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The Content Of Proteins In The Milk Of Cows Of Tatarstan Type With Their Mastitis.

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ABSTRACT

When the cows have mastitis in their milk, significant changes occur in protein composition, characterized by a decrease in the content of total protein (up to 40%) and casein (up to 65%), however, the concentration of whey proteins increases to 60%. The main changes are mainly in the main proteins of milk: αs1-, β-, κ-, αs2 - caseins, blood albumin, immunoglobulin, β-lactoglobulin, α-lactalbumin, the last two proteins have a counter-directional effect. As a result of these changes in the clinical form of mastitis, milk from the casein group passes into an abnormal albumin group with a ratio of caseins and whey proteins of 45: 55%. This indicator can be the main criterion for assessing the condition of the udder and the quality of dairy products in mastitis. Based on the results of the studies, it can also be concluded that curing mastitis cows does not completely restore the initial protein composition of milk in healthy udder, since the reduced content of total protein, casein and whey protein remains, however, the milk has a normal protein structure with the ratio of caseins and serum proteins 79: 21%. Studies have also shown that with the disease of one fraction of the udder, the protein composition of milk does not suffer in healthy lobes.
Keywords: cow, tatarstan type, milk, protein, mastitis.

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INTRODUCTION

Milk is a balanced, natural and healthy food. the fundamental role of milk in the human diet is based on the fact that it gives a complete protein of animal origin. The biological role of milk protein is hard to overestimate. It contains a full set of essential amino acids that are not synthesized in the human body and must come from outside with food [10]. In this respect, the protein of milk considerably exceeds the protein of beef, pork and eggs [5].

The biochemistry and genetics of milk proteins has been fairly well studied. The amino acid composition of most protein milk fractions is known [9, 16], while the main ones have the nucleotide sequences of genes responsible for their synthesis deciphered [15]. In previous studies, we have shown the effect of some of these genes on cow resistance to mastitis [1]. Mastitis is a disease of the mammary gland common in cows, it causes considerable economic damage to dairy cattle breeding [6, 7, 13], as the productivity of cows decreases, the chemical composition of milk changes, its important technological properties deteriorate, raw materials are undesirable [8,12].

In the scientific literature there are a number of reports on changes in the protein composition of milk with udder mastitis [2]. However, they contain data on the changes in mainly only complex proteins of milk, like total protein, casein, whey protein and some major fractions. Our present studies substantially supplement these data in the sense that we have carried out a more detailed analysis of the changes in milk protein for different forms of the disease with mastitis with the determination of up to 20 different protein fractions in milk.

MATERIALS AND METHODS

Studies were carried out in the breeding factory "Biruli" of the Vysokogorsky district of the Republic of Tatarstan. Protein composition of milk was determined in 52 cows of Tatarstan type, 8 of them were affected by subclinical mastitis, 9 - clinical mastitis, 8 - treated against mastitis and 27 - healthy animals that did not have mastitis during life. Samples of milk were taken from daily milk in individual sterile containers with a capacity of 100 ml.

