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Combination Of Polymorphism Of The TFAM Gene With Growth Dynamics, Milk Productivity And Reproductive Characteristics Of Cow-Heifers.

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ABSTRACT

The mitochondrial transcription factor A (TFAM) is central to regulating fat gain and energy metabolism, and is necessary for maintaining and biogenesis of the mtDNA.Physical development, growth dynamics, factors of milk productivity and reproductive characteristics depend on the effective functioning of the mitochondrial system, which reflects the importance of metabolic homeostasis.The introducedproject is devoted to DNA testing of allelic polymorphism of TFAM gene and establishment of associative connections of various genotypes with growth dynamics, dairy productivity and reproductive function in the population of Holstein cow-heifers.The study found that the dynamics of body weight does not have statistically significant differences depending on the polymorphic variants of the TFAM gene.Analysis of milk productivity showed that animals with the $TFAM^{AA}$ genotype significantly exceedthe other genotypes by milk yield, protein mass fraction, milk protein yield, milk ratio.The coordination of genotypes in lactation activity of the first-calf-cows and the lactation stability factor was also established. It is noted that TFAM, as a marker gene for lipid metabolism and energy balance, has an impact on fertility and reproductive function.

Keywords: gene, mitochondrial transcription factor A, TFAM, genotype, growth, body weight, productivity, yield, fat, protein, first-calf-cows (cow-heifers).

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INTRODUCTION

Body weight, milk production and reproductive characteristics are very important interrelated quantitative genetic traits that are potential targets for breeding using markers to improve the efficiency of dairy cattle. The main reasons for the rejection of heifers [3] are slow development and insufficient bodyweight for the first insemination, which increases the age of the first calving, that again leads to economic losses for the management of animals [1, 2].Incorrect, uneven development or excessive obesity of the first-calves cows are also risk factors for dystocia and can have a harmful effect on reproductive function and milking in the first lactation [4]. In addition, such factors as growth and body weight strongly correlate with the duration of economic use, which ultimately affects the productivity, fertility and health of the cow [5].

In recent years, the mitochondrial DNA (mtDNA), which is a small ring molecule with a size of about 16,500 nucleotide pairs, has become increasingly used as a genetic marker.MtDNA has unique qualities: strict maternal inheritance, high rate of mutation accumulation, absence of recombinations, a large number of copies of DNA molecules in cells, which allows using data ofmtDNA polymorphism for marking breed and inbreeding features of animals, and also to find links with economically useful factors [6].Mitochondria, which are maternal hereditary organelles, perform several cellular functions, for example, energy metabolism, homeostasis of calcium and iron, signal transduction, and apoptosis, and are considered important to the metabolic pathways involved in the biosynthesis of heme, lipids, amino acids and nucleotides [7].

Mitochondrial transcription factor A (TFAM) plays a key role in regulating fat gain and energy metabolism.Wilson-Fritch et al. (2004) found increased expression of mitochondrial genes encoded by nuclei during adipogenesis and, conversely, a 50% decrease in the expression of the transcription factors of these genes in obese mice.The involvement of mitochondria in adipogenesis confirms the localization of β-oxidation of fatty acids, the esterification of fatty acids into triglycerides and other similar processes that occur in mitochondria [8,9].

Mitochondrial transcription factor A (TFAM), a member of the high-mobility protein class having a molecular weight of about 25 kDa, and the first identified mitochondrial transcription factor [10], is required for management and biogenesis ofmtDNA.The TFAM gene of cattle is found on chromosome 28, consists of 7 exons and 6 introns, and the genomic DNA of this gene is 16,666 bp [11].Since this protein is directly involved in mitochondrial functionality, polymorphic gene variants can affect intracellular production, as well as subsequent processes.Two single nucleotide polymorphisms have been identified in the promoter region of the TFAM gene, namely, A/C-transversation and the C/T-transition [9].Polymorphisms in TFAM were previously associated with the thickness of subcutaneous fat, marbling of meat [9, 12], growth, milk yield and fertility [13] in cattle.All these studies have demonstrated the essential role and function of mitochondria in lipid metabolism.

Physical development, growth dynamics, signs of milk productivity and reproductive qualities depend on the effective functioning of the mitochondrial system, which reflects the importance of metabolic homeostasis.In this process after calving the decrease is obvious, when the mobilization of fat stores is necessary to provide additional energy at the beginning of lactation. This leads to a period of negative energy balance and loss of body weight.

