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Milk derived casomorphins and their health significance: A review

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Abstract
Milk is considered as an important source of nutrients for human population; specially good quality proteins, carbohydrates and some micronutrients. Casein is the major milk protein present in milk and remaining proteins are constituted by whey proteins and others. Beta casein is an important component of casein protein which has role in nutritional contribution of infant as well as adult; as milk is an important part of daily food habit consumed as fluid milk & different milk products in various parts of the country. However, it has been found that mutations in bovine beta casein gene resulted into several genetic variants. Out of the genetic variants reported till date, A1 and A2 are the most prevalent one. The basis of A1 and A2 milk is the difference in beta casein at 67th amino acid position where histidine is present in A1 and proline in A2 milk; this has been observed as a result of single nucleotide difference in beta casein gene. In gastrointestinal tract the proteolytic digestion of A1 variant of β-casein leads to formation of a bioactive peptide, beta-casomorphin (BCM-7). Different age groups consuming milk can be affected by BCM-7 leading several health implications. Initial studies have revealed that frequency of A1 allele is more common in cattle of exotic origin while native cattle and buffalo populations have only A2 allele; considered as a source for healthy and safe milk.

Keywords: Casein, casomorphins, A1 and A2 milk, health, BCM-7

Introduction
The only source of nutrition for babies in the first 6 months is milk, containing all the important micronutrients required for growth and development. Milk is in fact about 85 percent water, with the remaining 15 percent made up of fat, lactose, protein, minerals etc. Out of all constituents protein plays a vital role in growth and development of infants and an important source of animal protein for all those who are strict vegetarian. Casein is the most abundant in milk, accounting for about 80% of total milk protein. There are two primary genetic polymorphisms of casein: A1 and A2 (Kaminski *et al*., 2007) [20]. Depending upon the position of Proline or Histidine at 67th position out of 209 amino acid, the A1or A2 milk is classified. The mutation of codon 67 of beta casein gene results in variation of single nucleotide and hence the A1 and A2 milk casein (A2 Proline, A1 Histidine) (Kaminski *et al*., 2007) [20]. The commercial milk of different countries has a mix of A1 and A2 category (Mishra *et al*., 2009) [31]. At 67th position the histidine is not present in purebred Asian, hence classified as A2 type, however the same is present in many of the European breeds where mutation is there and are categorized as A1 type. A pair of genes on the sixth chromosome determine a cow's A1/A2 status (Rijnkels, 2002) [41]. The A2 allele gene is 100% in Indian milch cattle breeds (Red Sindhi, Sahiwal, Tharparkar, Gir, and Rathi) and buffaloes; it is around 94 percent in other Indian breeds, and over 60% in foreign breeds (Holstein Frisian and Jersey) (Joshi, 2011) [19].

Beta casein is the second major type of all the caseins and has an excellent nutritional properties with commendable balance of amino acids. About 30% of total casein in cow milk is considered as beta casein (Phelan *et al*., 2009) [37], and major beta casein genetic variants are A1 and A2 (Formaggioni *et al*., 1999) [11]. Because it existed before A1 beta-casein was developed in some European herds some 5000–10,000 years ago as a result of change in DNA sequence from proline 67 to Histidine 67, The A2 beta-casein form is believed to be the first beta-casein variety (Ng-Kwai-Hang and Grosclaude, 2002) [33].

The A1 milk has been implicated for several health issues due to certain components or peptides formed during digestion process involving several enzymes in the gastrointestinal tract (GI tract). ß-casomorphin-7 (BCM-7) is a bioactive peptide of seven-amino-acid, that

be released by digesting A1 ß-casein with pepsin, leucine aminopeptidase, and elastase in the small intestine, but the proline at position 67th position in A2 prevents a cleavage at this point (De Noni *et al*., 2009 and Pal *et al*., 2015) [8, 35]. The gastrointestinal functions, including mucus secretion, motility and hormone secretion, are regulated by opioid receptors. Met-enkephalin, leu-enkephalin, and dynorphin are three receptors found in the stomach. They can be found in neurons as well as cells of endocrine system. Beta casomorhin-7 derived from casein and its components, prevent mucus secretion in the gastrointestinal tract via opioid pathways, which may affect GI tract bacteria, medicine absorption, and other GI processes (Barnett *et al*., 2014) [1] These changes may cause the consumer to experience anomalous functioning and illnesses.

