Polymorphism of the leptin gene (a80v) and its effect on dairy productivity of cattle

Andrey Yaryshkin* and *Olga* Shatalina

Federal State Budgetary Scientific Institution "Ural Federal Agrarian Research Center of the Ural branch of the Russian Academy of Sciences", Yekaterinburg, Russia

Abstract. Modern technologies, including marker breeding, make it possible to identify genetically valuable animals and select individuals for breeding and raising, which in turn helps to increase productivity, improve the quality of milk and dairy products and reduce material costs for its production. The researchers also note the relationship of the leptin gene with an increase in live weight of cattle and lipid metabolism. The purpose of the research was to study the DNA polymorphism of the leptin marker gene and its possible use as a leptin marker gene to improve the productive performance of cattle. The animals were genotyped using PCR-RFLP analysis. Data on milk productivity and insemination indicators are taken from the ARM "Selex" (dairy cattle) program. Biometric processing of the obtained results was carried out, the reliability criterion was calculated. The results of the study showed that the AA genotype and the A allele are the most common in the Holstein breed. It was concluded that the AA genotype is associated with high milk yield, and the AV genotype is associated with an increase in fat and protein in milk and an increase in body weight. The milk yield of these individuals exceeds the indicators of their peers by 200-400 kg, and in terms of lifetime productivity reaches 4,600 kg. The AA genotype has a beneficial effect on the timing of economically beneficial use of cows. The difference in the duration of economic use was 0.6-2.1 lactation. The use of marker genes, such as leptin A80V, can help increase productivity and improve milk quality in cattle, which is an important task of the breeding process.

1 Introduction

Currently, both classical methods are used in cattle breeding: selection and matching, and more innovative ones, such as the determination of marker genes for economically useful traits [1-3].

The use of marker genes for dairy productivity of cattle is of great importance for modern cattle breeding. Thanks to these studies, it became possible to accurately determine the genetic characteristics of each animal and select the most promising individuals for breeding [4-6]. This makes it possible to increase the productivity of dairy cattle, improve the milk quality and reduce of its production cost. The use of marker genes also allows breeders to preserve and increase the genetic diversity of livestock, which is a key element for sustainable

* Corresponding author: x2580x@yandex.ru

and successful agricultural development. Thus, the use of marker breeding contributes to an increase in dairy production and contributes to the production of high-quality and competitive products [7-9].

One of the important marker genes is leptin, which plays a key role in regulating lipid metabolism and eating behavior. Moreover, it has been suggested that leptin may have an effect on immune responses and reproductive processes [10, 20]. The A80V polymorphism of the leptin gene is considered as a promising genetic marker [11-13]. The leptin gene is encoded by a protein produced by adipose tissue and comprising 167 amino acids [14, 15].

The leptin gene was found in mice, and then in a huge number of other vertebrate species [16]. The leptin gene is located on chromosome 4 and consists of three exons, one of which does not encode the protein sequence [12, 17, 18, 19].

In this research, the task was to study the polymorphism A80V of the leptin gene of Holstein cattle in the Sverdlovsk region and analyze its effect on productive indicators.

2 Materials and Methods

The study was conducted in the genetic research testing laboratory. The object of the study was cows of the third lactation and older, belonging to the Holstein breed of the Sverdlovsk region. DNA isolation was carried out according to the protocol of the company "Syntol" using a set of DNA-Extran-1.

The reaction mixture for performing PCR-RFLP according to SNP A80V had a volume of 20 µl and included: 10×SE buffer – 2 µl, dNTP – 2.5 µl, direct and reverse primers – 0.6 µl each, Taq DNA polymerase – 0.7 µl, deionized water – 9.6 µl and 4 µl DNA. Amplification took place using the BioRad PTC-225 DNA Engine Tetrad Cycle amplifier and primers (A80V F: CAAGCAGGAAATAGGGAGTCATGG; A80V R: CTGGTGAGGATCTGTTGGTAGGTC).

