Harnessing Full Potential of A1 and A2 Milk in India: An Update
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Preface

The potential beneficial health effects of milk proteins are recognized by the recent discovery of many bioactive peptides, exerting different pharmacological activities. Cow milk contains about 3.5 per cent proteins, and more than 95 per cent of these are constituted by caseins and whey proteins. Caseins, the main protein make up about 80 per cent of the milk proteins and comprise of alpha S1, alpha S2, beta, and kappa casein. β-casein is the second most prevalent protein in milk after α-casein. The nutritive value of bovine caseins is not only determined by their amino acid content but also by bioactive peptides which are released during in vivo, in vitro digestion or processing of milk. Different bioactive peptides are produced after digestion of beta casein and amongst these opioid β-casomorphin-7 (BCM7) generated from digestion of specific type of beta casein has been a focus of attention. β-casein, a 209 amino acid long major protein has different genetic variants with A1 and A2 genetic variants being the predominant ones. In the natural A2 variant (A2 milk) at 67th position, amino acid Proline is present. However, due to genetic mutation in A2 genetic variant (A2 milk), the Proline at 67th position in beta casein is replaced by amino acid Histidine which is known as A1 milk. This paper is primarily to address and educate consumers, policy makers, dairy farmers and researchers involved in dairy field, regarding A1 milk related controversy. Recently, concerns have been raised by different segments of society regarding consumption of A1 milk; however data are still not conclusive. Although majority of evidences are based on epidemiological data in European countries, no concrete proof of the adverse effect of A1 milk on human health is available till date. At the same time no beneficial effect has also been linked to A2 milk. Majority of the indigenous cows (more than 95%) reared in Indian subcontinent produce A2 milk. This has attracted attention of the research communities, health conscious individuals consuming milk, dairy farmers and dairy industry. India is largest producer of milk and this has been possible due to cross breeding of Indian breeds with exotic breeds. The crossbreeding program has increased milk production but at the same time has been responsible for introgression of A1 allele in Indian cattle which are primarily of A2 type. The current situation warrants a very clear picture on health attributes of A1 and A2 milk, which will come out from its indepth research study based on human as well as animal trials.

National Academy of Agricultural Sciences (NAAS), India organized one-day Strategic Workshop on “Harnessing full potential of A1 and A2 milk in India: An update" at NASC complex, New Delhi on May 19, 2018. This workshop was attended by more than 67 eminent scientists, policy makers and peer groups, dairy industry, NGOs and social activists. It is hoped that this strategy paper and subsequent recommendations will help the researchers and policy makers to decide a suitable strategy for future course of action in animal breeding programme. Further, the recommendations of this paper will also help to “rest” the unrest in society for A1 milk. Academy thanks all the eminent scientists and experts for their participation, in-depth interactions and deliberations. I especially compliment Dr A.K. Srivastava, Convener and Dr A.K. Mohanty, Co-convener for their initiative to organize the strategy consultation. The editorial support extended by Dr V.K. Bhatia and Dr Kusumakar Sharma is thankfully acknowledged.

(Panjab Singh)
President
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INTRODUCTION

Milk has been regarded as a wholesome food and also an essential part of diet for both infants and adults since ancient times (as early as 4000 BC). Worldwide the major sources of milk are cow, buffalo, goat, sheep, and camel (Fig. 1). Globally, cow contributes highest to the milk production i.e. 600 million tonnes (83%) of total milk produced every year. In India, the total milk production (approximately 176.3 million tons) is highest in the world with 190.90 million cattle that is around 12.5% of world cattle population. In India, 49% milk is produced by buffalo and 46.9% milk is produced by cattle. The detailed contribution of different category of bovines in milk production and categorization of Indian cattle population are given in Fig. 1A and Fig. 1B, respectively.

Milk is a complex biological fluid containing various components like proteins, lactose, fats, vitamins, minerals, hormones, immunoglobulins, growth factors, cytokines, nucleotides, peptides, polyamines, and enzymes. It is also a good source for calcium, magnesium, selenium, the vitamins B Complex (thiamin, riboflavin, niacin, vitamin B6, and folate), vitamin A, vitamin C, magnesium, and zinc as well. Summing up, milk is the only food that contains almost all substances needed for growth and development. With a protein content of ~3.5%, it accounts for the major portion of protein in diet of infants and vegetarians. The composition of milk from different lactating species differs in composition and cow milk is considered as best milk as its composition is very similar to human milk.
More than 95% of the cow milk proteins are constituted by caseins and whey proteins remaining 5% include peptones/low molecular weight peptides milk fat globule membrane proteins The casein (insoluble fraction) is the most abundant (80%) among the protein fraction, comprising of αS1 (39-46%), αS2 (8-11%), β-casein (25-35%) and κ-casein (8-15%) while whey (soluble fraction) comprising of α-lactalbumin and beta-lactoglobulin forms 20% of the milk protein content. With high protein content, it provides wide range of biologically active compounds such as immunoglobulins, antimicrobial proteins and peptides, oligosaccharides, lipids etc., which have a positive impact on human metabolism and health. Amongst these, proteins are key source of bioactive peptides encrypted in an inactive form within the proteins and released during in vivo or in vitro digestion. These bioactive peptides may exert regulatory activities in the human beyond nutrition also act as promoters of the physiological function.

