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Significance of Genetic Casein Polymorphism in Animal Husbandry

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Review Article

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ABSTRACT

Background: Milk proteins are a rich source of peptides which are bioactive. Beta Casomorphin-7 (BCM-7) is produced by successive proteolytic ingestion of beta- casein variants A1 and B, while not observed in A2 variant. The large amount of proteins in bovine's milk are caseins, which consist of four categories (αs1, αs2, β, and κ) encrypted by corresponding genes (CSN1S1, CSN1S2, CSN2, and CSN3, respectively). Ingestion of A1 beta-casein showed the way for the emergence of Beta Casomorphin-7 (BCM-7), a peptide that has been proposed to be a major cause of various human hazards.

Result: The relative profuseness of proline, methionine, and α-lactose were the highest in A2 variant of β-casein, while glycine, citric acid, choline, and cAMP (cyclic Adenosine Monophosphate) exhibited the highest abundance in A1 variant. These results thus come up with novel insights into the effect of casein variants, further expediting research into the biogenesis of milk ingredients and the prospective physiological activity of milk correlated with variants of β-casein.

Keywords: A1 Beta-Casein; A2 Beta-Casein; Beta-Casomorphin-7; Beta-lactoglobulin; Genetic polymorphism; Titratable acidity

INTRODUCTION

Milk is a well-balanced nutrient that consist of a carbohydrate, proteins, fat and minerals, of which the principle function is to certify growth of the mammals. The two major proteins in milk are primarily caseins and whey proteins. Caseins consist of 80% of cow milk protein, whereas whey proteins comprises of about 14% [1].

Caseins are divided into four groups, e.g., alphas1 (39%-40%), alphas2 (811%), beta (25-35%), and kappa (815%), respectively encoded by the *CSN1S1, CSN1S2, CSN2*, and *CSN3* situated on chromosome number 6 as proposed by Sebastiani, et.al., The ingredients of the case in fragments and the chemical structure of its discrete constituents are well studied, in some species, the close positioning of the four casein chains (alphas1, alphas2, and beta and kappa casein). A vast number of diversified models have been postulated by Schmidt, Walstra and Jenness and Holt based on the presence of submicelles which is now being considered. The main function of casein is to deliver calcium phosphate. Among the total protein content, 36% is constituted by beta casein which plays a pivotal role in curd making and it regulates the surface quality of micelles which are essential properties for cheese formation. In cow's milk, there are 12 beta casein variants *viz,* (A1,A2,A3,B,C,D,E,F,H1,H2,I and G).The A2 beta casein variant is contemplated to be the oldest variant from which the others evolved *via* genetic mutation [2]. Farrell, et al., demonstrated that the most common configuration of variants of beta-casein such as A1 and A2, whereas B is less common, and A3 and C are the least. Groves and Roginski proposed that at position 67 of the casein chain, amino acid proline A2 variant is replaced by the amino acid histidine in A1 variant. Throughout the world, the most popular variants are A1 and A2 which are found in dairy cattle such as Holstein-Friesian. The kappa casein gene constitute a 13 kb sequence subdivided into five exons as studied by Alexander, et al., Alipanah, et.al., enunciated that exon IV, pint mutation of the bovine κ-casein (*CSN3* gene) govern two genetic variants *viz,* A and B. The A variant and the B variant are differentiated in the amino acids 136 and 148. Alexander, et al., opined that at position 136, isoleucine replaces threonine, whereas at position 148, alanine replaces aspartic acid, for A and B variants respectively [3]. This variation which is related with processing qualities like cheese production technology as proposed by Alipanah, et al., and physiological processes such as antibacterial and cytotoxic effects, enhances the innate immunity which is advocated by Hamza, et.al., Azevedo, et.al., enunciated that the B allelic gene has some marvellous properties *viz.,* thermal resistance, brief coagulating time, superior curdles preparation, and micelles of various sizes which are highly suitable for cheese production [4]. The genotypes of beta case in have been discovered by estimating the existence of SNPs (Single Nucleotide Polymorphisms) of the CSN2 gene by the help of polymerase chain reaction and sequence analysis [5].

LITERATURE REVIEW

β-casomorphins

β-Casomorphins (BCMs) is a bio peptide which has been originated from β-casein having a chain length of four to eleven amino acids, all starting with amino acid tyrosine in position number 60. Meisel and Fitzgerald enunciated that there are various ranges of bio peptides which are having opioid like activity that is derived from all the beta-case in

groups (α-casein, β-casein and κ-casein), whey proteins (α-lactalbumin, β-lactoglobulin) and serum albumin. Nguyen, et al., identified that ceruloplasmin, S100-A9 protein, and cathelicidin-2 had the highest presence in A1 variant of bovine milk while antithrombin-III, S100-A8 protein, and S100-A12 protein had the highest cornucopian in A2 variant [6].

