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Comparative analysis of casein complex and amino acid composition in single-humped and double-humped camel milk: Implications for dairy camel breeding

Kuanysh Syman[1]*, Raissa Utegaliyeva[2], Abdreshov Serik Nauryzbaevish[3], Zharylkassynova Zhazira[4], Aytzhanova Mira Onlanbekovna[5], Kalekeshov Askar Maralovich[6], Mamataeva Aigul Tumaevna[7]

1. Biological Sciences, Institute of Natural Sciences and Geography, Abai Kazakh National Pedagogical University, Almaty, Kazakhstan, 13, Dostyk Av., 050010 Almaty, the Republic of Kazakhstan
2. Biological Sciences, Kazakh National Women's Pedagogical University, Almaty, Kazakhstan, 99, Aiteke Bi St., 090000, Almaty, the Republic of Kazakhstan
3.Biological Sciences, Institute of Genetics and Physiology CS MSHE RK, Laboratory of Physiology Lymphatic System, Almaty, Kazakhstan, 050060, 93 Al-Farabi Avenue, Almaty, the Republic of Kazakhstan
4.Master of pedagogical sciences, Institute of Natural Sciences and Geography, Abai Kazakh National pedagogical university, Almaty, Kazakhstan, 13, Dostyk Av., 050010 Almaty, the Republic of Kazakhstan;
5. Biological Sciences, Kazakh National Women's Teacher Training University, Almaty, Kazakhstan, 114/1 Gogol St. 050000, Almaty, the Republic of Kazakhstan
6. Biological sciences Kazakh National Women's Teacher Training University, Almaty, Kazakhstan, 114/1 Gogol St. 050000, Almaty, the Republic of Kazakhstan
7. Biological sciences Almaty Technological University, Almaty, Kazakhstan, 100, Tole Bi Av., 050000 Almaty, the Republic of Kazakhstan

** Corresponding author's Email: Syman71@mail.ru*

ABSTRACT

The purpose of this work is to study the casein complex of camel milk and their amino acid composition. including the chemical composition and physico-chemical properties of milk of single-humped and double-humped camels, as well as the fractional composition of milk proteins of two types of camels and combined milk, followed by the amino acid composition of the casein complex of milk. The protein composition of the casein complex and whey proteins can be used in breeding work in dairy camel breeding to select the most desirable types of animals. The electrophoretic picture of casein fractions on polyacrylamide gel can serve as a reference in the study of camel milk proteins.

Keywords: Camel, milk, Casein complex, Amino acid composition, bactrians, dromedaries, Electrophoretic analysis.
Article type: Research Article.

INTRODUCTION

Casein belongs to phosphoproteins. For a long time, it was considered as an individual protein substance. At first, Mellander (1939) reported that cow's milk casein consists of three electrophoretic components, called α-, β- and γ-caseins by mobility in an electric field. Further, Waugh and Hippel (Waugh & Hippel 1956) found that the casein micelle contains a complex of α_s-casein and another component, called χ-casein. Over time, it was considered that cow's milk casein consists of four main fractions: α_s-, χ-, β- and γ-caseins (Whitney *et al.* 1976). Fine electrophoretic studies using agar, starch and polyacrylamide gel made it possible to divide each casein

