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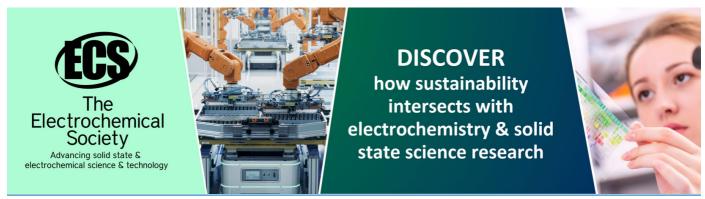
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Protein content in milk of holstein black-and-white cows

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Abstract. The most optimal method to solve the problem for cow's milk and protein content increase is to carry out zootechnical and breeding activities. The goal was to study the milk protein structure and content of the Holsteinized black-and-white breed cows of different genotypes, as well as to establish the relationship nature between the activity of transamination enzymes and the milk protein content of cows. For this purpose, 4 groups of experimental cows (15 heads in each) were formed according to the principle of father's belonging to the countries of origin (daughters of seed bulls of Canadian, Danish selection, Dutch and domestic selection). The superiority of cows-daughters of foreign breeding bulls in terms of milk protein content was established. Moreover, the milk of cows born from foreign producers contents high level of casein - the most important fraction from the technological point of view. Electrophoretic analysis of milk proteins isolated 16 fractions, including 9 casein and 7 whey ones. The highest content was found in such fractions as $\alpha s1$ -, β -, $\alpha s2$ -, κ -caseins and β -Lg. The calculated correlation coefficients between the alanine aminotransferase and aspartate aminotransferase and the milk protein activity of cows showed a direct relationship between them in cows of the studied groups. This is a favorable factor for increasing the cows milk protein content.

1. Introduction

One of the most important sectors of the national economy remains agriculture. It is the main supplier of food and raw materials for many industries, the basic source of income for the rural population. Its normal functioning allows providing jobs to other sectors of the economy. In this regard, the effective development of agriculture has important national, economic and social significance [1-3]. Estimates of genetic parameters for milk yield in dairy cows are abundant in the literature [4-8]. There is a strong belief that proteins are the most valuable constituents of milk. Their digestibility reaches 95%. Milk proteins play a huge role for young growing organisms participating in the creation of new cells and tissues as well as for mature people restoring obsolete cells [9]. Therefore, in all countries with developed dairy cattle breeding, the assessment and selection according to protein content in milk is a special direction in the practical selection of dairy cattle [10]. For the dairy industry, not only milk protein yield but also milk protein composition is important; for example, milk with higher casein content will result in greater cheese yield [11, 12]. Directional stock breeding to improve the structure of milk protein is very relevant. These indicators affect the consumption of raw materials, time and energy in the production of dairy products. The quality of products depends on them. It is well known that the fractional composition of milk proteins is a genetic factor [13]. Thus, the increase of content of technologically important protein fractions in milk and thereby improvement of technological properties can only be done through targeted breeding. The main role in the rapid improvement of the

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productive qualities of black-and-white cattle is assigned to Holstein seed bulls. In this case the assessment of seed bulls in a particular farm is one of the most productive and reliable elements in breeding with a high-producing dairy herd.

Currently, the requirements for quality of dairy products and, in particular, for protein content have increased. The dairy cattle breeding requires reliable and accurate indicators of its forecast. It is known that enzymatic blood systems under conditions of intensification of metabolic processes in animals play an important role. They are directly related to the course of milk formation. Therefore, the application of the interior characteristics of an animal as an innovative breeding method of modern biochemical genetics is quite promising. Learning the proportion of interdependence of indicators of protein metabolism and milk productivity in dairy cattle is relevant. It is an opportunity to raise milk productivity and to increase the content of fat and protein in cow's milk.

Such blood enzymes as alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) can be used in breeding with dairy cattle [14]. The purpose of the research is to study the content and structure of proteins in the milk of Holstein black-and-white cows of different genotypes as well as to establish the nature of relationship between the activity of transamination enzymes and the protein content in cow's milk.