All milk proteins have peptides either in latent form or active form. The majority of whey proteins are active; whereas the casein derived peptides are encrypted. β-casein, the second most prevalent protein in milk after α-casein, is considered as a resource for bioactive peptides. The focus in this paper will be on the bioactive peptides generated from beta casein.

**Gene polymorphism and bioactive peptides**

Bioactive peptides encrypted in the parent protein sequence are released and activated either by enzymatic proteolysis or during food processing (cooking, fermentation, ripening). The biological activity of these peptides is determined by the amino acid sequence and the hydrophobicity. The charge of bioactive peptides indicating that the sequence of the protein plays an important role in the type of bioactive peptides generated. The sequence of amino acids in proteins might get changed with the polymorphism in the coding region leading to generation of these diverse bioactive peptides. Thus, it becomes pertinent to study the polymorphism in protein genes which are a good resource of bioactive peptides. Change in the nucleotide sequence is responsible for the genetic polymorphism. Different alleles occurring at same locus may code for different amino acids. These modified amino acids subsequently alter the primary structure of peptides. Milk proteins exist in several forms due to the polymorphism exhibited by the coding genes. Till now nearly 50 variants have been identified for 6 main milk proteins.

**Protein variants of Beta casein gene**

Amongst the milk casein, β-casein holds special significance. It is reported that β-casein protein variants not only result in generation of different group of active peptides, but also has an influence on milk protein composition and milk-production traits according to its
genetic polymorphism. Till now 12 genetic variants (A1, A2, A3, B, C, D, E, F, H1, H2, I, G) have been reported for beta-casein. The most common forms of beta casein in dairy cattle breeds are A1 and A2, while B is less common and A3, C are rare (Farrell et al., 2004).

![Fig. 2: A1 and A2 variants of beta casein gene](image)

Among all the mutations in beta-casein, at 67th position a noteworthy mutation leads to replacement of proline by histidine with the replacement of codon CCT (proline) to CAT (histidine) leading to formation of variant A2 and A1, respectively (Fig. 2). On the basis of amino acid sequence variation, beta casein variants can be categorized as A2 type (with proline at position A3, E, D, H2 and I) and A1 type (with histidine at position 67: B, C, F, G and H1) (Roginski, 2003). Cow milk with A2 or A1 “like” variants of beta casein is known as A2 and A1 milk, respectively.

**Bioactive peptides from A1/A2 types of milk**

As discussed, milk proteins have peptides either in latent form or active form. The majority of whey proteins are active, whereas the casein derived peptides such as Opioids (morphin like) are encrypted. The different opioids released from beta casein are called beta casomorphins (BCMsin). These encrypted casomorphins are formed upon enzymatic proteolysis or some other procedures like fermentation using proteolytic cultures or proteolysis by microbial or plant derived enzymes. Several other factors determines the type of beta casomorphins formation within dairy products including thermal treatments, mammary health status of cattle and also the beta casein sequence.

The polymorphism proline to histidine leads to a change in the secondary structure of expressed β-casein protein and different bioactive peptides (opioids: Beta casomorphins) are released during *in vivo* or *in vitro* digestion of milk depending upon the specific genetic variant of beta casein (Fig. 3).
Gastrointestinal proteolytic digestion (leucine aminopeptidase, elastase, trypsin and carboxypeptidase Y) of A1 milk releases a 7 amino acid bioactive peptide ‘opioid’ called beta-casomorphin 7 (BCM-7) in small intestine, while proline in A2 milk at 67 position prevents the split at this particular site and generates nine amino acid peptide BCM-9 (Kostyra et al., 2004).

In hydrolyzed A1 type milk, the BCM7 content is four times higher than that of raw A1 type milk, while BCM7 has not been reported in fermented milk products which might be the result of degradation of the bioactive active peptides during processing (Cieœliñska et al., 2007). So far, the beta-casomorphines confirmed to be released from bovine raw milk are BCM-4, BCM5, BCM6, BCM7, BCM9, BCM10 and BCM11 (EFSA, 2009). The BCM-5 is primarily released from further proteolytic digestion of BCM7 and BCM9 by brush border peptidases.

**Mode of action of milk derived peptides**

When milk is ingested, it is gradually broken down to peptides at various sites of gastrointestinal tract. In oral cavity, milk components are sheared mechanically and then under acidic condition of stomach, milk coagulates and casein is separated as para-casein. After coagulation, the curd traps the fat globules and later dissolved by the pepsin gets converted into peptones and albumoses. Further, the bile present in pancreas emulsifies the fat and neutralizes the acidic effluent from stomach. At small intestine, in duodenum the concerned bio-active peptides are released by enzymatic digestion. BCM-7 generated from A1 type milk is further broken down to BCM-5 and BCM-3 by dipeptidyl peptidase IV (DPP IV) enzyme present on surface of enterocytes or in blood. If not degraded, the peptides (betacasomorphins), cross the epithelial layer and are then free to exert their physiological effect(s) on various tissue types and cells by participating in cellular pathway.