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fraction into several sub-fractions; α_s- casein was divided into 6 sub-fractions, χ- casein into 3, β-casein into 3 and γ-casein into 5 (Seitov & Zhumashev 1970). As a result of the brilliant work of a number of scientists (Waugh & Hippel 1956; Whitney *et al.* 1976; Abeiderrahmane 1997), the primary structures of all known casein variants have been established and their clear characteristics have been given. The main fraction of the casein complex is α_s-casein, which its content varies depending on many factors, in a fairly significant range (Pyanovskaya 1962; Morozov 1962; Nikitina 1965; Prokhorova 1969; Dilyanyan 1981). Thus, according to Alekseeva & Dyachenko [5], the fractional composition of casein varies within the following limits (electrophoresis on paper): α_s-casein (57-61%), β-casein (15-33%) and γ-casein (3-8%) to total casein. Whitney *et al.* (1976) divided casein complex into four fractions by gel electrophoresis in an alkaline-urea medium, depending on their mobility: α-casein, β-casein, χ-casein, γ-casein, and by electrophoresis on agar gel with urea at pH = 8.66 in the total casein of cow's milk. Seitov *et al.* (1970) identified 15-17 sub-fractions: α_s-casein included six, χ-casein three, β-casein three, sometimes five sub-fractions. Their relative content was in total casein: α_s-casein (41.9%), χ-casein (27.3%), β-casein (29.0%) and γ-casein (1.8%). The χ-casein fraction (Alekseeva & Dyachenko 1965; Seitov *et al.* 1970) plays an important role in stabilizing the entire casein complex in milk. Beta-casein with an average content of 23-35% (Gorbatova 1984) occupies the next place in the complex in quantitative terms. The fragment of β-casein formed during the cleavage of milk by proteases is gamma-casein containing about 3% (Hipp *et al.* 1952; Larson & Vendall 1957). Since the 50s of the 20th century, with the development of precise analytical methods, the study of the primary structure of protein molecules began. The founder of this epoch making work was the English biochemist Sanger (1953), who developed an original method for establishing the sequence of amino acid compounds in the polypeptide chain of a protein molecule. Sanger (1953) found the primary structure of the insulin molecule, a hormone whose deficiency is associated with the occurrence of a serious illness, i.e., diabetes mellitus. Then the study on the primary structure of enzymes and other biologically-active proteins began to be conducted rapidly. In 1970, the complete sequence of the amino acid compound, α- lactalbumin was the first to be established from milk proteins (Brew & Hill 1970). Furthermore, the structures of almost all cow's milk proteins and their genetic varieties have been described in (Whitney *et al.* 1976; Gorbatova 1993). Since 1992, the structure of the main casein fractions and the main whey proteins of camel milk has been studied mainly by Swiss scientists Kappeler (1998). The main casein fractions of both cow's and camel milk are α_{s1}-, α_{s2}-, β- and χ-caseins. The main chemical characteristics of these proteins are given in Table 1 (Kappeler 1998). The polypeptide chains of camel α_{s1}-casein contain six serine residues, and in cow- eight, which form esters with phosphoric acid. Nine and eleven phosphorylated serine residues were found in the α_{s2}-casein of camel and cow's milk, respectively. In these two casein fractions of milk of both animals there are no carbohydrates and also cysteine and cystine. β - casein is the most hydrophobic protein of all fractions of the casein complex. Camel casein contains three phosphorylated serines, while cow casein contains five. Their molecules contain a very high amount of proline amino acids, 35 residues, however, no cysteine and cystine. χ - casein has unique properties. It is hydrophilic and highly soluble in the aqueous part of milk. It stabilizes casein micelles in milk against precipitation by calcium ions and maintains their homogeneous dispersed state. The ability of milk to curdle under the action of rennet enzyme in cheese production is associated with the properties of χ-casein. However, the χ-caseins of camel and cow's milk differ greatly in their properties, which will be discussed as follow. One phosphorylated serine residue was identified in their molecules. Ten residues of threonine by its hydroxyl group are glycosed in camel milk, and twelve in cow's milk. There are sulfur-containing amino acids - cystine and methionine in two residues. From the data in Table 1, it can be seen that camel milk contains the most β-casein, 65% of the total amount of this protein, while in cow's milk its concentration is only 39%. The amount of α_{s1}-casein in camel milk (22%) is significantly less than in cow's milk (38%). The content of α_{s2}- casein in both types of milk is almost the same. Of interest is the meager content of hydrophilic protein, χ-casein in camel and cow's milk are 3.5% and 13% respectively. According to the nomenclature (Whitney *et al.* 1976) there are three fractions of γ-casein in cow's milk. Information regarding its content in camel milk is not yet available in the literature. One of them, from cow γ-casein, consists of 181 amino acid residues with one phosphoric acid residue by molecular weight 20.560 kDl. The other two fractions include 104 and 102 amino acid residues with molecular weights of 11,821 kDl and 11,550 kDl. Notably, the study of the primary structure of the fractions of γ-casein shows its identity with the structure of the polypeptide chain of β-casein. Therefore, the fractions of γ-casein are considered as fragments of the proteolytic hydrolysis of β-casein (Hipp *et al.* 1952; Larson & Vendall 1957).