BetaCasomorphin-7

When milk or milk products are ingested, enzymes from digestive system in the gut break down A1 type beta-casein, releasing the betacasomorhin-7 that human body does not metabolise (Boutrou *et al*., 2013, UlHaq *et al*., 2015) [3, 50]. Under typical gut conditions, A2 beta-casein, on the other hand, releases far fewer and perhaps negligible levels of BCM-7 (De Noni *et al*., 2009) [8]. However, A2 beta-casein releases much less betacasomorhin-7 under normal gut conditions (Cielinkska *et al*., 2012) [5]. Although 13 genetic variants of β-casein have been reported: A1, A2, A3, A4, B, C, D, E, F, H1, H2, I and G where milk from cattle generally contains only two major types i.e. A1 and A2.

Many DNA-based techniques are used to screen these alleles, including single stranded conformational polymorphism (SSCP), (Baroso *et al*., 1999) [2] Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) (Miluchova *et al*., 2009) [30], allele-specific PCR (AS-PCR), (Keating *et al*., 2008) [21], real time PCR TaqMan, (Manga and Dvorak,2010) [25] and PCR-amplification created restriction site (PCR-ACRS) (Raies *et al*., 2012) [39]. There are some proofs, however, that the B variant from A1 family causes significant release of Betacasomorphin-7 (De Noni, 2008) [7]. A1 ß-casein has only been discovered in European breeds of cattle. Crossbred ancestry could be attributed to some cattle which are otherwise phenotypically resembling as Asian or African cattle but may produce A1 beta-casein, however cattle from pure breeds produce milk containing only A2 ß-casein. The prevalence of A1 and A2 ß-casein in cattle depend upon breed, from North region of Europe types having higher A1 beta-casein levels than South region of Europe varieties. The A2 allele frequency is thought to be especially high in the Guernsey and Fleckvieh breeds. The ratio of A1:A2 in many Western countries' herds is roughly 1:1 (De Noni *et al*., 2009) [8]. Process of determining individual characteristics, which is commercially available in specific countries, can be used to test herds for beta-casein alleles. Use of semen from A2A2 bulls is the simplest approach to convert the existing herd, it is possible to speed up conversion of A2 beta-casein, but it is more likely to take 5–8 years or longer (Mencarini *et al*.,2013) [26].

Betacasomorphin-7 is documented to be secreted from a variety of sources, including milk and other dairy items like yoghurt and cheese (De Noni *et al*., 2010) [9]. The cheese- and yoghurt-making processes may lead to BCM-7 release, but that specific bacteria found in yoghurt may hydrolyze BCM-7 (Janer *et al*., 2005, Nguyen *et al*., 2014) [15, 34]. For the quantification of BCM7, RPHPLC-UV (Muehlenkamp and Warthesen, 1996) [32] and ion-exchange chromatography (Jarmolowska *et al*., 1999) [16] are used, while HPLC-UV has been utilized to assess BCM7 in human milk (Jarmolowska *et al*., 2007) [7]. In human milk beta-casein is of the A2 type, with a proline in the same place on the beta-casein protein chain (Hamosh *et al*., 1989) [12]. Human BCM-7 differs from bovine BCM-7 in amino acid sequence, with homology in five of seven amino acids (Hamosh *et al*., 1989, Wada and Lonnerdal, 2015) [12, 51] and significantly lower opioid activity (Koch *et al*., 1985, Brantl, 1984) [22, 4]. Wada and Lonnerdal (2015) [51] observed *in vitro*-digested human milk and found human betacasomorphin-9 (with a proline at position eight), but neither human betacasomorphin-7 nor betacasomorphin-5 found (i.e., BCM-5 is a shortened version of BCM-7). BCM-5 and BCM-7 were found in human colostrum (averaging 5 and 3 g/mL, respectively) but at 2 months in lactation period much lower quantities reported by Jarmolowska *et al*. (2007) [17]. Casomorphin functionality in new born babies has been linked to motherly bonding, gastro- intestinal function, mucous membrane development, and sleep induction (Jarmolowska *et al*. 2007) [17].