The size of the PCR product was 424 bps. The program for PCR-RFLP of the leptin A80V gene consisted of the following stages: step 1 – 95°C for 3.5 minutes, step 2 – 95°C – 30 s, 65°C – 20 s and 72°C – 30 s, with a total number of cycles of 27.

The restriction mixture consisting of 5 µl of amplification, 2 µl of restriction buffer, 0.2 µl of PspEI restrictase, 12.8 µl of deionized water and 3 µl of DNA was kept in a Binder thermostat for 3 hours at a temperature of 37°C.

Restriction products were visualized on a Gel Doc transilluminator (BioRad) after electrophoresis at a voltage of 100V for 1 hour. The data on the economically valuable characteristics of cattle are taken from the ARM "Selex" program (for dairy cattle). Biometric processing was carried out with the participation of IBM SPSS Statistics 23 and Microsoft Excel programs. The arithmetic mean values and the error of the arithmetic mean (X ± Sx) are calculated using the methods of E.K. Merkurieva (1983) according to indicators such as milk yield, fat and protein content in milk and body weight. To assess the reliability of the difference in the data obtained, the Student's criterion was used and Spearman's correlation analysis was performed to identify reliable dimensions of leptin gene polymorphism effect on productive indicators.

3 Results and Discussion

A study of the A80V polymorphism of the leptin gene was conducted on a sample of 120 cattle from two breeding plants with 60 heads each.

The A80V polymorphism of the leptin gene consists of alleles A and V, forming three possible genotypes: AA, AV, and VV. The AA genotype is determined by a single band

located at the level of 424 nucleotide pairs. The AV genotype has two bands at levels 424 and 398 bps, VV has one band at the level of 398 base pairs (Fig. 1).

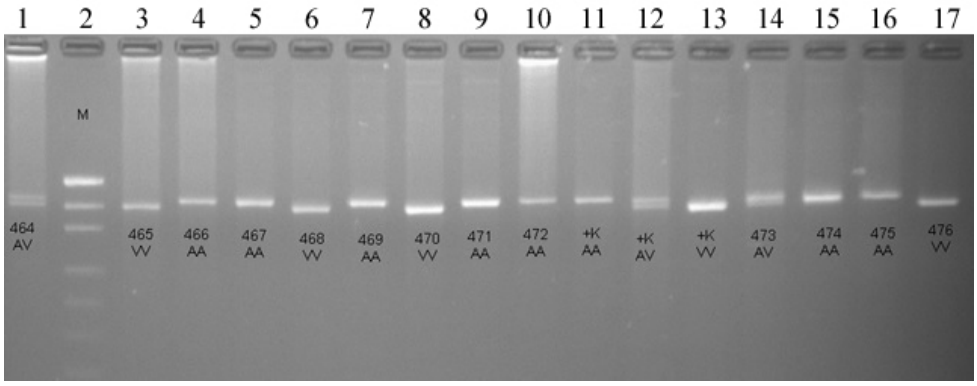

Fig. 1. Electrophoregram of restriction products of the leptin gene amplification site (polymorphism A80V).

Note: 4, 5, 7, 9, 10, 11, 15, 16 – genotype AA; 1, 12, 14 – genotype AV; 3, 6, 8, 13, 17 – genotype VV; 2 is a molecular weight marker.

Figure 2 shows the frequency of occurrence of various leptin A80V genotypes in "Breeding Plant 1" and "Breeding Plant 2".