Table 1. Physico-chemical characteristics of the main protein fractions of milk casein

Animal	Casein	Number of amino acid residues	Molecular weight kD	Isoelectric point, pH	Relative amount in total casein
Female camel	$\alpha_{s\,1}$- casein	207	24.275	4.78	22%
Cow	α_{s1}- casein B	199	22.975	4.76	38%
Female camel	α_{s2} – casein	178	21.266	5.81	9.75%
Cow	α_{s2} – casein A	207	24.348	8.68	10%
Female camel	β - casein	217	24.651	5.17	65%
Cow	β - casein A_2	209	23.583	5.01	39%
Female camel	χ- casein	162	18.254	8.27	3.5%
Cow	χ- casein	169	18.974	5.97	13%

MATERIALS AND METHODS

Isolation and drying casein for electrophoretic analysis

The milk was separated twice into skimmed milk (fat content 0.05%) with a temperature of 20-21 ºC with constant stirring, 1 M HCI was added to pH 4.6. The pH was stirred and adjusted again, equal to 4.6. The suspension was defended for an hour. The infusion fluid was siphoned, and the precipitate was filled with water with a pH of 4.6 to the initial volume. They were stirred with an electric mixer for 10 min; the sediment was settled; the supernatant fluid was siphoned. Washing casein with water was repeated 6 times. In order to obtain purified casein, the precipitate was filled with water and an alkali solution was added to pH 7.5. The dissolution of casein was carried out at pH 7.5-7.6, stirring with an electric mixer for 1.5-2 hours. The solution was filtered through a Buchner funnel. Casein was again precipitated from the filtrate with acid at pH 4.6. The resulting casein precipitate was washed with water at pH 4.6 at least 6 times. In conclusion, the suspension was filtered on a Buchner funnel and the casein was dried with cold pure acetone. About 20 g of chilled casein was ground in a porcelain cup and 100 mL cold acetone was poured. The casein particles were well crushed and left for 30 minutes in the refrigerator evaporator. The casein treatment was repeated two more times. The mixture was filtered on a Buchner funnel. The casein on the filter was washed with 100 mL cold acetone and the acetone was removed using a water jet pump. The protein was dried in a vacuum desiccator over potash for a day. At the same time, a dry white casein powder was obtained.

Determination of casein fractions by electrophoresis in agar gel

For electrophoretic separation of the casein complex of milk, the method of electrophoresis in agar gel was used (Seitov & Zhumashev 1970). Agar gel was chosen as the supporting medium for electrophoresis. It is known that due to the very small pore sizes, the gel acts as a molecular sieve, contributing to a more complete separation of proteins than is possible on paper (Seitov & Zhumashev 1970). Consequently, in gel electrophoresis, the separation of proteins, in addition to the magnitude of their charge, depends on their molecular weights, shape and size. Hence, electrophoresis in gel has a high resolution of protein mixtures. From agar gel, it is easy to elute the paint used for staining protein strips on an electropherogram. This is very important for the quantitative determination of the protein fraction. The gel from Korsakov agar was used in the work, the camera for electrophoresis was an apparatus made of plexiglass. A borate-acetate buffer (pH 8.6) was used as the main buffer solution. Denaturation of the associated fractions of the casein complex was carried out by introducing 4.5 M urea into the buffer solution, and the casein preparation was dissolved in the same buffer solution with urea. Such a concentration of urea completely destroys hydrogen bonds, hydrophobic interactions between casein fractions, and the casein complex breaks down into separate protein components. Further electrophoretic separation of casein was carried out according to the recipe described in the method proposed by Seitov & Zhumashev (1970).