Opioid characteristics of casomorphins

The peptides bind to μ-receptors found in the CNS, GI tract, and few immune cells. μ-opioid receptor are the molecular target of morphine and other opioid drugs or beta-casomorphins (Jinsmaa and Yoshikawa, 1999) [18]. Teschemacher (2003) [48] found that Betacasomorhin-5, Betacasomorphin-7, and Betacasomorphin-9 are relevant natural casomorphins. BCM-5 is the most prominent of these natural opioids. The human analogue of the enzyme carboxypeptidase Y might hypothetically release Betacasomorhin-5 from Betacasomorphin-7 within the human living system (Henschen *et al*., 1979) [13], and there is data to confirm this (De noni, 2008) [7]. Wasilewska *et al*. (2011a) [53] discovered human infants who were exclusively breastfed and whose moms drank bovine milk have bovine Betacasomorphin-5 in their bloodstream. BCMs were proposed to play a role in the endocrinic regulation of pregnancy (Teschemacher *et al*., 1994) [47]. BCM-5 demonstrated a cardio protective effect due to its favourable inotropic and antiarrhythmic effects (Mentz *et al*., 1990) [27]. In mice, with a high dose of bovine Betacasomorphin-5 intracerebroventricular injections, temporary memory loss was induced and in step-down type passive avoidance tests, a little dose of scopolamine effectively nullifies scopolamine-induced temporary loss of memory (Sakaguchi *et al*., 2003) [43]. Following milk protein consumption, Human jejunal contents include amounts of cattle Betacasomorphin-7 that are consistent with pharmacological actions, after 2 hours of digestion, 4mg Betacasomorphin-7 was released from 30g of casein, with more released later (Boutrou *et al*., 2013) [3]. It has also been found in human newborns' blood (Wasilewska *et al*., 2011b, Kost *et al*., 2009) [54, 23] and children's urine (Sokolov *et al*., 2014) [45]. BCM-9 is also a natural casomorphin of importance. The A2 form of beta-casein produces betacasomorphin-9 (Boutrou *et al*., 2013, Wada and Lonnerdal, 2015) [3, 51], although it is not of significance in reference to A1 beta-casein due to the presence of histidine at position 67. This supports the idea that BCM-9 with a histidine at 67th position is easily split down into BCM-7 at the histidine cleavage point in the GI system, whereas BCM-9 with a proline at 67th position does not breaks. These results are in line with those of Barnett *et al*. (2014) [1]; they

discovered that whereas the -opioid receptor antagonist naloxone prevented a variety of gastrointestinal symptoms in A1 beta-casein, no similar effects were observed in A2 beta-casein after naloxone treatment. BCM-9 has been found to have antihypertensive characteristics, which is interesting (Saito *et al.*, 2000) [42].

Delayed intestinal transit

µ-Opioid receptors can be found all over the human body, especially in the gastrointestinal system (Pleuvry, 1991) [38]. It is well established that µ-opioid receptor activation affects the intestinal activity (Ward and Takemori,1983) [52] and to perform an important physiological role in GI function regulation, including mucus formation, motility and hormone synthesis (Zoghbi *et al.*, 2006) [57]. In humans, µ-opioid receptor agonists induce a naloxone-reversible delay in gastrointestinal transit time. According to trials casein and its peptides decrease GI motility, reducing the intestinal contractions (Mihatsch *et al.*, 2005) [28]. Casein was seen to delay gastrointestinal emptying time when compared to whey protein, suggesting that casein's opioid activity delays transit time (Daniel *et al*., 1990) [6] where naloxone was seen reversing these effects. In rat small intestine transit time was delayed when fed casein or its hydrolyzed products, (Mihatsch *et al.*, 2005) [28], and naloxone treatment prevented the impact of intact casein on delaying emptying time. These findings imply that when intact casein is digested, peptides with opioid action are produced, potentially causing gastrointestinal transit time delays. In comparison to A2 beta-casein, A1 beta-casein delays gastrointestinal emptying time (Barnett *et al*., 2014) [1]. Wistar rats were administered milk-based diets containing A1 or A2 beta-casein, respectively, and it was revealed that the diets having A1 beta-casein lowered GI emptying time compared to the diets containing A2 type beta-casein. (Barnett *et al.*, 2014) [1].

Post drinking effect

The digestive system is slowed by the milk we are consuming, which is A1 milk. In comparison to A2 beta casein, A1 beta casein slows food transit through the digestive system, which is an opioid action.

1. In the colon, A1 beta casein exhibits both a pro-inflammatory and an opioid impact.
2. In comparison to A2 beta casein, A1 beta casein promotes upregulation of the enzymes DPP4 (Dipeptidyl peptidase 4) in the small intestine, which appears to be a non-opioid effect.
3. When it comes to food transit delays or pro-inflammatory effects there is no record of opioid effects caused from A2 beta casein.