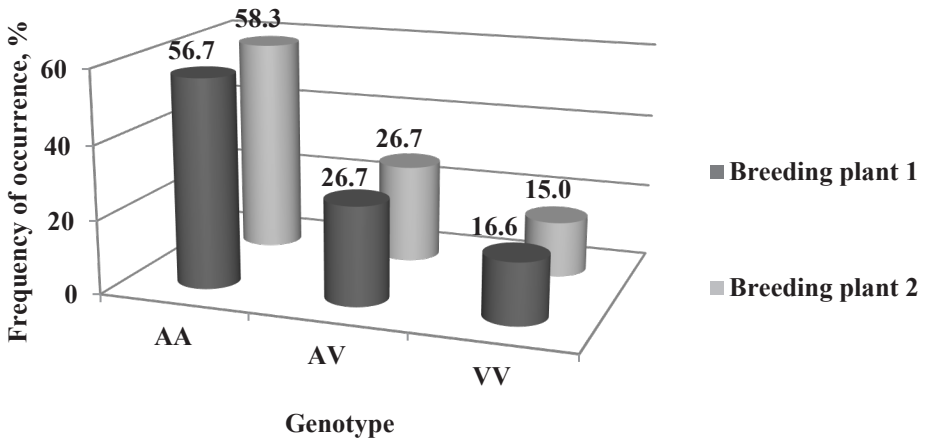

Fig. 2. The occurrence of various leptin A80V genotypes.

In the studied population of cows, SNP leptin A80V has the most widespread AA genotype, amounting to 56.7-58.3%. The frequency of the AV genotype occurrence is less than 26.7%. The VV genotype is less common in the Holstein breed and its frequency does not exceed 17%. The results obtained are consistent with the work of A.I. Ganja et al. [17], made on Holstein breed. The distribution of leptin A80V alleles in animals of breeding organizations was also studied (Figure 3).

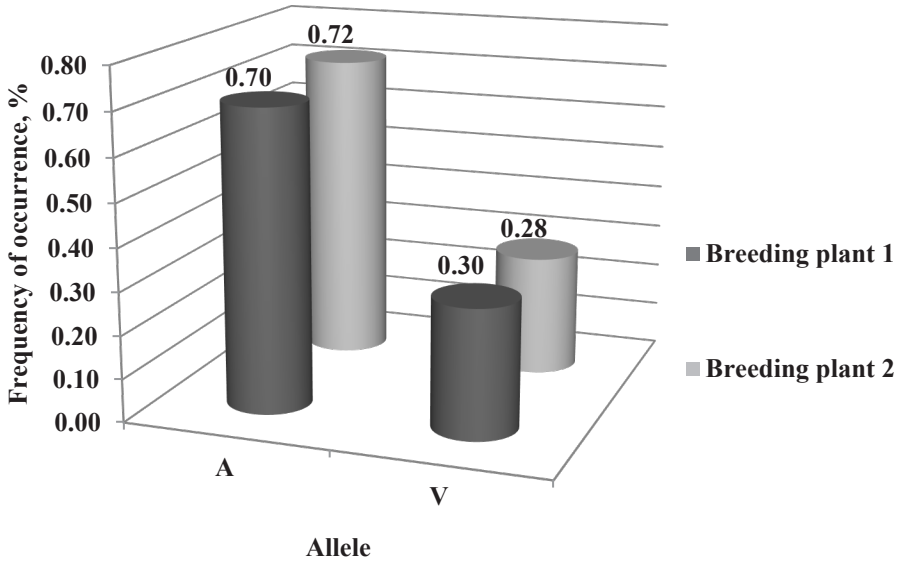

Fig. 3. The occurrence of various leptin A80V alleles.

In the SNP A80V, the A allele is dominant, with a frequency of 70-72%.

Table 1 shows the productive indicators of cows during the first lactation, depending on the polymorphism of leptin A80V.

Table 1. Indicators of dairy productivity and live weight of cows for the first lactation with different leptin A80V genotypes (n=120) (X ± Sx).

	LEP	Protein mass fraction, %	Fat mass fraction, %	Milk yield, kg	Live weight, kg
Breeding Plant 1	AA	3,13±0,01*	3,95±0,02***	6806±111	591±3*
	AV	3,17±0,02*	4,03±0,01	6594±239	582±9
	VV	3,10±0,03	3,98±0,03	6398±127	571±10
Breeding Plant 2	AA	3,10±0,02*	3,96±0,02*	9398±201	579±12
	AV	3,14±0,01	4,00±0,01	9145±186**	575±5
	VV	3,09±0,03	3,94±0,04	8285±504	570±5

Cows carrying the AV genotype showed the best results in terms of fat and protein content in milk. The difference with carriers of the AA and VV genotypes was 0.05-0.08% ($p \leq 0.05$), ($rs=0.44$-0.45, $p \leq 0.001$). At the same time, the AA genotype is associated with increased milk yield and higher body weight. The difference in milk yield was 500-900 kg ($p \leq 0.01$), in live weight – 9-20 kg ($p \leq 0.05$). This pattern is present in both breeding plants.