Quantitative determination of fractions

The electropherogram was cut along all fractions on its both sides, then each fraction was cut out of this tape

separately and its length was measured in mm. To control the space between the electropherogram tapes, a piece is cut out in a light area with a width equal to that of the tape. Fractions after measuring their length were crushed, transferred to test tubes and filled with 2 mL 061 M solution. NaOH containing 0.5 g EDTA in 1 liter. The contents of the tubes were shaken during extraction (30 min), the paint solution was poured into another tube, and the pieces of agar were again filled with 2 mL alkali solution, stirred and left for 30 min. The paint solution was drained and the paint was extracted again with another 2 mL alkali solution. The eluate was combined and the optical density was measured on SF-26 at 590 mmc. The percentage of casein fractions was calculated based on the sum of optical densities.

Sample calculations

Suppose, the optical density (D) of the solution of α_2-casein is 0.42, the length of the forogram is 12 mm, the control length is 0.02 and the length is 10 mm. We found the correction for the background 10 - 0.02

12-x

$$X = \frac{12 \times 0.02}{10} = 0.024$$

Then, from the D solution of α_2- casein, we calculated the correction for the background and found the true D: 0.42-0.024 = 0.396. Having calculated in this way the true D of solutions of all casein fractions, we sum them (suppose, it is 1.6) and took it as 100%. The amount of α_2- casein was found from the ratios:

1.6-100

0.396-α_2

$$\alpha_2 = \frac{0.396 \times 100}{1.6} = 24.7\%$$

According to the sum of optical densities, the percentage of each casein fraction was calculated. Electrophoretic separation of milk whey proteins in agar gel was performed according to the recipe of the method used to determine casein fractions (Seitov & Zhumashev 1970), without introducing urea into the borate-acetate buffer and into the protein solution. At the same time, some of the techniques described in the methodology of Seitov & Mustafin (1973) were taken into account, due to the characteristics of the properties of whey proteins.

Determination of casein and whey protein fractions in polyacrylamide gel

For a detailed study of the individual characteristics of the protein composition of camel milk, the content of casein complex and whey proteins in the milk of individual camels and dry saumal in polyacrylamide gel were studied. The study was carried out in an AVGE-1 apparatus with a vertical arrangement of glass plates. Electrophoretic separation of casein (Seitov & Zhumashev 1970; Seitov & Toktamysova 2002), includes the preparation of 7% fine-pored and 3.125% coarse-pored polyacrylamide gels in a TRIS-glycine buffer, in the presence of 4.5 M urea, polymerization of gels in a cell, introduction of a gel cell into an electrophoresis apparatus, dissolution of casein in a buffer with sucrose, introduction of the resulting solution into the wells of the cell, pouring into electrode compartments of the electrode TRIS-glycine buffer, followed by electrophoresis, staining of the resulting electrophoregram. For a more complete determination of whey proteins, we developed a method for their electrophoretic separation in polyacrylamide gel in the presence of sodium dodecyl sulfate, which is adsorbed on the surface of the polypeptide chain of the protein molecule. The protein acquired a negative charge. Different concentrations of polyacrylamide gel were tested, from 6.0 to 16.0%, for the separation of whey proteins. The best results were at a gel concentration of 12.0%. The method is recognized as an invention, and a preliminary patent of the Kazakhstan Institute of Patent Examination of the Republic of Kazakhstan was obtained under application No. 11274. 2002. Electrophoresis of whey proteins is carried out in an AVGE-1 apparatus with a vertical arrangement of glass plates.

Preparation of a solution of the protein under study

Milk is precipitated with 0.1 M HCl, then centrifuged at 10000-12000 rpm for 5 min. The infusion fluid was filtered and put on dialysis against distilled water for 24 h. Water was changed every 3-5 h. Dialysis took place at

4 ºC (in the refrigerator). One volume was taken from the protein solution and 1 volume of sodium dodecyl sulfate solution was added to it. Then we kept the resulting mixture in a water bath for 5 minutes at 40-60 ºC.

