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Composizione in acidi grassi ed in proteine del latte di cavalla e di asina. Implicazioni per l'utilizzazione del latte

Composition of mare and donkey milk fatty acids and proteins and consequences on milk utilisation

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ABSTRACT – This review summarises ancient and recent knowledge on milk fatty acids and protein fractions in mare and donkey milk. Fatty acids are characterised by their high content in α -linolenic acid, especially when mares are on pasture. No other fatty acids beneficial to human health, as conjugated linoleic acid, are found in significant amounts. Mare and donkeys milk proteins are characterised by a high proportion of whey proteins. Their structure and their polymorphism have been studied mainly for α -lactalbumin and β -lactoglobulin, and also for minor proteins as lactoferrin. The identification and the characterisation of all classes of casein has also been made. In particular, κ -casein has been evidenced in mare milk. The implications of these compositional data for the different uses of mare and donkey milk is discussed. A lack of demonstrative experiments showing for these milks a better value than cow milk is noticed.

KEY WORDS: mare, donkey, milk, fatty acids, linolenic acid, CLA, casein, whey proteins

INTRODUCTION - Although mare milk is mainly devoted to foal feeding, the use of milk from dairy mares is increasing. In the former USSR and in Mongolia, there is a tradition of milk consumption based on therapeutic qualities, suggested by empirical knowledge, but which have never been scientifically assessed (Doreau and Martin-Rosset, 2002). In western Europe, mare milk utilisation is developing, on one hand for drinking, on the other hand for cosmetics. The scientific bases of understanding the nutritional qualities of mare milk are certainly related to the specificity of its composition, in particular for fat and protein. These two milk fractions will thus be described in this review.

FAT

Milk fat content

It is well known that horse milk is very poor in milk fat. Among mammals, only milk of white rhinoceros is known to have a lower fat content than mare milk (Jenness, 1974), even though, as milk is richer in fat at the end of milking, it can be supposed that the milking was incomplete in rhinoceros which is known to be bad-tempered. Data on other *equidae* are scarce. No difference between domestic or feral horses, donkeys and zebras has been evidenced (Ofstedal and Jenness, 1988). Variations in total milk fat content have been widely described in the review of Doreau and Boulot (1989), and complementary data have been provided in the reviews by Solaroli et al. (1993) and Martuzzi et al. (2001). The main features are 1) a decrease throughout lactation from 15 – 20 g/kg to 10 – 15 g/kg ; 2) low effects of age, parity, breed and individual variations 3) a higher value when mares are fat at foaling but no variation with energy or nitrogen intake; 4) a strong decrease with high-concentrate diets (Doreau et al., 1992) and a trend to an increase with dietary fat supplementation (Davison et al., 1987). In the above mentioned reviews, some data are available on the classes of lipids in milk fat, and on the properties of fat globule. The main specificity of mare milk fat compared to cow or human milk is the lower proportion of triglycerides, compensated for by high percentage of free fatty acids (9 – 10%) and of phospholipids (5 – 19 %). The present review will be focused on fatty acid (FA) composition in mare milk.

Milk fat synthesis

In horses as in other mammals, milk fat has a double origin: on one hand, circulating FA, transported in lipoproteins, and on another hand FA synthesised . Circulating FA mainly come from dietary 16- and 18-carbon FA which, in horses as in other species, are absorbed in the small intestine. As in other monogastric species, they are not transformed before absorption, contrary to ruminants, in which FA are transformed, essentially by isomerisation and hydrogenation, and to a low extent synthesised, in the rumen, that is prior to absorption. In the large intestine, FA metabolism occurs due to bacterial activity, but in ruminants they are thought not to be absorbed (Doreau and Ferlay, 1994). The same hypothesis is done in horses. As a consequence, in horses milk long-chain FA composition is close to dietary FA composition. *De novo* synthesis arises from glucose in

monogastric species (Figure 1). On the contrary in ruminants it arises from two of the three main volatile fatty acids: acetate and butyrate, this latter being transformed in β -hydroxybutyrate in the rumen wall. The amount of glucose absorbed in ruminants is very low in most dietary conditions. In horses, FA are synthesised from acetate and β -hydroxybutyrate, which are produced in the large intestine mainly from forage carbohydrates (Linzell et al., 1972). Glucose is absorbed in the small intestine with diets rich in concentrates, but glucose does not contribute to *de novo* FA synthesis. Moreover in ruminants, microbial ruminal metabolism leads to the absorption of trans monounsaturated FA, whereas in horses a possible similar production in the large intestine does not result in a significant absorption. The main difference in the mechanisms of *de novo* synthesis between ruminants and horses is that in ruminants *de novo* synthesis accounts for 4- to 14-carbon FA, and about half of 16-carbon FA, whereas in horses *de novo* synthesis results in addition in a part of 18-carbon FA (Linzell et al., 1972).