Fig. 3: Enzymatic digestion of A1 and A2 variants of bovine beta-casein (adapted from Jinsmaa and Yoshikawa, 1999)
by virtue of being “atypical” opioid peptides or in intact form as immunomodulatory peptides. Several modes of transport transcellular, transporter mediated and paracellular have been suggested for the peptides across intestinal epithelial barrier. The transport is more common in neonates due to high permeability of intestine or improper function of DPP (IV) enzymes. Adults with compromised digestive health or conditions such as celiac disease, stomach ulcers or autism have increased intestinal permeability and consequently the probability of BCM-7 entering the blood stream is more in such cases (UlHaq et al., 2015).

Both in vitro and in vivo studies have suggested the absorption and transport of beta-casomorphine-7 across epithelial barrier of small intestine. The BCMs have opiate properties similar to morphine that includes affinity to opioid receptors, especially the MOR (µ-opioid receptor) and opioid receptors (µ and Δ opioid receptors) are widely distributed over the intestinal lining. In the bovine beta-casomorphine-7, presence of tyrosine residue at the N terminal and a phenylalanine residue at the 3rd or 4th forms the motif enabling it’s binding to the opioid receptor, which are pharmacophoric residues. The proline present at the 2nd position is crucial for the formation of bioactive conformation of the peptide in order to bind receptor. The proline at 2nd position along with the pharmacophoric residues forms the “messenger sequence”. The remaining C terminal sequence forms the “address sequence” (Janecka et al., 2004). The BCM9 is also an opioid agonist but with lesser affinity for µ opioid receptor. The BCMs binds to opioid receptors with high affinity for MORs which have numerous effects on physiological processes upon activation by endogenous or exogenous opioid ligands. These have been identified on cell surfaces of gastrointestinal tract, immune cells, pancreatic cells and various cells and tissue types in central nervous system. The MORs have seven transmembrane spanning domain coupled to G inhibitory protein and after binding of exogenous ligand such as BCM-7, the Gβ/ sub-unit dissociates from Ga and various intracellular pathways are initiated (Fig. 4) that may lead to different health disorders (Sobczak et al., 2013; Deth et al., 2016). The another class of mechanism is by the virtue of non-opioid mediated effects of milk derived peptides. Among the β-casein derived peptides, beta-casomorphine-7 (YPFP GPI), beta-casokinin (YQQPVLGPR), caseinophosphopeptides (RELEELNVPGEIV ESLSSSEESITR and KNTMEHVSSSEESIQETYKQEkNMAINPSK) and immunopeptides (PGPIPN and LLY) have immunemodulatory function. These peptides are reported to inhibit lymphocyte proliferation and inflammatory responses (Singh et al., 1989). Several studies have indicated the physiological effects of β-casomorphin immuno-reactive material (BCMIR) in neonates (canines), but not in the adults (Umbach et al., 1989; Singh et al., 1989). This indicates the physiological significance of beta-casomorphins in early developmental stage of infants. In the either way, the beta-casomorphins and other bio-peptides from milk are able to elicit responses apart from the nutritional aspects. So, it is must to evaluate the effect of these peptides on different tissue types and metabolism. Another point need to be clarified is, whether there are any combitorial effect of various beta-casomorphines
and other immunopeptides derived from milk. Through this postulated mode of action, role of BCM7 have implicated in many illnesses, including heart disease, type I diabetes, and sudden infant death syndrome.

![Mechanism of action of BCM](image)

**Fig. 4: Mechanism of action of BCM**

### Effect of A1 milk consumption on human health

Milk consumption has been linked to various beneficial as well as adverse health effects. There are literature to the evidence linking A1 beta-casein to different diseases such as type I diabetes and CVD (Laugesen and Elliott, 2003), arteriosclerosis (Tailford et al., 2003), schizophrenia and autism (Woodford, 2006), and sudden infant death syndrome (Sun et al., 1999).

#### Epidemiological Studies

The debate about probable association of A1 with diseases started in 1990 after publication of some papers in Australia. In one of such papers, incidence of mortality rates due to chronic heart diseases (CHD), juvenile insulin-dependent type I diabetes mellitus (DM-I) and other diseases across 20 developed countries was compared with food consumption trends. In the survey, strong association between high A1 β-casein consumption and CHD/DM-I was reported. As compared to other countries, the association was found to be stronger in high altitude countries, such as North America and North Europe wherein consumption of A1 milk and milk products is higher. These surveys were based on epidemiological data and above all these were based only on the assumption that all the individuals are exposed to equal level of risk factor i.e. A1-casein-milk, which may not be valid, keeping in view the socio-economic status of the consumers.
Another example in this area is for Samoan children, with higher incidence of type 1 diabetes in Samoan children living in New Zealand compared to Samoan children living in Samoa. The reason might lie in the diet pattern, Samoan children in Samoa consumed very much less milk than Samoan children in New Zealand. Conversely, Masai people in Kenya drink large amounts of milk but the incidence of childhood diabetes is extremely rare. The situation explains that, factor is not only the quantity of milk but the type of milk and essentially the caseins in the milk. The milk, the Masai people consumed was A2 type whereas the people in New Zealand had A1 milk, supporting the hypothesis that A1 beta-casein might be the casual factor in type 1 diabetes. One of the broadest ecological studies has been conducted by Laugesen and Elliot (2003) among nineteen European countries, which involved estimation of A1/A2 allele distribution among cattle breeds and was, correlated with type I diabetes incidences. The study concluded a strong relationship between A1 beta casein per capita consumption with type I diabetes.