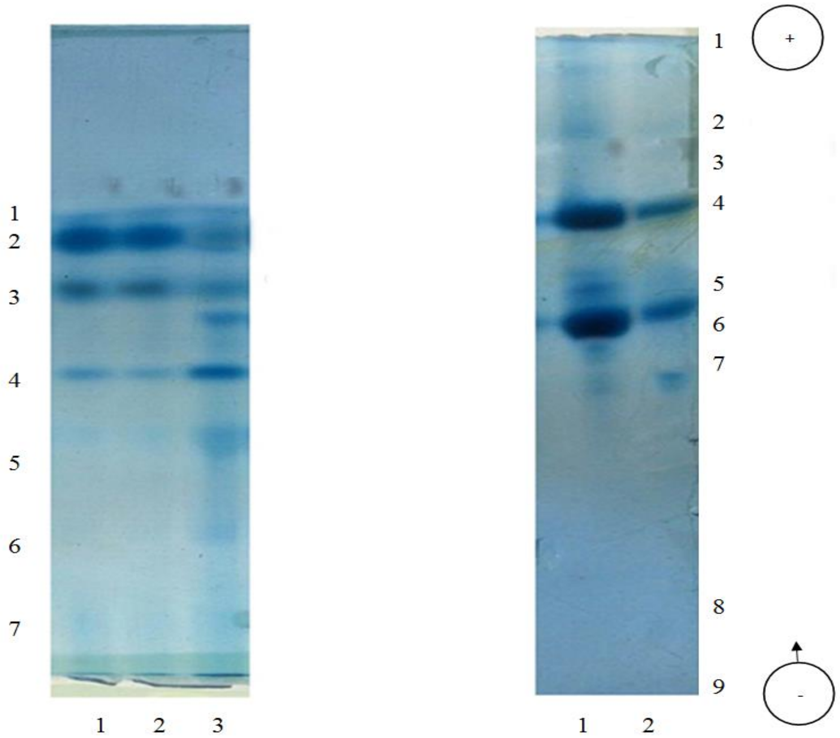
The preparation of milk from caseins and serum was carried out according to R.A. (1989), for which 5 ml of skim milk was poured into centrifuge tubes and caseins were precipitated by acidifying the milk with 0.25 ml of acetate buffer (25 ml of acetic acid, 35 ml of sodium tetrahydrate, 100 ml of water, pH 4.6). The mixture was stirred until the casein was completely precipitated and left in a water bath at 38-40 ° C for 30 minutes. The mixture was then centrifuged for 20 minutes at 8000 rpm. Serum was poured into another dish, and precipitated caseins were dissolved in a buffer consisting of 86 ml of acetic acid and 25 ml of formic acid, 4.5 M urea and 1 L of water, pH 3.0. The buffer was added to the previous volume of milk. In the same solution, a standard sample of casein was prepared according to Hammersten at a concentration of 2.5 g per 100 ml. It was stirred until the caseins were completely dissolved

Separation of casein into fractions was carried out by electrophoresis in 7.5% - polyacrylamide gel No.1 system according to MaurerH.R. (1968), with the addition of 9 M urea and 2-mercaptoethanol to the gel. The identification of fractions of casein was carried out according to RA. Haertdinovu (2009). To separate whey proteins, the same gel system No. 1 as caseins was used, but without urea and 2-mercaptoethanol. Identification of protein fractions in the serum of milk was carried out according to E.N. Reimerdes, H.A. Mehrens (1978). Densitometry of the received foregrams was carried out on an IFO-451 microphotometer. The amount of protein in the fractions was determined by comparison with standard samples (caseins according to Hammersten). By standard samples, the conversion factors of conventional units (mg of paper under the peaks of the corresponding fractions) were calculated. For this purpose, the protein content in a standard sample was divided by the weight of the paper under the peaks of all the protein fractions of this sample. Further, the mass of paper under the peaks of the corresponding protein fraction in the test samples was multiplied by a conversion factor and the protein content in g / 100 ml was obtained. Summarizing the protein content in the fractions, the total number of caseins, whey proteins and total protein in the sample of milk was determined. The relative content of protein fractions was determined as the percentage of protein in the fraction to be analyzed for the total protein in milk.

Evaluation and statistical processing of data was carried out on a personal computer using formulas and algorithms, using the program EXCEL-2017 [3]. In this case, the well-known biometric values were calculated as the arithmetic mean (M), the arithmetic mean error (m), the standard deviation (σ), the coefficient of variation (Cv), the reliability criterion (t), and the reliability (P).