2. Materials and methods

The Federal State Unitary Enterprise Scientific-Experimental Farm "Privolzhskoye" of the Chuvash State Agricultural Academy where the studies have been conducted ranches Holstein black-and-white cows. The herd is represented by the daughters of Holstein bulls from different countries of origin. Four groups of experimental cows (15 animals each) were composed according to the principle of father's belonging to countries of origin. The experimental groups of cows were made up by the method of pair-analogues taking into account blood relationship, live weight, age and physiological state. The daughters of Canadian seed bulls formed the first group, Danish – the second one. Their herdmates of Dutch and domestic selection were included into the third and fourth groups, respectively. The primary research materials have become cows breeding cards, automated system of livestock zootechnical registration on PEVM ARM "SELEX" and other documents of zootechnical registration. The experimental animals were in the same conditions of feeding and keeping.

Analytical studies were conducted in the laboratory of the Department of Private Zootechnics of the Chuvash State Agricultural Academy using modern instruments and equipment. The total protein content was determined in an average daily milk sample taken in proportion to the yield of milk (by the method of formol titration). Milk proteins were fractioned by electrophoretic method in a synthetic polyacrylamide gel which makes it possible to carry out qualitative and quantitative analysis of milk proteins faster and more qualitatively in the modern mini-chamber for vertical electrophoresis with glass size of 20×20 VE-20 (Helicon) [15].

The gels were examined using the high-frequency Vilber Lourmat TFP-L/WL transilluminator (Helicon). Quantitative analysis of phoregrams of the studied samples was carried out by scanning using the computer video system Gel Imager-2, designed for capturing and processing images of luminescent DNA in gels (Helicon).

Blood for research was taken from the jugular vein located in the jugular gutter. Pre-sterilized special injection needles were used for the collection. The activity of the transamination enzymes ASAT and ALAT in non-homolized blood serum was determined photometrically with a unified Wright-Frenkel method using set of reagents AlAT-Vital and AsAT-Vital.

3. Results and discussion

The studies have shown that the milk of cows from different experimental groups had some differences. Animals of group II (3.24%) were characterized by the highest protein content in milk. It was significantly higher than in animals of group IV by 0.24% (P < 0.05). The protein content in milk of cows of experimental groups I and III made up 3.15 and 3.16%, respectively. The highest casein content (2.52%) was found in the milk of cows of experimental group II. Moreover, the descendants of

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Canadian and Dutch bulls (group I and III) were characterized by an equal casein content of 2.46%. It is 0.12% more than cows of group IV. However, the difference is not reliable. No significant differences in the level of whey protein in milk of cows of different origin were found. The Danish cows (group II) showed 0.72% of whey protein content in milk which was 0.06% higher than in domestic cows (group IV) (P <0.05). It is 0.04% higher in Dutch cows (group III), and 0.03% higher in daughters of Canadian bulls (group I) than in animals of group IV (the offspring of domestic selection bulls). The established superiority of cows of imported bulls in terms of protein content in milk allows us to recommend using them as improvers of protein content in milk of Holstein blackand-white cows.

The studies of electrophoretic mobility have identified individual protein fractions of milk: 9 casein and 7 serum. α_{s1} -, β -, α_{s2} - and κ -caseins identified on the electrophoregram of casein and β -Lg - on the electrophoregram of whey proteins had the highest concentration. The share of other fractions was insignificant.

It is determined that the electrophoretic presentation of milk proteins separated on the carrier material - polyacrylamide gel - in cows of different origin is different. According to the current classification a detailed analysis of milk protein fractions is shown in figure 1.

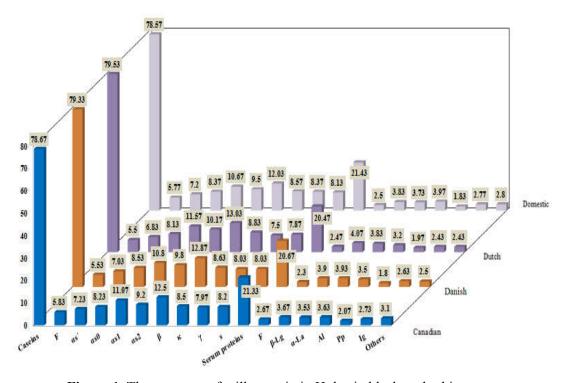


Figure 1. The structure of milk protein in Holstein black-and-white cows.