Average composition

Mare milk composition is very different from ruminant milk composition, because in ruminants the ruminal microbial metabolism prior to absorption leads in particular to an extensive hydrogenation of dietary FA. The main difference in composition with other monogastric species is that horses consume grass, which is rich in α -linolenic acid, whereas feedstuffs consumed by non herbivorous monogastric are most often poor in this acid. Compared to human milk, mare milk is rich in short- and medium-chain FA and in α -linolenic acid, and poorer in oleic acid (Table 1). Concentrations in individual FA in milk fat, based on literature data, are very variable. These results are often biased by many factors: 1) the loss of short-chain FA when methyl esters are used instead of butyl esters; 2) imperfect chromatographic conditions; 3) the fact that composition of FA is expressed generally in weight percentages, but sometimes in molar percentages; when this is expressed in weight, it can be on FA basis or on ester basis, which changes the result, especially for butyl esters of very short-chain FA. In many published papers, the mode of expression is not specified. Mare milk is rich in short- and medium-chain FA (Table 1). Butyric and caproic acid are present in low amounts (less than 1%), caprylic acid represents about 3%, capric, lauric and myristic acids about 7.5% each. Palmitic acid represents 12 to 28% of total FA. This is less than in cow milk and human milk. A specificity of mare milk fat is the very low concentration of stearic acid, between 0.3 and 3.0% of total FA. Among mammals, only seals have such a low concentration. For mares, it can be attributed to 1) the low content of stearic acid in the diet, and 2) a possible high activity of $\Delta 9$ desaturase, which converts stearic acid into oleic acid (C18:1 n-7, i.e. $\Delta 9$) in the udder. This latter hypothesis is enhanced by the high content in palmitoleic acid (C16:1 n-7, i.e. $\Delta 9$), which varies between 4 and 8% in most data. Palmitoleic acid, present in low quantities in feedstuffs, arises from the desaturation of palmitic acid by $\Delta 9$ desaturase, but this enzyme is less active on 16-carbon FA than on 18-carbon FA. Mare milk is rich in polyunsaturated FA. The important proportion of

linoleic acid, between 5 and 20% of total FA in most data, is due to the presence of this acid in the diet, which is the major FA in cereals and their by-products. However, a major specificity of mare milk is the high content in α -linolenic acid, on average 12%, between 2 and 26% according to the authors. A high concentration of α -linolenic acid can be reached in milk from animals with are both nonruminants and herbivorous, that is when the diet contains large amounts of α -linolenic acid, as grass, and when no hydrogenation occurs before absorption. The effect of diet on concentration of α -linolenic acid will be detailed below. Very few data are available for other *equidae*, as donkey and zebra, so that the comparison with horse is of low value. Few differences between these species have been found by Glass et al. (1967).

Non dietary effects

The effect of the availability in end-products of digestion, and thus of dietary composition, is the major determinant of FA composition for a given animal species. Non-dietary factors generally play a minor role. Milk fat decreases with lactation stage, on average from 15 - 20 g/kg at the beginning of lactation to 10 - 15 g/kg at 6 months of lactation, with forage-rich diets (Doreau and Boulot, 1989). Colostrum is richer in long-chain FA than milk (Doreau et al., 1993, Csapo et al., 1995, Curadi et al., 2001), but after 1 week of lactation, the composition of fat is not modified to a large extent when mares are fed a constant diet (Doreau et al., 1993, Hoffman et al., 1998, Curadi et al., 2001). Some results have shown an effect of lactation stage (Jaworski et al., 1982), but feeding is not specified. It is likely that the diet changed in the course of the experiment, so that the proper effect of lactation stage cannot be determined. Milk of fat mares is slightly higher in long-chain FA and poorer in short- and medium-chain FA than milk of thin mares (Doreau et al., 1993). This is a consequence of a lower mobilisation of the long-chain FA of body reserves in thin mares which ate more, so that acetate and β -hydroxybutyrate, the precursors of *de novo* FA synthesis, are available in higher amount. Genetic effects have been seldom studied. Csapo et al. (1995) found no difference in fat composition between Breton, Boulonnais, Haflinger and Hungarian Draught. Differences obtained by Kulisa (1977) between Arabian, Malopolski and Huculski breeds are likely due to their situation in different herds, with different feeding conditions.

Dietary effects

The most evident dietary effect on FA composition is the effect on α -linolenic acid, of which proportions in milk fat can be 5 times higher with herbage than with winter diets. Fresh grass contains 1 to 3% FA, of which more than 50% is made of α -linolenic acid. Almost all measurements made with fresh grass result in proportions of α -linolenic acid comprised between 15 and 25%, whereas all measurements made with winter diets give values lower than 10% (e.g. Goryaev, 1970, Jamsranjav, 1982, Csapo et al., 1995, Hoffman et al., 1998). Among winter diets, a hay diet gives a higher proportion of α -linolenic acid than a concentrate

diet (Doreau et al., 1992) due to a higher linolenic content in hay than in concentrate. The replacement of a concentrate rich in starch and sugar by a concentrate rich in fibre and fat from maize oil resulted in an increase in linoleic acid from 10 to 15% of total FA, and to a lower extent in a decrease in linolenic acid and in an increase in transvaccenic acid, the other FA being unmodified (Hoffman et al., 1998). The high content in linoleic acid is a consequence of maize oil addition, but other variations cannot be explained, especially because of concomitant variations in starch, sugar, fibre and fat in the diet. The addition of soybean oil in the diet also increased linoleic acid in a trial by Zeyner et al. (1996) but to a lower extent, due to a low amount of oil given to animals.

