

Relationships among urea, protein and macro-mineral concentrations in colostrums and whole milk in lactating mares

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Abstract

The concentrations of urea, protein and macro-minerals and their interrelationships were investigated in lactating mares. Fifty-six samples including 17 colostrum and 39 whole milks were collected in 2011-2012 in Uremia, Iran. Milk parameters were evaluated by auto-analyzer using commercial kits, after separation of the milk casein in 0.1 normal HCl. The overall means for urea, protein, calcium (Ca), phosphorus (IP), magnesium (Mg), sodium (Na) and potassium (K) concentrations were 26.4 mg/dl, 4.66 g/dl, 43.2, 20.2, 7.33, 39.7, and 13.1 mmol/l, respectively. The concentrations of urea, Ca, IP and Na in whole milk were 24.4%, 2.8%, 14.3% and 9% greater than in colostrum and protein, and the Mg and K in colostrum were 505%, 91% and 9% greater than in whole milk of mares. Mean comparison between colostrum and whole milk parameters revealed significant differences ($P < 0.01$) in the concentrations of urea, protein and Mg. The highest concentrations of urea, Ca, IP and Na were found in whole milk and for protein, Mg and K in colostrum. With the exception of the differences ($P < 0.05$) in colostrum Ca concentrations between 5, 9 and 10 year olds, no significant differences were observed among the six age groups in milk and colostrum parameters. There were significant correlations between urea/protein ($r = -0.19$, $P < 0.01$), urea/Ca ($r = 0.37$, $P < 0.01$), urea/IP ($r = -0.35$, $P < 0.01$), urea/Mg ($r = -0.32$, $P < 0.01$), Ca/IP ($r = 0.47$, $P < 0.01$), Na/K ($r = -0.49$, $P < 0.01$) and protein/Mg ($r = -0.72$, $P < 0.01$), of which the greatest and strongest correlations were seen in urea and protein/magnesium, respectively. It is concluded that the concentrations of protein and Mg in colostrum of mares were higher and urea was lower than in whole milk. Milk urea revealed the greatest relationships with the other milk parameters, and can be considered as a useful index in studies related to milk production in lactating mares.

Keywords: Mares, milk, colostrum urea, protein, macro-minerals

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Introduction

Milk is considered as the most complete and perfect food due to its abundance of energy, protein and minerals, and many studies have been carried out in relation to the quality and quantity dimensions on it. Consumption of milk and milk products is considered an

important rule for human health. Milk protein in the form of whole milk, yoghurt, and cheese are not only regarded as one of the prominent pillars in milk economy, but is also vital in human life (18). Milk contains many antibodies which protect children against infectious and zymotic factors. Consumption of

ruminants and camels' milk is common but cow's milk is the most suitable and, it is cheap. Nowadays, as a result of cow's milk allergy, donkeys and mares milk are now widely consumed in many countries (9).

Conditions for a suitable milk is based on containing valuable nutrients, the industrial production available, lack of milk allergy, and being economical and cheap. Mares and donkeys' milk contain some of these conditions and medical indications as well. The priority of equine compared to ruminants' milk is producing milk from 5 to 15 kg/day (5), the same or even more than small ruminants, buffaloes and camels. Although the protein and fat contents (8) of the mare's milk is low, essential amino-acids (4), lactose (22) and dry matter (14) contents are more than cow's milk. The presence of intensive lactating mare herds in the Netherlands (27), and over 230000 lactating mares in Kyrgyzstan and other countries indicates the importance of equine milk. Allergy to mares' milk is less than cows' milk (9) and finally, consumption of the mares' milk is effective in the coalescence of human lesions (30), thus these advantages support the notion that mare's milk may replace cow's milk in many countries in future.

Protein and macro-minerals of mare's colostrum' are reported more often than in whole milk (3, 29), but they gradually decrease and reach whole milk values within 24 hours (16). Among livestock species, milk protein in sheep is the highest and mares the lowest, while mares milk macro-minerals are higher than in ruminants (7, 14). The authors reported small amounts of urea in ruminants' milk as a result of protein metabolism, the same as reported in blood, sweat and urine (18), which is unknown in Iranian mares' milk. Milk also contains 0.9% macro-minerals, 80% of which is soluble in the milk serum and 20% is conjugated to milk casein. Calcium is the main and magnesium the least among macro-minerals. Macro-minerals are considered an essential factor in providing the calcium and magnesium requirements of children, their growth and weight gain, casein coagulation in abomasum and prevention of

metabolic and nutritional diseases. The literature mentions different values and variations in the macro-minerals of colostrum and whole milk of mares (25, 7), which are unclear in the mares of this region.

