MILK PROTEINS GENETIC ASPECTS RELATED TO HUMAN HEALTH

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Abstract

In general, milk quality depends on its chemical composition and on some key proteins' genetic variants. Cow`s milk shows a various protein profile based on specific polymorphisms, which are related to human health, throughout positive associations with different diseases occurrence or prevention. Caseins are the main proteins of cow's milk and β-casein of the caseins, showing a variable genetic profile, but with two most common variants, A1 and A2, which are still under debate on their effect on human heath, in the context of a growing global interest for A2-milk. Also, other milk proteins with genetic polymorphic variation show potentially benefic effects for human health. Dairy cattle genetic management could be directed for using favorable milk genetic profiles, by genotyping means. Since many countries, including Romania still need to make progress towards implementing and using widely available genotyping facilities for such purposes, the present paper aims to highlight some relevant aspects and the necessity of knowing the prevalence of milk proteins variants in indigenous as well as cross bred cattle population for targeting adequate breeding strategies based on health concerns of milk proteins genotypes.

Key words: milk protein, genotypes, cattle, human health

INTRODUCTION

Disease prevention, including cancer is a major concern all over the world. World Health Organization reported an increasing global share of noncommunicable diseases deaths ranging from 60.8% in 2000 to 73.6% in 2019 [25], meanwhile GLOBOCAN a global cancer burden of 19.3 million cases and about 10.0 million cancer deaths for 2020 with a higher overall incidence in transitioned versus transitioning countries (from 2-fold to 3-fold higher) for and a higher mortality in men, and an increasing trend of 47% from 2020 i.e., of 28.4 million cases also with a higher increase in transitioning (64% to 95%) versus transitioned (32% to 56%) countries [21]. Since many factors related globalization and growing economy are responsible for such effects, prevention measures are critical for disease and also cancer control and management, especially in transitioning countries. In terms of reducing mortality and improving global life expectancy and healthy life expectancy recommendations related all implied factors are addressed to overcome this issue, including dietary.

Milk and dairy products are highly recommended in dietary guidelines as health-promoting foods [14]. Milk and milk-based products, especially from cow's milk are essential in human daily diet due to their components, being widely consumed by humans. Moreover, regular intake of milk and milk products was proved to be inverse associated with development of some cancer types [3]. Also, it is well known the debate over milk proteins quality, due to their genetic variants and disease occurrence and progression [1].

Additionally, an increased special interest for some milk components and their effects is of interest lately mainly related to their potential association to various diseases [13].

Milk proteins such as caseins have a variation profile based on the encoding genetic background showing a spectrum of effects related human heath not fully

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understood. Identification of milk protein genetic variants in various cattle breeds population and defining breeding strategies related to health concerns should be considered a strategy to be started and implemented at national level. This paper aims to highlight some relevant aspects and the necessity of knowing the prevalence of milk proteins variants in indigenous as well as crossbred cattle population for targeting adequate breeding strategies based on health concerns of milk proteins genotypes.

COW`S MILK COMPOSITION

Cow`s milk contains water (86–88%), milk fat (3–6%), protein (3–4%), lactose (5%), and minerals (0.7%) [7].

Milk contains 2 major protein groups: caseins and whey proteins [11]. Milk composition shows a 3.2 % of protein content and in proteins about 80% are caseins and 20% are whey proteins comprising α-lactalbumin and β-lactoglobulins.

Bovine milk contains 4 caseins: alpha s1 (CSN1S1), alpha s2 CSN1S2), beta (CSN2), kappa (CSN3), and also gamma casein, which is a product of degradation of beta-casein. Of the casein class of proteins harboring several protein types (α- casein, β-casein, κ-casein and γ -casein), β-casein is the most abundant of total milk protein (25-35%) [13]. Whey protein content harbors: beta-lactoglobulin (LGB), alphalactoalbumin (LALBA), immunoglobins (IgGs), glycomacropeptides (GMP), bovine serum albumin (BSA), and minor proteins, such as lactoperoxidase, lysozyme and lactoferrin (table 1).

COW`S MILK PROTEINS GENETICS RELATED TO HUMAN HEALTH

Caseins from milk form macromolecular aggregates i.e., casein micelles between 30-300 nm. The casein phosphoproteins can be classified in four families based on their primary amino acid structure homology, each showing several variants according to the genetic heterogeneity [8].

Casein and whey protein fractions as α-, β-, k-caseins, lactapin (k-casein fragment), PGPIPN (β-casein fragment), INKKI (β-casein fragment), casomorphin, casein phosphopeptide and others are known for antitumor activity [13].

Moreover, depending on cattle breed, all casein families show various genetic variants resulting in a many different biologically active protein fragments after gastrointestinal digestion or food processing processes as hydrolysis or fermentation [15].

