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Mare's milk: composition and protein fraction in comparison with different milk species

Klemen Potočnik[1], Vesna Gantner[2], Krešimir Kuterovac[3], Angela Cividini[1]

[1]Univerza v Ljubljani, Biotehniška Fakulteta, Oddelek za zootehniko, Groblje 3, 1230 Domžale, Slovenia
[2]Poljoprivredni fakultet Sveučilišta J.J. Strossmayera u Osijeku, Trg Svetog Trojstva 3, Osijek, Croatia
[3]Agrokor d.d., Trg D. Petrovića 3, Zagreb, Croatia

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Summary

The usage of the mare's milk as functional food especial for children intolerant to cow's milk, with neurodermitis, allergies and similar disorders desiring to improve the quality of life is fiercely debated for last decades but there were no scientific studies to suggest such use of mare's milk based on scientific research. The objectives of this study were to determine similarities of mare's milk in comparison with milk of ruminants (cattle, sheep and goat) and human milk in terms of milk composition and protein fraction as whey proteins, caseins and micelles size. All differences were discussed regarding usage of mare's milk in human diet and compared to milk which is usually used in human nutrition. Regarding composition, the mare's milk is similar to human milk in of crude protein, salt and lactose content, but it has significantly lower content of fat. Fractions of main proteins are similar between human and mare's milk, except nitrogen casein (casein N) which has twice lower content in human than in mare's milk. Content of casein N from all ruminants' milk differ much more. Just for true whey N and non-protein nitrogen (NPN) similar content as human and mare's milk has also goat milk. The casein content is the lowest in human milk; this content is three times greater in mare's milk and six to seven times greater in goat's and cow's milk, while in sheep's milk it is more than 10 times grater. In many components and fractions mare's milk is more similar to human milk than milk of ruminants. A detail comparison of protein fraction shows quite large differences between milk of different species. More study and clinical research are needed that can recommend usage of mare's milk in human diet as functional food on scientific bases.

Key words: mare's milk, human milk, ruminant's milk, composition, protein fraction

Introduction

Milk represents the essential source of nourishment of mammals during the neonatal period. Mare's milk represents the essential source of nourishment of foals during the first months of life. Around 30 million people consume mare milk regularly throughout the world. In areas of central Asia steppes: Turks, Bashkirs, Kazakhs, Kyrygyz, Mongols, Yakuts and Uzbeks a lactic-alcoholic beverage called Koumiss is traditionally produced through fermentation, it is also one of the most important basic foodstuffs for the human populations (Orskov, 1995; Montanari et al., 1996; Montanari et al., 1997). This ancient beverage which Scythian tribes used to drink some 25 centuries ago was also consumed throughout Eastern Europe, particularly in Hungary and Asiatic regions (Koroleva, 1988). Tamime et al. (1999) reported that Koumiss is now produced at industrial level. In Western Europe, where the most

*Corresponding author/Dopisni autor: Phone/Tel.: +385 1 4605 037; E-mail: vgantner@pfos.hr

important product of equine breeding is foals, studies on mare's milk have been concerned mainly with the growth and health of the newborn horse.

In the last several years, interest in the use of mare's milk for human nutrition particularly in France and Germany increased (Drogoul et al., 1992). The studies about equine milk regarded especially in protein compound which is indicator of amount caseins and whey proteins with some interest for a possible use as a substitute of cow's milk for children with intolerance or allergy (Businco et al., 2000; Curadi et al., 2001). Another goal of studies like this was to find new way of utilisation for local equine breeds (Pinto et al., 2001). The objectives of this study were to determine similarities of mare's milk in comparison with milk of ruminants (cattle, sheep and goat) and human milk in terms of milk composition and protein fraction as whey proteins, caseins and micelles size as well as to discuss parameters that could be of interest in terms of human nutrition.

Composition

Secretion of mammary gland in terms of milk composition is physiologically and structurally correlated to the nutritional requirements of the newborns of each species. Therefore, milk composition highly depends on animal species. Composition comparison of mare's, ruminant's (cow, sheep and goat) and human milk is presented in Table 1.