Minor fatty acids

The composition of milk fat is generally much more complex than that of feedstuffs from vegetal origin, suggesting transformations in animal organism. This is particularly true for cow milk, due to the intensive microbial metabolism in the rumen, which results not only in an extensive hydrogenation of dietary FA, but also in specific FA (Doreau and Ferlay, 1994). In particular, odd-chain FA, with 15 and 17 carbons, are characteristics of bacteria. Moreover trans monounsaturated 18-carbon FA and conjugated linoleic acids (CLA) are intermediate compounds of hydrogenation of polyunsaturated FA. On another hand, specific dietary FA, as 20- and 22-carbon FA, present in fish and algae fat, can be found in milk. Most milk FA analyses have been carried out with rather rough methods in order to analyse main FA. For a few years, the increasing interest for minor FA leads to analyse FA with more accuracy, but until now few data are available. Odd-chain FA, which are characteristic of bacteria, are present in small amounts. From 12 publications in which they are determined, their concentration is generally comprised between 0.2 and 0.9% for 15:0, between 0.3 and 0.6% for 15:1, between 0 and 1.2% for 17:0, and between 0.0 and 1.1% for 17:1. The concentration seems to be reduced by dietary fat (Zeyner et al., 1996). This concentration is only 2 to 3 times lower than in cow milk fat for saturated FA, and in the same range of concentration for monounsaturated FA (Jensen, 2002), whereas absorption of FA of microbial origin occurs in ruminants and not in monogastric animals. Transvaccenic acid has been found in mare milk, at concentrations of 0.9% (Curadi et al., 2001) or 0.2% (Hoffman et al., 1998). Other data (Zeyner et al., 1996, Doreau and Chabrot, unpublished, table 2) suggest that the main isomer of trans monounsaturated 18-carbon FA is elaidic acid (n-9) and not transvaccenic acid (n-7) as it occurs in cow. In fact, in ruminants transvaccenic acid arises from the ruminal hydrogenation of polyunsaturated FA; there is no reason to assume the same importance among trans isomers in horses. In cow, the main isomer of CLA (18:2 cis 9 trans 11) is produced in the rumen but is mainly synthesised in the udder from transvaccenic acid (18:1 trans 11, i.e. n-7) of ruminal origin; its concentration is within 0.5% for normal diets to 2% with diets rich in lipids (Doreau et al., 1999). It has been first observed by Park and Pariza (1998) that CLA was present in very low amount in mare plasma, strengthening the hypothesis of the absence of absorption of FA of

microbial origin produced in the large intestine. In mare milk, its concentration has recently been found as 0.05% by Curadi et al. (2001) and as 0.09% by Martuzzi et al. (2001). A more specific determination by separation of isomers has shown a mean concentration of 0.05%, mainly composed of the cis 9 trans 11 isomer, and of different trans trans isomers (Doreau and Chabrot, unpublished, table 2). This repartition is similar to that of absorbable CLA in ruminants (Lor et al., 2002), after the action of rumen microbes but before the mammary desaturation of transvaccenic acid in the cis 9 trans 11 isomer. The very low concentration in CLA in mares compared to cows is due to the quasi absence of absorption of both CLA and transvaccenic acid in mares. An isomer of α -linolenic acid, γ -linolenic acid of the n-6 series, is generally present in very small amounts (0.02% according to Curadi et al., 2001) but Csapo et al. (1995) observed a concentration of 0.6%. This could be due to the intake at pasture of plants rich in γ -linolenic acid, as borage or evening primrose. This FA was present as traces in determinations by Doreau and Chabrot (unpublished). Very long-chain FA have also been found in mare milk. In normally fed mares, the two acids of the n-3 series, eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) are present in very small amounts (less than 0.02%) but partially hydrogenated 20-carbon FA are present in higher amounts, especially 20:3, 20:2 and 20:1 (0.3 to 0.5% each) (Curadi et al., 2001, Doreau and Chabrot, unpublished). On the contrary, among 22-carbon FA, only 22:0 (Intrieri and Minieri, 1970) and 22:1 have been found, and in very low amounts. EPA and DHA are present as traces (Doreau and Chabrot, unpublished). All these results have been obtained with classical diets. Supplying mare diets with sources of EPA and DHA, as fish oil, may increase in these two FA in milk as it occurs in human (Jensen et al., 1990), unless they are not incorporated in milk triglycerides. A trial by Bontempo et al. (2000) showed an moderate increase in n-3 FA with a cod liver supply, but authors do not specify the chain length.

PROTEINS

Milk protein content

Milk crude protein content is the highest in colostral period (25 – 30 g/kg) then declines from 20 – 25 g/kg in the first month of lactation to 15 – 22 g/kg in the following months. No effect of breed on protein content has been evidenced; protein content is not modified by other animal factors as age and parity. An increase in energy supply to mares results in a lower protein content (Pagan and Hintz, 1986, Doreau et al., 1992); an increase in nitrogen supply to mares sometimes did not modify protein content (Peltonen et al., 1980) but in other cases increases protein content (Doreau et al., 1988). Crude protein is calculated as N x 6.38, and is made of about 10% of nonprotein N, of which urea represents about 50% (Doreau and Boulot, 1989). Among proteins, casein represents 50 to 65% of true protein, according to the experiments (Solaroli et al., 1993, Martuzzi et al., 2001). This is less both in the ratio casein:protein and in concentration than in milk cow, in which casein represents almost 80% of protein, crude protein content being on average 32 g/kg. In comparison, human milk is poor in protein (9 – 17 g/kg) and

shares with elephant the lowest ratio casein:protein among mammals, since human milk contains more whey proteins than caseins (Jenness, 1974). The proportion of casein in mare milk may be underestimated because most determinations have been made by precipitation at pH 4.6 as in bovine milk, whereas for mare the maximum of casein precipitation has been obtained at pH 4.2 (Egito et al., 2002). Amino acid composition is different from that observed in other species (Doreau and Boulot, 1989). Mare milk is richer in Cys and Gly than most other milks. Variations according the lactation stage are low (Doreau et al., 1990, Csapo-Kiss et al., 1993).