Milk quality can be related beta-casein genetics, which shows various genetic variants, one is believed to be linked to disease progression i.e., cardiovascular, metabolic and nervous. β-caseins show 13 different genetic variants (A1, A2, A3, A4, B, C, D, E, F, H1, H2, I, G) the most common being A1 and A2, B variant being less common and A3 and C variant being rare [11], data related to the variation of β-casein in the amino acid sequence being presented in table 2. Beta-casein is encoded by CSN2 gene has a SNP in exon 7 of CSN2 which is believed to be associated with human health named as A1A2 SNP. A1 and A2 variant variation consist in a cytosine (C) to adenine (A) nucleotide substitution changing histidine (present in A1 type) with proline (present in A2) at 67th position of beta casein polypeptide [4]. A1 beta-casein is mostly found in European cattle`s milk, while cattle of Asian or African origin secretes A2 beta-casein [1]. Related beta-casein variant presence in A1 and A2 variant of beta-casein can be assessed in milk or in animals at genetic level, related the homozygous or heterozygous state of A1 and A2 genotype, so that milk resulted from A2 genotype will contain only A2 variant of beta-casein, meanwhile heterozygous genotypes will lead to both A1 and A2 variant of beta-casein. ``A1 and A2 Milk`` is still a global debate over milk quality and its relevance for disease occurrence. A1 milk consumption is associated with occurrence of many diseases including type 1 diabetes, cardiovascular diseases, autism, schizophrenia and auto immune disorders [26].

Milk proteins show different physiological functions related to their potential to release bioactive peptides after enzymatic digestion, which in the gastrointestinal environment are activated. Some milk proteins represent a source of active peptides named opioids, being chemical substances with a morphine-like activity in human body, playing a role in the response to stress and pain and food intake regulation, which bind to opioid μ-receptors, mainly found in the central nervous system and the gastrointestinal tract [23]. Milk proteins are in an inactive state within the sequence of the parent protein, but they can be released during gastrointestinal digestion (hydrolysis) or food processing (digestion or maturation throughout technological processing), or produced by the body itself, leading to the formation of various bioactive peptides [11].

Casein phosphopeptides result by gastrointestinal trypsin digestion from αs1-, αs2-, or β-caseins (varying based on their extent of phosphorylation) and are carrier molecules for minerals (as magnesium, iron, and trace elements such as zinc, barium, chrome, nickel, cobalt, and selenium) by forming complexes, mainly with calcium, and modulate their bioavailability [12].

Beta-casomorphins (BCMs) are bioactive peptides originating from β -casein, the most common forms being BCM-7 and BCM-5.

During gastrointestinal digestion, the beta-casomorphin-7 result from A1 bovine milk proteins, being considered the primary causative factor for health and digestive disorders associated with A1 milk. BCM-7 shows a strong opioid activity having a role in stimulating human lymphocyte T proliferation in vitro, cytomodulatory properties, etc. The hydrolyzed milk with A1 variant of beta-casein contains a 4-fold higher level of BCM-7 than in A2 milk [23].

Moreover, BCM-7 may be a risk factor for various human diseases such as ischemic heart disease, atherosclerosis, type 1 diabetes, sudden infant death syndrome [20, 22], autism, schizophrenia [5, 18]. Moreover, there is no proved association between the presence of A2 β-casein and cow milk protein allergy (CMPA) or health problems [15]. A2 milk generates another peptide called BCM-9 [10].

The northern European cattle breeds such as Holstein, Friesian, Ayrshire and British Shorthorn in general produce milk with a high A1 β-casein content, meanwhile breed from the Channel Islands and southern France, such as Guernsey, Jersey, Simmental, Charolais and Limousin produce A2 β-casein milk [11]. and indigenous cows and buffaloes from India and other Asian countries mostly show A2 β-casein milk, too [18, 15].

αs1-casein milk leads to a firmer curds production and a higher cheese yield, on opposite to milk having higher αs2-casein, which leads to a softer curd and a lower cheese yield, but with an enhanced digestibility, showing benefic effects in human nutrition. Also, milk showing high αs1-casein is linked to higher incidences of milk allergy, digestive problems and CMPA patients [16, 15].

Other bovine milk proteins such as kappa-casein (CSN3) and beta-lactoglobulin (β-LG) are linked to important economically traits, genetic variants of this genes being associated with milk composition, quality, production, and also cheese production [19].

CSN3 gene in bovine shows 11 genetic variants associated with milk composition, quantity, quality and also processing, of which A and B alleles being the most common. Allele A in CSN3 gene is related to milk quantity and a lower protein content, meanwhile allele B is related to a lower milk quantity and a higher protein and fat content [6, 2].

