Effect of Protein Genotypes on Physicochemical Properties

Subjects: Agriculture, Dairy & Animal Science

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High protein content was reported by Ikonen et al.in milk genotyped A1A1-BB, A1A2-AB and A1A1-AB, while a low protein content was related to the A1A1-EE genotype. For the composite genotype of αS1-β-κ-CN, B-A1-B was reported to be positively correlated with percentages of fat and protein in Holstein cows, Brown Swiss cowsand Finnish Ayshire cows, as well as in a local Italian Reggiana cows, but negatively correlated with milk yield. Haplotype C-A2-B has similar effects to B-A1-B, and also leads to low milk yield and high protein concentration.

protein genetic variants genotype frequency milk physiochemical properties milk functionality

1. Introduction

As the demand for milk and milk products increases continuously, and since milk provides essential nutrients in the human diet [1][2], studies on milk and dairy products have generated a lot of attention in dairy related research.

Protein is a macronutrient for the human body [1], and accounts for about 3.5% of milk mass, typically comprising approximately 80% casein and 20% whey protein [2]. Four forms of casein are found in milk protein, including α S1 -CN, α S2 -CN, β-CN, and κ-CN, and their genes are found at bovine chromosome 6 [3][4], coded as CSN1S1, CSN1S2, CSN2 and CSN3, respectively [1][5]. These proteins have several genetic variants, as described by Caroli et al. [6] and Farrell et al. [7]. The gene of α-lactalbumin (α-lac) in the whey protein fraction is located on bovine chromosome 5, coded as LAA [3], and that of β-lactoglobulin (β-lg) is coded by the PAEP gene (or LBG gene) [1], which is situated on bovine chromosome 11 [8]. Polymorphisms of CSN1S1, CSN2, CSN3 and PAEP have widely been studied [6][9], but only a few polymorphs of LAA and CSN1S2 have been identified, mainly in French breeds [10]. The selection of milk protein phenotypes is regarded as a practical way for altering the composition of milk protein, and traditional methods for improving milk quality included estimating the bull breeding values by the phenotypes of their numerous female offspring [10].

2. Milk Protein Genetic Variants and Genotyping Frequency

Establishment of protein genetic variants discussed above is shown in **Table 1** ; methods used to determine genotypes are listed, except where these were not clearly stated in the entry.

Table 1. Establishment of main protein genotypes in bovine milk.

Protein	Genotype	Methodology	Date
β-lg	Variant A, variant B	Electrophoresis	1958 [11], 1959 [12], 1961 [13]
	Variant C	Electrophoresis	1962 [14]
	Variant D	-	1966 [15]
	Variant E, variant F, variant G	Electrophoresis	1957 [16], 1963 [17] 1970 [18], 1973 [19], 1976 [20], 1981 [21]
	Variant H	IEF-IPG	1988 [22][23]
	Variant W	chromatofocusing	1990 [24]
	Variant I, variant J	Ion-exchange chromatography	1996 [25]
α-lac	Variant A, variant B	Electrophoresis	1963 [26][27]
	Variant C	Electrophoresis	1981 [28]
	Variant A, variant B, variant C	Electrophoresis	1962 [29][30]
	Variant D	Electrophoresis	1965 [31]
	Vaiant E	Electrophoresis	1963 [17], 1971 [32], 1976 [20]
α_{S1}-CN	Variant F	pI	1993 [33]
	Variant G	Endonucleases	1992–1994 [34][35][36]
	Variant H	pI	1999 [37]
	Variant I	IEF, PCR	2009 [38]
α_{S2}-CN	Variant A, variant B, variant C, variant D	Electrophoresis	1984 [39]
β-CN	Variant A, variant B, variant C	Electrophoresis	1961 [40], 1963 [41], 1964 [42]
	Variant A_1, variant A_2, variant A_3	Electrophoresis	1966 [43][44]
	Variant D	Amino acid composition	1969 [45]
	Variant E	-	1972 [46], 1974 [47]
	Variant A_4	Electrophoresis	1981 [28], 1995 [48]
	Variant B_Z (special case)	Peptide profiling	1970 [49]

Protein	Genotype	Methodology	Date
	Variant F, variant G	RP-HPLC	1995 [50], 1998 [51]
	Variant H_1	Electrophoresis, PCR	2000 [52]
	Variant H_2	LC-MS	2002 [53]
	Variant I	PCR	2002 [54]
	Variant A, variant B	Electrophoresis	1966 [55], 1975 [56]
	Variant J	RP-HPLC	1999 [37]
	Variant B_2	Nucleotide sequencing	1987 [57]
	Variant C, variant E	RP-HPLC	1993 [58]
κ-CN	Variant F_1	PCR	1992 [59]
	Variant F_2	PCR	1996 [60]
	Variant G_1	IEF	1996 [61]
	Variant G_2	PCR	1996 [62]
	Variant H, Variant I	DNA sequencing	1999 [63]

In several studies, frequencies of these protein genetic variants have been reported, as discussed below.