Major whey proteins

α -Lactalbumin (α -LA) and β -lactoglobulin (β -LG) are the two major whey proteins and generally represent in proportion about 40 - 55% for β -LG, 25 - 35% for α -LA (Martuzzi et al., 1999), the remainder being minor proteins. The primary structure of major proteins is known both in donkey and mare milk. Like bovine whey proteins, genetic polymorphism occurs in the *equidae* milks. The occurrence of three genetic variants of α -LA, named A, B, C, was reported for mare milk (Godovac-Zimmermann et al., 1987). As far as, only one α -LA genetic variant has been isolated from donkey milk (Giufrida et al., 1992). The sequence identity percent of donkey α -LA with equine A-type variant is 99%, whereas it is 96.7% with equine B/C type variants. More recently, glycosylation was evidenced for equine α -LA, as already found for different species especially human α -LA. β -Lactoglobulin exists predominantly as monomer in *equidae* milks whereas this protein is dimeric in ruminant milks. Two distinct molecular forms of β -LG named I and II, have been evidenced both in horse and donkey milks (Conti et al., 1984, Halliday et al., 1991, Godovac-Zimmermann et al., 1988a, 1990). The β -LGI represents the major form in both milks. The two *equidae* β -LG I and β -LG II show 98.8 and 97.0% identity, respectively, in these two milks. Recently, three new genetic variants of donkey β -LG were identified (Herrouin et al, 2000): one form close to β -LG I (named β -LG I B) presents three amino acid substitutions at positions 36, 97, and 150; two other forms close to β -LG II presents amino acid substitutions at positions 110, 118 and 96 (β -LG I B), and 110, 118 (β -LG I C). The percent of identity with the horse homologue is higher than 96%.

Minor whey proteins

Recently, much attention has been paid to minor whey proteins. These proteins are gaining interest because of their potential benefic role in the prevention and disease consequences. These minor whey proteins included lysozyme (LYS), lactoferrin (LF), immunoglobulins (Ig), serum albumin and various growth factors and enzymes. The latter will not be developed here. LYS content, between 6 and 11% of whey proteins is higher than in human (1.5 – 2%) whereas only traces are found in cow milk (Pagliarini et al., 1993, Martuzzi et al., 2001). LYS concentration is higher at the beginning of lactation, then decreases, and the concentration is the

same in mare and donkey (Faccia et al., 2001). Two forms of horse LYS have been shown by electrophoresis, but they were not identified as genetic variants (Bell et al., 1981). In contrast, two genetic variants, named A and B, have been identified in donkey milk (Godovac-Zimmermann et al., 1988b, Herrouin et al., 2000). These two LYS differ by three amino acid substitutions at positions 49, 52 and 61 and present 97.7% identity. LF content in equine milk is reported to be 0.2 - 2 g/L (Masson and Heremans, 1971), i.e. on average 10% of whey proteins, much than cow (8%) but much less than human (30%) (Martuzzi et al., 2001). In donkey milk, its content is not well known but it might represent about 7.4% of whey proteins (Iacono et al., 1992). For both species, the concentration in colostrum is much higher than in the normal milk. LF is a glycoprotein and is a member of the family of iron-binding proteins including transferrin from blood and ovotransferrin from eggs. The primary structure of equine LF has been determined (Paramasivam et al., 1998) as well as its three-dimensional structure. The primary structure of donkey LF is not known. All LF structures such as human, cow, buffalo and horse, established by X-ray crystallographic, have the same general organization. The polypeptide chain of about 690 residues (~80 kDa) is folded into two globular lobes, each of which contains one iron binding site and one bicarbonate or carbonate ion. In all these milks, LF is only 6 - 8% saturated. In mare, the N-terminal sequence of lactoferrine has been determined (Jollès et al., 1984). One of the biological role of immunoglobulin (Ig) is to provide passive protection to the newborn until its own immune system becomes mature. In ruminants and *equidae*, there is no *in utero* transfer of Ig, what increases the interest for Ig in these species. Ig represent between 8.3 and 26.8% of whey proteins in mare milks (8 publications) (Martuzzi et al., 1999) but a value of 4.3% has been reported. In donkey, a value of 7.4% has been reported (Iacono et al., 1992). Like for LF, the higher Ig content is observed in the colostrum with about 11 - 271 g/l during the first hours after parturition (Hugon, 1996). At the present time, process for extraction of Ig from colostrum for domesticated species exists and supplements based on Ig concentrates are potentially tradable. These concentrates may be used for the protective effect of newborn animals. Equine serum albumin represents between 1.9 and 8.6% of whey proteins, according to most published data (Martuzzi et al., 1999). Its sequence has been deduced from cDNA (Ho et al., 1993). Its sequence shows 74 and 76% identity with bovine and human serum albumin, respectively. The presence of serum albumin has been evidenced in donkey milk (Conti et al, 1989) but this protein has not been yet characterized.

General considerations on caseins

Caseins vary as total protein with lactation stage (Mariani et al., 1993, 2000, Csapo-Kiss et al., 1995). It is thought to be composed in equivalent parts of α - and β -casein (Martuzzi et al., 2001). The size of casein micelles is about 170 - 225 nm, that is higher than in cow milk (80 - 100 nm) and than in human milk (11 - 55 nm) (Welsch et al., 1988). The electrophoretic profiles of both horse and donkey caseins showed a great heterogeneity suggesting a high level of post-translational

modifications. The primary structure of different caseins in donkey milk have not been yet determined. On the basis of similarity of electrophoretic patterns between the horse and donkey milks; it can be suggested a great homology for these proteins in the two milks.

