

Impact of LEP gene allele's polymorphism on functional traits in Holstein-Friesian cattle.

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ABSTRACT

The problem of short functional longevity of cattle remains one of the major unsolved problems of agriculture. PCR / RFLP method allows identification of genetic markers that have an advantage in the selection based on functional longevity. Hormone leptin gene is such a marker.

Dairy cattle breeding is an important branch of modern world agriculture. In the world there are about 1.5 billion head of cattle (cattle). Therefore, the search for a solution to the problem associated with increasing profitability and production is a priority for workers in agriculture [2].

At the same time, functional longevity is the limiting factor, since it directly affects the lifetime milk yield, the number of offspring and, ultimately, the improvement of breeds and herds [1]. Therefore, in the modern livestock sector, highly productive cows with a long period of use play an extremely important role.

To increase the term of functional longevity of cattle by breeding methods will help to search for genetic markers and studies on the evaluation of the connection between SNP markers localized in selected genes and functional longevity of dairy cows.

These markers include polymorphisms of the leptin hormone (LEP) gene: R25C, Y7F, A80V [5]. In addition to the influence of the LEP genotype on functional longevity, energy metabolism also depends on food behavior. It may affect the functioning of the immune system and reproductive function, as well as the growth and constitution of animals [4]. It is believed that leptin has a pleiotropic effect on the body [3].

Keywords: Cattle, LEP gene, polymorphism, the frequency of occurrence

INTRUCTION

Leptin is a globular protein synthesized by adipose tissue. The leptin molecule (16 kDa) consists of 167 amino acids with the N-terminal secretory signal sequence of 21 amino acids [Leptin, the obese ..., 1996]. Refers to cytokines - signaling proteins. The leptin molecule has four spiral chains with five to six full turns. The main physiological role of leptin is manifested by a decrease in the synthesis of macroergs and an increase in energy costs [Agarwal, Rout, Singh, 2009]. Its mechanism of action is to

transmit to the hypothalamus information about body weight and fat metabolism. The interaction of leptin with specific receptors located in the hypothalamic region activates the production of nerve impulses directed to areas of the brain responsible for the regulation of appetite. In addition, the action of leptin stimulates the sympathetic nervous system, which in turn leads to increased blood pressure, heart rate and thermogenesis processes, by separating the processes of oxidation (cellular respiration) and phosphorylation (synthesis of ATP molecules) in the mitochondria of white adipose tissue. In modern animal breeding genes are involved in biochemical and physiological pathways and have SNP polymorphisms in coding sequences (exon) leading to amino acid replacements in proteins, and in regulatory elements. Significant attention is given to the study of relationships between polymorphisms in lipid metabolism genes such as leptin (*LEP*), thyroglobulin (*TG*), diacylglycerol O-acyltransferase 1 (*DGATI*) with milk and beef productivity traits in cattle. The problem of short functional longevity of cattle remains one of the main unsolved problems of agriculture. The PCR / RFLP method makes it possible to identify genetic markers that have an advantage in selection based on functional longevity. These markers include the hormone leptin gene.

The achievements in modern molecular genetics allow us to study the genes correlated with the useful traits in cattle. The leptin gene alleles (*LEP*) may be considered as the potential markers for milk and meat production [1, 2]. Approximately 60 SNP polymorphisms in this gene are described [3]. The leptin gene and its polymorphism have been generally studied owing to the energy metabolism in the beef cattle and the milk production in the Holstein animals [2, 4]. It is reported that the *R25C* polymorphism is associated with the milk fat and protein contents, the ease of calving, and the length of gestation [5]. In addition, the length of a cow productive life depends on the *LEP* genotype. Thus, it was ascertained that the culling risk is 3.14 times greater in the cows with the *CC* genotype (SNP *R25C* in the leptin gene) than that in the animals with the heterozygous genotypes. It is 3.64 times greater in the cows with *FF* genotype (SNP *Y7F* in the leptin gene) than that in the cows with the *YY* genotype [6]. The effects of the *LEP-A80V* polymorphism on the productive lifetime and the profitability level in cows are reported [6, 7]. The objective of the surveys is studying the leptin gene polymorphisms (*R25C*, *Y7F*, and *A80V*) associated with the Holstein cattle productive longevity as reported in the related literature data.