RESULTS

Electrophoresis in agar gel

In fresh milk, casein is contained in the form of a caseinate calcium phosphate complex consisting of calcium caseinate in combination with calcium phosphate. It belongs to complex proteins- phosphoproteins and makes up 80-82% of the total amount of cow's milk proteins. In its pure form, casein is a white amorphous powder without taste and odor, with a density of 1.26-1.30, slightly soluble in water and insoluble in alcohol, ether, well soluble in solutions of some salts. Electrophoretic and chromatographically, cow's milk proteins were well studied (Kumar *et al.* 2016; Adel *et al.* 2016; Alavi *et al.* 2017; Abderrahmane *et al.* 2017; Izadi *et al.* 2019; Ayman *et al.* 2021; He *et al.* 2022) The electrophoregrams identified 4 main fractions and several sub-fractions, as well as whey proteins. Since we have not been able to detect electrophoretic identification of camel milk proteins in the literature, in this work, camel milk casein was separated in comparison with cow's milk casein by electrophoresis in agar gel. This method is well established at the Department of Biochemistry of the Kazakh National Agrarian University. Fig. 1 shows electrophoregrams of camel and cow's milk casein obtained in agar gel with urea.

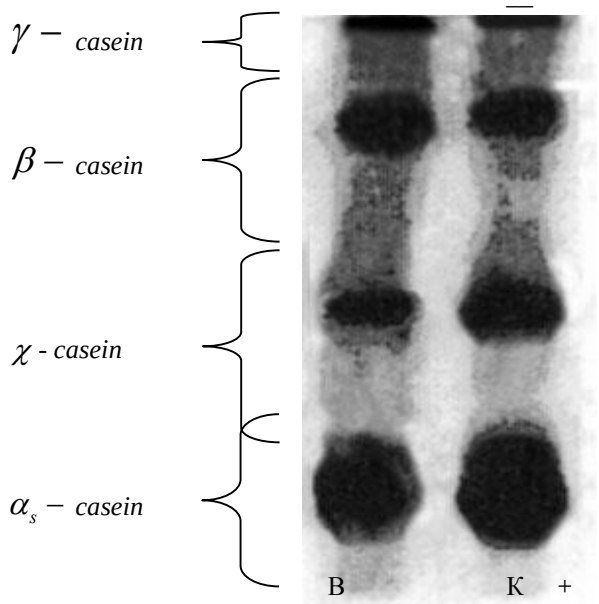

Fig. 1. Electrophoregram of camel casein (B) and cow's milk (K) in agar gel with 4.5 M urea in borate-acetate buffer, pH 8.6.

Now let's decipher the protein bands on the agar electrophoregram (Fig. 1), taking as a basis the work of Seitov & Zhumashev (1970). In this work, cow's milk casein fractions were identified using homogeneous β- and χ-caseins isolated by the authors and taken as marker proteins. On the electrophorogram as the electric mobility increased, the fractions of cow's milk were arranged in the following sequence: γ-casein, β-casein, χ-casein and α_s-casein (Seitov & Zhumashev 1970)

Electrophoresis in polyacrylamide gel

To identify and decipher electrophoretic separation of camel milk casein we used electrophoretic study in comparison with cow's milk casein in agar gel. At the same time, it turned out that the zones of location of all casein fractions of both types of milk on the electrophoregram coincide. To obtain a more complete picture of the protein composition of the casein complex of camel milk, electrophoresis in a polyacrylamide gel with a high separating property was used in the work. In addition, in this case, electrophoresis of camel milk casein was performed in comparison with cow's milk casein. The identification of camel milk casein fractions was carried out based on the works of Seitov & Zhumashev (1972), Seitov & Zhumashev (1971) and Seitov & Zhumashev (1970) in comparison with the materials of other studies (Seitov & Zhumashev 1971; Kumar *et al.* 2016; Izadi *et al.* 2019; He *et al.* 2022). As shown in electrophorogram 2 (Fig. 2), cow's milk casein exhibited 18 protein bands.

Of those, seven bands with Rf from 0.05 to 0.38 are deciphered as the β-casein zone; from 0.46 to 0.71 as α_s-casein- two forms 0.76 to 0.80 as χ-casein and one band as γ-casein. The zones of location of camel milk fractions and their identification were carried out in accordance with these experimental results.