The purpose of this study was to evaluate the dynamics of growth, milk productivity and reproductive qualities of the first-calf cows of Holstein breed with different TFAM genotypes.

MATERIALS AND METHODS

The study was conducted on 172 original Holstein cows in the "Plemzavodnamed after Lenin "of the Atninsky district of the Republic of Tatarstan.The animals participating in the experiment were kept in the same environmental conditions on a standard diet.Sampling of blood was carried out in vacuum tubes EDTA K-3 (APEXLAB, China) from the tail vein of animals.DNA extraction from the obtained biological material was carried out using a ready-made kit for extracting AmpliPraym DNA-Sorb B (InterLabService, Russia), according to the manufacturer's instructions.For the genotyping of animals at the TFAM-Hae III gene locus (801 bp), the PRTR-RFLP method was used (polymerase chain reaction - restriction fragment length polymorphism).The

reaction mixture was a final volume of 15 µl containing 1.5 µl of purified DNA, 0.2 µlTaq-DNA polymerase with 1.5 µl SE buffer, 1.5 µl DNTPs and oligonucleotide primers with the sequence:

TFAM F: 5'– GTTGTTGCAGAAATCAGCTAAAATG – 3'
TFAM R: 5' – CATCCACTGAGACTATCGCTGACCT – 3' [9],

were amplified in a thermal cycler T100 Thermal Cycler (Bio-Rad, USA) under optimized temperature-time conditions: denaturation for 3 minutes at 94 ° C; annealing 34 cycles 94 ° C - 25 seconds, 65 ° C - 25 seconds, 72 ° C - 30 seconds; elongation for 7 minutes at 72 ° C.PCR products were digested with Hae III restriction endonuclease for 16 hours at 37 ° C.The resulting fragments were electrophoretically separated in an agarose 3% gel at field strength of 20 V/cm for 25 minutes in the presence of ethidium bromide in 1xTBE buffer.The effect of electric current divides the amplified fragments by the molecular weight (fragments with a large nucleotide sequence are heavier, so they move more slowly in the gel than short ones) [14].Visualization, video recording and documentation were realized with the help of UV transilluminator and Gel&Doc system (Bio-Rad, USA).Analysis of the development, milk production, lactation activity and reproductive qualities of the first-calves cows was established according to the data obtained from the official electronic card index of the herd "SELEKS 5.63" (ARM Plinor, Russia).

The frequency of individual alleles was determined according to the formula of E.K. Merkurieva [15]:

$$p_A = \frac{2nAA+nAB}{2N}, \quad q_A = \frac{2nBB+nAB}{2N}, \text{where(1, 2)}$$

p_A – thefrequency of allele A,
q_A – the frequency of allele B,
n – thenumber of heads of a given genotype,
N – thenumber of heads in the studied population.

Calculations of the occurrence of genotypes were made according to the formula of E.K. Merkurieva and G.N. Shangin-Berezovsky (1983) [15]:

$$f = \frac{n}{N}, \text{where} \qquad (3)$$

f – thefrequency of the genotype,
n – thenumber of heads of a given genotype,
N – thenumber of heads in the studied population.

The relative increase and the absolute average daily growth during a certain period were calculated by the formulas [16]:

$$O = W_1 - W_0 \text{ and } A = \frac{W_1 - W_0}{t} \times 1000, \text{ where} \qquad (4, 5)$$

O - relative increase, kg;
A - absolute average daily gain, g;
W_1 - body weight final, kg;
W_0 - body weight initial, kg;
t - interval between weightings, days.

The stability factor of lactation (CFA) was calculated by the formula:

$$CFA = \frac{y_1 - y_2}{y_1}, \text{ where} \qquad (6)$$

CFA - coefficient of stability of lactation,%;
y_1 - milk yield for 305 days, kg;
y_2 - milk yield for the first 100 days, kg.

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The yield of live calves (YLC) per 100 heads was calculated by the calculation method according to the formula [17]:

$$YLC = \frac{365 - SV}{285} \times 100, \text{ where} \quad (7)$$

YLC - calves' output, goal;
SV - service period (time between calving and fertilization), days;
365 - the number of days in a year;
285 - average duration of pregnancy, days.

The coefficient of the reproductive capacity (CRC) of livestock was calculated taking into account the period of the calving interval(CI) according to the formula [17]:

$$CRC = \frac{365}{CI}, \text{ where} \quad (8)$$

CRC - the coefficient of reproductive ability;
365 - the number of days in a year;
CI - averagecalving interval, months.