Table 2 shows the productive indicators of cows during the third lactation, depending on different genotypes.

Table 2. Productive characteristics of cows with different leptin genotypes (n=120) (X ± Sx).

	LEP	Protein mass fraction, %	Fat mass fraction, %	Milk yield, kg	Live weight, kg
Breeding Plant 1	AA	3,14±0,02	3,96±0,02	7897±154	649±6*
	AV	3,18±0,01*	3,97±0,02	7491±196	633±3
	VV	3,13±0,02	3,93±0,02	7383±301	630±9
Breeding	AA	3,15±0,02	3,92±0,02*	10261±1500	667±4**

Plant 2	AV	3,18±0,01***	3,97±0,01*	9985±310	658±9***
	VV	3,13±0,01	3,91±0,03	9645±440	622±12

Analyzing the data in Table 2, it can also be noted that the AV genotype is associated with the fat and protein content in milk. In breeding plant 1, the difference between peers was 0.04-0.05% ($p \leq 0.05$-0.001) ($rs=0.48$). In breeding plant 2, the fat and protein content in the milk of cows with the AV genotype was higher by 0.05-0.06% ($p \leq 0.05$) ($rs=0.52$). The AA genotype is associated with live weight and milk yield. The difference in live weight was: in the breeding plant 1 – 19 kg ($p \leq 0.05$), in the breeding plant 2 – 36 kg ($p \leq 0.001$). The difference in milk yield was: in the breeding plant 1 – 500 kg; in the breeding plant 2 – 600 kg.

The indicators of milk productivity and live weight of cows with different leptin genotypes for the last completed lactation were also studied (Table 3).

Table 3. Polymorphism of the leptin A80V gene and its effect on the productive indicators of cows after the last completed lactation (n=120) (X ± Sx).

	LEP	Protein mass fraction, %	Fat mass fraction, %	Milk yield, kg	Live weight, kg
Breeding Plant 1	AA	3,20±0,01**	3,90±0,01	8428±322	630±4
	AV	3,27±0,02**	3,95±0,03	7749±494	619±13
	VV	3,21±0,01	3,92±0,03	7080±792	615±10
Breeding Plant 2	AA	3,18±0,02*	3,91±0,03	11041±322***	670±20*
	AV	3,25±0,01**	3,94±0,02	10902±156***	649±9
	VV	3,21±0,02	3,92±0,03	9365±317	634±5

In breeding plant 1, cows with the AA genotype demonstrate the highest milk yield, which exceed the values of their peers by 679-1348 kg. As for cows with the AV genotype, their milk contains 3.95% fat, and they are also prone to an increased protein content ($rs=0.43$, $p \leq 0.001$), the difference is 0.06-0.07%. The live weight of cows with the AA genotype is also higher, exceeding the indicators of their peers by 11-15 kg.

In breeding plant 2 cows with the AA genotype demonstrate the highest milk yield, the difference with their peers reaches 1676 kg ($p \leq 0.001$). Carriers of the AV genotype have an increase in the level of fat and protein in milk, where the mass fraction of fat is 3.94%, and the mass fraction of protein is 3.25%.

Table 4 illustrates the pattern of changes in the productive indicators of cows with different genotypes of the leptin A80V gene throughout their lives.

Table 4. Lifetime indicators of productive characteristics of cows depending on the leptin gene carrier (n=120) (X ± Sx).