Regarding the fat content, mare's milk has noticeably less fat when compared to ruminants and human. The protein fraction of the milk of the ruminant species shows a remarkable similarity (with highest content determined in sheep) comparing to the mare's and human milk that are much poorer in protein substance. The lactose content of mare's milk is similar to that of human milk and higher than content in ruminant's milk indicating that mare's and human milk are quite similar. This also includes galactose which is a constituent of the myelinic sheath of the central nervous system cells. The structural complexity of the minor carbohydrate fractions (Alais, 1974; Kunz et al., 1999) of human milk makes a functional comparison with ruminants and mare's milk difficult. Nakano et al. (2001) reported that sialic acid is a component that affects intestinal flora development as well as, most probably, the level of glycosylation of gangliosides of the brain and central nervous system. The values of 100 mg/100 mL found in human milk are significantly higher than that found in cow's 20 mg/100mL, and mare's 5 mg/100 mL, milk (Kulisa, 1986; Heine et al., 1993). Mare's and human's milk are poorer in mineral salt content when compared to ruminant's milk. Gross energy supply of mare's milk is clearly lower than that of human milk, which is comparable to that of ruminant's milk (Jenness and Sloan, 1970; Alais, 1974; Doreau and Boulot, 1989; Solaroli et al., 1993; Simos et al., 1996; Salimei, 1999). Similarities regarding the crude protein, lac-

Table 1. Composition of mare's, cows, sheep's, goat's and human milk - mean value, minimum-maximum values reported in literature (Mitić et al., 1987; Solaroli et al., 1993; Simos et al., 1996; Salimei, 1999; Martuzzi et al., 2000)

Component	Value	Mare	Cow	Sheep	Goat	Human
Fat, g/kg	Mean	12.1	36.1	75	41	36.4
	(Min-Max)	(5-20)	(33-54)	(50-90)	(30-60)	(35-40)
Crude protein, g/kg	Mean	21.4	32.5	54.5	34	14.2
	(Min-Max)	(15-28)	(31-39)	(45-70)	(30-36)	(9-17)
Lactose, g/kg	Mean	63.7	48.8	49	47	67.0
	(Min-Max)	(58-70)	(44-49)	(41-59)	(42-50)	(63-70)
Ash, g/kg	Mean	4.2	7.6	8.5	7.7	2.2
	(Min-Max)	(3-5)	(7-8)	(8-9)	(7-8)	(2-3)
Gross energy, kcal/kg	Mean	480	674	-	670	677
	(Min-Max)	(390-550)	(650-712)		(660-690)	(650-700)

Table 2. Main nitrogen fractions of mare's, cows, sheep's, goat's and human milk - mean value, minimum-maximum values reported in literature (Boland et al., 1992; Doreau, 1994; Martuzzi et al., 2000; Park et al., 2007)

Fractions	Value	Mare	Cow	Sheep	Goat	Human
Total N, g/kg	Mean	21.4	32.5	55.5	34	14.2
	(Min-Max)	(15-28)	(31-38)	(45-70)	(30-36)	(9-17)
True whey N, g/kg	Mean	8.3	5.7	11	7.4	7.6
	(Min-Max)	(7.4-9.1)	(5.5-7.0)	(9-13)		(6.8-8.3)
Casein-N, g/kg	Mean	10.7	25.1	43	24	3.7
	(Min-Max)	(9.4-12.0)	(24.6-28.0)	(35-50)		(3.2-4.2)
NPN x 6.38, g/kg	Mean	2.4	1.7	1.5	2.6	2.9
	(Min-Max)	(1.7-3.5)	(1.0-1.9)			(2.6-3.2)
True whey protein, %	Mean	38.79	17.54	20	21.7	53.52
Casein, %	Mean	50.00	77.23	77.5	70.6	26.06
NPN x 6.38, %	Mean	11.21	5.23	2.5	7.7	20.42

tose and salt content between the mare's and human milk, make mares' milk more suitable replacement for human milk than milk from ruminants (Marconi and Panfili, 1998).