The fertility index of cows was determined by the formula proposed by the Hungarian scientist I. Doha (1961):

$$FI = 100 - (A + 2 \times CI), \text{ where} \quad (9)$$

FI - fertility index
A - age of the first-calf-cow, month;
CI - average calving interval period, months.

Data processing was performed in the MS Excel program using biometric analysis formulas; reliability was checked according to the t-Student's criterion.

RESULTS AND DISCUSSION

Based on the results of the visualization, fragments of the TFAM gene, which has 801 bp (Figure 1), were recorded for three genotypes: AA - 152, 187 and 426 bp; AC - 83, 104, 187 and 462 bp; SS - 83, 104, 152, 462 bp.

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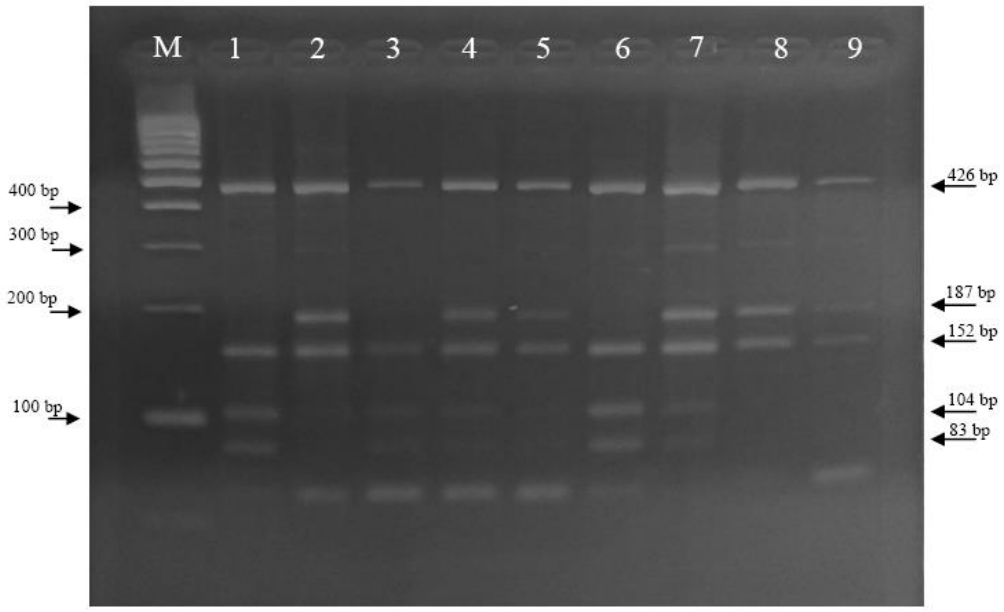

Figure 1: Electrophoretogram of PCR-PDRF products of the TFAM gene in a 3% agarose gel (M-DNA marker, AA-2, 8, 9, AS-4, 5, 7, CC-1, 3, 6)

As a result of identification of polymorphic variants of alleles and genotypes atthe TFAM-Hae III gene locus, the following distribution was observed (Table 1), indicating the predominance of individuals with heterozygous AC genotype in the studied Holstein cattle population.

Table 1: Frequency of occurrence of alleles and genotypes

Gene	Genotype	n	Occurrence	Allele	Occurrence
TFAM	AA	43	0,250	A	0,555
	AC	105	0,610		
	CC	24	0,140	C	0,445

The variability of alleles and genotypes recorded earlier by other researchers who studied the polymorphism of the TFAM gene has similar values: Kaplanová et al. (A-0.59, C-0.41), Moradgholi et al. (A-0.53, C-0.47) [18, 19].Moradgholi also established the maximum number of animals with the $TFAM^{CC}$ genotype [19]. Rezende and Ayres noted the prevalence of the A allele over C, their data of 0.873 and 0.127, and 0.840 and 0.160, respectively.Homozygous CC-specimens were 2.0% and 2.2% of the total herd in the populations studied by them [20, 21]. The insignificant prevalence of the C allele (0.560) over the A (0.440) allele is described in Jiang et al. [9, 11].

Mitochondrial transcription factor A is considered to play a significant role in the development of obesity and diabetes, affecting lipid metabolism, energy balance and body weight regulation [22, 23].Thereby, further research was conducted with the aim of discovering the associative links of the polymorphic variants of the TFAM gene with the dynamics of growth of first-calf cows.