α_{s1} - and α_{s2} -caseins

Recently, the primary structure of equine α_{s1} -casein has been deduced from gene sequence (GenBank database accession number: AY049939). Equine α_{s1} -casein presents 42, 38 and 37% identity with the rabbit, human and bovine caseins, respectively. However, it has just been demonstrated that α_{s1} casein is quite similar between mare and other species, the apparent difference being due to different patterns of splicing from one species to another (Milenkovic et al., 2002). A high polymorphism exists in α_s -casein of mare milk (Chiofalo et al., 1983, Ono et al., 1989). Fractionation and purification by anion-exchange chromatography and by reverse-phase HPLC evidenced for α_{s1} -casein at least 7 electrophoretic bands (Egito et al., 2002) which probably differ by their degrees of phosphorylation. Amino-terminal sequences are homologous to α_{s1} - and α_{s2} -caseins in cow milk (Ochirkhuyag et al., 2000, Table 3). The composition in amino acids differs from that of cow milk. Mare α_{s1} -casein is lower in Gly and Met and higher in Arg than in cow; mare α_{s2} -casein is lower in Gly, Val and Thr, and higher in Arg, Leu and His than in cow (Ochirkhuyag et al., 2000). α_{s1} -casein is hydrolysed in different sites by chymosin and trypsin. The microsequencing of peptides shows high percentages of identity with regions of α_{s1} -casein of other species: 51 and 48% identities with sow and camel, but also 43 and 40% with cow and human (Egito et al., 2002).

β -casein

β -casein is the major casein component in equid milk as it is in human milk. This is the first casein fraction which has been isolated in mare milk (Visser et al., 1982). Recently, the primary structure of mare β -casein has been deduced from gene sequence (GenBank database accession number: AAG43954) and the amino- and carboxy-terminal regions of the β -casein polypeptidic chain has been microsequenced (Egito et al., 2001). Equine β -casein presents 48, 47 and 45% identity with the human, porcine and bovine caseins, respectively. Visser et al. (1982) found in European breeds 5 fractions of β -casein, all of them showing quite similar amino acid compositions and identical amino- and carboxy-terminal sequences. Recently, six fractions of β -casein have been evidenced in Mongolian breeds, occurring in the ratios 1:5:18:20:15:10, and differing in their degree of phosphorylation (Ochirkhuyag et al., 2000), but other factors as the content in some amino acid residues could also be responsible of this polymorphism. The amino acid composition of the different forms of β -caseins has been determined by Visser et al. (1982) and Ochirkhuyag et al. (2000). These determination both show a higher arginine content and a lower methionine content than in cow (Ribadeau-

Dumas et al., 1972). However, differences between the forms are observed, concerning some amino acids as GLx, Asx, Pro or Ala. The presence of plasmin in mare milk leads to the formation of γ_2 and γ_3 casein-like, which are not phosphorylated (Visser et al., 1982). They have also been found by Egito et al. (2002), together with proteose-peptone 5-like (Figure 2). These peptides are in fact intermediate products of hydrolysis, since they disappear with longer hydrolysis; other peptides are then formed (Egito et al., 2002).

κ -casein

For a long time, the existence of κ -casein in mare has been ignored. For example, Ono et al. (1989) and Ochirkhuyag et al. (2000) did not detect it. However, the presence of N- acetyl neuraminic acid, characteristic of κ -casein, was evidenced in mare milk (Morrissey, 1973) and glycosylated casein was shown in mare and donkey casein (Iametti et al., 1998), and it is known that κ -casein is the only casein to be glycosylated. Moreover, products of chymosin hydrolysis were similar to those found in cow milk (Morrissey, 1973, Kotts and Jenness, 1976). At least, κ -casein has been isolated by reverse phase HPLC and the N-terminal sequence has been determined (Miranda et al., 2000) then its primary structure was entirely determined (Iametti et al., 2001). Equine κ -casein has 56, 55, 52 and 47% identity with the caprine, camel, human and bovine caseins, respectively. The κ -casein has also been prepared by affinity chromatography on immobilised wheat germ agglutinin, due to a high degree of glycosylation (Egito et al., 2001). This allows to calculate a proportion of κ -casein of 6.8% in whole casein, whereas its proportion in cow represents 13% of bovine caseins (Ribadeau-Dumas and Grappin, 1989). As cow milk contains more than 4 times more total caseins than mare milk, the total amount of κ -casein in milk is much less in equine than in bovine. The equine κ -casein possesses 9 putative O-glycosylation consensus sequences (Egito et al., 2001) whereas bovine κ -casein displays only 6 consensus sequences (Pisano et al., 1994). Glycosylated κ -casein has apparent molecular masses of approximately 30 – 32 kDa by SDS-PAGE (Egito et al., 2002), against 28 kDa for the bovine one (Rasmussen and Petersen, 1991). Non glycosylated κ -casein (i.e. κ_1 -casein) has not been evidenced in mare milk (Egito et al., 2001). κ -casein has the ability to be hydrolysed by chymosin. This has been confirmed in mare, where hydrolysis results in an almost complete disappearance of the κ -casein, with generation of a glycosylated macropeptide and a non-glycosylated peptide, para- κ -casein (Egito et al., 2001, figure 3). Logically, the cleavage occurs at a Phe-Ile site, as other nonruminants, whereas the cleavage occurs at a Phe-Met site in ruminants.

MILK UTILISATION

Apart foal feeding, mare milk is used for human consumption, but also for cosmetology (Doreau and Martin-Rosset, 2002). Only the use for human consumption and the functionality of mare milk will be developed here.