It can be seen that cows of group III show significant differences in the concentration of the most important protein fraction of casein (79.53% of the total protein). Whereas the domestic origin herdmates (group IV) have a casein concentration of 78.57%. The concentration of such casein fractions as α_{s1} -, β - and κ -fractions form the quality milk products containing protein. Milk of the domestic origin cows (experimental group IV) in comparison with milk from other groups is characterized by a lower concentration of α_{s1} -casein (10.67%).

By α_{s1} -casein content, the daughters of Canadian, Danish and Dutch bulls exceeded domestic breeding cows (group IV) by 0.4, 0.13 and 0.9%, respectively. The domestic origin cows also had a low β -casein content of 12.03%. The difference in the content of this fraction in the descendants of foreign (I, II and III group) and domestic (IV group) bulls made up 0.47, 0.84 and 1.0%, respectively. The concentration of κ -casein at the level of 8.63 and 8.83% was determined in the milk of cows in the

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II and III experimental groups (Danish and Dutch breeding). Such a milk protein fraction as γ -casein is considered to be unimportant from a technological point of view. Its share in the structure of milk protein was greater in daughters of the domestic (group IV) bulls than in other groups of animals. Whereas the concentration of γ -casein was insignificant in cows of Dutch origin (experimental group III), at the level of 7.50% of the total milk protein content.

Immunoglobulins are a fraction of whey proteins. The results of the analysis showed that their relative content in milk of daughters of the domestic bulls (group IV) was the highest (2.77%). The lowest concentration was noted in milk of Dutch cows (group III) - at the level of 2.43%. The β -Lg and α -La fractions are identified between the fast fraction (F) and serum albumin. The concentration of β -Lg is at the level of 3.67% in Canadian cows which is 0.23, 0.4 and 0.16% less than in groups II, III and IV, respectively. The smallest concentration of α -La was also found in cows of experimental group I - daughters of Canadian bulls.

Currently, the issue of selection of dairy cattle to increase the content of high-value milk protein is relevant. Therefore, the identification of the interdependence of the content of protein fractions will solve this difficult problem [1].

It is discovered that protein content in milk of all the studied groups of animals is closely related to the level of α_{s2} -casein (the correlation coefficient was in the range from 0.50 to 0.97). This creates suitable conditions for practical selection. A negative relationship between the relative content of total protein and γ casein in the studied groups of cows (the correlation coefficient ranged from -0.28 to -0.99) indicates an opposite relationship. The higher the total protein value is, the lower the γ value of the casein fraction and vice versa. The relationship of total protein and other protein fractions in these studies is not found.

The absolute values of the correlation coefficients of the total protein and the relative content of complex proteins in animals of groups I and IV make up 0.57 and 0.68 for casein, -0.57 and -0.68 for serum proteins. It is interpreted as follows: the selection of dairy cattle by content of protein in milk in the group of Canadian and domestic animals will lead to an increase in the proportion of casein in the total protein. In this case, the concentration of whey proteins will decrease. This connection is desirable for cheese production technology as it will lead to an increase in the yield of cheese mass. The data of the correlation analysis of the dependence of the total protein and the relative content of complex proteins in animals of groups II and III turned out to be opposite to what was observed in animals of groups I and IV. The correlation of the total protein and the main fractions of casein ($\alpha_{\rm sl}$ -, β - and κ) is positive in the daughters of Canadian and domestic fathers (I and IV groups). The interdependence of total protein and casein β-fraction was also established in Danish cows - group II (with the increase of one, the other criteria also increases). Moreover, the relationship between the total protein content and α_{s1} and κ fraction of casein in this group of cows is inverse (an increase in one criteria leads to a decrease in the other). The correlation analysis revealed a positive relationship between the concentration of total protein and α_{s1} -fraction and a negative relationship with its β - and κ-fractions in daughters of Dutch bulls (group III).