Risk of heart disease

A1 type beta casein was found to induce fat buildup in wounded blood arteries in studies. Fat build-up has the potential to obstruct blood arteries and lead to heart disease. However, the result's human significance has been questioned. Ischemic heart disease (IHD) and stroke are the clinical manifestations of multi-factorial pathogenic processes that unfold over decades, and various risk factors have been identified. The findings that A1 beta casein is linked as a risk factor for IHD is based primarily on ecological data similar to that used in the DM1 (Diabetes Mellitus^{-1}) case. The connections are impressive for such a multi-factorial condition, even if they aren't as strong as they are for DM1. In one study, the direct effect of A1 and A2 milk consumption on the development of atherosclerosis was investigated in a rabbit model. The study found no evidence of a significant negative impact on heart disease risk factors. In comparison to beta casein A2, beta casein A1 is found to be atherogenic (Tailford and Berry, 2003) [46].

Risk of type 1 diabetes

Type 1 diabetes is caused by a shortage of insulin in the body and is most commonly diagnosed in youngsters. According to the research, drinking A1 milk as a child increases the likelihood of developing type 1 diabetes (Pelto *et al*., 1999) [36]. One or more environmental triggers are thought to be the etiology of type 1 diabetes. The link between A1 beta casein intake and DM 1 incidence rate in different nations is extremely strong. Although such correlations can't prove cause and effect, they are prone to bias. It is known that A1 beta casein is split in the GI tract to form a compound with morphine-like effects in the body, which is thought to modulate immune surveillance. The relationship between the consumption of beta-casein variants and diabetes incidence could be explained by beta casomorphin-7's opioid characteristics, which include immune-suppression (Yin *et al*., 2010) [56]. DM Type 1 is an insulin-dependent diabetes mellitus in which the body does not manufacture insulin. The death of pancreatic beta cells could be the reason, as the pancreas produce insulin to keep blood sugar levels in check. BMC-7, produced in A1 milk consumption exhibits cross reactivity with the epitope of the pancreatic beta cell (glucose transporter) GLUP-2, resulting in the destruction of pancreatic beta cells by autoantibodies to GLUP-2. In prediabetic people, a gut-related immune response in some conditions can be treated with an opioid antagonist (naloxone) (Eliott *et al*., 1997) [10].

Death of Infants by A1 Milk

Sudden Infant Death Syndrome (SIDS) is the most common cause of mortality in children below the age of one year. It is the prominent reason of death in seemingly healthy children in developed countries. According to an analysis of healthy newborns, variation of BCM 7 was positively associated with higher DPP4 (Dipeptidyl peptidase 4) activity. In other words, when BCM 7 is high, the body naturally increases DPP4 activity in healthy youngsters. However, the increased level of DPP4 is not favorable. This shows that the at-risk newborns lack the ability to promptly respond to high BCM 7 levels by making enough of the sole enzyme that can break it down. In a study babies were fed different diets (Kreil *et al*., 1983) [24]; infants who were fed casein-rich milk formula had significantly higher BCM levels than those who were administered primarily whey-based formula. Because BCM 7 can only arise from casein and not whey, this was to be expected (Tiruppathi and Miyamoto, 1990) [49]. However, babies aged one to four months who appeared to be eating only breakfast had protein fragments, such as but not limited to bovine BCM. Can it make their way into breast milk from the mother's stomach? It's possible that BCM7 is transmitted through the blood (Iwan *et al*., 2008) [14]. However, it is becoming increasingly likely that it could also be through other mechanisms, such as the lymphatic system. Whatever mechanism at work, there is little doubt that bovine BCM7 can enter human breast milk and trigger life-threatening events in infants. As a result of the research, it appeared that not only babies, but also breastfeeding moms, require A1 beta

casein-free cow's milk. These findings suggest that some youngsters are hypersensitive to A1 beta casein, which is contained in cow's milk. However, more research is required for any concrete conclusions and there are very few options for lowering the likelihood of them happening (Shimizu *et al*., 1997) [44].