Protein fractions: main components

Protein contents vary widely within species, and are influenced by breed, stage of lactation, feeding, climate, parity, season, and udder health status. Main nitrogen fractions of mare's, cows, sheep's, goat's and human milk are reported in Table 2. Regarding the crude protein, the highest values are noticed in ruminants, especially in sheep, while mare's and human milk contain significantly less amount of proteins. The whole protein system of mare's milk is quite similar to that of human milk. Both, whey proteins in total (casein content and whey protein/casein ratio) and non-protein nitrogen (NPN) concentrations are comparable. Goat and sheep milk contains about 0.7-1.0 % and 0.4-0.8 % N, which is distributed in fractions. Mares' milk has a higher level of non-protein N and less casein-N than milk of cows, sheep and goats. On the other hand cows', sheep' and goats' milk has much higher casein content (caseineux milk). Ruminants' milk proteins are comprised of about 80 % caseins and 20 % whey proteins (Alais, 1974; Boland et al., 1992; Pagliarini et al., 1993; Doreau, 1994; Csapó-Kiss et al., 1995; Martuzzi et al., 2000; Park et al., 2007).

The whey protein fraction represents almost 40 % in mare's milk, more than 50 % in human milk and less than 20 % in ruminant's milk. Cow's milk protein features, like other ruminant milks (e.g. goat and sheep), are quite different, as characterised by an acid-enzymatic, mixed coagulation. From this point of view mare's milk is more similar to human milk, which could be defined typically as *albumineux* milk. The richness in whey protein content of mare's milk makes it more favourable to human nutrition than cow's, sheep's and goat's milk, because of the relatively higher amount of essential amino-acids (Hambræus, 1994).

Protein fractions: whey proteins

The analysis of whey protein structure was objective of many researchers (Boland et al., 1992; Pagliarini et al., 1993; Solaroli et al., 1993; Doreau, 1994; Martuzzi et al., 2000). Determined results clearly showed the physiological specificity of different mammary secretions; as seen by both the concentration and distribution of the single proteins and whey enzymes (Table 3).

The content of α-lactalbumin is the highest in cow's milk which could explain the fact that cow's milk causes allergic response in many individuals. This could be a serious problem, especially for young children, who are often able to consume goat milk f without suffering from that reaction, which could

Table 3. Whey protein fractions of mare's, cows, sheep's, goat's and human milk - mean value, minimum-maximum values reported in literature (Boland et al., 1992; Doreau, 1994; Casper et al., 1998; Martuzzi et al., 2000; Moatsou et al. 2005)

Whey protein fraction	Value	Mare	Cow	Sheep	Goat	Human
True whey protein, g/kg	Mean	8.3	5.7	11	7.4	7.6
	(Min-Max)	(7.4-9.1)	(5.5-7.0)	(9-13)		(6.8-8.3)
α-lactoalbumin, %	Mean	28.55	53.59	8.97-17.00	13.31-34.70	42.37
	(Min-Max)	(27.5-29.7)	(52.9-53.6)			(30.3-45.4)
β-lactoglobulin, %	Mean	30.75	20.10	59.24-77.70	43.54-63.80	0
	(Min-Max)	(25.3-36.3)	(18.4-20.1)			
Immunoglobulins, %	Mean	19.77	11.73	-	-	18.15
	(Min-Max)	(18.7-20.9)	(10.1-11.7)			(15.1-19.7)
Serum albumin, %	Mean	4.45	6.20	3.6-5.1	1.8-5.5	7.56
	(Min-Max)	(4.4-4.5)	(5.5-76.7)			(4.5-9.1)
Lactoferrin, %	Mean	9.89	8.38	-	-	30.26
Lysozyme, %	Mean	6.59	-	-	-	1.66

be explained through the dissimilarities in structure of the two proteins. The enzymes of goat milk are similar to those of the cow, although some specific differences have been described. Of primary interest, it has been shown that the level of alkaline phosphatase is slightly lower than that found in work with dairy cattle, but the enzyme demonstrates the same degree of heat susceptibility and therefore serves equally well as a pasteurization marker. Peroxidase activity in the milk of both species is the same in all respects, while the xanthine oxidase level is lower in the milk of the goat. Higher levels of activity are observed for both ribonuclease and lysozyme. Human milk is free of β-lactoglobulin, while this protein is the major whey protein of sheep's, goat's and mare's milk. Businco and Bellanti (1993) reported that β-lactoglobulin is responsible for the onset of allergic forms to milk proteins that affect a significant percentage of infants nourished with maternal milk replacements (cow milk formulas). Konig (1993) and Businco et al., (2000) reported that this problem seems to occur less often when infants are nourished with mare's milk. Antimicrobial defence in mare's milk seems to be due mainly to the presence of lysozyme (as in human and goat milk) and, to a lesser degree, to lactoferrin, which is preponderant in human milk (Solaroli et al., 1993). These antimicrobial factors are scarce in cow's milk, where immunoglobulins represent the principal defence against microbes and are particularly abundant in colostrum (Boland et al., 1992; Solaroli et al., 1993).

