566	Ehrmann et al., 1997
Jersey	Germany	43	0.093	0.721	Ehrmann et al., 1997
	Denmark	157	0.070	0.580-0.650	Bech and Kristiansen, 1990
	New Zealand	1328	0.123	0.591	Winkelman and Wickham, 1997
Red	Sweden	392	0.48	0.51	Gustavsson et al., 2014
	Denmark	169	0.71	0.23	Bech and Kristiansen, 1990
	Poland	201	0.53	0.47	Cieślińska et al., 2019
Swedish Brown	Germany	232	0.108	0.705	Ehmann et al., 1997
	USA	259	0.140-0.180	0.660-0.720	Eenennaam and Medrano, 1991
Guernsey	USA	3861	0.010-0.060	0.880-0.970	Eenennaam and Medrano, 1991
Black-and-White	Denmark	223	0.550	0.390	Bech and Kristiansen, 1990
Red-and-White	Sweden	394	0.460	0.531	Lunden et al., 1997
Ayrshire	New Zealand	37	0.432	0.527	Winkelman and Wickham, 1997
	Finland	686	0.509	0.490	Ikonen et al., 1997
	USA	45	0.720	0.280	Swaissgood, 1992
Pinzgau	Slovakia	89	0.562	0.438	Miluchová et al. (2014)
Turkish Grey	Turkey	34	0.426	0.544	Dinc et al. (2013)
Eastern Anatolian Red		34	0.118	0.824	
Anatolian Black		34	0.132	0.765	
Southern Anatolian Red		30	0.485	0.456	

A2 milk

Because intensive dairy cattle breeding may have emphasized the genetic variant of milk which had adverse effects in humans, further studies in animals and clinical tests in humans are needed to compare the risk of disease in the case of A1-free milk (A2 milk) and regular milk. A2 milk is primarily intended for autistic persons and allergy sufferers. There is a potential target group that is a sales niche for dairies, which in the future would like to target part or all of their production to this group of consumers. For reasons of health, consumers increasingly choose substitutes for cow's milk, which may result in its lower consumption; therefore, A2 milk could be attractive for a small group of consumers but with a limited market. In this respect, it is essential to provide adequate marketing backed by reliable research. Today, A1 and A2 milk is already marketed in some countries, and consumers may choose without giving up drinking milking or eating milk products only because they have milk protein intolerance or are allergic to milk proteins. A leading producer of A2 milk is New Zealand, where screening tests and breeding programmes have been implemented to promote the desirable A2 variant among dairy breeds. This has led breeders to choose herds of milk cows producing only A2 milk, resulting in the marketing of A2 milk in 2003 (Kamiński et al., 2007). Cow's A2 milk is already marketed in many countries, including Australia, Great Britain, United States, New Zealand and The Netherlands, and it is recommended for milk intolerant people. An infant formula containing A2 β-casein milk is available in China and Australia, where it is marketed as easier on the infant's digestive system (Brooke-Taylor et al., 2017).

To sum up, it would be beneficial to market A2 milk also in Poland; this would expand the range of milk products while increasing the choice for consumers, especially conscious buyers. Thus, it is essential to promote knowledge about this subject. Production of A2 milk could provide an opportunity to increase the income of small family farms, farms with local or conservation breeds, and organic farms. A new dedicated product on the market would allow obtaining a higher price, which is economically important for milk producers and processors.

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