Another study conducted at University of Florida indicated that after removal of BCM7 sources (milk and gluten) from diet, the patients with childhood autism showed considerable improvement and this pointed towards association of BCM7 with autism and schizophrenia. Further, the ecological data (Tonstad et al., 2002) have indicated comparative advantage of soy based diet over the casein in reducing the risk for heart diseases. Following these hypothesis, number of studies were carried out to get the insight of the hypothesis and logic behind the association of A1/A2 milk/BCM with health disorders.

**Experimental Evidences**

Although several epidemiological/ecological data positively correlated different diseases with A1/A2 milk consumption, but there are scanty in vivo and in vitro data supporting the same. Animal model based experiments clearly support the role of BCM-7 in affecting physiology and immune status of an animal. Intra-cerebro-ventricular or intra-peritoneal injection of BCM-7 have been observed to result in analgesic activity, change in sleep cycle as well as amnesia in rat and mouse model. Studies have also indicated that consumption of A1 type milk (A1A1 and A1A2) significantly increase the levels of inflammation-associated molecules (MPO, MCP-1, IL-4), humoral immune response (total IgE, IgG, IgG1, IgG2a) and leukocyte infiltration in intestine with concurrent up-regulated expression of TLR-2 and TLR-4 mRNA. These changes may reflect the induction of inflammatory response in gut through activation of Th2 pathway on administration of A1 type milk. Another study by Jianquin et al., (2016) indicated the A1 milk to be a causative factor for worsening the gastrointestinal discomforts in Han Chinese. In this study, 45 Han Chinese subjects were fed with only A2 type and mixed milk (A1 and A2 type), and observed the effect on digestive discomfort, gastro intestinal transit time, inflammation and cognitive behavior. The study indicated that A2 fed subjects did not show any of the above adverse effect. On
the other hand, subjects fed with A2 along with A1 milk in a ratio 3:2, displayed symptoms of gastrointestinal discomfort with increased gastrointestinal transit time, inflammatory serum marker level and retarded cognitive processing speed.

The potential role of cows’ milk in diabetes is still debated and there is no consensus on the diabetogenicity of individual milk proteins. However, there are some evidences that BCM-7 released during digestion of A1 beta casein activate µ receptor that compress our immune system and produce autoantibody against pancreatic beta cells that leads the progression towards diabetes. Suppression of body’s immune system by BCM-7 may also enhance the survival of pathogens such as enteroviruses or bacteria (Mycobacterium avium). These pathogens are ultimately involved in triggering of type 1 diabetes like symptoms. Some of the bioactive peptides sequences generated from β-casein mimic the sequence of GLUT-2 transporter (involved in glucose transportation in cell). T cells recognize them as an antigen and activate B cells for the production of antibodies, which target the peptides sequences as well as insulin producing beta cells, causing type 1 diabetes (Cavallò et al., 1999). Also, leaky gut syndrome is postulated to increase the permeability of submucosal layer to dietary antigens. The altered mucosal immunity leads to inflammation and subsequently the autoimmune destruction of pancreatic beta cells.

The preliminary findings of a study at University of Florida showed that 95% of 81 autistic children had 100 times more than the normal levels of milk protein in their blood and urine. When these children were put on a milk free diet, at least 8 out of 10 no longer had symptoms of autism or schizophrenia. The scientific reports confirm that in some children biopeptides of casein can leak through the gut wall into the blood, and from there into the brain causing significant behavioural problems. Similar neural disorders have also been observed in rats dosed with varying levels of BCM-7. Furthermore, a range of erratic behaviors’ has been observed in rats dosed with BCM-7, which did not occur when the rats were pre-treated with naloxone, an opioid antagonist. These studies suggest that BCM-7 may play a role in behavioural disorders such as schizophrenia and autism. Further, an in vivo study on rabbit, Tailford et al., (2003) has also demonstrated the atherogenic property of A1 based diet over A2 diet. Allison et al., (2006) also suggested linkage of cardiovascular diseases with the consumption of milk and dairy products with the A1 variant. On one hand, β-Casomorphins (opioid peptides) originating from milk have been associated with various health issues, while on the other hand, these peptides are potential modulators of various regulatory processes as well. Later in 2015, the detailed review paper on effect of A1 beta Casein protein on health attributes was also published (Parasher and Saini, 2015).

Prof. Truswell, Human Nutritionist, University of Sydney, Australia thoroughly reviewed all the studies conducted so far on A1 and A2 milk in a paper entitled ‘A2 milk case: a critical review’ (Truswell, 2005) and reported that the release of BCM-7 has not yet been
demonstrated in human. The correlation of DM-I incidence and estimated national average of A1 β-casein consumption was only a suggestive evidence. He observed that no mechanism was presented for any differential effect of cows’ milk β-casein types on the pathogenesis of CHD and pointed that CHD mortality had declined considerably in countries like USA, Australia and Switzerland without reduction in milk and cheese protein consumption. The available human prospective epidemiological studies showed no increased CHD in people who drink more milk. As the evidence relating autism and schizophrenia to A1 or A2 β-caseins in milk were more speculative and the evidence was more unsubstantial than that for DM-I and CHD, he concluded that there was no convincing or even probable evidence that A1 β-casein of cow milk had any adverse effect in human beings. Further, European Food Safety Authority (EFSA) also reviewed all research conducted on A1 and A2 type milk so far. The scientific report of EFSA on the review of the potential health impact of β casomorphines and related peptides was issued in January 2009 (EFSA, 2009). In the report, it was recommended that a cause effect relationship between to oral intake of BCM7 or related peptides and etiology of cause of any suggested non communicable diseases could not be established.