β-casomorphin-7 (BCM-7)

Brantl, et al., first identified a bio peptide which having morphine like function and christened that peptide as Beta Casomorphin 7 (BCM7). It has been shown to invigorate human T lymphocyte expansion *in silico* [7]. According to the epidemiological evidence from New Zealand, it can be suggested that A2 variant is much healthier for consumption than A1 variant and also much better for human health. Relationship as postulated by McLachlan that A1 variant of beta-casein, or possibly its residue of the bio peptide, beta casomorphin-7, may be a remarkable source for the chronic heart disease [8].

Metabolomics

Zhong, Wang et al., have identified several metabolites having large quantities of two variants *i.e.,* A1 and A2 consisting of choline, phytosphingosine, and cyclic adenosine monophosphate which has been at remarkably higher levels, while oleamide, methionine, phenylalanine were analysed at notably lower level sat A1 beta-casein variant. Numerous metabolites *viz.*, cAMP, acetylcholine, and choline were identified at strikingly lower presence, while cytosine, uric acid, oleamide were significantly more profuse in heterozygote A2 variant than A1 variant of milk. Myo-inositol and methionine were notably less copious albeit, cytosine, uric acid, tryptophan, phosphocholine were significantly more plenteous invariant A2 than A1 variant [9].

Genetic polymorphism

Some scientists have opined that the A2 allele of the beta casein (*CSN2*) has a positive association between milk yield and protein. The beta casein A1 allele differs from the A2 allele due to a substitution of the amino acid at position 67 from a histidine (CAT) to a proline (CCT), caused by SNP (Single Nucleotide Polymorphism) [10]. The presence of the beta-casein A1 in milk causes the cleavage of the peptide bond resulting in the digestion process, releasing bioactive peptides such as β-Casomorphin-7 (BCM-7); the presence of the A2 allele prevents the hydrolysis of the peptide bonds between residues 66a and 67a which prevent the release of BCM-7 as proposed by Sharma, et al., According to Deth, et al., consumption of A2 milk increases the production of Glutathione (GSH), and antioxidant that is widely reputed for its nexus with myriad of health benefits. The study showed that the consumption of milk containing only the A2 variant of bovine milk elevates the GSH level in the blood to approximately twice the level which is associated with the consumption of milk consisting of both the variants A1 and A2. The allele variants that are regarded as risk factors for human health are those that contain histidine at position 67, while those that consist of proline, *i.e.,* the A3 and variants which behave like the variant A2 although they contain other Single Nucleotide Polymorphisms (SNP) in position 106 and position 93 respectively [11].

Caldow, et al., demonstrated that glycine, choline and cAMP were the most plenteous metabolites identified in variant A1 milk of beta casein. Additionally it operates as an activator of mammalian target of rapamycin signalling pathway for protein biosynthesis, whereas glycine also participates in the synthesis of glutathione, nucleic acids, and uric acid.

Glutathione is one of the antioxidant agents in cells [12]. Choline has been regarded as a primary nutrient. Choline concentration in human milk was shown to be stable not withstanding of maternal age and intake. For the biosynthesis of acetylcholine, choline acts as a precursor (a major transmitter for nervous system and leukocyte) and various phospholipids, which are dispensable components of the cellular membranes, and further provides for modulating macrophage interleukin (IL)-1β and IL-18 production. Farrel, et al., reassessed the composition of amino acids as well as genetic variants of six milk proteins *viz.,* (αs1-casein, αs2-casein, β-casein (β-CN, CSN2), κ-casein (κ-CN, CSN3), β-Lactoglobulin (β-LG,LGB), and α-lactalbumin) which are permeated in cattle [13]. There are innumerable scientific reports which corroborated the pertinence of different variants such as β-casein, κ-casein, and β-lactoglobulin genetic polymorphisms to milk production and milk composition characters. Numerous scientists enumerated that the joint venture of *CSN2, CSN3*, and *LGB* genotypes, and it is generally regarded more suitable to peruse haplotypes or composite genotypes preferable than single genes in bovine cattle selection. Kyaclova, et al., studied that the influence of *β-LG* and the two most polymorphic *β-CN* and *κ-CN* genotypes on acid-persuaded gelation of cattle milk [14].