Table 2: Dynamics of body weight of first-calf cows with different TFAM genotypes

Data	GenotypesTFAM		
	AA (n=45)	CA (n=105)	CC (n=24)
Body weight, kg			
Birth weight	31,4±0,6	31,1±0,4	31,4±1,0
6 months	174,8±3,6	168,3±2,3	168,1±3,9

12 months	309,4±4,2	307,8±3,4	303,3±5,5
18 months	426,6±6,3	416,7±4,4	418,9±6,4
1 calving	512,7±6,4	525,8±3,4	529,6±5,5*
Growth rate, kg			
0 – 6 months	143,4±3,7	137,2±2,3	136,7±3,6
6 months– 12 months	134,6±3,3	139,6±2,5	135,3±4,6
12 months – 18 months.	117,2±4,0	108,8±3,6	115,5±5,9
18 months – calving	481,3±6,3	494,8±5,4	498,2±6,6*
Average daily weight gain			
0 – 6 months	796,8±20,3	762,3±12,6	759,3±20,1
6 months – 12 months	747,9±18,3	775,3±14,1	751,4±25,3
12 months – 18 months	651,0±22,5	604,6±20,0	641,9±32,8

*Note:** - P ≤ 0,05

According to the Table 2 it is evident that during the control weighings in different age periods, except for the moment of the first calving, the predominance of body weight is demonstrated by the first-calf cows with the AA genotype.In measurements after calving, an insignificant advantage was assigned to a group of individuals with the CC genotype. The difference between these animals and their peers with other genotypes was: CC to AA - 16.9 kg (3.2%, P ≤ 0.05), CC to CA - 3.8 kg (0.7%).Concerning thegrowth rate, minor differences were observed.

Reliability in the difference in body weight is fixed in the period from 18 months. up to the first calving in the same group, the first-calf cows with the genotype CC: CC to AA - 16.9 kg (3.4%, P ≤ 0.05), CC to CA - 3.4 kg (0.7%).In average daily growth, the differences were of a nature of trend and were not significant. According to data published by Aryes et al., animals with the CC genotype had a maximum body weight during weighings of 12 months at 303.9 kg, compared to AA and AC (293.0 and 298.0 kg) [21].In the results obtained by Kaplanová et al., the advantage of a group of first-calf cows with the AC genotype at this age was measured at the age of 18 months - 553.7 kg, in contrast to AA and CC (550.5 and 353.0 kg) [18].Moradgholi found no significant differences between the genotypes of the TFAM gene and signs of growth in native Iranian cattle [19].Thus, it follows that the dynamics of the body mass has a slight associative connection with the polymorphic variants of the TFAM gene.

Milk production strongly depends on the synthesis of mitochondrial ATP [24]. Alex et al. observed an increase in mitochondria in mammary epithelial cells of milk cows, first lactation, along with an increase in milk yield in highly productive animals [25].Based on this, the indicators of milk productivity of the examined livestock by lactation activity and total milk yield, the percentage content of fat and protein, as well as the yield of milk fat and protein were studied.

Table 3: Association of polymorphism of TFAM gene and indicators of dairy productivity of first-calf cows

Data	Genotypes		
	AA (n=43)	AC (n=105)	CC (n=24)
Milk yield for lactation (305 days), kg	7235,3±164,0	6921,2±111,2	7047,3±303,2
Fat mass fraction, %	3,72±0,10	3,86±0,06	4,02±0,20
Mass fraction of protein, %	3,46±0,03**	3,34±0,03	3,30±0,04
Milkfatyield, kg	269,1±9,7	267,9±5,8	277,6±13,8
Milkproteinyield, kg	250,8±6,6*	231,6±4,6	233,2±11,1
Stabilitycoefficientoflactation,%	105,3±2,4**	101,9±1,8	97,5±1,9
Milkcoefficient	1426,6±41,7*	1321,8±23,3	1335,7±59,6

*Note: * - P ≤ 0,05; ** - P ≤ 0,01*

Investigation of the connection between the signs of milk productivity in the context of polymorphism of the TFAM gene showed that the largest milk yield for the first lactation was obtained from first-calf cows with the AA genotype.The advantage to other groups was: AA to AC - 314.1 kg (4.34%), AA to CC - 188 kg (2.30%). The same animals carrying the homozygous allele A at the locus of the TFAM-Hae III gene significantly differed in many characteristics.The difference with the animals with the lowest genotype of the CC genotype

