THE GENETIC STRUCTURE OF LACTO-PROTEINS AT KARAKUL SHEEP

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Abstract

Sheep farmers in Moldova pay particular attention to milk production. This is economically motivated. Regardless of race and destination, all the sheep are milked, and the obtained milk is processed by artisanal methods. The study of the genetic structure of lacto-proteins allows homozygous (BB) animals to be identified in the kappa-casein locus, which, according to the bibliographic data, have an increased yield in the manufacture of cheeses. Research on the "Botnari V.Gh." farm showed that from 75 of milking sheep 3 sheep or 4.0% are holders of homozygous alleles BB, 10 or 13.3% heterozygous alleles of AB and 62 or 82.7% of AA alleles.

According to the chemical analysis of the milk, it was found that the sheep with the genotype BB exceeded those with the genotype AA by the amount of: fat with 21.9%, proteins with 19.04%, including casein 22.7%, lactose with 5.9 % (P<0.001) and serum proteins with 7.6% (P <0.01). Sheep with heterozygous genotype AB also genuinely exceed those with AA genotype by the amount of fat with 12.3%, protein with 10.6%, casein with 12.3% (P <0.001) serum protein with 5.4% (P <0.01).

In order to increase in flock the number of sheep with the homozygous alleles BB and heterozigote AB were selected from the sheep- mothers the possessors of these alleles, 4 gray lambs for breeding and reproduction, which will allow to increase the weight of sheep with the required genotype.

Key words. Sheep karakul, k-casein, genotype, milk, chemical composition

INTRODUCTION

Milk - a valuable food product rich in nutrients, considered indispensable for life. It is the only food for newborns that fully covers life requirements over a specific period of time. "Milk is an amazing food created by nature," said the famous Russian physiologist I. Pavlov. But at the same time, milk is relatively perishable because of its contamination with microorganisms, still from milking. In order to reduce the number of germs in milk, it is necessary to minimize the period from milking to processing. In particular, under the conditions of the Republic of Moldova, sheep's milk is entirely used for the production of cheese, curd. It is known that in the world only 35% of the quantity of the obtained milk is used in the manufacture of cheeses [4]. The advantages resulting from the possibility of transforming the main components of milk into cheese constituted arguments for the development of this production: storage stability, relatively easy transport and diversification of human diet [2, 3]. The analysis of milk as a raw material for manufacture of cheeses is more widely studied at cattle. In the work Bugeac T. and others. [1] are presented data on genetic variants from the loci of the six major milk proteins which were identified at cattle of breed Montbeliard exploited in the eastern part of Romania. Researchers Ahmetov T.M. [5], Barshinova F.V. [6], Dâmani T.N., Glazco V.I.. [7] report on the positive influence of the genetic structure in the kappa-casein locus on the technological properties of the milk used in cheese production. Depending on the productive orientation, were selected according to the

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genetic structure of the alleles in the animal kappa-casein locus with a high yield of cheeses especially at cattle. The results of this research have contributed to the initiation of the respective direction of scientific investigation on milk lacto-proteins of karakul sheep's milk.

MATERIAL AND METHOD

The research was carried out at the breeding farm of the "Botnari V.Gh." GI in the village Elizaveta, Balti municipality. In the study were taken 75 milking- sheep from which were collected samples of individual milk on the control milking day. Samples were collected from morning and evening milking. At the end of the day we got the average sample, which had a volume of 100 ml. Before the analysis, the samples were warmed to 20°C, then homogenized well. The physico-chemical indicators were determined with the milk analyzer Ekomilk Bond Total. This ultrasonic analyzer determines the physico-chemical indices of cow, sheep, and goat milk. Measured parameters: fat 0.5% -12% with accuracy ± 0.1%, solids without fat (SNF) 6% - 12% with accuracy ± 0.2%, proteins 2% - 6% with accuracy ± 0.2%, lactose 0.5% - 7% with accuracy ± 0.2%, milk density 1.0260 g / cm^3 – 1.0330 g / cm^3 ± 0.0005 g / cm^3, freezing point -1000 - 0 ° C ° accuracy ± 0.015°C, water added to milk 0% - 60% with accuracy ± 5%, pH 0.00 - 14 pH with accuracy ± 0.02, temperature 0-50°C with an accuracy of ± 0.1°C. Protein and casein content was determined by introducing into the balls 10 ml of milk, 10 drops of phenolphthalein, titrated with NaOH (0.1 n) until the total acidity was neutralized (colour pale-pink), then we added 2 ml of formaldehyde freshly neutralized and again titrated with NaOH (0.1 n) till the obtaining the pink color (as in the previous titration). The amount of NaOH (ml) consumed at II nd titration, multiplied by the coefficient 1.94 corresponds to the total protein content but multiplied by the coefficient 1.51 corresponds to the casein content. The content of serum proteins was determined by the difference from the total protein content and casein content. By the calculation method were determined the total dry substance (TDS).