Leaky gut syndrome

What is the fate BCM7 once it is formed into the gut is a piece of the puzzle in determining how it enters the bloodstream. Because BCM7 is a big molecule, it is not easy for it to get through the gut wall and reach the blood stream. Some patients, however, have leaky gut syndrome, which allows BCM7 and other peptides to cross the intestinal barrier reach into the bloodstream quite quickly. According to Woodford (2007) [55], BCM7 can be detected in the urine of persons with a leaky gut. He claimed that this illness has been linked to autism and schizophrenia symptoms due to BCM7's recognized opioid effects. It has been found that patients who have stomach ulcers or untreated celiac disease absorb BCM7 through the GI wall. Babies are likely to absorb BCM7 in the same way as adults; in fact, newborns are supposed to pass bigger molecules through the GI wall. This phenomenon helps them receive colostrum from their mother's milk (Woodford, 2007) [55].

Effects of BCM7 on babies

If neonates can pass big molecules through the gut wall, they are more vulnerable and susceptible to the effects of BCM7 in A1 milk. Opioids, especially BCM7, are known to slow the transit of food in the GI tract and it explains the reason of constipation in babies fed with cow milk formula. Woodford (2007) [55] proposed that the slower passage of A1 milk through the digestive tract due to the release of BCM7, enhances the lactose intolerance problems. Early and persistent exposure to BCM7 in newborn formulae could thus play a role in the increased rates of Type 1 diabetes, heart disease, autism and a variety of other auto-immune diseases. There hasn't been any research done on the existence of BCM7 in newborn formula, and it's desperately needed. Mothers should breastfeed their newborns for as long as possible and insist on breast milk substitutes prepared with A2 milk rather than A1 milk.

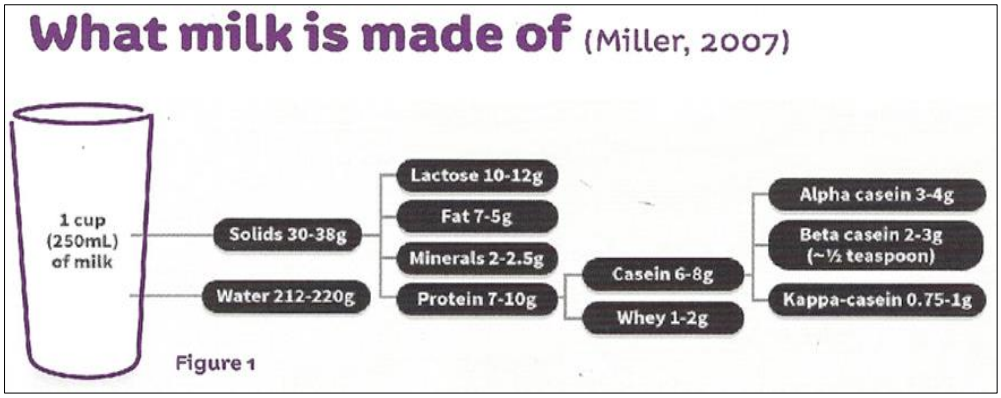

Fig 1: Important constituents of milk (Miller, 2007)

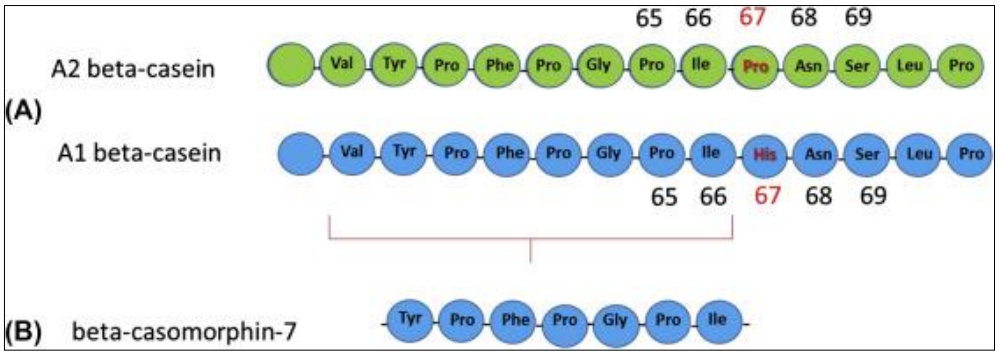

Fig 2: (A) The difference between A1 and A2 beta-casein; (B) release of beta-casomorphin-7 (Rashidinejad *et al*., 2017) [40].

Conclusion

People are unaware of the differences between A1 and A2 milk and as A2 milk is produced by all indigenous milk breeds we are blessed with a safer option. Continued research to explore the role of BCMs in human health and time to time risk assessment with survey study and laboratory trials is required to produce a vivid picture of the milk derived casomorphins and their associated health issues. Improved diagnostic tools to establish the link of casomorphins derived from milk with different ailments is needed. To avoid consumer confusion about the safety of A1 type and A2 type milk, a detailed understanding of the milk-protein derived peptides specially with opioid effect, their release during digestion, absorption through gut wall and further effect is essential.