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was fixed at the level of: 0.16% (P ≤ 0.01) by the protein mass fraction and 17.6 kg (7.01%; P ≤ 0.05) by the milk protein yield; 7.8% (P ≤ 0.01) for the coefficient of lactation stability.According to data obtained by other researchers, in cows with high productivity and stable milk yields, the lactation resistance coefficient is above 90%, and in cows with declining milk yields, 70-80% [26]. The difference between the groups of individuals $TFAM^{AA}$ and $TFAM^{AC}$ reached 104.8 (7.3% P ≤ 0.05) for the coefficient of milkness.However, the content and yield of milk fat prevailed in the milk produced by the primordia with the CC genotype. The trend towards the mass fraction of fat (%) was 0.16% and 0.30% compared to peers with the genotypes of AC and AA, and the yield of milk fat (kg) was 9.7 kg (2.49%) and 8.5 kg (3.06%) to animals with AC and AA genotypes, respectively.Heterozygous animals distinguished by a full value of lactation, by 2.6 and 2.5%, ahead of groups with homozygous genotypes AA and CC.

The concepts of dairy productivity for the first lactation of first-calf cows with different TFAM genotypes will be more complete when constructing lactational curves and assessing lactation activity during different periods of milking. Thus, we studied the indicators of the average daily milk yield (Fig. 2).With the beginning of lactation, the energy needs of high-yielding cows increase rapidly due to the production of milk. Adapting to lactation requires careful regulation and coordination of energy metabolism between key organs, such as the liver and breast [27, 28].Mitochondria are the main source for energy production in mammalian cells, and the number of copies of mtDNA that reflects the abundance of mitochondria in the cell can adapt to energy consumption and the physiological state of each organism [29].During lactation in cows, the concentrations of ATP in the mammary gland change during periods of peak milk production [30].The increased uniformity of milk yield during the whole lactation provides the opportunity of using milking machines, as well as receiving a stable amount of raw milk for the processing industry, and as a result, the uninterrupted supply of dairy products to the population and the increased financial performance of dairy production [31].Figure 2 shows how the average monthly milk yield changed during lactation within 10 months.

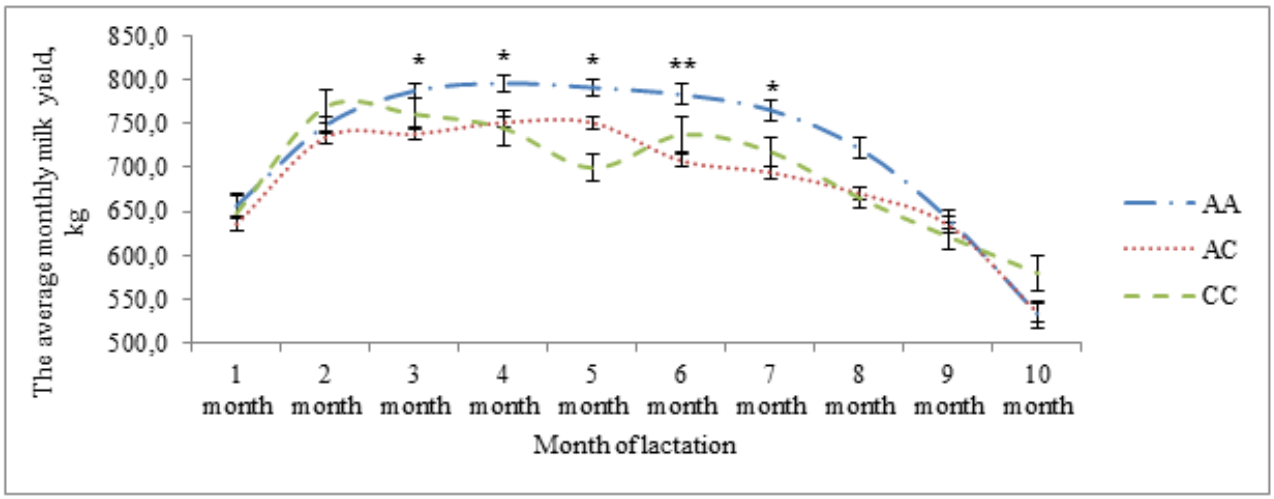

Figure 2: Lactation curves of the milk productivity of first-calf cows with different TFAM genotypes (* - P ≤ 0,05, ** - P ≤ 0,01)

According to the classification of lactation activity proposed by Yemelyanov (1953), there are four types of lactations: strong, stable lactation activity with high yield; strong unstable lactation activity, which falls after receiving higher daily milk yields and again rising in the second half of lactation (a two-vertex lactation curve); high, but unstable, rapidly disintegrating lactation activity; stable low lactation activity [32].