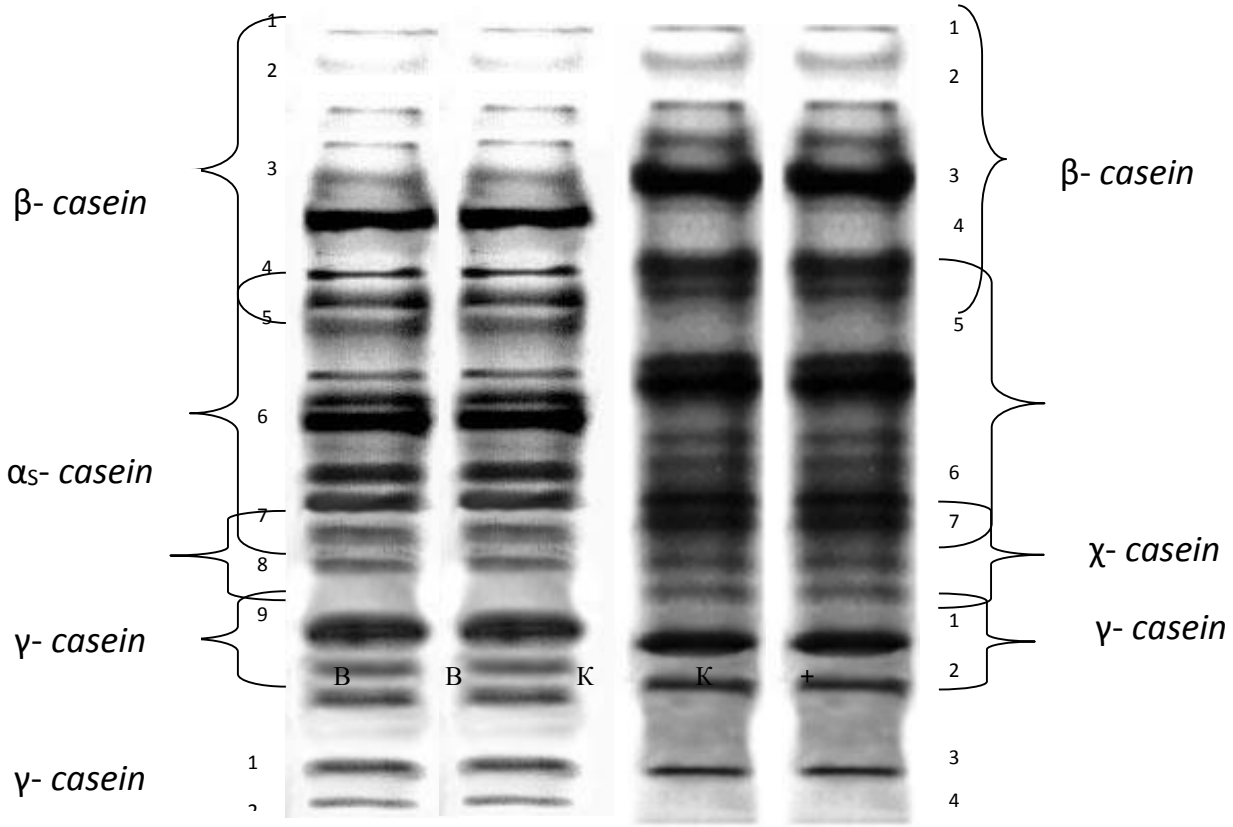

Fig. 2. Electrophoregram of bactrian casein (B) and cow's milk (K) in a 7% polyacrylamide gel in the presence of 4.5 M urea in a tris – glycine buffer with a pH of 8.6.