The main variants of β-CN are A 1, A 2, A 3, B and C [54][64]. The A 2 variant is regarded as the ancient original variant, while A 1 is the product of mutation through natural selection [65][66]. It is important to note that the A 1 variant is only found in bovine milk [65][67] and commercial bovine milk often contains both variants [68].

Genotype frequencies of β-lg among breeds vary, where the A variant is more frequent than B in Holstein-Friesian cows, while B is more frequent than A in Jerseys cows [10][69][70] and Norwegian Red cows [71]. BB is more common than AB or AA in Norwegian Red cows [71], while AB is more common than AA and BB in Czech cows [72]. In Finnish Ayrshire cows, the AA variant is the rarest [73].

3. Impact of Protein Genotype on Milk Protein Structure

Protein structure and functionality are closely linked [74] and are the basis of its interaction with other milk components [75]. In product processing, some undesirable behaviours are associated with protein structures, or changes in structure during processing, such as gelling in processing equipment, or non-coagulation in milk curd processing, i.e., cheese-making [76].

The structures of the main proteins in bovine milk, including β-CN, α S1 -CN, α S2 -CN, κ-CN, α-lac and β-lg are influenced by genetic variants, as these lead to modifications of amino acid sequences [77]. These structural differences affect milk composition and quality, as well as the isoelectric points and electric charges of the proteins [7][9], and ultimately influence the physicochemical properties of milk [69].

However, it has been concluded in an European Food Safety Authority (EFSA) science report in 2009 that no relationship exists between the consumption of A 1 milk and reported illness [78], while Küllenberg de Gaudry et al. [79] reported that the correlation between the consumption of A 1 or A 2 milk and negative effects on human health are not significantly or clinically different, and that results of relevant studies are inconclusive due to the insufficient evidence or uncomprehensive study design.

In addition, the substitutions at position 67 and 122 of the A 1 and B variants exist in the hydrophobic part of β-CN, which could affect milk functionality, i.e., emulsifying properties [80]. The B variant has one or two more positive charges compared to the A 1 and A 2, respectively, which allows it to more easily bind with other functional proteins [80].

4. Milk Coagulation

Milk coagulation properties, including rennet coagulation and acid coagulation properties, are the basis of cheese-making, and cheese yield and quality depend on rennet and acid coagulation properties of milk [70][81]. These properties are influenced by milk composition [71], casein micelle size [82][83], milk protein genotypes [70], milk protein content and composition [70][82], proportion of caseins and whey proteins [84], mineral and total salts contents and their distributions [70][83], as well as cow's health status [85][86], lactation stage [87], breed [81][88], season [89] and feeding [90].

To define milk rennet coagulation properties, some key parameters may be measured using a Formagraph, including rennet coagulation time (RCT), curd firming time (k 20, in min) and curd firmness (a 30, in mm) [91]. Gel formation can also be determined using rheology, through measurement of G', the storage modulus, with RCT being determined from the time when G' begins to increase [92].

Milk composition is an important parameter which affects milk coagulation properties [71]. Higher protein content improves a 30, GFR and G 30, and impairs k 20; higher casein content has a positive effect on a 30, GFR and G 30, and a negative effect on k 20 and GT; higher fat content leads to shorter RCT but produces weak acid gels, and higher lactose content is associated with better rennet and acid coagulation properties [93][71][94]. An optimal fat-to-casein ratio is also important for good milk coagulation properties [95].

Casein micelle size and fat globule size could affect milk rennet and acid coagulation properties; larger fat globule size leads to poorer acid coagulation properties, and larger casein micelles are associated with weak acid and

rennet gels [71][82][96]. The beneficial effect of small micelle size on coagulation might be due to the large surface area for gel network formation [71], which leads to faster aggregation and stronger gel formation [82].

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