Different relationships have reported among protein, urea and macro-minerals in sheep, goat and cow's milk (18) which are unclear in equine milk. The importance of milk urea evaluation is evidenced as an indicator in the diagnosis and prediction of livestock nutritional management, milk yield and its component, and finally, reproduction performances in ruminants (28), however, no evidence was found in the mares' milk of Iranian equines in spite of the special advantages mentioned previously for their milk. The outcome of this investigation not only enriches authors and consumers' knowledge regarding mare's milk, but may also motivate farmers to encourage and enhance the efficiency of the newly introduced livestock breeding called mares. The purpose of this study is the determination of protein, urea and macro-mineral values in colostrum and whole milk in mares according to their ages, and then the determination of the relationships among milk parameters in mares.

Materials and methods

Animals and sampling method:

Fifty-six samples including 17 colostrum and 39 whole milk were prepared from mares located in Urmia, Torkaman and Maragheh cities in 2011-12. Age frequency in lactating mares of 5, 6, 7, 8, 9 and 10 years of age for whole milk were 4, 7, 10, 5, 6, 7 mares and for colostrum were 5, 3, 3, 2, 2, 2 mares, respectively. Ten ml colostrum or whole milk was collected in a test tube from teats. Supplementary information including the owners' name, address of horse breeding and date of sample collection for further consideration were recorded. The mares were bred individually in the stable or in small groups, and condensed or intensive as a big herd. Colostrum samples were collected from foaled

mares up to 24 hours after foaling and for whole milk during the lactation period up to six months' lactation. Samples were collected under very difficult situations with the cooperation of the owners, with some unsuccessful attempts. Horses were fed a diet containing hay, roughage, barley and Lucerne. Milk samples were delivered to the relevant laboratory in groups of 5 to 7 samples and then the related tests were run on them. Mares were all healthy and mastitis was not observed in any of the mares.

Laboratory Methods:

Five ml colostrum or whole milk was collected in a test tube. Milk samples were first defatted by placing them in a cool area (4C°) and/or centrifugation in 3000g for 5 minutes. Milk casein was separated by 0.1 N HCl in pH 3.6. Milk sera were used to determine the milk urea, protein and macro-mineral concentrations. The concentrations of urea, protein, calcium, magnesium and inorganic phosphorus were assessed in an auto-analyzer machine (RA-1000, USA), milk sodium and potassium in a flame photometer using an appropriate kit for each measurement (Pars Azmon, IR, Rumbut, UK). Results were calculated in standard units.

Statistic

Data were analyzed by SPSS₁₃ statistical program and Mean±SEM were determined for all parameters in the milk samples. Student t-test was carried out to understand the differences in the parameters under study. Pearson correlation tests were used to evaluate the relationships among parameters in different milking yields.

Results

Concentration of protein in colostrum was higher and urea lower than in whole milk of lactating mares (Fig 1). The range for urea and protein in colostrum of

lactating mares was 12-32 mg/dl and 0.8-1.62 g/dl, and for whole milk was 27-108 mg/dl and 1-4/8 g/dl, respectively. The mean overall for urea and protein was 26.35 mg/dl and 4.66 g/dl, respectively. There were no significant differences between the concentrations of urea and protein in colostrum and whole milk of the six age groups (Tables 1 and 2), while differences were observed for milk urea (df=1, t=9.6, P<0.01) and protein (df=1, t=99.9, P<0.01) of colostrum' and whole milk for the overall samples.

Mean for Ca, IP and Na in colostrum was lower and Mg and K higher than in whole milk of lactating mares (Fig 2). The ranges for Ca, Mg, IP, Na and K in colostrum were 2.16-55, 6.13-17.17, 7.44-28.3, 32-49, and 9.1-21 mmol/l and in whole milk were 1.44-9.13, 35-60.4, 5.2-54.8, 20-78 and 6.3-23.4 mmol/l, respectively. The concentrations for overall were 43.2, 7.3, 20.2, 39.7, 13.1 mmol/l. The highest and lowest concentrations in mares' milk belonged to Ca and Mg, respectively (Fig 2). With the exception of Ca concentration in mares with less than 5, 9, and 10 years old (P<0.05), the statistical analysis did not show any significant differences in the concentrations of macro-minerals in colostrum (Table 1) and whole milk of the lactating mares (Table 2). Overall, no significant differences were found between macro-minerals in colostrum and whole milk of mare.