Genetic investigations were performed on the milk serum, in which the genetic structure of lacto-proteins was studied by the starch gel electrophoresis method. As a result, the following fractions were determined: kappa-casein (k-Cn), beta-casein (β-Cn) and beta-lactoglobulin (β-Lg). The research was carried out jointly with the collaborators of the Zootechny and Biotechnology Faculty of ASUM in Chisinau, including the laboratory for assessing milk quality and dairy products, the genetics laboratory of the Department of Biotechnologies in Zootechny.

The numerical material obtained from the research was statistically processed using the Microsoft Excel program, and the degree of certainty of the difference was determined by using the Student criterion [8].

RESULTS AND DISCUSSIONS

The study of the genetic structure of the sheep's milk serum showed that from the lot of animals subjected to the investigations, only 3 heads (accession no. 2014024, 1599266, 451789) possess the homozygous BB alleles in the k-casein locus, which represents a share of 4% (Tab. 1). The number of sheep found with the heterozygous alleles AB were 10 heads, representing a weight of 13.3 %. The other sheep - 62 heads are animals possessing AA alleles, being numerically dominant with a maximum weight of 82.7 %. The results of the control milk (milk production per day) showed that the maximum milk quantity was recorded at the sheep lot with heterozygous alleles AB – 468.3±60.5 ml. This group is followed by those with homozygous AA alleles with 362.2±25.6 ml, and those with BB alleles had the lowest production per day – 318.3±72.0 ml/milk. After analyzing the authenticity of media differences for milk production in the studied lots, it was found that although this exists mathematically, the values are not statistically true (P≥0.5).

Analyzing the beta-casein locus (β-Cn), it was determined that the homozygous BB alleles were found in 6 sheep heads, the weight being of 8.0%, and with the heterozygous AB alleles were found 7 heads, making 9.3% of the lot. Sheep the owner of

the homozygous AA alleles were registered 62 heads which constitutes 82.7%. It should be noted that average milk production per day in sheep lots with different gene structure locus (β-Cn) does not have significant differences (P≥0.5). However, the maximum value of milk production was recorded on the heterozygous sheep lot with the AB alleles – 395.9±58.2 ml. It should be noted that homozygous BB alleles in the beta-casein locus are references to the increased concentration of retinol and are in a positive correlation with the level of immunity of the organisms. According to data obtained from the sheep lot subject to the study 13 heads or 17.3 % are genetically predisposed for a higher amount of vitamin A (Retinol) in milk.

Table 1 Genetic structure and milk production of Karakul sheep

Specification	Genotype		
	AA	**AB**	**BB**
the k-Cn locus			
n (head)	62	10	3
Weight (%)	82.7	13.3	4.0
Milk production / day, ml	362.2±25.6	468.3±60.5	318.3±72.0
the β-Cn locus			
n (head)	62	7	6
Weight (%)	82.7	9.3	8.0
Milk production / day, ml	377.3±24.8	395.9±58.2	322.3±125.0
the β-Lg locus			
n (head)	42	18	15
Weight (%)	56.0	24.0	20.0
Milk production / day, ml	375.45±28.39	322.67±57.22	434.53±46.89

Research on the genetic structure in the β-lactoglobulin locus (β-Lg) has shown that 15 sheep or 20% are the possessors of homozygous alleles BB, 18 sheep or 24.0% are the possessors of heterozygous alleles AB and 42 sheep or 56.0 possess homozygous AA alleles. It is known that lacto-proteins in the beta-lactoglobulin locus and, in particular, BB alleles are genetic markers for enhanced milk production. This is also confirmed in our research. It is worth mentioning that at this Karakul sheep farm over several years have been made selection works after milk production. Thus, sheep's milk production with BB alleles tends to be superior to milk production. The average on the lot, in this case, is 434.53ml/day. Selecting the progeny of sheep with BB alleles in the beta-lactoglobulin locus can serve as a reference for increasing the production of milk in flocks with which such work is carried out.