In compliance to above division, the first-line genes with the AA genotype have a more lined curve without jumps and sharp drops in the lactation curve related to the first type. From the third to the seventh month of lactation, this group of animals has a significant advantage over those with other genotypes of the TFAM gene.The first-calf cows with heterozygous genotype AC are high, but unstable indicators of milk yield and are of the third type. Cows with a genotype of CC, demonstrating a strong decline in the milk yield in the

fifth month and rise in the second and sixth, can be described as animals with unstable two-peak lactation.The predominance between groups of animals with different TFAM genotypes by average monthly milk yield was: in the third month between AA and AC - 49.3 kg (6.5%, P ≤ 0.05); in the fourth month between AA and AC - 44.5 kg (5.6%, P ≤ 0.05), AA and CC - 51.1 kg (6.4%); in the fifth month between AA and CC - 91.6 kg (11.6%, P ≤ 0.01); in the sixth month between AA and AC - 75.4 kg (9.6%, P ≤ 0.05); in the seventh between AA and AC - 71.0 kg (9.3%, P ≤ 0.05).It should be noted that the studied population is characterized by stable milk yield and high productivity throughout the first lactation, which indicates the usefulness of the diet and good preparation for calving.

The milk productivity of the first-calf cows is significantly influenced by the following factors as the body weight of the animal and the age of the first productive insemination.Too early insemination leads to a slowdown in development and pushes back the period of maximum expansion. In the first months of lactation, the exchange energy obtained with food cannot cover the body's expenses for milk production, so that the energy balance becomes negative, trying to recover from internal reserves.At which point in animals there is a loss of body weight, fatness, and metabolic disorders. Characteristics of reproductive capacity and reproductive qualities are presented in Table 3.

Table 3: Association of polymorphism of the TFAM gene and the reproductive qualities of the first-calf cows

Data	Genotypes		
	AA (n=43)	AC (n=105)	CC (n=24)
Age of the first insemination, months	17,3±0,4	17,8±0,3	17,0±0,7
Body weight in the first insemination, kg	420,7±3,5	421,5±2,6	406,4±9,4
Body weight in the first calving, kg	512,7±6,4	525,8±3,4	529,6±5,5*
Calving interval, days	364,2±11,3	396,6±7,9*	461,4±28,7**
Interlactation period, days	59,3±3,7	51,8±1,8	50,1±4,8
Calf crop, animals	82,3±4,0	77,3±2,9	73,5±5,4
Coefficient of reproductivity	1,02±0,03	0,95±0,02*	0,84±0,05**
Index of calf-producing capabilities	59,8±1,2**	56,2±0,7*	52,2±1,8

*Note: * - P ≤ 0, 05; ** - P ≤ 0, 01*

According to our data, the entire heifer sat the time of the first insemination had a sufficient body weight and a suitable age, without statistically significant differences. However, at the time of calving, animals with the CC genotype outnumbered peers with the AA genotype by 16.9 kg (3.2%, P ≤ 0.05), and with the AC genotype by 3.8 kg (0.7%).The smallest calving interval was recorded in animals with the AA genotype. The difference in this indicator with other groups was: with AC - 32.4 days (P ≤ 0.05), with CC - 97.2 days (P ≤ 0.01). On the basis of the reproductive capacity factor (1.02) and the fertility index of the cow (59.8), the first-calves cows with the AA genotype were also statistically significant. They had the best data on calves' output (goal) - 82.3 calves per 100 cows, compared to other animals. In general, the obtained data on reproductive qualities of the studied livestock testify to a good level of fertility and reproductive ability of the herd.

CONCLUSION

In the study of the Holstein cows was found that the studied population is polymorphic in the gene of the mitochondrial transcription factor A and has a significant variability in alleles and genotypes. The dynamics of body weight does not have statistically significant differences depending on the polymorphic variants of the TFAM gene. Analysis of milk productivity showed that animals with the $TFAM^{AA}$ genotype significantly excels peers with other genotypes by milk yield, protein mass fraction, milk protein yield, milk ratio. The connection of genotypes to lactation activity of the first-calf cows and the lactation stability factor was also established. It is noted that TFAM, as a marker gene for lipid metabolism and energy balance, has an impact on fertility and reproductive function. Heterozygous AA-animal units had a positive difference in some indicators characterizing reproductive qualities. These established associations can be useful in drawing up plans for breeding and breeding activities in the context of improving the genetic potential of cattle.

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