RESULTS AND DISCUSSION

Data on the content of proteins in the milk of healthy cows and patients with subclinical and clinical forms of mastitis are presented in Table 1 (see also Figures 1 and 2). Studies have shown that the udder disease of mastitis affects all protein fractions in milk, starting with the total protein ending with its "small" fractions, all of which are subject to change. Thus, in udder disease, the content of total protein decreased from 3,311 g / 100 ml to 2,949 (subclinical form) and 2,041 g / 100 ml (clinical form, $P < 0.001$). The decrease in total protein was mainly due to the content of caseins, their amount decreased very significantly, from 2.602 g / 100 ml to 2.042 and 0.923 g / 100 ml ($P < 0.001$). Major fractions of casein have undergone the greatest changes: αs1, β, κ. They lost 24 to 75% protein in mastitis ($P < 0.001$). Similar changes occurred with middle-level fractions and some "small" fractions: αs0, αs2, αs' and s. In mastitis, the decrease was somewhat less pronounced than that of the "main" proteins and was 19 to 65% (P <0.01 ... 0.001). With udder mastitis the most stable fraction was F-casein, in which the concentration had a constant value of 0.027 g / 100 ml. Another distinctive fraction was γ-casein, its concentration, in contrast to other fractions of casein, in mastitis, on the contrary, increased from 0.0070 g / 100 ml in normal milk to 0.094 g / 100 ml in mastitis ($P < 0.05$). In this regard, the change in the concentration of γ-casein with udder mastitis turned out to be similar to the change in the content of whey proteins, in which the increase was up to 16%. The increase in the concentration of serum proteins was mainly due to the fractions: albumin and immunoglobulin, their content in virgin milk as compared with the normal was 3-5 times higher.



Samples of whey:
1, 2 - healthy cows
3 - cow with subclinical mastitis

Samples of casein:
1- healthy cow;
2 - cows with subclinical mastitis

Figure 1: Forogram of whey proteins and casein in healthy cows and patients with subclinical mastitis after electrophoresis in the 7.5th polyacrylamide gel, pH 8.9.

On the left are protein fractions of whey. 1 - F-fraction, 2 - β-lactoglobulin, 3 - α-lactalbumin, 4 - serum albumin, 5 - other, 6 - proteose-peptone fraction, 7 - immunoglobulin. On the right are the fractions of casein: 1 - F, 2 - αs', 3 - αs0, 4 - αs1, 5 - αs2, 6 - β, 7 - κ, 8 - γ, 9 - S.

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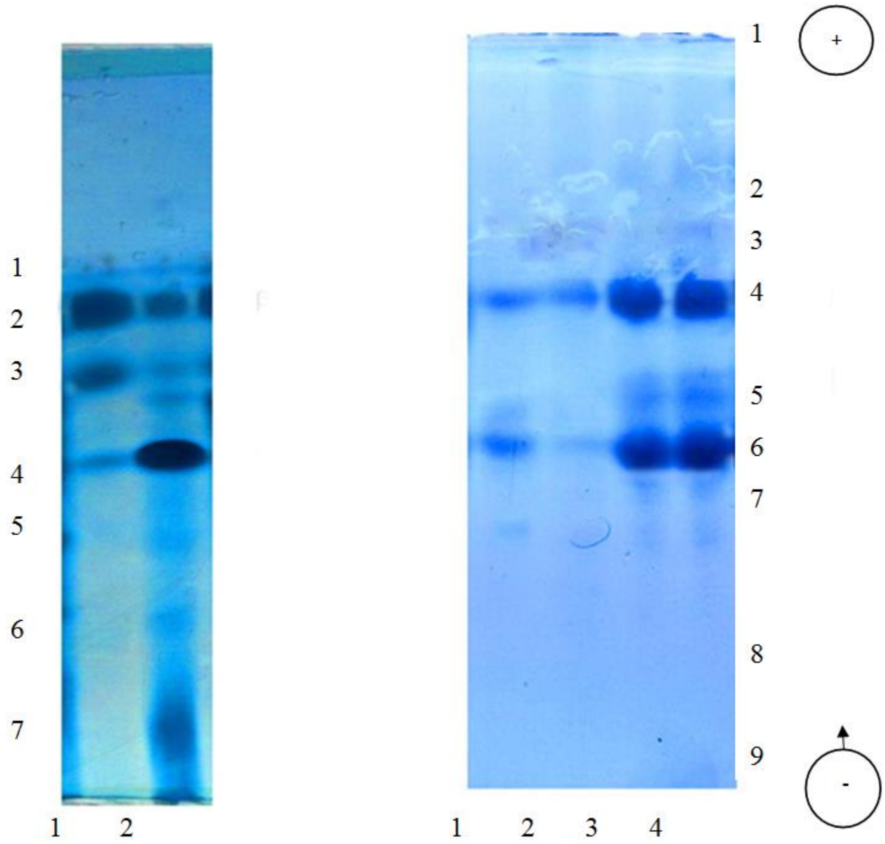
Table 1: Protein content in milk in healthy cows and patients with subclinical and clinical forms of mastitis