The most abundant genotypes at the *CSN2* and *LGB* loci were both the heterozygotes A1 A2 and AB respectively. No cattle were found to carry A3 allelic gene. At the *CSN3* loci, AA and AB were the most customary genotypes. The lowest presence among all the three genes were explored, *viz.,* CSN2BB, CSN3BE, and LGBAA genotypes. The highest, and therefore in terms of milk characteristics, the most advantageous TA (Titratable Acidity), was linked with the A1A2 BB AA genotypes, whereas the lowest TA was discovered in the milk samples with the A1A1 AE BB genotypes. Mayer, et al., enumerated that the interconnection between *CSN2*, *CSN3*, and *LGB* loci was well coordinated to the total protein, casein and whey protein ingredients of bovine milk [15]. Gencurova, et al., emphasized that TA was positively interconnected with the protein as well as phosphorus. The acidity of milk criterion *viz.,* pH, TA, and milk protein genetic polymorphism have been enunciated as being highly correlated to the coagulation characteristics for cheese production. Rangel, et al., observed that the appearance of more than one animal with A1SNP in the *CSN2* gene was identified, while on the other hand these animals were heterozygous (A1A2), whereas the existence of homozygous animals for the A1 allelic gene was not detected [16].

Effects of a single gene on the casein variants of bovine milk

Based on the statistical analysis of Kyaclova, et al., the effects of *CSN2, CSN3* and *LGB* were detected on the investigated parameters, which also includes the F test values. Amidst all the evaluated physiochemical and fermentative characteristics, it was found that just the Alcohol Stability (AS) was remarkably influenced by the CSN3 genotype, whereas neither the *CSN2* nor the *LGB* gene exhibited any particular effect. The milk constancy with the *CSN3BE* genotype was the highest; contrarily, the BB genotype exhibited the lowest stability. The most common genotype, *CSN3AA* performed a middle value for the stability of alcohol. Much attention should be paid on breed, milk yield, seasonality, mineral content, and milk composition. Feable, non-significant relationships were investigated between both variants of *CSN3* and milk phosphorus and between calcium content and *LGB*. The highest content of phosphorus was cognate with *CSN3BE* whereas the lowest phosphorus was connected with *CSN3AE*. There was a commendatory influence of the *CSN3B* allelic gene on the phosphorus content of milk, while the negative effect of the E allele was partially approved [17].

Effects of composite genotype on the characteristics of milk

It was observed that the effects of the composite genotypes mostly indicated the effects of the individual genotypes. The most influenced was milk TA (Titratable Acidity) which was accompanied by Y-pH (Yoghurt pH) and log LC (log of total count of lactobacilli (cfu/mL)). On the contrary, the significant effect of CSN3 gene and the observed proclivity for LGB, no relation between AS and the composite genotype was determined. No nexus was observed between the genotype and the content of Y-TA (Yoghurt Titratable Acidity), pH, other minerals or log SC (log of total count of Streptococci (cfu/ml)) [18].

Type 1 Diabetes Mellitus (DM-1)

It was observed by Elliot that very low level of prevalence of DM-1 among children in Polynesian islands like W Samoa, in contrast with Polynesian children in Auckland. It was found that prolonged breast feeding was highly protective against DM-1. Cow's milk antibodies were detected at higher levels in diabetic children than in placebo. Cow's milk beta-casein contains 209 amino acids. The two most important variants of beta casein *viz.,* A1 and A2 were found to differ at position 67, which is histidine in A1 or proline in A2 milk. A comparison was done by Elliot, et.al., with DM-I incidence in between 0 to 14 year old children from 10 different developed countries such as (Australia, Canada, Denmark, Finland, Germany, Iceland, New Zealand, Norway, Sweden and USA) with the national annual cow milk protein utilization and they established the consumption of total milk protein and A1, Bβ casein variants of bovine milk. FAO data was obtained by them for breed conformation, information and milk protein ingestion of the cows and their genetic milk protein polymorphism. They observed that the total milk protein did not corroborate significantly with DM-1 but A1 beta casein did an equivalence which was even vigorous with A1 and B β-casein variant of bovine milk [19].

Coronary Heart Disease (CHD)

From New Zealand, McLachlan assembled data for a significant relation of mortality from CHD in 16 countries with the consumption of A1 beta casein variant. The evidence of McLachlan's contended that consumption of a particular common variant of the milk protein beta-casein (A1) aggravates the development of coronary heart diseases in human beings [20-23]. During this second world war, CHD mortalities in different countries have been closely investigated, rates have come down in some countries such as Australia, Finland, and USA significantly elevated in other countries particularly in Eastern Europe. In 2002, Ness, et al., established the outcome for CHD mortality and all causes mortality in a group of 5765 men enrolled from work places in the west of Scotland and the result was scrutinized for 25 years. BCM-7 (β-casomorphin-7) has a physiological effect in A1 beta-casein variant on the oxidation of LDL (Low Density Lipoprotein) or peroxidation of a lipid component of LDL, considered as a confirmative as pectin the initiation of CHD has been shown by Elliot, et al.