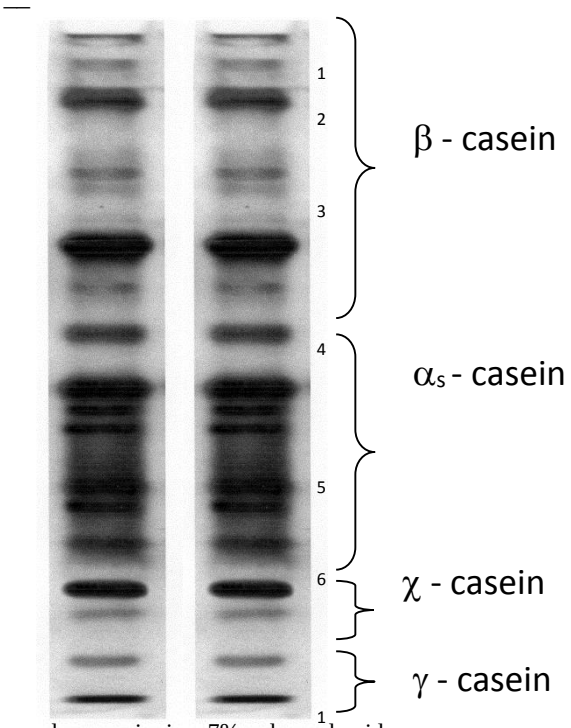

Fig. 3. Electrophoregram of dromedary casein, in a 7% polyacrylamide gel with 4.5 M urea in a tris-glycine buffer with a pH of 8.6.

Table 2. Quantitative content, electrophoretic mobility of casein fractions of bactrian and cow's milk.

Camel milk			**Cow milk**		
Casein Fractions	**content (%)**	**Rf**	**Casein Fractions**	**content (%)**	**Rf**
β - casein					
1.	0.9	0.05	1.	0.9	0.05
2.	0.7	0.06	2.	1.1	0.07
3.	0.9	0.09	3.	1.2	0.09
4.	0.9	0.12	4.	2.1	0.13
5.	1.1	0.19	5.	14.3	0.17
6.	13.4	0.22	6.	5.1	0.35
7.	5.1	0.25	7.	3.5	0.38
8.	5.0	0.36			
9.	4.5	0.39			
		α_s- casein			
1.	4.2	0.45	1.	4.4	0.46
2.	5.0	0.49	2.	13.8	0.49
3.	13.6	0.58	3.	2.5	0.55
4.	8.3	0.62	4.	2.5	0.59
5.	8.4	0.66	5.	8.1	0.62
6.	1.3	0.68	6.	8.1	0.65
7.	1.4	0.70	7.	2.5	0.67
			8.	2.5	0.71
		χ - casein			
1.	14.3	0.72	1.	13.9	0.76
2.	2.7	0.80	2.	7.8	0.80
3.	2.7	0.82			
		γ - casein			
1.	4.5	0.97	1.	6.1	0.85
2.	1.1	0.98			
β - whole casein	32.5		28.2		
α_s- whole casein	42.2		44.4		
χ - whole casein	19.7		21.7		
γ - whole casein	5.6		6.1		

Table 3. Quantitative content and electrophoretic mobility of dromedary casein fractions.

Casein fractions	**Content (%)**	**R_f**
β - casein		
1.	0.9	0.04
2.	0.2	0.08
3.	13.1	0.11
4.	0.3	0.19
5.	17.3	0.26
6.	0.1	0.35
α_s - casein		
1.	6.3	0.40
2.	14.9	0.42
3.	4.5	0.46
4.	4.7	0.51
5.	5.6	0.59
6.	5.3	0.62
7.	3.3	0.69
χ - casein		
1.	13.5	0.72
2.	5.3	0.78
γ - casein		
1.	1.1	0.85
2.	3.6	0.92
β-whole casein	31.9	
α_s-whole casein	44.6	
χ-whole casein	18.8	
γ-whole casein	4.7	

Table 2 shows the quantitative content of casein fractions of bactrian camel milk and their relative electric mobility on the PAAG-electrophoregram. It is very difficult to decipher the sub-fractions inside the main casein fraction, especially since each fraction gave up to 9 protein bands. Therefore, we limited ourselves to bringing the total sum of the components for the four main casein fractions. Thus, in quantitative terms, casein fractions in the milk of camels from bactrians had the following indicators (in % of total casein): β-casein-32.5, α_s-casein-42.2, χ-casein-19.7 and γ-casein-5.6. The electrophoregram of milk casein taken from individual dromedary camels is shown in Fig. 3. The decoding of the electrophoregram was carried out similarly with previous experience (Fig. 2). The casein of the milk of one bactrian camel consists of 21 protein fractions, while that of dromedaries under similar conditions was divided into only 17 fractions, which include six fractions of β-casein, seven - α_s - casein, two- χ-casein and two -γ-casein. According to the coefficient (Rf) of relative electrophoretic mobility, the distribution zone of the four main types of milk casein of dromedaries coincides with the corresponding boundaries of the casein fractions of bactrians.