Proteins	Protein content in whole cow milk					
	in healthy, n=27		in patients			
			subclinical mastitis, n=8		clinical mastitis,n=9	
	g/100 ml	%	g/100 ml	%	g/100 ml	%
Total protein	3,311±0,053	100	2,949±0,046***	100	2,042±0,176***	100
Caseins:	2,602±0,039	78,6	2,042±0,066**	69,2	0,924±0,022***	45,2
F	0,027±0,001	0,8	0,027±0,001	0,9	0,027±0,001	1,3
αs'	0,091±0,002	2,8	0,077±0,005*	2,6	0,046±0,004**	2,2
αs_0	0,158±0,009	4,8	0,134±0,011***	4,6	0,055±0,004***	2,7
αs_1	0,857±0,029	25,9	0,652±0,036***	22,1	0,216±0,012***	10,6
αs_2	0,318±0,006	9,6	0,258±0,017**	8,7	0,136±0,006***	6,7
β	0,766±0,016	23,1	0,579±0,018**	19,7	0,212±0,009***	10,4
κ	0,232±0,005	7,0	0,169±0,013*	5,7	0,094±0,011***	4,6
γ	0,070±0,003	2,1	0,082±0,011*	2,8	0,095±0,002*	4,6
s	0,082±0,006	2,5	0,063±0,003*	2,1	0,043±0,003**	2,1
Whey proteins:	0,709±0,010	21,4	0,907±0,030**	30,8	1,118±0,060	54,8
F	0,026±0,001	0,8	0,026±0,006	0,9	0,026±0,001	1,3
β-Lg	0,306±0,006	9,2	0,125±0,016*	4,2	0,101±0,009***	4,9
α- La	0,138±0,003	4,2	0,111±0,010*	3,8	0,085±0,005*	4,2
Al	0,056±0,003	1,7	0,204±0,018***	6,9	0,298±0,015***	14,6
Pp	0,047±0,003	1,4	0,073±0,006*	2,5	0,129±0,010**	6,3
Ig	0,088±0,003	2,7	0,240±0,021***	8,1	0,331±0,023***	16,2
other	0,046±0,001	1,4	0,129±0,011**	4,4	0,148±0,013**	7,3

Note: *p<0,05. **p<0,01; ***p<0,001.



Samples of whey:
1 - healthy cow
2 - a cow with clinical mastitis

Samples of milk casein:
1, 2 - cows with clinical mastitis
3,4 - healthy cows

Figure 2: Forogram of milk whey proteins and casein in healthy cows and patients with clinical mastitis after electrophoresis in the 7.5th polyacrylamide gel pH 8.9.

On the left are protein fractions of whey. 1 - F-fraction, 2 - β-lactoglobulin, 3 - α-lactalbumin, 4 - serum albumin, 5 - other, 6 - proteose-peptone fraction, 7 - immunoglobulin. On the right are the fractions of casein: 1 - F, 2 - αs', 3 - αs0, 4 - αs1, 5 - αs2, 6 - β, 7 - κ, 8 - γ, 9 - S.