Materials and Methods.

The research was carried out in 2016 in the period from 20.06.14 to 27.07.14 and from 02.09.16 to 12.10.16 on the basis of the biotechnology laboratory of KSZA (Kharkov Ukraine). The object of the study was bulls-producers and cows of Holstein-Friesian cattle. The material of the study was the sperm of bulls-producers, the blood of young cows and cows of the fifth to ninth lactation, as well as the blood of heifers, descendants of one of the genotyped bulls. A total of 272 samples were analyzed. To extract DNA from sperm and blood, Diatom™ DNA Prep 100 reagents were used. The DNA yield was 3-5 mg / 100 µL with an OD of 260/280 from 1.6 to 2.0. Samples were examined by the PCR / RFLP method for the presence of polymorphisms *R25C*, *A80V*, *Y7F* in the *LEP* gene. Based on the results of the analysis, the frequency of genotypes of the *LEP* gene was determined by the polymorphism *R25C*: *RR*, *RC* and *CC*; by polymorphism *A80V*: *AA*, *AV* and *VV*; and by the polymorphism *Y7F*: *YY*, *YF* and *FF*; and a link was established between the genotypes of *LEP* and the functional longevity of cattle.

Results and Discussion.

Cows with the maximum period of use (5 - 9 lactations) were included in one sample to determine the advantage of different *LEP* genotypes, and in the other - cows-recorders for the first finished lactation (cows with milk yield over 7800 kg for the first complete lactation) usually the term of their functional longevity is on the average in the herd lower.

Table 1.

LEP genotypes by polymorphism R 25 C , Y 7 F , A 80 V Holstein-Friesian cattle.

SNP LEP	Genotype	Holstein-Friesian cattle.			
		Cows 5-9 lactation (n = 104)	Cows 1-3 lactation (n = 102)	descendants of a bull with the genotype RRFFAA (n = 34)	in the group of bulls-producers (n = 32)
R25C	RR	0.28	0.26	0.86	0.53
	RC	0.49	0.53	0.14	0.40
	CC	0.23	0.21	0.00	0.06
Y7F	YY	0.51	0.61	0.00	0.50
	YF	0.46	0.34	0.41	0.41
	FF	0.03	0.5	0.59	0.09
A80V	AA	0.96	0.98	1.00	1.00
	AV	0.04	0.02	0.00	0.00
	VV	0.00	0.00	0.00	0.00

For example, the incidence of LEP-R25C genotypes (replacement of C → T at 96590050 bp LEP), which directly affects the functional longevity of Holstein-Friesian cattle, does not have significant differences in long-lived cows and cows. Attention is drawn to the genotype of RR, which occurs in long-lived cows more often (0.28) than in recorders (0.26). The calculation of the criterion showed that none of the genotypes has a breeding advantage. **Interesting single nucleotide polymorphisms are located in different zones of the leptin gene. Their location is shown in Table 2.**

Table 2

Description of the individual polymorphisms of the LEP gene given in this study

The name SNP (and other names used in the literature)	Nucleotide substitutions	Gene Area	The leptin protein region
C (-963) T	C / T	promoter	-
Y7F (A252T)	A / T	2 exon	Signal sequence
R25C (C305T, R4C, C73T, LepKpn2I)	T / C	2 exon	b-helix A
A80V (LepHphI, A59V)	C / T	3 exon	b-helix B

The leptin gene is a highly polymorphic gene. In it, in addition to the SNP polymorphisms of interest, there are still about sixty one-nucleotide substitutions. Moreover, most of them are located in the intron regions, which reduces their effect on the conformation, amino acid composition and function of the leptin protein. However, the SNP polymorphisms we are considering are located in the coding regions of the gene. The second feature of the breed is the lack of polymorphism of LEP-A80V alleles in most airshares (replacement of C → T at position 95689996 bp LEP). The frequency of occurrence of allele V in Holstein-Friesian cattle. does not exceed 0.03, and the homozygous genotype over it has not been identified by us in any animal. When analyzing the frequencies of occurrence of LEP-Y7F polymorphisms in Holstein-Friesian cattle, it was noted that in all samples the frequency of FF

homozygotes was very low despite the high incidence of YF in the long-lived cattle groups (0.46) and heterogeneity of origin (the descendants of 20 bulls were analyzed) . Heterozygous animals (YF) among the record cows met with a slightly lower frequency (0.34).