The calculation of the coefficients of dependence (correlation) between the total protein content and the main fractions of whey proteins – β -Lg and α -La – showed the presence of a positive relationship in cows of groups I, II and IV. The values of the correlation coefficients ranged from 0.33 to 0.95. Moreover, experimental group of animals No. 3 was characterized by a weak (–0.38) and average (–0.73) dependence of the total protein content and the relative content of β -Lg and α -La. Moreover, the reliability of the correlation is absent.

The breeding work can also use such transaminase enzymes as alanine aminotransferase and aspartate aminotransferase. The biochemical profile of enzymes also depends on the stage of lactation of animals. The change in the activity of the enzymes ALAT and ASAT during lactation is presented in figures 2 and 3.

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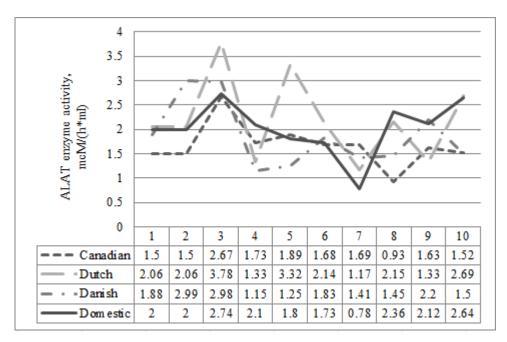


Figure 2. Change of ALAT activity in serum during lactation.

The analysis shows that the catalytic activity of the enzyme ALAT in the first three months of lactation in cows of all experimental groups increases. Then, at the 4th month of lactation, a sharp decline in enzyme activity is observed. From the fifth month of lactation, daughters of foreign bulls (groups I, II and III) have a significant decrease in its activity with some fluctuations (fluctuations are unreliable). It should be noted that animals of group IV (daughters of domestic bulls) are characterized by a decrease in enzyme activity up to the 7th month of lactation.

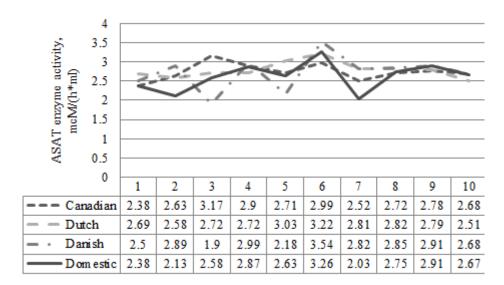


Figure 3. Change of AsAT activity in serum during lactation.

According to the change in the activity of ASAT enzyme during lactation in cows of different origin, the following was noted: increases until the 6th month of lactation, then decreases by the end of lactation.

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Since the biochemical composition of the blood is to some extent interconnected with the breeding and productive qualities of animals, the correlation coefficients of two values - the activity of ALAT and ASAT enzymes and the protein content in milk - were calculated (table 1).

Table 1. The relationship of ALAT and ASAT with protein content in milk of cows (X±Sx).

	Enzyme Activity, mcM/(h*ml)	
Animal Groups	ALAT	ASAT
I	0.98±0.22***	0.99±0.13***
II	0.78 ± 0.44	$0.97 \pm 0.16***$
III	$0.92 \pm 0.39 *$	$0.96\pm0.26***$
IV	0.27 ± 0.56	0.37 ± 0.54

^{*}P < 0.05

The studies conducted on Holstein black-and-white cows of different origin showed that a positive statistically significant correlation was established between the activity of ASAT and ALAT in the blood and the protein level in cow's milk. The maximum value of correlation was found in the group of cows originating from bulls of Canadian selection. The second in correlation are cows of group III of Dutch origin. It is a favorable factor for increasing protein content in milk of cows.

4. Conclusion

As a result of the studies, the superiority of cows-daughters of the imported bulls- in terms of the protein content in milk was established. It allows us to recommend using them as improvers of protein content in milk of Holstein black-and-white cows. The detailed analysis of the fractional composition of the milk protein of cows of different origin indicates that the female ancestors of Holstein producers of Dutch companies are distinguished by the highest content of the main protein fractions that affect the technology of milk and dairy products. The protein content in milk of cows positively correlates with the enzymatic activity of serum transaminases. It is a favorable factor for increasing protein content in milk of cows.

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