The study of the chemical composition of milk according to the genetic structure showed that in the k-casein locus the sheep with the BB and AB alleles based on the basic components (fat, lactose, protein including casein) accurately exceeded the sheep with the AA alleles (Tab. 2). According to the obtained results, it was observed that at sheep with BB alleles, on average per lot, the fat content constituted 12.6±0.35% or 21.9 % more in comparison to the sheep the possessors AA alleles. The same as the amount of protein, which constituted at sheep with the BB alleles 9.19±0.22% or 19.04 % more than those with AA alleles. It should be noted that a maximum value at sheep with homozygous BB alleles was found in the amount of casein, which constituted 7.2±0.15%, being with 22.7% more in comparison with sheep the possessors of AA- alleys. The amount of lactose at sheep with BB alleles constituted 6.47±0.02%, which by 5.9% exceeds the established value at sheep with AA alleles. Respectively, after the amount of total dry substance (24.27±0.35%) and of degreased

dry substance (11.67±0.04%), sheep with the BB alleles increase with 13.4 and 5.5% corresponding to sheep with AA alleles. The differences between the milk components mentioned above are statistically authentic with P≤0.001. After the amount of serum proteins, with the exception of the sheep lot with the AB alleles, at sheep with BB alleles is observed a tendency towards increase by 7.6 percentage points. Similarly after the amount of mineral salts we have a tendency to increase at sheep with BB alleles in relation to those carrying AA alleles.

Table 2 Chemical composition of milk at sheep with different genotypes

Specification	Genotype of sheep (alleles)		
	AA	**AB**	**BB**
the k-Cn locus			
Fat, %	10.34±0.13	11.61±0.17***	12.6±0.35***
Protein, %	7.72±0.08	8.54±0.11***	9.19±0.22***
Casein, %	5.87±0.08	6.59±0.10***	7.20±0.15***
Serum proteins,%	1.85±0.02	1.95±0.04*	1.99±0.08
Lactose, %	6.11±0.05	6.25±0.15	6.47±0.02***
Mineral salts, %	0.90±0.01	0.95±0.02	0.96±0.00
TDS, %	21.40±0.18	23.17±0.37***	24.27±0.35***
DDS, %	11.06±0.09	11.56±0.25	11.67±0.04***
the β-Cn locus			
Fat, %	10.55±0.13	11.73±0.48	9.8±0.36
Protein, %	7.85±0.09	8.62±0.31*	7.37±0.23
Casein, %	5.99±0.08	6.68±0.29*	5.61±0.22
Serum proteins, %	2.03±0.16	1.94±0.07	1.76±0.03
Lactose, %	6.12±0.05	6.33±0.19	6.16±0.17
Mineral salts, %	0.90±0.01	0.93±0.03	0.91±0.03
TDS, %	24.65±0.19	23.15±0.76	20.96±0.56
DDS, %	11.12±0.09	11.42±0.34	11.16±0.31
the β-Lg locus			
Fat,, %	10.64±0.16***	9.58±0.10	11.72±0.24***
Protein, %	7.91±0.10***	7.22±0.07	8.62±0.16***
Casein, %	6.04±0.08***	5.45±0.06	6.66±0.15***
Serum proteins, %	1.86±0.01**	1.77±0.03	1.96±0.04**
Lactose, %	6.10±0.05	6.01±0.09	6.42±0.10**
Mineral salts, %	0.90±0.01	0.89±0.01	0.94±0.02**
TDS, %	21.75±0.21***	20.47±0.23	23.32±0.34***
DDS, %	11.10±0.10	10.89±0.17	11.60±0.18**