Koumiss, but not cheese

Koumiss is a fermented horse milk produced traditionally in Russia, West Asia and Mongolia. The fermentation is both lactic and alcoholic, and the product contains between 0.6 and 3% alcohol. Its FA composition is close to that of milk. It is not possible to obtain cheese from mare milk, due to the failure of coagulation with chymosin. The ability of horse milk to coagulate in acidic conditions suggests that the low amount of casein in milk is not a limiting factor to coagulation. The absence of coagulation may be due to the low concentration of κ -casein; indeed the action of chymosin on κ -casein allows coagulation to be initiated. It has also been suggested that the absence of coagulation was due to the low interaction between calcium and casein. The pH value, which sometimes reaches 7.0, may also limit the activity of chymosin.

Mare milk, a food with good nutritional value

In some countries of western Europe, mare milk is sold, at a high price, frozen or lyophilised. One of the main arguments (at least the less questionable) in advertising is the high content in polyunsaturated FA of the n-3 series. It appears that this content is widely dependent on feeding, and that winter feeding does not allow to obtain a high amount in these acids. By comparison with results obtained in cows receiving lipids protected against hydrogenation, it can be expected that a supply in linseeds, rich in α -linolenic acid, in mare feeding, may lead to an increase in this FA in milk (Doreau et al., 1999). An increase in 20- and 22-carbon FA of the n-3 series could in the same way be reached from fish oil supplementation, but this practice both is unacceptable for many consumers, and may impair milk flavour (Chilliard et al., 2001). The high level of FA unsaturation could make mare milk oxidisable. In fact, it is much less sensitive to oxidation than expected (Sidhu et al., 1976), perhaps due to natural antioxidants in milk. On the contrary, mare milk is sensitive to lipolysis and thus tends to become rancid, because of a lipase (Chilliard and Doreau, 1985).

Mare milk for infants

A widely spread idea is that mare milk composition is close of that of human milk. It is not exact, and many differences have been stated above. Despite these differences, mare milk has often been given to infants instead of maternal milk. When it is possible, it is clear that maternal milk is the best one. Moreover, recent progresses have been made in infant formulas, especially for hypoallergenic milks. The use of mare milk is thus limited. However, its advantages are a higher digestibility than cow milk, measured in rats, and a faster evacuation of stomach. At least, studies on the allergenicity of mare milk in children suffering of IgE-mediated cow's milk allergy have shown that mare milk is much better tolerated than cow milk (Businco et al., 2000). As an exception, one case of intolerance to mare milk by adult has been mentioned (Fanta and Ebner, 1998).

Mare milk against diseases

For centuries mare milk has been known to be beneficial for human health. An abundant literature from the former USSR is available, but the experimental procedures are seldom rigorous. However, empirical knowledge must be taken into account, and main properties have been summarised by Lozovich (1995). Horse milk, like camel milk, is more efficient than cow's milk against peptic ulcer and hepatitis (Sharmanov et al., 1981, 1982). The higher content in phospholipids and vitamin A is assumed to favour healing. Horse milk also has antiacid properties. Tuberculosis has often been treated in sanatoria. Horse milk may increase the number of erythrocytes and lymphocytes, and restore erythrocyte sedimentation rate. Stimulation of the immune system has been thought. The presence of FA of the n-3 series may promote the synthesis of prostaglandins, explaining some actions of mare milk. However, fish is the main source of n-3 FA in human feeding, and thus consumption of mare milk is not the easiest way to increase the sources of n-3 FA. Various digestive diseases are also thought to be cured by mare milk or koumiss, this latter being more efficient probably because of the presence of some products of microbial metabolism, such as peptides and bactericidal substances. The high content in LYS and LF compared to cow milk could be involved in the beneficial aspects of mare milk. Many studies have suggested that LF plays an important role in the natural non-specific defense system in the organism. Besides, LF may provide nutritional effects, e.g., by increasing the bioavailability of iron, and exerts trophic activity on the intestinal mucosa (Nuijens et al., 1996). The biological activities shown for LF are: antimicrobial activity, regulation of immune system, regulation of iron transport, control of cell proliferation, prevention of oxidative functions (Sanchez et al., 1992). Due to its multifunctional role, more and more interest are devoted to LF in view of its applications. LF has been commercialized in milk-based infant formulas and in the area of functional foods such as sport nutrition, health supplements, cosmetics, chewing gums and food supplements. Until now, these actions have been shown on human and/or cow LF, and remain to be evaluated for mare LF. The specific activity of mare milk LF is not higher than that of cow's, goat's or ewe's milk (Nam et al., 1998). Besides, Ig have also numerous biological activities of which interesting activities against oral pathogens, the human-specific gastric pathogen *Helicobacter pylori*, rotavirus infection in children. This remains to be shown for mare Ig. Among the hypotheses, it has been suggested that peptides produced by hydrolysis of β -casein could have a positive action on human health (Maubois and Léonil, 1989). It has been recently shown that mare milk and koumiss contained peptides with hypotensive activities (Ochirkhuyag, 1999, Egito, 2002), but the effect on tuberculosis and hepatitis cannot be explained by such factors. Moreover, it has not been shown that cow milk does not have the same properties.

CONCLUSION - There is a need for a better knowledge of mare milk characteristics for analysing the origin of the positive effects for human health. Until now, few attention has been paid to specific fatty acids. The main way is the

increase in n-3 FA, or at least the guarantee of a minimal content, which could be achieved by nutritional means. For a few years, there is a development of studies on the properties of caseins and whey proteins, especially on their primary structure, both in mares and donkeys. The comparison with proteins of other species could allow to understand some effects of mare milk. LYS, which is absent in cow milk, and LF, which is known for health value, have to be more studied. However, it is necessary to carry out experiments with a rigorous design, allowing to show the particularities of mare or donkey milk compared to cow milk.