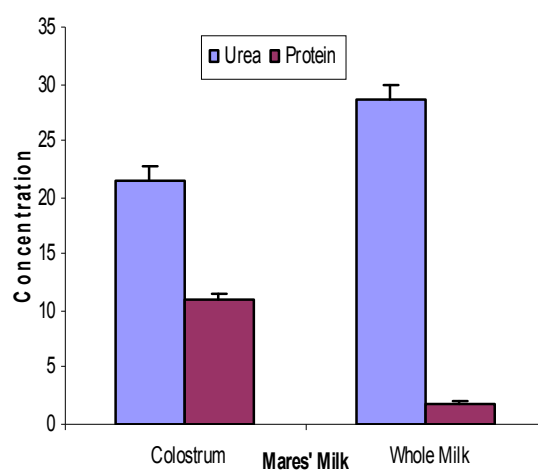
The results of correlations (Pearson) among urea, protein and macro-minerals in the overall mares' milk revealed the relationships between urea and protein (r=-0.19, p<0.05), urea/Ca (r=0.37, P<0.01), urea/Mg (r=-0.32, P<0.01), Ca/P (r=0.47, P<0.01), urea/IP (r=0.35, P<0.01), Protein /Mg (r=0.72, P<0.01) and Na/K (r=0.49, P<0.01). Urea showed the greatest relationships with the other milk parameters and the strongest correlation was observed between protein and Magnesium.

Table 1: Mean age comparison of urea (mg/dl), protein (g/dl) and macro-minerals (mmol/l) in colostrum of lactating mare.

Age (year)	Numbers	Urea	Protein	Ca	P	Mg	Na	K
5	5	20.4	12.2	22.2	10.8	12.1	40.3	14.9
6	3	22	9.42	44.2	20.1	12	36.8	12.6
7	3	21.5	10.63	45.9	21.3	12.5	33.8	14.4
8	2	22.25	11.85	35.8	18.1	11.9	35	10.2
9	2	22.75	10.73	51.7	19	8.8	40.3	15.7
10	2	21	11.23	52.7	17	8.6	40.3	15.1
Overall	17	21.44	11.1	42.4	18	11.1	37.4	13.8

Table 2: Mean age comparison of urea (mg/dl), protein (g/dl) and macro-minerals (mmol/l) in whole milk of lactating mare.

Age (year)	Numbers	Urea	Protein	Ca	P	Mg	Na	K
5	4	26.5	1.5	42.5	20.2	5.93	39.7	13.19
6	7	30.3	1.86	41.6	20.3	5.49	41.1	10.73
7	10	30.9	2.05	41.8	20.6	6.23	36.7	13.28
8	5	27.4	1.72	52.9	19.6	6.32	37.5	15.27
9	6	24.7	1.83	44.7	21.1	5.18	54	12.7
10	7	28.4	1.9	44	24.6	5.34	42	12.35
overall	39	28.5	1.86	43.6	21.1	5.75	41	12.72

**Fig 1.** Mean comparison of the urea (mg/d) and protein (g/d) in colostrum and whole milk of mares

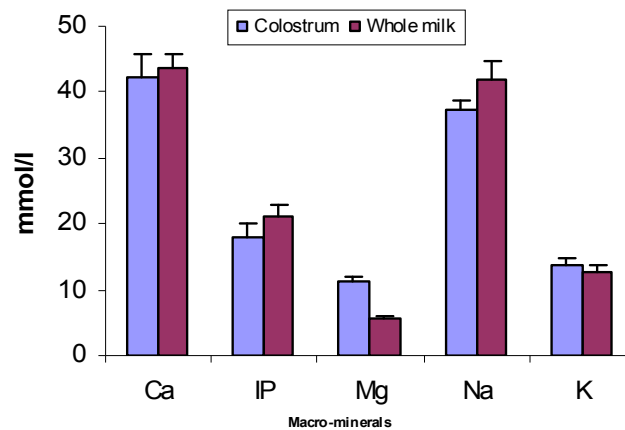


Fig 2. Mean comparison of the macro-mineral concentrations in colostrum and whole milk of mares

Discussion

Reports on the mares and donkeys' milk quality and quantity and industrial milk production have increased during the last two decades. The low mares' milk allergy in comparison to cows' milk allergy (20, 9) and its mass production for specific consumption specific consumption was the main focus in equine milk. The presence of the big dairy mare herds in the Netherlands (27) and over 230000 lactating mares in Kyrgyzstan, Turkmenistan, Russia and other countries demonstrates the necessity of commercial and scientific investments in this novel phenomenon. Daily mare's milk yield is reported from 5 to 15 kg/day (5), which is apparently less than the yield from dairy cows (19), but comparable with buffaloes, sheep, goats and camels milk yield (Medhammar et al. 2011), however it was too low in the mares of this study due to the lack of intensive breeding knowledge in Urmia and because it is used only for foal feeding. The composition of the donkey's milk is not only similar to human milk, but also better than mares milk (8). There are several advantages of mare's milk including high lactose up to 40% more than cow's milk, so it can be fermented and converted to alcoholic drinks (22), increased health status, beauty lotion production, shampoos, recovery of skin lesions, prevention of atherosclerosis, increased immunity status and finally, its use as a probiotic and growth promoter (21).