The data based on epidemiological studies or available limited reports on humans or animal trials are not strong enough and need deep investigations. Hence, it is necessary to continue research in exploring the role of BCMs in human health. Till then, it is important to assess the frequency distribution of A1/A2 allele across the native as well crossbred population to ascertain that milk being consumed is safe for health.

Demographic distribution pattern of A1/A2 allele of Beta casein gene

The distribution of A1/A2 allele of Beta casein gene varies across the globe and across different breeds. Large variation has also been recorded between the species of Bos genus for the A1 and A2 allele frequency. It carries an evolutionary significance with A2 being the primitive type. Asian and African cattle, goats, sheep, yaks and camels all produce A2 milk. Even human beta-casein is also of the A2 type as defined by the relevant amino-acid sequence.

It is postulated that all animals belonging to Genus Bos, which includes Bos indicus (Indian humped Zebu), Bos taurus (Exotic) and Yak were initially of A2A2 type only and due to mutation some animals became A1. Majority of taurine breeds originally domesticated in America, Europe and Australia (excluding Indian sub-continent, most of the African zebu and few Far-East countries) have been bred selectively for higher milk production. While making selection for high milk production unconsciously A1 allele carrying bulls, that were genetically superior, were chosen in breeding programs and hence A1 mutation became
prominent in European cattle some thousands of years ago. Conversely, Indian cattle (*Bos indicus*) evolved naturally without any selection, carry higher frequency of A2 allele.

The percentage of modern European cattle producing A1 beta-casein varies from breed to breed. A1 β-casein is a major variant of β -casein in the milk of the common dairy cows of north European origin: Friesian, Ayrshire, British Shorthorn, and Holstein. Also, the frequency of A1 has increased over the last century as the Holstein Friesian (HF) has become predominant breed in many countries due to its high milk production character. Artificial insemination of large number of cows with semen of HF bulls carrying A1 gene multiplied the gene and genotypic frequency over time. Several reports (EFSA 2009; Demirel et al., 2018) indicate that relative distribution of A1/A2 allele of β-casein is not only due to breed but also area specific (Fig. 5 and 8). For example, in German Holstein the frequency of A1 is less than 0.05 while in Holstein Friesian from North America and North Europe, the frequency is more than 0.70. Frequency of A1 allele in HF in countries other than Germany ranges between 0.33-0.72. The Jersey, another widely spread breed has lower A1 gene frequency ranging from 0.12-0.22 with an average of 0.12. On the contrary, many other breeds from USA and Europe e.g., Guernsey breed from USA / Europe; Kerry breed from Ireland; Spotted breed from Hungary; Charolais and Limousin breeds from Southern France; Channel Island cows; Brown Swiss, Brown Italian, Guernsey and Fleckvieh breeds have comparatively much higher frequency of A2 (>0.80).

![Fig. 5: Beta-casein allele frequency distribution in Holstein and Jersy across different countries (EFSA 2009).](image)

**Status of β-casein variants in Indian cattle breeds**

The status of A1/A2 alleles of β-casein gene was delineated systematically at ICAR-NBAGR, Karnal. In a comprehensive analysis, more than 1500 animals representing 25 breeds of Indian cattle and more than 500 breeding bulls and crossbred animals were genotyped to understand the distribution of A1/A2 variants.
Majority of the Indian native cattle (91%) showed A2A2, the desirable genotype, followed by heterozygous A1A2 (0.09) genotype. None of the animal showed homozygous A1A1 genotype. Only few animals of Malnad Gidda, Kherigarh, Ladakhi and Badri cattle showed heterozygous A1A2 genotype with frequency of 0.191, 0.218 and 0.24 respectively (Mishra et al., 2009; Sodhi et al., 2012; Sodhi et al., 2018). The overall frequency of favorable A2 allele across all the 1500 analyzed animals was 0.95 and its distribution across the different utility types were 1.0, 0.94 and 0.92 for milch, dual and draft purpose breeds, respectively (Fig. 6). Interestingly, none of the milk breeds viz., Gir, Tharparkar, Rathi, Red Sindhi, and Sahiwal showed A1 allele or A1A1/ A1A2 genotypes. The results indicated the preponderance of A2 β-casein variant and also pointed towards the Indian origin of A2 allele.