Table 1 Milk fat from mares, cows and humans. Mean and extreme values of the literature, in % of total FA

Fatty acid	Mare milk * Mean and extreme values	Cow milk ** Mean value	Human milk ** Mean value
C4:0 butyric acid	0.6 (0.3 – 0.9)	3.3	-
C6:0 caproic acid	0.7 (0.3 – 1.4)	1.6	traces
C8:0 caprylic acid	3.1 (0.8 – 6.1)	1.3	traces
C10:0 capric acid	7.2 (2.3 – 16.7)	3.0	1.3
C12:0 lauric acid	7.6 (3.8 – 14.6)	3.1	3.1
C14:0 myristic acid	7.5 (4.7 – 19.2)	14.2	5.1
C14:1 n-5 myristoleic acid	0.7 (0.1 – 2.6)	1.3	0.5
C15:0 pentadecanoic acid	0.4 (0.2 – 0.9)	1.3	traces to 0.4
C15:1 pentadecenoic acid	0.4 (0.3 – 0.6)	0.3	traces
C16:0 palmitic acid	20.8 (12.4 – 28.5)	42.7	20.2
C16:1 n-7 palmitoleic acid	5.6 (2.2 – 9.7)	3.7	5.7
C17:0 margaric acid	0.4 (0.0 – 1.2)	0.8	0.6
C17:1 heptadecenoic acid	0.4 (0.0 – 1.1)	0.3	0.3
C18:0 stearic acid	1.2 (0.3 – 3.0)	5.7	6.0
C18:1 n-9 oleic acid	19.8 (9.4 – 31.6)	16.7	46.4
C18:2 n-6 linoleic acid	11.3 (3.6 – 20.3)	1.6	13.0
C18:3 n-3 linolenic acid	11.9 (2.2 – 26.2)	1.8	1.4

* From 17 publications quoted by Doreau and Boulot, 1989, and 8 more recent publications: Doreau et al. (1992), Doreau et al. (1993), Csapo et al. (1995), Zeyner et al. (1996), Martuzzi et al., 1998, Hoffman et al., 1998, Curadi et al., 2001, Doreau and Chabrot (unpublished).

** From Jensen et al., 1990

Table 2 Concentration in isomers of some minor FA of nutritional importance, as % of total FA methyl esters (Doreau and Chabrot, unpublished).

	Extreme values (6 milks)
Isomers of conjugated linoleic acid	
cis 9 trans 11	0.021 – 0.043
trans 8 cis 10	0.003 – 0.006
trans 9 cis 11 + cis 10 trans 12	0.004 – 0.006
cis 9 cis 11	0.005 – 0.015
trans 11 trans 13	0.000 – 0.003
trans 8 trans 10 + trans 9 trans 11 + trans 10 trans 12	0.013 – 0.043
Isomers of trans octadecenoic acid	
trans 5	0.001 – 0.007
trans 6 + trans 7 + trans 8	0.002 – 0.059
trans 9 (elaidic acid)	0.028 – 0.180
trans 10	0.001 – 0.077
trans 11 (transvaccenic acid)	0.002 – 0.063
trans 12	0.009 – 0.067
trans 13 + trans 14	0.008 – 0.028
20- and 22-carbon FA	
20:0	0.022 – 0.037
20:1 n-3	0.124 – 0.291
20:2	0.071 – 0.204
20:3 n-3	0.155 – 0.515
20:5 n-3 (eicosapentaenoic acid, EPA)	0.009 – 0.043
22:0	0.004 – 0.010
22:1 n-9	0.013 – 0.033
22:6 n-3 (docosahexaenoic acid, DHA)	0.004 – 0.019

Milks were 5 commercial milks and 1 milk of INRA research centre, from herds of different regions of France, composed of Haflinger (3 milks), Breton (1 milk), Auxois (1 milk) and Merens (1 milk). Fatty acids have been determined by gas chromatography, using a 100-m column CPSil 88, with temperatures from 70°C to 225°C with a step at 175°C.

Table 3. N-terminal sequence of obtained fractions of α_{S1} - [CP-6 Ib (peak CP-6₁)], α_{S2} - [CP-4 Ia (peak CP-4₁)] and β - [TP-4 to TP-8; CP-4 II (peak CP-4₂) and CP-6 II (peak CP-6₂)] of *Equus caballus*. Comparison with α_{S1} -, α_{S2} - and β -caseins of cow*, goat*, mouse*, pig*, mare*, *Camelus bactrianus*[§] and *Camelus dromedarius*[†].

α_{S1} -casein

Cow	R - P-K-H-P-I-K-H-Q-G-L-P-Q-E-
Goat	R - P-K-H-P-I-N-H-R-G-L-S- P-E-
Mouse	R - L-H-S-R-N-A-V-S-S-Q-T-Q-Q-
Pig	R - P-K-P-P-L-R-H-Q-E-H-L-Q-N-
<i>Camelus bactrianus</i>	R/K-P-K-Y-P-L-R-Y-P-E-V-F-Q-N-
<i>Camelus dromedarius</i>	R - P-K-Y-P-L-R-Y-P-E-V-F-Q-N-
Mare CP-6 ₁	R - P-K-L-P-S-R-Q-Q-E-I-I-Q-N-
Mare	R - L-K-L-P-H-R-Q-P-E-I-I-Q-N-