	LEP	Protein mass fraction, %	Fat mass fraction, %	Milk yield, kg	Live weight, kg
Breeding Plant 1	AA	3,16±0,02	3,97±0,02	33056±1041**	650±9**
	AV	3,19±0,03	4,00±0,03	29440±1210	626±7
	VV	3,13±0,01	3,99±0,02	28950±2144	618±5
Breeding Plant 2	AA	3,16±0,03**	3,93±0,02**	53863±901	659±8
	AV	3,21±0,01**	4,00±0,02*	52687±2080*	647±11
	VV	3,14±0,02	3,94±0,01	46120±1300	645±7

When studying the lifelong productive indicators of cows, depending on the polymorphism of the leptin gene, the same pattern is present that can be traced during the first, third, and last completed lactation. In terms of protein content, cows with the AV genotype show the best results. In breeding plant 1, the difference with non-residents was 0.03-0.06%, in breeding plant 2 – 0,05-0,07 % ($p \leq 0.01$) ($rs=0.34$). The AV genotype is also

associated with fat content. In the breeding plant 1, the difference was 0.01-0.03%, in the breeding plant 2 – 0,06-0,07 % (p≤0.05-0.01) (rs=0.47).

The AA genotype can be called a marker of high milk yield and body weight gain. In breeding plant 1, the difference in milk yield was 3600-4100 kg (p≤0.01), in breeding plant 2 – 1100-7700 kg (p≤0.05) (rs=0.42). Also, the AA genotype is associated with the live weight of cows. The difference in breeding plant 1 was 25-30 kg (p≤0.01), in breeding plant 2 it reaches 12 kg.

Polymorphisms of the leptin gene play an important role in determining the duration of productive longevity of cows (Figure 4).

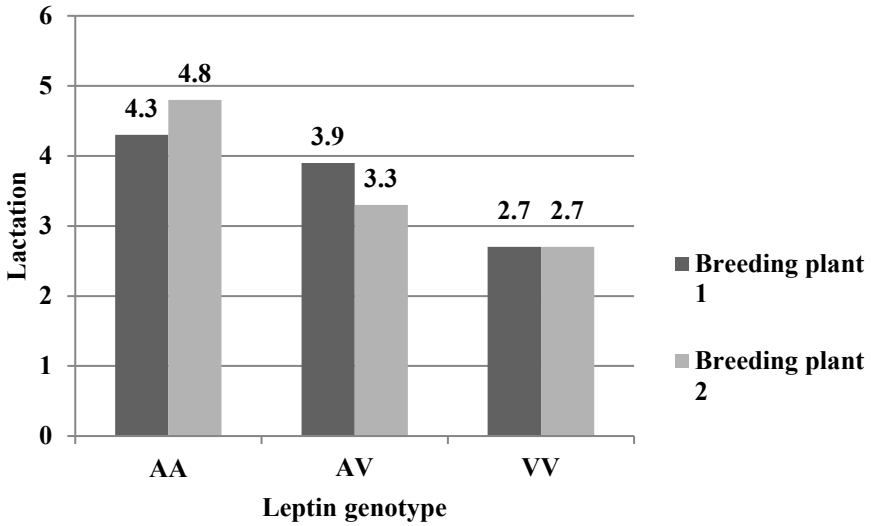

Fig. 4. Terms of productive longevity of cows with different genotypes A80V- polymorphism.

Cows with the VV genotype show a short duration of productive longevity. The difference with cows carrying the AA and AV genotypes was 0.6-2.1 lactation. On the contrary, cows with the AA genotype demonstrate high rates of economic use in both agricultural organizations (rs=0.52, p≤0.001). This suggests that the presence of the A allele has a beneficial effect on animal health. [20].

4 Conclusions

As a result of the conducted research, it was found that the AA genotype is a marker of high milk yield, since throughout the life of the carrier cows of this genotype show the best results. The difference between genetically distinct cows by the leptin gene, in some cases, reaches 7700 kg over a lifetime. Also, the AA genotype is associated with a large set of body weight. The AV genotype is a marker of protein and fat in milk. Cows with the AV genotype exceed the indicators of their peers by 0.05-0.07%. The presence of the A allele in the polymorphism of the A80V leptin gene is associated with an increase in the economically-beneficial life, which is on average 1.6-2.1 more lactation compared with carriers of the VV homozygote. Thus, it can be concluded that it is undesirable to spread animals with homozygous genotype VV in herds.

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