The bovine β-LG gene located on chromosome 11 has 15 alleles determined and of the two most common variants investigated (A and B variants), variant B is related to milk quality and variant A to milk quality [27]. β-lactoglobulin, which is the most abundant non-casein protein in cow`s milk, have a molecular weight is 18.3 kDa (162 amino acid residues) and shows two main genetic variants A and B, based on two-point mutations under physiological pH and ambient temperature and function as a carrier protein for a variety of hydrophobic molecules, like retinoids (e.g., vitamin A), lipids, and polyphenols protecting them against oxidative damage or increasing their solubility [9].

Milk proteins genes such as CSN1S1, CSN2, CSN3, LGB show potential to be used in animal breeding programmes for improving or changing milk proteins profile and in this way the precursors for bioactive peptides. Implementing a simple selection of both genotype B in β-lactoglobulin gene and the A2B haplotype β–κ-casein for a higher milk protein content, of BB and AB genotypes in CSN1S1 for a increasing milk protein percentage could stand for such purposes [24].

Milk bioactive peptides content shows differences related to the immune system between cattle breeds. For example, Holstein has lower ectonucleotide pyrophosphatase and chitinase domain-containing protein 1, and higher lactoferrin concentrations compared to Jersey, meanwhile it seems that osteopontin, lactoperoxidase, and growth factors including insulin-like growth factor (IGF) and transforming growth factor-β does not show differences between these breeds [24].

ROMANIA STATUS RELATED TO MILK PROTEIN VARIANTS SELECTION IN CATTLE

Dairy cattle farming in Romania produce milk, which is processed and marketed, but a special interest should be addressed to the genotype status of cattle livestock, for further adequate certification based on specific genotypes of milk proteins related human health.

β-casein polymorphisms assessment along with other milk proteins variants is important due to their ability to influence the casein micelle structure and thus milk properties and milk products i.e., yoghurt, cheese.

Microarray technologies and systematic genotyping for beta-casein or other milk proteins are useful tools for monitoring milk proteins frequencies in bulls and also in cows, and also for selection of desirable genotypes of milk proteins, specifically A2 genotypes and selective commercialization of bull semen for obtaining cattle herds producing A2 milk. All together highlight the milk proteins variants importance for human health, especially for those at high risk for various susceptible diseases.

In Romania semen genetic resources commercialized show description of allelic variant of milk proteins, including those of kappa- and beta-caseins genotypes, for various bulls, but also bull semen not offering such specification can be found on market. Genotyping current cattle livestock population and using genetic resources with known and targeted genotypes could stand for such purposes and could contribute to a healthier milk production in our country. Local cattle breeds in Romania could be a valuable genetic pool for healthy milk protein variants. Further studies in cattle livestock genotyping still need to be assessed to establish the genetic polymorphic profile of milk protein in local breeds. Trade promotion and development of animal breeding sector in Romania by choosing heathy milk protein variants and targeted campaign for farmers could have an important impact for health sector too.

Table 1 Cow`s milk composition (data adapted from [24])

Cow`s milk components							
Water	86-88 %						
Fat	3-6 %						
Protein	3-4 %	Insoluble casein proteins	80 %	α - casein	α_{s1} - casein	39-46 %	% in total casein fraction
					α_{s2} - casein	8-11 %	
				β - casein		25-35 %	
				k - casein		8-15 %	
				γ -casein		3 %	
		Soluble whey proteins	20 %	α - lactalbumin		5 %	% in total protein fraction
				β - lactoglobulin		10 %	
				lactoferrin		traces	
				serumalbumin		1 %	
				immunoglobulins	mainly Ig_G	3 %	
Lactose	5 %						
Minerals	0.7 %						
Vitamins	<0.5 %						

Table 2 Polymorphic profile of β-caseins (data adapted from [11])

No.	β-casein genetic variants	Polymorphic sites in amino acid sequence													
		18	25	35	36	37	67	72	88	93	106	117	122	137	138
1	**A2**	Ser-P	Arg	Ser-P	Glu	Glu	Pro	Glu	Leu	Gln	His	Gln	Ser	Leu	Pro
2	**A1**						His								
3	**A3**										Gln				
4	**A4**	change not yet recognized													
5	**B**						His						Arg		
6	**C**			Ser		Lys	His								
7	**D**	Lys													
8	**E**				Lys										
9	**F**						His								Leu
10	**G**						His						Leu		
11	**H1**		Cys						Ile						
12	**H2**							Glu		Leu					Glu
13	**I**									Leu					

CONCLUSIONS

Changing milk protein profile associated to animal genetics is still challenging and may drive further research to develop new dairy products with healthy and benefic properties for humans.

Future studies and animal breeding programs should use and implement milk protein genetics knowledge for improving the healthiness of milk protein next to prevention of human related diseases, also in our country.

Romanian cattle breeds should be assessed for such purposes to find out its encoded genetics related healthy variants of milk proteins.

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