References

1. Barnett MP, McNabb WC, Roy NC, Woodford, KB, Clarke AJ. Dietary A1β-casein affects gastrointestinal transit time, dipeptidyl peptidase-4 activity, and inflammatory status relative to A2 β-casein in Wistar rats. International Journal of Food Science and Nutrition. 2014;65:720-727.
2. Barroso A, Dunner S, Canon J. Technical Note Use of

PCR single strand conformation polymorphism analysis for detection of bovine ß-casein variant A1,A2,A3 & B. Journal of Animal Science. 1999;77:2629-2632.
3. Boutrou R, Gaudichon C, Dupont D, Jardin J, Airinei G, Marsset-Baglieri A, *et al*. Sequential release of milk protein-derived bioactive peptides in the jejunum in healthy humans. American Journal of Clinical Nutrition. 2013;97:1314–1323.
4. Brantl V. Novel opioid peptides derived from human beta-casein: Human beta-casomorphins. European Journal Pharmacology. 1984;106:213–214.
5. Cielinkska A, Kostyra EB, Kostyra H, Olenski K, Fiedorowicz E, Kaminski SA. Milk from cows of different-casein genotypes as a source of-casomorphin-7. International Journal Food Science Nutrition. 2012;63:426–430.
6. Daniel H, Vohwinkel M, Rehner G. Effect of casein and beta-casomorphins on gastrointestinal motility in rats. Journal of Nutrition. 1990;120:252–257.
7. De Noni I. Release of β-casomorphins 5 and 7 during simulated gastro-intestinal digestion of bovine β-casein variants and milk-based infant formulas. Food Chemistry 2008;110:897–903.
8. De Noni RJ, Fitz Gerald HJT, Korhonen Y, Le Roux CT, Livesey I, Thorsdottir D *et al*. Scientific Report of EFSA prepared by a DATEX Working Group on the potential health impact of casomorphins and related peptides. EFSA Science Report. 2009;231:101–107.
9. DeNoni I, Cattaneo S. Occurrence of β-casomorphins 5 and 7 in commercial dairy products and in their digests following *in vitro* simulated gastro-intestinal digestion. Food Chemistry. 2010;119:560–566.
10. Elliott R, Wasmuth H, Bibby N, Hill J. The role of β-casein variants in the induction of insulin dependent diabetes in the non-obese diabetic mouse and humans, in Seminar on milk protein polymorphism, ID special. 1997;9702: 445–453.
11. Formaggioni P, Summer A, Malacarne M, Mariani P. Milk protein polymorphism: Detection and diffusion of the genetic variants in Bos genus. Ann. Fac. Medicine Veterinary University Parmacology. 1999;19:127–165.
12. Hamosh M, Hong H, Hamosh P. Beta-Casomorphins: Milk-β-casein derived opioid peptides. In: Lebenthal E (ed.) Textbook of Gastroenterology and Nutrition in Infancy, 2nd Edn. Raven Press, New York; c1989. p. 143–150.
13. Henschen A, Lottspeich F, Brantl V, Teschemacher H. Novel opioid peptides derived from casein (β-casomorphins). II. Structure of active components from bovine casein peptone. Hoppe Seylers Z. Physiology Chemistry. 1979;360:1217-1224.
14. Iwan M, Jarmolowaska B, Bielikowicz K. Transport of micro opioid receptor agonists and antagonist and antagonist peptides across Caco 2 monolayer. Peptides. 2008;29(6):1042-1047.
15. Janer C, Arigoni F, Lee BH, Peláez C, Requena T. Enzymatic ability of *Bifidobacterium animalis* subsp. lactis to hydrolyze milk proteins: Identification and characterization of endopeptidase O. Applied Environment Microbiology. 2005;71:8460–8465.