α_{S2} -casein

Cow	K-N-T-M-E-H-V-S-S-S-E-E-S-I-I-
Goat	K-H-K-M-E-H-V-S-S-S-E-E-P-I-N-
Mouse	K-Q-R-M-E-Q-Y-I-S-S-E-E-S-M-D-
Pig	K-H-E-M-E-H-V-S-S-S-E-E-S-I-N-
<i>Camelus bactrianus</i>	K-H-E-M-D-Q-G-X-X-X-E-E-Q-N-I-
<i>Camelus dromedarius</i>	K-H-E-M-D-Q-G-S-S-S-E-E-S-I-N-
Mare CP-4 ₁	K-H-K-M-E-H-F-A-P-X-Y-X-Q-V-L-

β -casein

Cow	R-E-L-E-E-L-N-V-P-G-E-I-
Goat	R-E-Q-E-E-L-N-V-V-G-E-T-
Mouse	R-E-T-T-F-T-V-S-S-E-T-D-
Pig	R-A-K-E-E-L-N-A-S-G-E-T-
<i>Camelus bactrianus</i>	R-E-K-E-E-F-K-T-A-G-E-A-
<i>Camelus dromedarius</i>	R-E-K-E-E-F-K-T-A-G-E-A-
Mare TP-4 to TP-8	R-E-K-E-E-L-N-V-S-S-E-I-
Mare CP-4 ₂	L-E-K-E-E-L-N-V-P-K-E-Y-
Mare CP-6 ₂	R-E-K-E-E-L-N-V-I-S-Q-I-
Mare	R-E-K-E-E-L-N-V-S-S-E-T-

* Data on cow, goat, mouse, pig and mare caseins are from the SwissProt database.

§ according to Ochirkhuyag *et al.* (1997).

† according to Kappeler *et al.* (1998).

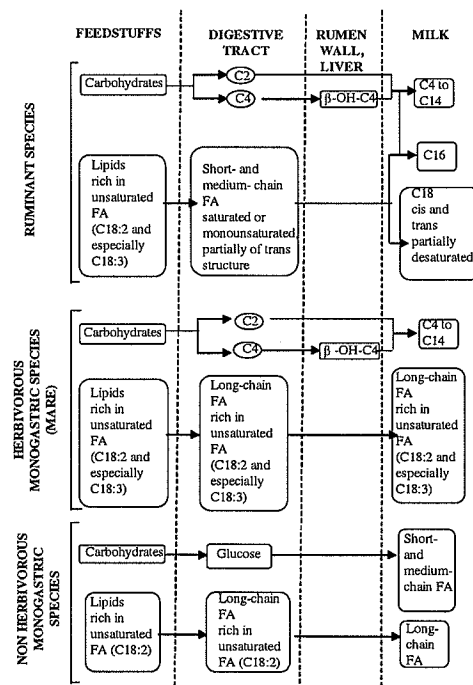


Figure 1. Simplified relationships between feedstuffs and milk FA composition in different species (from Doreau, 1994).

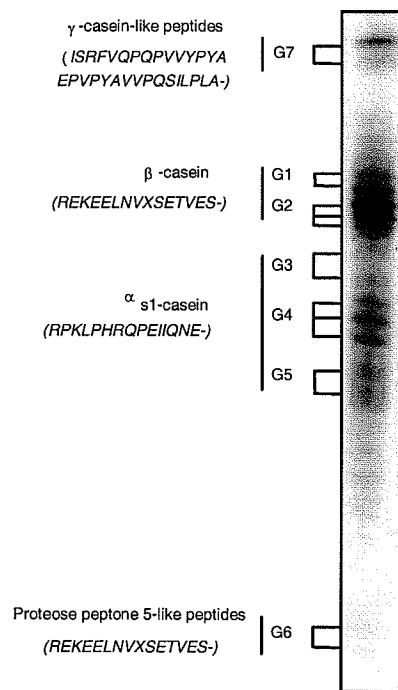


Figure 2: Urea-PAGE of whole equine casein. Proteins were loaded in the gel and stained by Coomassie blue. The 16 principal bands (classified in seven groups G1-G7) were identified by amino-terminal microsequencing after electrotransfer (Egito et al., 2002).

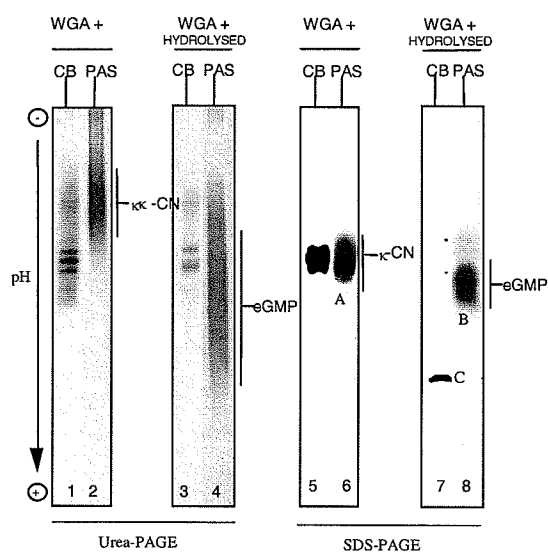


Figure 3: Urea-PAGE and SDS-PAGE of the wheat germ agglutinin (WGA)-bound fraction and of its chymosin hydrolysate. Gels were directly stained with Coomassie blue (CB). Glycoproteins were revealed by PAS staining after electrotransfer.

Legend: WGA+: WGA-bound fraction; WGA+'hydrolysed': WGA-bound fraction hydrolysed by chymosin; eGMP, equine glycomacropeptide; κ -CN: glycosylated κ -casein; A, B, C: bands recovered after electrotransfer and microsequencing (Egito et al, 2001).

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