CONCLUSION

The protein composition of the casein complex of the milk of individual bactrian and dromedary camels and combined milk were studied by electrophoresis in polyacrylamide gel with urea. To decipher casein fractions, electrophoresis of camel milk casein was carried out together with cow's milk casein in agar and polyacrylamide gels. As known, cow casein has been well studied by electrophoresis in agar gel and all its fractions have been identified on electrophoregram (Yaguchi *et al.* 1968; Seitov & Zhumashev 1970; Abd Lehia 1987). In the present study, on the electrophoregram, the zones of the location of the four main fractions of camel milk corresponded to similar zones of cow casein fractions (Fig. 1). Of the four main fractions in the casein of both individual camels and combined milk, α_s -casein was quantitatively predominant (38.4-44.6%). In cow casein, it was the largest of all caseins (44.4%), slightly less contained β-casein (31.9-36.8%), more than half as much α_s-casein, contained χ-casein (no more than 20%). The differences in the protein composition of the casein complex between bactrians and dromedaries illustrated that the casein of dromedaries consisted of three fractions of β-casein and one fraction of χ-casein less than the milk of bactrians, with an equal number of fractions of α_s -and γ-casein. As a result of the study of different milk samples from individual camels and combined milk, clear electrophoregrams of casein fractions on the PAAG were obtained and their location bands were indicated. They were deciphered and identified in comparison with cow's milk casein, literature materials and by the Rf coefficient. From this it can be concluded that the resulting PAAG electrophoregram of casein can serve as a reference in the study of camel milk proteins. In camel milk casein, 38.56 g essential amino acids were found in 100 g protein and 6.46 g of partially interchangeable in whey proteins, 39.41 and 6.51 were calculated respectively. The caseins of bactrian milk and precast milk were electrophoretically divided into 21 fractions on polyacrylamide gel, of those 9 were attributed to β-casein, seven to α_s -casein, three to χ-casein and two to γ-casein. The casein of dromedary milk contained three fractions of β-casein and one fraction of χ-casein less than the milk of bactrians. Quantitatively, the predominant fraction in camel milk was α_s -casein, same as in cow's milk.

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Kuanysh Syman
ORCID 0000-0003-0313-1391
1.PhD (Biological Sciences), Institute of Natural Sciences and Geography, Abai Kazakh National pedagogical university, Almaty, Kazakhstan
13, Dostyk Av., 050010 Almaty, the Republic of Kazakhstan

Raissa Utegaliyeva
ORCID 0000-0002-1145-5655
2.PhD (Biological Sciences), Kazakh National Women's Pedagogical University,
Almaty, Kazakhstan, 99, Aiteke Bi st, 090000, Almaty, the Republic of Kazakhstan

Abdreshov Serik Nauryzbaevish
0000-0002-8527-921X
3.Biological Sciences) Instute of Genetics and Physiology CS MSHE RK
Laboratory of Physiology Lymphatic System, Almaty, Kazakhstan
050060, 93 Al-Farabi Avenue, Almaty, the Republic of Kazakhstan

Zharylkassynova Zhazira
ORCID 0000-0002-3416-9336
4.Master of pedagogical sciences, Institute of Natural Sciences and Geography, Abai Kazakh National pedagogical university, Almaty, Kazakhstan
13, Dostyk Av., 050010 Almaty, the Republic of Kazakhstan;

Aytzhanova Mira Onlanbekovna
Candidate of biological sciences (PhD)
0000-0002-7067-9296
5. Kazakh National Women's Teacher Training University, Almaty, Kazakhstan,
114/1 Gogol st. 050000
Almaty, the Republic of Kazakhstan

Kalekeshov Askar Maralovich,
0000-0001-6925-5552
6. Biological sciences Kazakh National Women's Teacher Training University, Almaty, Kazakhstan, 114/1 Gogol st. 050000
Almaty, the Republic of Kazakhstan

Mamataeva Aigul Tumaevna
Associate Professor ()
ORCID 0000-0002-6758-2768
7. Biological sciences Almaty Technological University, Almaty, Kazakhstan
100, Tole Bi Av., 050000 Almaty, the Republic of Kazakhstan;

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