16. Jarmolowska B, Kostyra E, Krawczuk S, Kostyra H. Betacasomorphin- 7 isolated from Brie cheese. Journal Science Food Agriculture. 1999;79(13):1788.
17. Jarmolowska B, Sidor K, Iwan M, Bielikowicz K, Kaczmarski M, Kostyra E *et al*. Changes of β-casomorphin content in human milk during lactation. Peptides. 2007;28:1982-1986.
18. JinsmaaY, Yoshikawa M. Enzymatic release of neo-casomorphin and β-casomorphin from bovine beta-casein. Peptides. 1999;20:957–962.
19. Joshi BK. Indian Cow, Buffalo Breeds Give Healthier Milk. Outlook Report. 2011, New Delhi.
20. Kaminski S, Cieslinska Kostyra E. Polymorphophism of bovine beta casien and its potential effect on human health. Journal Applied Genetics. 2007;48(3):189-98.
21. Keating AF, Smith TJ, Ross RP, Cairns MT. A note on the evaluation on beta –casein variant in bovine breeds by allele specific PCR and relevance to beta-casomorphin. Irish Journal of Agriculture and Food Research. 2008;47:99-104.
22. Koch G, Wiedemann K, Teschemacher H. Opioid activities of human beta-casomorphins. Naunyn Schmiedebergs Architecture Pharmacology. 1985;33:351-354.
23. Kost NV, Sokolov OY, Kurasova OB, Dmitriev AD, Tarakanova JN, Gabaeva MV, *et al*. β-casomorphins-7ininfants on different type of feeding and different levels of psychomotor development. Peptides. 2009;30:1854–1860.
24. Kreil G, Umbach M, Brantl V, Teschemacher H. Studies on the enzymatic degradation of beta casomorphins. Life Science. 1983;33:137-140.
25. Manga I, Dvorak J. TaqMan allelic discrimination assay for A1and A2 alleles of the bovine CSN2 gene. Czech Journal of Animal Science. 2010;55(8):307-312.
26. Mencarini IR, Woodford KB, Old KM. Comparing herd selection strategies for A2 beta-casein. Proc. New Zealand Society of Animal Production. 2013;73:149–154.
27. Mentz P, Neubert K, Liebmann C, Hoffmann S, Schrader U, Barth A. Pharmacological effects of β-casomorphins on the cardiac function. In:β-Casomorphins and Related Peptides, Weinheim VCH, Germany; c1990. p. 133-139.
28. Mihatsch W A, Franz AR, Kuhnt B, Hogel J, Pohlandt F. Hydrolysis of casein accelerates gastrointestinal transit via reduction of opioid receptor agonists released from casein in rats. Biology of Neonates. 2005;87:160–163.
29. Miller GD, Jarvis JK, McBean LD, Boca Raton FL. National Dairy Council; Handbook of dairy foods and nutrition; c2007.
30. Miluchova M, Trackovika A, Gabor M. Analysis of polymorphism of beta-casein Slovak pinzgau cattle by PCR-RFLP for alleles A1 and A2. Lucrari stitifice Zootehniesi Biotechnologii. 2009;42(2):288-292.
31. Mishra BP, Mukesh M, Prakash B, Sodhi M, Kapila R, Kishore A, *et al*. Status of milk protein, β-casein variants among Indian milch animals. Indian Journal of Animal Sciences. 2009;79 (7):722–725.
32. Muehlenkamp M R, Warthesen JJ. Beta- Casomorphins: Analysis in cheese and susceptibility to proteolytic enzymes from *Lactococcus lactis* ssp. cremoris. Journal of Dairy Science. 1996;79(1):20-26.
33. Ng-Kwai-Hang KF, Grosclaude F. Genetic polymorphism of milk proteins. In: Fox PF, Mc Sweeney PLH (ed) Advanced Dairy Chemistry: Volume 1: Proteins, Parts A & B, Kluwer Academic/Plenum Publishers, New York; c2002. p. 739–816.
34. Nguyen DD, Solah VA, Johnson SK, Charrois JW,