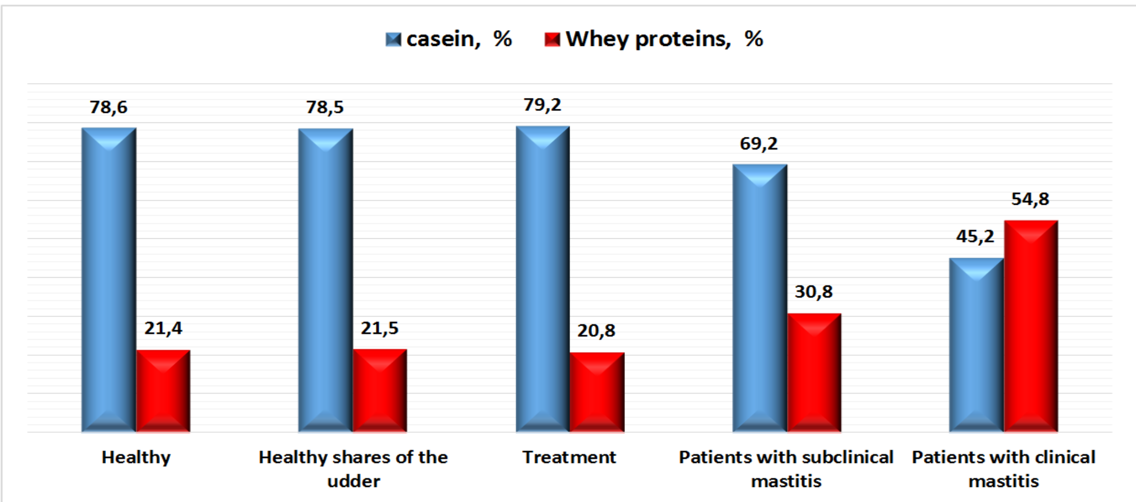


Figure 3: Diagram of percentage of caseins and proteins of whey in healthy cows and patients with mastitis

Table 2: Protein content in milk in healthy cows and patients treated and in a healthy fraction of the udder

Proteins	Protein content in whole cow milk					
	in healthy,n=27		in patients			
			subclinical mastitis, n=8		subclinical mastitis, n=8	
	g/100 ml	%	g/100 ml	%	g/100 ml	%
Total protein	3,311±0,053	100	2,860±0,073***	100,0	3,239±0,069	100,0
Caseins:	2,602±0,039	78,6	2,266±0,060***	79,2	2,541±0,065	78,4
F	0,027±0,001	0,8	0,027±0,001	0,9	0,027±0,001	0,8
αs'	0,091±0,002	2,8	0,094±0,006	3,3	0,093±0,007	2,9
αs_0	0,158±0,009	4,8	0,140±0,006*	4,9	0,154±0,006	4,8
αs_1	0,857±0,029	25,9	0,678±0,045**	23,7	0,831±0,041	25,6
αs_2	0,318±0,016	9,6	0,287±0,017**	10,0	0,315±0,014	9,7
B	0,766±0,026	23,1	0,683±0,032**	23,9	0,742±0,028	22,9
K	0,232±0,005	7,0	0,216±0,010*	7,6	0,230±0,008	7,1
Г	0,070±0,003	2,1	0,070±0,004	2,4	0,073±0,003	2,3
S	0,082±0,006	2,5	0,072±0,003*	2,5	0,076±0,009	2,3
Whey proteins:	0,709±0,010	21,4	0,594±0,015**	20,8	0,698±0,016	21,6
F	0,026±0,001	0,8	0,026±0,001	0,9	0,026±0,001	0,8
β-Lg	0,306±0,006	9,2	0,252±0,017*	8,8	0,295±0,012	9,1
α- La	0,138±0,003	4,2	0,117±0,008*	4,1	0,137±0,010	4,2
Al	0,056±0,003	1,7	0,044±0,005*	1,5	0, 056±0,005	1,7
Pp	0,047±0,003	1,4	0,036±0,002*	1,3	0,049±0,006	1,5
Ig	0,088±0,003	2,7	0,085±0,003	3,0	0,090±0,005	2,8
Other	0,046±0,001	1,4	0,034±0,002*	1,2	0,045±0,003	1,4

Note: *p<0,05. **p<0,01; ***p<0,001.