Conversely, the scenario in exotic cattle (Bos taurus) from different countries has been reported to be quite different with much higher frequency of A1 allele i.e. 0.435 (Fig. 7).
Status of β-casein variants in crossbred cattle/bulls

Considering the widespread use of taurine germplasm in our country’s cross-breeding program and fact that these cattle could be the potential source for A1 allele, status of A1/A2 alleles in breeding bulls was also analyzed at NBAGR, Karnal. As per latest data, India has 190.1 million cattle population of which nearly 20% is of crossbred cattle. For cross-breeding, exotic dairy breeds primarily used are Holstein Friesian, Jersey and Brown Swiss breed. Jersey is being used for the genetic improvement of local cattle in Assam, Arunachal Pradesh, Bihar, Goa, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Manipur, Odisha, Rajasthan, Tamil Nadu, Tripura and West Bengal. Holstein Friesian is widely used across the country and specifically in Delhi and Punjab. Both Holstein Friesian and Jersey are being used in Gujarat, Haryana and Uttar Pradesh in cross-breeding program currently (Mishra et al., 2017). As such, 400 crossbred breeding bulls, being used across the country, were genotyped. Out of these, 48.1% were heterozygous for A1A2 followed by A2A2 (40.5%) and A1A1 (11.4%). The mean frequency of A1 and A2 alleles was 0.355 and 0.645, respectively. Data generated for cross-bred, Holstein Friesian and Jersey bulls being used in different AI centers in India have also been screened (Sodhi et al., 2012). The findings indicated higher frequency of A2 allele (0.702) in cross-bred bulls as compared to Jersey and Holstein Friesian bulls wherein it was 0.675 and 0.559, respectively. It shows that about 33-45% of the animals of Jersey and Holstein cattle being currently used in AI program are having A1 allele. Among the A1A1, A1A2 and A2A2 genotypes, heterozygous genotype A1A2 had higher frequency in Holstein Friesian and Jersey bulls compared to crossbreds bulls (Fig. 8). The frequency of A2 allele

![Fig. 8: Overall status of A1/A2 variants in AI bulls of different breeds (ICAR-NBAGR data)](image-url)
in cross-bred and exotic bulls was lower than indigenous bulls (0.88). In continuation, Karan Fries (KF) population was also screened to assess the frequency of A1/A2 allele of beta casein. KF was developed using Holstein Friesian (exotic breed) and Tharparkar (indigenous breed) with the ultimate motive to enhance milk production coupled with high disease resistance and adaptability to local conditions. The genetic profiling with respect to A1/A2 allele revealed that amongst KF samples frequency of A1 was 0.12 whereas A2 predominated by 0.88.

Apart from NBAGR data, other studies also indicate similar trend for allelic and genotypic frequencies with respect to A1/A2 allele across breeding bulls and crossbred cattle population. Frequency of A2 allele in Holstein Friesian crossbred males has been reported to be 0.706 with genotypic frequencies of 0.17, 0.46 and 0.37 for A1A1, A1A2 and A2A2 genotypes, respectively. Frequency for A2 allele in HF crossbred cow has been observed to be 0.595 (Malarmathi et al., 2014), while in the Frieswal cattle it was 0.65 with genotypic frequencies of 0.15, 0.41 and 0.44 for A1A1, A1A2 and A2A2 genotypes, respectively (Ganguly et al., 2013). Crossbred cattle of Kerala were also reported to have higher frequency of A2 allele (0.56) with genotypic frequencies of 0.32 (A1A1), 0.28 (A1A2), 0.40 (A2A2) (Muhammed and Stephen, 2012). On the contrary, Hardhenu crossbred cattle maintained at LUVAS, Hisar revealed genotype frequencies of 0.32 (A1A1) and 0.68 (A1A2) with frequency of 0.34 for A2 allele (Ramkaran et al., 2017). In another crossbred cattle, Vrindavani, observed genotypic frequencies of A1A1, A1A2 and A2A2 were 0.11, 0.47 and 0.42, respectively while frequency of A1 and A2 alleles was 0.35 and 0.65, respectively (Kumar et al., 2018).

**Summing up, in India the average frequency of A2 allele in crossbred bulls is ~ 0.68 while in exotic bulls it is nearly 0.60. Similarly for different crossbred populations, the percent of A2 frequency is nearly 74 indicating the abundance of A2 variant even in Indian crossbred population. Apparently, it is a happy scenario for India even if the adverse effects of A1 allele are authenticated.** However, the contribution towards this high percentage of A2 allele in exotic and crossbred populations including breeding bulls from homozygous A2A2 genotype is less than 40% and rest is from the heterozygous A1A2 genotype. It clearly demands the screening of all the breeding bulls being currently used in AI program for status of A1/A2 allele of β-casein.

To date, the debate about A1 and A2 β-casein has largely been occurring in New Zealand and Australia. New Zealand has been typing all breeding bulls for A1/A2 status for approximately 10 years, and the national herd is now slowly drifting towards A2. In most countries, the national herds could be converted to A2 over about a 10 year period, or more rapidly using new technologies of A1/A2 allele tested semen. A project at Pennsylvania State University, USA is going on wherein dams are typed for A1/A2 status. New Zealand
has established ‘A2 Corporation Ltd.’ in 2000 to test cows and market the milk with only the A2 variants of β-casein. The company is selling A2 milk as a premium brand in New Zealand and Australia and has started commercialization of A2 milk in Asia and USA, too.

India, though continues to be the largest milk producing country in the world, no such concrete effort has been made to type the semen for A2 allele. Crossbreeding programme of dairy cattle has played significant role in attaining India’s top position as highest milk producer country of the world. Total crossbred cattle in India accounts for only approximately 21%, but if we observe the share towards total milk production, it is 26 % while indigenous cattle breeds contribute 20.7%. Owing to the contribution of crossbred milk in India, it is important to type such high producing animals. Though, the present data indicated predominance of the desirable A2 allele across all studied breeding bulls, still there is a need to draw a sound breeding policy for careful screening of sire lines being used in the breeding programs. Delineation of the status of β-casein A1/A2 alleles in AI bulls (Exotic, Crossbred or Indigenous) would help to minimize the risk of disseminating the A1 allele in Indian cattle. As many of them are heterozygous with A1A2 and percentage of homozygous A1A1 animals is not very high, it is easy to accomplish the task and drift the herds towards A2.