Protein fractions: caseins and micelles size

Caseins distribution of mare's, cows, sheep's, goat's and human milk are presented in Table 4. Abd El-Salam et al. (1992) and Ochirkhuyag et al. (2000) noticed that mare's milk casein is composed mainly of equal amounts of α_s-casein and β-casein. Malacarne et al. (2000) and Egito et al. (2002) reported that the proportions of the main α_s-casein fractions, i.e. α_{s1} - and α_{s2} -casein, are still under study. Iametti et al. (2001) and Egito et al. (2002) stated that lately mare κ-casein has also been identified and characterised. It shows several biochemical properties similar to that of bovine and human κ-casein, such as the presence of carbohydrate moieties and susceptibility to hydrolysis by chymosin-group II (Egito et al., 2001). The proportion of κ-casein in mare's milk appears to be lower compared to that of ruminants and human milks (Egito et al., 2001).

Creamer (1991) and Boland et al. (1992) determined that bovine casein composition is relatively richer in α_{s1}-casein. Whitelaw et al. (1990) reported that α_{s1}-casein fraction is probably responsible for the onset of allergic forms in children. Both, cow's and sheep's casein differ from that of human milk (Creamer, 1991; Boland et al., 1992; Cuilliere et al., 1999) by a highest content of α_s-casein compared to human that are characterised by a clear prevalence of β-casein. Highest content of β-casein compared to α_s-casein and κ-casein was also determined in goat's milk. Mare's casein could be considered relatively rich in β-casein (similar portion of

Table 4 Caseins distribution of mare's, cows, sheep's, goat's and human milk - mean value, minimum-maximum values reported in literature (Creamer, 1991; Boland et al., 1992; Abd El-Salam et al., 1992; Cuilliere et al., 1999, Pirisi et al., 1999; Ochirkhuyag et al., 2000; Malacarne et al., 2000; Bramanti et al., 2003; Park et al., 2007; Moatsou et al., 2008)

Parameter	Value	Mare	Cow	Sheep	Goat	Human
Casein, g/kg	Mean	10.7	25.1	44	24	3.7
α_s-casein, %	Mean	46.65	48.46	50.23	21.2-32	11.75
	(Min-Max)	(40.2–59.0)	(48.3–48.5)			(11.1–12.5)
β-casein, %	Mean	45.64	35.7	39.95	48-60	64.75
	(Min-Max)	(40.1-51.4)	(35.8–37.9)			(62.5-66.7)
κ-casein, %	Mean	7.71	12.69	9.82	12-20	23.50
	(Min-Max)		(12.7-13.8)			(22.2-25.0)
Micelles size, nm	Mean	255	182	210	260	64

α_s-casein and β-casein) and thereby able to supply children with abundant amounts of casomorphins (Clare and Swaisgood, 2000). Mare's milk micelles are the largest as compared to both human, and cow's milk micelles (Buchheim et al., 1989). Structurally, the milk protein casein of the goat's and sheep's milk is sufficiently different from that found in cow's milk. The casein micelles typically exist either as much larger or much smaller aggregations as are found in cow milk. Because of this it has been suggested that, although the quantity and distribution of amino acids in the casein fractions of the milks of the ruminant species are similar, the sequence of assembly is almost certainly different. This difference is further substantiated by the fact that goat casein is associated with a lower mobility in an electrophoretic field. Micellar structure varies considerably from species to species. In cow's and mare's milk it has a spongy structure, while in human milk it is reticular, fairly regular and very loose, due to numerous canals and caverns (Jasiska and Jaworska, 1991). This affects susceptibility to pepsin hydrolysis, which, however, depends mainly on the high β-casein micellar content. The different protein composition in total and the different micellar structure (caseins distribution and micelles size) determine marked differences in the rheological properties of the curds obtained from each of the milks under consideration, and consequently influence the digestive utilisation of milk nutrients. Kalliala et al. (1951) and Solaroli et al. (1993) reported that mare's and human milk form a finer, softer precipitate, which is physiologically more suitable for infant nutrition because it is moreeasily digestible than the firm coagulum of cow's milk.