The most resistant to the inflammatory processes of the udder was the F-fraction, its concentration was constant in normal and abnormal milk - 0.026 g / 100 ml. The change in the content of the main proteins of the serum: β-lactoglobulin and α-lactalbumin was the opposite of what was observed with the rest of the whey protein, their concentration similarly to casein fractions decreased in mastitum milk from 40 to 70% (P <0.001).

As a result of the above changes in protein content in milk with udder disease, normal casein milk turned into abnormal albumin with a ratio of caseins and whey proteins of 45: 55%, respectively. This indicator should become the main criterion for assessing the condition of the udder in mastitis and the quality of dairy products, milk with this indicator can be considered abnormal. Its entry into the marketable marketable milk must be excluded.

We also studied the protein composition of milk in cows who had recovered mastitis and the influence of the diseased portion of the udder on the protein composition of milk in its other healthy lobes. The results of these studies are presented in Table 2 (see also Figure 3). Studies have established that in mastitis infected with cows, the protein composition of milk, which was in healthy cows, is not fully restored. First of all, this is manifested in a decrease in the content of the total protein and its fractions. Thus, in the milk of cows recovered from mastitis, the content of total protein was significantly lower (14%) than in healthy cows and amounted to 2,860 and 3,311 g / 100 ml (P <0,001), respectively. Decrease in total protein occurred proportionally, both due to caseins and serum proteins, respectively, 2,266 and 0,594 g / 100 ml vs. 2,602 and 0,709 g / 100 ml in normal milk (P <0,01 ... 0,001). At the same time, the synthesis of the main proteins of milk: αs1 -, αs2 -, β -, κ - caseins and β - lactoglobulin, α - lactalbumin of the whey suffered the greatest damage. Their concentration decreased from 7 to 21% (P <0.05 ... 0.01). However, it should be noted that in this case the structure of the milk protein is restored completely and the ratio of caseins and whey proteins reaches the value of normal milk, i. E. it is 79: 21% like the milk of healthy cows.

Another important result in our studies is that when the disease of one fraction of the udder, the protein composition of milk in other healthy lobes does not suffer.

Thus, in milk from healthy parts of the udder, almost equal amounts of total protein (3.239 g / 100 ml), casein (2.541) and whey protein (0.698) were contained in the milk of absolutely healthy cows, respectively, 3.311: 2.602: 0.709 g / 100 ml. Small differences are not reliable. Similar data were obtained for individual protein fractions. For example, the content of αs1 - casein in milk from healthy udder shares was 0.831 g / 100 ml, and in the milk of absolutely healthy cows - 0.857 g / 100 ml; respectively, β-casein-0,742 and 0,766 g / 100 ml; κ-casein-0.230 and 0.232 g / 100 ml; β-lactoglobulin - 0.295 and 0.306 g / 100 ml; α-lactalbumin 0.137 and 0.138 g / 100 ml, etc.

CONCLUSIONS

Thus, when the cows are mastitis, significant changes occur in the protein composition of their milk, which is characterized by a decrease in the total protein content (up to 40%), casein (up to 65%), however, the concentration of whey proteins increases to 60%). The main changes are mainly in the main proteins of milk: αs1-, β-, κ-, αs2 - caseins, blood albumin, immunoglobulin, β-lactoglobulin, α-lactalbumin, the last two proteins have a counter-directional effect. As a result of these changes in the clinical form of mastitis, milk from the casein group passes into an abnormal albumin group with a ratio of caseins and whey proteins of 45: 55%. This indicator can be the main criterion for assessing the condition of the udder and the quality of dairy products in mastitis.

Based on the results of the studies, it can also be concluded that curing mastitis from cows does not completely restore the protein composition of milk, which is original in healthy udder, the reduced content of total protein, casein and whey protein is preserved, however, the milk has a normal protein structure with the ratio of caseins and whey proteins of 79: 21%.

Studies have also shown that with the disease of one fraction of the udder, the protein composition of milk does not suffer in healthy lobes.

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