The normal values for protein in mares' milk were reported 2.2 g/dl (8) and in this study 1.86 g/dl which was similar to the results of Medhammar et al (14), Santoz and Silvestre (22) and Doreau and Martin (2011), but less than that reported by Uniacke et al (2010) and Csapo-kiss et al (1995), up to 4.13 g/dl. Protein is considered one of the economic advantages of milk, and low milk protein results in an imbalance of dietary protein in the ration (12), so to compensate this deficiency the concentrate feeding containing 14% protein must be applied. Variations in milk protein during the lactation period is reported in mares the same as confirmed in ruminants. Santos and Silvestre (22) demonstrated that the protein and fat amounts of mares' milk decreased during the 180 days of lactation, while Pecka et al (2011) mentioned it increased during this period. Milk protein value in mares is more than in human but less than in ruminant's milk (26). The values for milk protein in this study were at the normal range and indicate a reasonable feeding program in mares.

Colostrum are the first production of breast cells following parturition and its consumption is strictly recommended for children. The colostrum production in foaled mares is limited, but its nutritional value is higher than in whole milk due to the high quality of proteins, albumins and immunoglobulin (3). In this study the concentration of the colostrum protein was 505% of

whole milk (30) but less than reported by Csapo-Kiss et al (1995) up to 16.4 g/dl. However, the protein level of colostrum is not constant and sharply decreases within 12 hours after parturition (29). The quality of colostrum in pure breeds is more than in hybrids (29), its essential amino acids are higher than in whole milk and in mares is more than in cows (4). The role of colostrum in the recovery of human lesions is greater than in whole milk of mares (30). The mechanism of milk and colostrum protein production was described by Tanner et al (24). Milk protein contains nutritional and economic value, and is responsible for infant growth, milk prices, and finally, by products such as yoghurt and various cheeses. The amounts of κ -casein in mare's milk are very low, thus milk cannot coagulate and producing cheese is not possible from mares' milk (6, 5,8).

The amount of urea in colostrum and whole milk has not been investigated in Iran. Milk nitrogen is classified into total nitrogen (TN), protein nitrogen (PN) and non-protein nitrogen (NPN), and in this study the NPN was the target (17). Approximately 90% of milk nitrogen is located in the milk protein and casein is the most important one (5). The low NPN in colostrum in comparison to whole milk is reasonably reported by authors in that it increased up to 13% during the lactation period (4). Milk urea in mares was lower than in buffaloes, cows, sheep and goats (18), meaning that the mares of this study were not high producing animals, and therefore, urea supplementation was not included in their ration. Milk urea increases following urea supplementation and wheat gluten in diet (17). Adding 20 g urea in the diet results in an increase in milk urea up to 20% (23), thus foals will refuse to drink milk and their growth will be retarded (12). Milk urea was used as a diagnostic and prediction index to improve the production and reproduction performances in dairy cows and sheep. Milk urea index is widely related to hematological, biochemical parameters, milk quality and quantity, estrus cycles, pregnancy and parturition

status, which is not exactly defined in mares; so establishing the relevant information is critical (1).

In this study the mean for Ca, IP and Na in colostrum was higher and Mg and K lower than in whole milk mares. These results disagreed with the reports of Grace et al (1999) for Ca and IP and Ciesta et al (2009) results for Mg, but in other minerals the results were quite similar. Variations in the amount of minerals, especially for Ca in the mares' milk is not clear during the lactation period, the same as described in ruminants (2). Tischner et al (25) reported that the Na concentration in colostrum was more than in whole milk, which decreases up to 40% within 24 hrs. after parturition. In this study, the same as in other reports, milk Ca was the highest while milk Mg was the lowest. The role of milk IP and Mg in the mares' milk is not as predominant as in ruminants, while much attention has been paid to the roles of Ca, IP and Na. Milk Ca in mares starts to increase seven days before foaling (12) and when it reaches to over 10 mmol/l it is accompanied by an increase in milk K, indicating the maturation of foal and foaling time (15). Ji et al (Ji et al. 2011) have demonstrated the role of vitamin D in Ca absorption from breast into the milk and acceleration of the foal growth.

The results of the correlations among milk parameters revealed the interrelationships among protein, urea, and macro-minerals in the mares' milk. These results did not support the results of urea and protein in cows, buffaloes, sheep and goats (18) but were in agreement with the results for milk minerals in ruminants (7, 19). Milk urea and protein in the mares' milk were in contrast to each other. Jonker et al (11) demonstrated the possibility of a relationship between milk urea and protein. The complementary study is essential for determination of the relationships between urea and protein in milk and blood in order to designate the diagnostic value of these parameters in milk. In conclusion, the colostrum protein is higher and urea lower than in whole milk of the mares. The differences in the colostrum and whole milk parameters were

significant. Among all milk parameters urea showed the greatest relationship with others. Finally, urea in the mares' colostrum and whole milk is confirmed, which is probably related to milk protein and macro-minerals.

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