**P>0.01; ***P>0.001

On the sheep lot with the heterozygous AB alleles in the k-casein locus we observe that chemical composition indices of the milk have an intermediate position between the sheep indexes with homozygous BB and AA alleles. Thus, the amount of fat at these sheep constituted 11.61±0.17% or with 12.3% higher in the in relation with those with AA alleles. The amount of protein at heterozygous sheep constituted 8.54±0.11% or with 10.6% higher than in sheep AA alleles. Similarly, the amount of casein in sheep's milk with AB alleles constituted 6.59±0.1% or with 12.3% higher than at sheep with AA alleles. The amount of DDS on the heterozygous sheep lot was 23.17±0.37% or with 8.3% higher towards the value established at sheep with AA alleles. The difference between the analyzed components at

these heterozygous AB sheep in relation to the indices at sheep with homozygous AA alleles is statistically true after the superior authenticity threshold - P≤0.001. It should be noted that unlike the BB alleles, the sheep lot with the AB alleles, the serum protein content, which constituted 1.95±0.04%, true (P≤0.01), exceeds the index determined in sheep with AA alleles. It should be noted that unlike the sheep with BB alleles, on the sheep lot with the AB alleles, the serum protein content, which constituted 1.95±0.04%, truthfully (P≤0.01), exceeds the respective index determined at sheep with AA alleles.

The study of the chemical composition of milk at sheep formed in dependence on the genotype- in the β-casein locus has shown that it is not strongly infuenced by the genetal status of the animal. In the data obtained from the sheep lots, in most milk components is not observed a regularity at the analyzed indices and at the same time on the heterozygous sheep lot in the β-casein locus to the protein content and casein were found to be truthful differences (P≤0.01) of 17.0 and 19.1% in relation to respective indices at sheep with homozygous BB alleles. On the heterozygote sheep lot in the β-casein locus, three heterozygous AB sheep were detected and one homozygous sheep with the BB alleles in the k-casein locus. This has led to a difference in protein and casein content in their milk. Otherwise, conclusive findings cannot be deduced from the observed differences between some indices.

The results of the study of the sheep's milk composition at lots in the β-lactoglobulin locus (β-Lg) demonstrated that the sheep with the homozygous BB alleles truthfully exceed (P≤0.01, P≤0.001) after all indications of sheep with heterozygous alleles AB. The chemical composition of milk at sheep with homozygous AA alleles, according to the indices: fat, protein, casein, TDS (***P>0.001) and serum proteins (*P>0.01) also exceed the heterozygote sheep indices. And in this case, these differences between the compared lots are influenced by heterozygous and homozygous sheep in the k-casein locus. Thus, in the homozygous sheep lot with the AA alleles in the β-lacto globulin 4 sheep were detected with the AB alleles and 2 sheep with the BB alleles in the k-casein locus. In the sheep lot with BB alleles in the β-lacto globulin locus, 6 sheep were detected with AB alleles and one sheep with BB alleles in the k-casein locus. No animal was found in the sheep lot with heterogeneous alleles AB in the β-lacto globulin locus with higher genotype in the k-casein locus. Therefore, the data analyzed allows to conclude that the chemical composition of the milk in the locus β-lactoglobulin cannot be a benchmark for selection work.

In order to increase in the flock, the sheep with the homozygous BB alleles and heterozygous AB were selected from the mothers the possessors of these alleles 4 gray lambs for growth at breeding fact which will contribute to increasing the share in flocks of sheep with the required genotype.

CONCLUSIONS

Research on the "Botnari V.Gh." breed farm has shown that from 75 milking sheep 3 sheep or 4.0% are the possessors of BB alleles, 10 sheep or 13.3% of the AB alleles and 62 sheep or 82.7 % with the AA alleles in the k-casein locus.

Following the chemical analysis of the milk, it was found that at sheep with the BB alleles in the k-casein locus truthfully exceed those with AA alleles after the amount of: fat with 21.9%, lactose with 5.9% lactose, protein with 19.04% including casein with those homozygous with AA alleles after the amount of: fat with 12.3%, protein with 10.6%, including casein 12.3% (P<0.001), serum proteins with 5.4% (P≤0.01) .

In order to increase the number of sheep with BB homozygous and heterozygous AB alleles, were selected descendants from the sheep- mothers the possessors of these alleles.

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