DISCUSSION

The method suggested for the appraisal of beta-casein genetic polymorphism resulting in a simple typing system, which grants genotyping of the most beta-casein variants. The combination of fairly low cost and rapid implementation, along with the high sensitizing conscientiousness, make it highly acceptable for routinely and large scale selection of beta-casein variants in dairy cattle cows. Several previous studies have revealed that genetic

variants of beta-casein were remarkably related with major milk proteins, e.g., αs1-casein, β-casein, κ-casein, and β-lacto globulin. Beta-casein variants A1 and A2 have been evaluated to contribute to a difference in casein micelle ensemble and molecular chaperone achievement. These results enumerated that beta-casein variants can contribute milk constituents, even from a part of the fraction of milk [24].

To establish the usability of milk for fermented milk products, the acid fermentation test (*i.e.,* yoghurt test) is generally considered. A fermented gel made from milk is called yoghurt which is formed by mixing a starter culture mix of lactic acid bacteria, which usually includes Lactobacillus sp and Streptococcus sp. The assessment of the prevalence of occurrence of beta-casein *CSN2* gene in dairy cows is the main objective of the scientists. The evaluation of allelic gene and genotype frequencies is the most essential for genetic scrutinizing programme for these animals with the final objective for the production of A2 cow's milk. The A2 beta casein variant was the most abundant form found with a frequency of 60.65%, followed by the A1 variant with a frequency of 30.39%, the B allelic gene at 5.68%, the I variant at 3.10%, the A3 allelic gene at 0.15% and the C allele with a frequency of 0.03%. More work should be done on F variant, E variant, D, G, H1, and A2 variants. Kaminski, et al., enunciated that both A1 and A2 beta casein variants are the most abundant in the Holstein Friesian breed. A breed is considered as ideal for the selection of A2 variant its frequency is approximately close to 50%. If we consider the Mendelian inheritance of the trait and contemplating that the population is in the Hardy Weinberg equilibrium in the cattle group, we would anticipate to perceive 25% homozygous A1 variant animals, 50% A1A2 heterozygotes, and 25% A2 variant homozygous cattle, and that the manufactured bulk milk consist of 50% A1 beta casein variant and 50% A2 beta casein variant as proposed by Canavesi [25].

There are some specific problems associated with the A1 beta-casein-DM-1 correlations. The most unpredictability lies with national figures for A1 beta-casein variant than for total milk casein protein and more uncertainties for these than for average milk utilization. Several scientists have delineated that addition of skim milk or proteins of cows' milk or casein to basal diets elevates the diabetes preponderance in Bio Breeding (BB) rats or Non-Obese Diabetic (NOD) mice. Incorporation of milk protein or bovine serum albumin to a diet which is based on hydrolysed protein did not influence the occurrence of DM-1 in BB rats. According to the postulation of Laugesen and Elliott, Austria and France were evaluated to have equivalent beta-casein consumption (0.93 g/day) but heart disease mortalities of 88 and 33 per 100000 respectively. Following countries like Iceland, island of Jesey, Germany, Sweden, Israel, Australia, Canada all had heart disease mortalities ranging from 70 and 80 per 100000 but their A1 casein intake lies between nearly the highest in the world (2.8 g/day in Sweden) to the lowest (0.3 g/day in island of Jersey). Plasma cholesterol-raisin effect of beta casein when resembled with soya protein, which is best studied in rabbits [26]. The concept of a cholesterol-decreasing milk factor arising from the monitoring on the Masai tribe in East Africa, some of whom ingested immense amounts of fermented milk but have low plasma cholesterols and rarely experience CHD. A list of 23 dietary factors as tabled by the World Health Organization's (WHO) Expert Consultation on chronic diseases, nutrition, and diet as associated (or not associated) to CHD. The appearance of milk does not occur in the summary table. After all milk in most developed countries consists of considerable proportions of A1 beta-casein variant, it follows that this distinct variant of a crucial cow milk protein is not generally considered as a risk factor for CHD.

CONCLUSION

The effect of beta-casein variants on the metabolites of cow milk were evaluated using NMR (Nuclear Magnetic Resonance) and LC-MS (Liquid Chromatography Mass Spectroscopy) which is dependent on metabolomics techniques. Numerous metabolites such as alpha lactose, proline, and methionine were found to be more copious in A2 milk, while citric acid, glycine, choline and cAMP glycine, were more profuse in A1 milk variant of beta casein. These studies give novel perceptions into the differentiation of milk constituents amid beta-casein variants at the metabolite level. The A2 variant of beta casein is desirable in milk because it elevates the milk digestibility. There is a relation between genetic polymorphism and the AS, milk TA, calcium in milk, and the contents of phosphorus, yoghurt pH, and the number of fermenting Lactobacilli. Recently, the genetic polymorphism of beta-casein has accepted appreciable interest in dairy industry as well as in animal breeding because of their action innumerous aspects of human health and nutrition.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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AUTHOR CONTRIBUTIONS

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