Busetti F. Isotope dilution liquid chromatography-tandem mass spectrometry for simultaneous identification and quantification of beta-casomorphin 5 and beta-casomorphin 7 in yoghurt. Food Chemistry 2014;146:345-352.
35. Pal S, Woodford K, Kukuljan S, Ho S. Milk intolerance, beta-casein and lactose. Nutrients. 2015;7:7285-7297.
36. Pelto L, Impivaara O, Salminen S, Poussa T. Milk hypersensitivity in young and adults. European Journal of Clinical Nutrition. 1999;53(88):620-624.
37. Phelan M, Aherne A, Fitz Gerald RJ, O'Brien NM. Casein-derived bioactive peptides: Biological effects, industrial uses, safety aspects and regulatory status. International Dairy Journal. 2009;19:643-654.
38. Pleuvry BJ. Opioid receptors and their ligands: Natural and unnatural. British Journal of Anaesthia. 1991;66:370-380.
39. Raies M, Kapila R, Shandilya U Kapila, S. Detection of A1 and A2 genetic variants of beta casein in Indian crossbred cattle by PCR-ACRS. Milchwissenschaft. 2012;67(4):396-398.
40. Rashidinejad A, Bremer P, Birch J, Oey I. Nutrients in Cheese and Their Effect on Health and Disease. In: Watson RR, Collier R J, Preedy V R (ed), Nutrients in Dairy and Their Implications on Health and Disease; c2017. p. 177–192.
41. Rijnkels M. Multispecies comparison of the casein gene loci and evolution of the casein gene family. Journalof Mammary Gland Biology Neoplasia. 2002;7:327-345.
42. Saito T, Nakamura T, Kitazawa H, Kawai Y, Itoh T. Isolation and structural analysis of antihypertensive peptides that exist naturally in Gouda cheese. Journal of Dairy Science. 2000;83:1434–1440.
43. Sakaguchi M, Koseki M, Wakamatsu M, Matsumura E. Effects of beta-casomorphin-5 on passive avoidance response in mice. Bioscience, Biotechnology and Biochemistry. 2003;67(11):2501-2504.
44. Shimizu M, Tsunogai M, Arai S. Trans-epithelial transport of oligopeptides in the human intestinal cell, Caco 2. Peptides. 1997;18(5):681-687.
45. Sokolov O, Kost N, Andreeva O, Korneeva E, Meshavkin V, Tarakanova Y, *et al*. Autistic children display elevated urine levels of bovine casomorphin-7 immunoreactivity. Peptides; c2014. p. 56-68.
46. Tailford K A, Berry CL. A casein variants in cow milk is atherogenic. Atherogenic atherosclerosis. 2003;170:13-19.
47. Teschemacher H, Koch G, Brantl V. Milk protein derived atypical peptides and related compounds with opioid antagonist activity (review). In: Brantl V, Teschemacher H (Eds.), β-Casomorphins and Related Peptides: Recent Developments; VCH-Weinheim Germany; c1994. p. 3-17.
48. Teschemacher H. Opioid receptor ligands derived from food proteins. Current Pharmaceutical Design. 2003;9:1331-1344.
49. Tiruppathi C, Miyamoto Y. Hydrolysis and transport of proline containing peptides in renal brush border membrane vesicles from dipeptidyl peptidase strians. Journal of Biology and Chemistry. 1990;265(3):1476-1483.
50. UlHaq MR, Kapila R, Kapila S. Release of β-casomorphin-7/5 during simulated gastrointestinal digestion of milk β-casein variants from Indian cross bred cattle (Karan Fries). Food Chemistry. 2015;168:70-79.
51. Wada Y, Lonnerdal B. Bioactive peptides released from *in vitro* digestion of human milk with or without pasteurization. Pediatrician Residence. 2015;77:546-553.
52. Ward SJ, Takemori AE. Relative involvement of receptor subtypes in opioid-induced inhibition of gastrointestinal transit in mice. Journal of Pharmacology and Experiental Therapeutics. 1983;224:359–363.
53. Wasilewska J, Kaczmarski M, Kostyra E, Iwan M. Cow's-milk-induced infant apnoea with increased serum content of bovine β-casomorphin-5. Journal of Pediatrition Gastroenterology Nutrition. 2011a;52:772-775.
54. Wasilewska J, Sienkiewicz-Szlapka E, Kuzbida E, Jarmolowska B, Kaczmarski M, Kostyra E. The exogenous opioid peptides and DPPIV serum activity in infants with apnoea expressed as apparent life threatening events (ALTE). Neuropeptides. 2011b;45:189–195.
55. Woodford K. Devil in the Milk:Illness, Health and Politics: A1 and A2 Milk;Craig Potton Publishing: Wellington, New Zealand; c2007.
56. Yin H, Miao J, Zhang Y. Protective effect of beta casromorphin -7 on type 1 diabetes rats induced with streptozotocin. E. Pub Jan. 2010;31(9):1725-1729.
57. Zoghbi S, Trompette A, Claustre J, El Homsi M, Garzon J, Jourdan G, *et al*. β-Casomorphin-7 regulates the secretion and expression of gastrointestinal mucins through a μ-opioid pathway. American Journal of Physiology Gastrointestinal and Liver Physiology. 2006;290:G1105–G1113.