Conclusions

Compared to the characteristics of human and ruminant's milk, mare's milk, due to a lower fat content, has a lower energy value. The sugar content, whole protein and salt supply of mare's milk is similar to that of human milk, whereas ruminant milk, richer in salts, is less suitable as a replacement for mother's milk. Regarding the main nitrogen fractions, mare's milk, is similar to human, while ruminant milk differs from both for higher casein content (*caseineux* milk). The richness and pattern of the whey protein of mare's milk make it more favourable than cow's and sheep's milk for human nourishment. Mare's milk casein is composed of nearly equal parts of α_s-casein and β-casein; human and goat's milk is characterised by a prevalence of β-casein; while cow's and sheep's milk is characterised by a prevalence of α_s-casein. Cow casein is relatively richer in α_{s1}-casein, which is believed to be responsible for the onset of allergic forms in nursing infants. Regarding the structural characteristics, mare's and human milk form a finer and softer precipitate, which and more easily digestible than the firm coagulum of ruminant milk.

In many components and fraction the mare's milk is more similar to human milk than milk of ruminants. But, before recommendation of usage of mare's milk in human diet as functional food, more studies and clinical researches are needed.

Kobilje mlijeko: sastav i frakcije proteina u usporedbi s drugim vrstama mlijeka

Sažetak

Upotreba kobiljeg mlijeka kao funkcionalne hrane, s ciljem poboljšanja kvalitete života, osobito djeci netolerantnoj na kravlje mlijeko s utvrđenim neurodermatitisima, alergijama te sličnim poremećajima, tema je mnogih rasprava posljednjih godina. No, nedovoljno je znanstvenih studija koje bi predlagale upotrebu kobiljeg mlijeka baziranu na znanstvenim činjenicama. Ciljevi ovoga rada bili su utvrđivanje sličnosti između kobiljeg mlijeka i mlijeka preživača (goveda, ovce i koze) te humanog mlijeka, obzirom na kemijski sastav mlijeka te proteinske frakcije odnosno proteine sirutke, kazeine te veličinu micela. Utvrđene razlike analizirane su s aspekta upotrebe kobiljeg mlijeka u prehrani ljudi te komparirane s kravljim mlijekom. Obzirom na sastav, kobilje mlijeko slično je humanom glede sadržaja sirovih proteina, minerala i laktoze, no sadrži značajno manji udio mliječne masti. Obzirom na glavne proteinske frakcije, kobilje je mlijeko slično humanom uz izuzetak kazeinaskog N, čiji je sadržaj u humanom dvostruko niži, dok su u mlijeku preživača utvrđene znatno veće razlike. Sadržaj ukupnih proteina sirutke i neproteinskog dušika (NPN) sličan onome u humanom i kobiljem utvrđen je i u kozjem mlijeku. Sadržaj kazeina u mlijeku najniži je u humanom mlijeku, tri puta je viši u kobiljem, šest do sedam puta viši u kozjem i kravljem, te više od deset puta u mlijeku ovaca. Obzirom na mnoge uspoređene komponente i frakcije, kobilje je mlijeko sličnije humanom u odnosu na mlijeko preživača. Detaljna usporedba proteinskih frakcija ukazuje na vrlo veliku razliku između mlijeka različitih vrsta domaćih životinja. Prije znanstveno utemeljene preporuke upotrebe kobiljeg mlijeka kao funkcionalne hrane u prehrani ljudi potrebne su dodatne znanstvene studije te klinička istraživanja.

Ključne riječi: kobilje mlijeko, humano mlijeko, mlijeko preživača, kemijski sastav, frakcije proteina

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