With the sound cattle breeding policy, not only our Indian cattle that naturally harbor the preferred A2 allele, but the total cattle population will have an edge over their European counterpart that possess comparatively higher proportion of A1 variant. Even if the undesirable effects of β-casein A1 allele on health are validated and demand for A2 milk increases, our native cattle breeds as well as crossbred population would be the best resource to meet the global demands for A2 milk in the international dairy sector.

Conclusion

Dairy husbandry has been a successful development story in India, where the country which was importing milk in spite of having highest bovine population in the world was transformed into highest milk producer, within a span of 2-3 decades. At that time as upgrading the progeny of nondescript cattle was not attractive option due to low production potential, crossbreeding was promoted using selected western dairy breeds. This helped to produce high yielding crossbred cows at their doorsteps, without high capital investment and risk. As such, during the process of crossbreeding, A1 allele was also introduced in Indian milch herd.

The A1/A2 hypothesis is both intriguing and potentially very important for public health, if it is proved correct. The BCM-7 released from A1 type has been the central theme for hypothesis, and needs to be investigated for the claimed health ailments. The epidemiological and in vitro data suggests the potential health hazards of consuming A1 type milk derived
BCM-7, but the *in vivo* studies of the same are very few to support the findings. Although a clear link between A1 β-casein and a disease state has not yet been confirmed, it raises question and doubt in the mind of consumers. Similarly, there is also no evidence to suggest that consumption of A2 type milk would provide protection or sparing effects from these diseases. The importance of monitoring the status of A1/A2 alleles in Indian dairy animals as a precautionary measure has been realized recently. We are still to prove the link between intake of A1 milk (BCM7) and associated disease problems. Till then, screening of all breeding bulls for their A1/A2 variant status would be a promising way to check the flow of undesirable alleles in our native breeds, which are natural resource for A2 milk.

**Recommendations**

1. In order to reiterate strengths and good qualities of indigenous cows, there is no need to denounce crossbreds and crossbreeding programme in India.

2. The hypothesis of A1 milk association with few chronic diseases drawn from a survey, done in population of western countries should not be extrapolated to India without credible evidence.

3. Considering the contribution of buffalo, indigenous cows and crossbred population in India's milk pool and based on the assumption that β-casein constitutes 45% of total protein and that around 25% of the β-casein may be from A1 allele cows, the average consumption of A1 milk in India would be around 0.24g/day, which is 5-10 times lower than countries surveyed. Therefore, there is no cause to show undue concern and panic.

4. The Indian scientists should not consider the issue as buried but remain vigilant and monitor the situation periodically.

5. The vested interest groups should not whip up passions and mis-information campaign but should leave such matters to the wisdom of scientists and the scientists should become more vocal in stating facts.

6. If there is demand from consumers for A2 milk as a matter of choice, the Government of India and State Governments should come up with policy guidelines and entrust the certification powers with the Registered Agency to protect the interest of consumers.

7. The huge potential of indigenous buffalo milk to cater to the growing demand of A2 milk and A2 milk based value-added products in the international market may be exploited by enterprising dairy units.

8. Further research, especially involving human trials, is needed before it can be said with confidence that the A1/A2 composition of milk is important in human health. A strong partnership between ICAR and ICMR is needed.
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LIST OF PARTICIPANTS