Allele F is quite common. Its frequency is from 0.21 to 0.34. However, the frequency of occurrence of homozygotes FF is not more than 0.5, which may indicate a reduced viability of animals with this genotype. However, this phenomenon can be the result of other processes, for example, gene drift.

In order to verify at which of the stages of ontogenesis of the animal the allele F can leave the herd, we genotyped the descendants of the bull-producer with the genotype RRFFAA. It was found that the frequency of allele F is also quite high (0.79), but it is found not only in heterozygous genotype, but also in homozygous genotype in 59 percent of cases. This suggests that animals do not retire in the prenatal period of ontogenesis, because at two - three months of age, chicks with the FF genotype occur with a high frequency. After the genotyped selection of heifers, an observation will be established to determine the period of economic use of each of them.

Another interesting feature is the cohesion of genotypes in the Holstein-Friesian cattle. Thus, in a sample of animals (n=38) with the genotypes CCYY, CCYF, CCFF, a combination of CCYY occurs in 90% of cases, and the CCYF genotype in 10%. At the same time, combinations of YF with non-CC genotypes (RR and RC) are possible and occur at a high frequency.

The genotype FF also has the same tendency to cohesion. According to the obtained data, in 33 animals with the FF genotype it occurs in combination with the RR genotype in 100% of cases for possible combinations of the genotypes CCFF, RCFF and RRFF. As a result, we came to the conclusion that genotypes are linked according to the principle of a combination of "weak" plus "strong" and "strong" plus "weak" in terms of functional longevity (CC genotype worse than RR, and FF worse than YY). However, such "weak" combinations as CCFF do not occur.

Conclusions

These results indicate the relationship of LEP with functional longevity and are well supported by other studies in the field of health of eating behavior and dairy cattle reproduction, which also determine functional longevity. In view of the growing importance of functional signs of dairy cattle, LEP polymorphisms should be considered as markers determining the decision in favor of a choice in cattle breeding. Thus, we concluded that when selecting breeding bulls for breeding on the basis of functional longevity, genotypes should be avoided, which when crossed can give combinations: RRFF, RCFF, CCYF, CCFF. These results indicate the relationship of LEP with functional longevity and are well supported by other studies in the field of health of eating behavior and dairy cattle reproduction, which also determine functional longevity. In view of the growing importance of functional signs of dairy cattle, LEP polymorphisms should be considered as markers determining the decision in favor of a choice in cattle breeding. that the polymorphism with the highest degree of influence on the functional longevity of cattle is LEP-R25C, located inside the leptin gene.

References

1. Sivkin N.V. The experience of breeding the Swedish red breed in Central Russia. Zootechnology. - 2011. - No. 2. - P. 58-60.
2. Surovtsev VN, Nikulina Yu.N. Economic aspects of productive longevity of cows / [Electronic resource]. - Access mode: URL: <http://fs-8F.pdf> (date of circulation: 19.11.2015).
3. Giblin L., Bytler S., Kearney B., Waters S. All Association of bovine leptin polymorphisms with energy output and energy storage traits in progeny Holstein-Friesian dairy cattle sires // BMC Genetic. - 2010. - No. 11. - P. 73.
4. Komisarek J. Impact of LEP and LEPR gene polymorphisms on functional traits in Polish Holstein - Friesian cattle // Animal Science Papers and Reports. - 2010. - No. 10. - P. 133-141.
5. Scyda J. Evaluating markers in selected genes for association with functional longevity of dairy cattle // NCBI. BioMed central genetics. [Electronic resource]. - Access mode: URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3061949> (date of circulation: 01.19.2014).