1. Prof Panjab Singh, President, NAAS, New Delhi
2. Prof R.B. Singh, Ex-President, NAAS, and Chancellor, CAU, New Delhi
3. Dr A.K. Srivastava, Vice President, NAAS & Chairman ASRB, New Delhi
4. Dr V.K. Bhatia, Editor, NAAS, New Delhi
5. Dr Kusumakar Sharma, Editor, NAAS, New Delhi
6. Dr P.K. Agrawal, ADG, NASF, New Delhi
7. Shri Mukesh Anand, Keshav Gaushala, Anand Ashram, Junjhunu, Rajasthan
8. Dr C. Anandharamakrishnan, Director, IIFPT, Min of Food Processing Industries, GOI, Thanjavur, Tamil Nadu
9. Dr Sumit Arora, PS, ICAR-NDRI, Karnal, Haryana
10. Shri Sanjay Bhalla, Prop., The Way We Were, Nizamuddin (W), New Delhi
11. Dr M.S. Chauhan, Director, ICAR- CIRG, Mathura, Uttar Pradesh
12. Shri Badri Narayan Chowdary, General Secretary, Bharatiya Kisan Sangh, New Delhi
13. Dr T.K. Dutta, PS, ICAR-NDRI, Karnal, Haryana
14. Dr Sitangsu Mohan Deb, PS, ICAR-NDRI, Karnal, Haryana
15. Dr (Ms) Rekha Dahiya, Assistant Professor, Lala Lajpat Rai University of Veterinary & Animal Science, Hisar, Haryana
16. Shri Parvesh Kumar Gupta, Partner, Gouamrit, Faridabad, Haryana
17. Shri Sharad Gupta, Editor & Publisher, Dairy India Yearbook, New Delhi
18. Dr Narayan G. Hegde, Trustee, BAIF, Pune, Maharashtra
19. Dr B.K. Joshi, Former Director, ICAR-NBAGR, Karnal, Haryana
20. Prof (Dr) Ashok Kale, Head, Ulhas Patil Medical College, Jalgaon, Maharashtra
21. Dr R.S. Kataria, PS, ICAR-NBAGR, Karnal, Haryana
22. Dr Sudarshan Kumar, Scientist, ICAR-NDRI, Karnal, Haryana
23. Dr Ashok Kumar, ADG (Animal Health), ICAR, New Delhi
24. Prof Sushil Kumar, INSA Honorary Emeritus Scientist, New Delhi
25. Dr S.S. Lathwal, PS, ICAR-NDRI, Karnal, Haryana
26. Dr M.L. Madan, Ex-DDG (AS), ICAR, Karnal, Haryana
27. Dr R.K. Malik, Emeritus Scientist, ICAR-NDRI, Karnal, Haryana
28. Dr Bimlesh Mann, JD (Res), ICAR-NDRI, Karnal, Haryana
29. Shri Sunil Mansinghka, AWBI-Member, Bharatiya Kisan Sangh, Nagpur, Maharashtra
30. Prof A.K. Misra, VC, GBPUA&T, Pantnagar, Uttarakhand
31. Dr Ashok K. Mohanty, PS, ICAR-NDRI, Karnal, Haryana
32. Dr A.K. Pande, Group Vice President (L.S. Devp.), BAIF Devp Res. Foundation, Pune, Maharashtra
33. Dr Ashish Motiram Paturkar, VC, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra
34. Dr G.S. Rajorhia, President, Indian Dairy Association, Karnal, Haryana
35. Dr Y.S. Rajput, Emeritus Scientist, ICAR-NDRI, Karnal, Haryana
36. Dr K.P. Ramesha, Head, ICAR-NDRI, Bengaluru, Karnataka
37. Dr S.K. Rana, Group Head (Animal Health), NDBB, Anand, Gujarat
38. Dr D.K. Sadana, Retd. PS, ICAR-NBAGR, Karnal, Haryana
39. Dr S.K. Sharma, Professor, GADVASU, Ludhiana, Punjab
40. Dr Ashish Kumar Singh, PS, ICAR-NDRI, Karnal, Haryana
41. Dr P.K. Singh, PS, ICAR-NBAGR, Karnal, Haryana
42. Dr R.R.B. Singh, Director, ICAR-NDRI, Karnal, Haryana
43. Dr Inderjeet Singh, PS, ICAR-CIRB, Hisar, Haryana
44. Dr Dheer Singh, PS & Head, ICAR-NDRI, Karnal, Haryana
45. Dr Gurdial Singh, VC, LUVAS, Hisar, Haryana
46. Dr Rajeev Singh, Assoc. Professor, SVBPUA&T, Meerut, Uttar Pradesh
47. Dr Rameshwar Singh, VC, Bihar Animal Sciences University, Patna, Bihar
48. Dr Monika Sodhi, PS, ICAR-NBAGR, Karnal, Haryana
49. Dr Girish Sohani, President and Managing Trustee, BAIF Dev. Res. Foundation, Pune, Maharashtra
50. Shri Rajendra Tamboli, A2 Milk Research Corporation, Tamboli Bhavan, Raipur, Chhattisgarh
51. Prof P.K. Uppal, Former Adviser, Govt. of Punjab, Department of Animal Husbandry, Dairying and Fisheries, Gurgaon, Haryana
52. Dr Chindi Vasudevappa, VC, NIFTEM, Kundli, Sonepat, Haryana
53. Dr Pawan Verma, Assistant Professor, SKUAST, Jammu
54. Dr Vikas Vohra, PS, ICAR-NBAGR, Karnal, Haryana
55. Dr M.P. Yadav, Ex-Director, ICAR-IVRI, Gurgaon, Haryana
56. Shri Ramesh Rawal, Director, Kisan Vikas Milk Producers Co. Ltd., Noida, Uttar Pradesh
57. Dr Sachinandan De, PS, ICAR-NDRI, Karnal, Haryana
58. Shri Tarun Jain, Partner, Gouamrit, Faridabad, Haryana
59. Shri Badri Norain Choudhary, All India General Secretary, Bhartiya Kisan Sangh
60. Shri Kapil Sood, Share Holder, Breeder Farmer Producer SICS, Ludhiana, Punjab
61. Dr B.S. Prakash, ADG(AN&P), ICAR, New Delhi
62. Prof Ramesh K. Yadava, Chairman, Haryana Kisan Ayog, Panchkula, Haryana
63. Dr Chandra, Professor, AAU, Anand, Gujarat
64. Dr Suresh S. Honnappagol, Animal Husbandary Commissioner/ CVO, DADF, MOA&FW, New Delhi
65. Shri Praveen Tyagi, President, Godhuli Godham Samiti, Ballabgarh, Faridabad, Haryana
66. Shri Subhash Chand, Palwal, Haryana
67. Shri Ravi Verma, Milk Research Corporation

Note: The designations and affiliations of the participants are as on the date of Strategy Workshop.