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Expression of candidate genes of lipid metabolism in the Kazakhstani breeding simmental cattle

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ABSTRACT

This research paper describes a gene polymorphism of growth hormone (GH), leptin (LEP) and diacylglycerol *O*-acyltransferase (DGAT) in Simmental cows of LLP "Galitskoe" of Pavlodar oblast. The frequency distribution of alleles and genotypes of the studied genes was held: at GH gene locus it was revealed that 19% of the 123 cows had VV genotype; 77% of the cows had VL genotype; 4% - LL genotype, at that the allele frequency was V - 58% and allele L - 42%.At Lep gene locus the genotype distribution was as follows: CC - 42%; CT - 48% and TT-10%. The frequency of allele C and T in the study was 66 and 34%. In DGAT gene locus genotype KK had 25%; genotype AA - 75%. The allelic frequency of K genotype was 63%, and A genotype - 37%.The study of GH gene expression showed that the minimum and maximum milk yield indicator of the test animals varied from 3089 to 8017 kg, the fat content ranged from 2.20 to 6.20%. Expression of Lep gene showed that the maximum milk yield was received from cows with CT genotype (9056 kg) and a minimum in the CC genotype (7407 kg), maximum fat content was observed in cows with CT genotype (6,1%), and the minimum in CC (2,2%) genotype. The variation of the studied milk yield indicators was from 2544 to 9056 kg of milk, fat content in the milk varied from 2.2 to 6.1%. According to DGAT gene milk production levels ranged from 3377 kg to 7985 kg of milk. The maximum yield of high fat content was obtained from cows with AK genotype (7985 kg - 5.9%), the minimum milk yield result was received from cows withKK genotype (3377 kg) and minimum fat cow result was received from cows with AK genotype (2.2%).

Introduction

Success in breeding is largely dependent on the accuracy of the breeding value of animals. In this regard, the value of methods helping to identify the best animals and predict their breeding qualities at an early age is increasing. The achievements of modern molecular genetics make it possible to determine the genes that control the economic traits. In addition to the traditional selection of animals, detection of gene variants will allow to carry out selection directly at the DNA level. DNA technologies advantage is that it is possible to determine the genotype of the animal regardless of sex, age and physiological state, which is an important factor in breeding. As a potential marker of milk production alleles of genes milk proteins and hormones can be considered [1].

Intensification in the livestock breeding process is impossible without use of modern molecular genetic techniques and use of DNA markers associated with economic traits of animals. Many researchers analyzed the distribution of allelic variants of a number of structural genes, polymorphism of which is often associated with the core indicators of cattle milk production. Emergence of allelic variants in regulatory and structural regions of these genes can affect diversification of the amount and composition of milk [2].

The central role in regulation of mammalian growth and metabolism, either directly or indirectly affecting numerous aspects of animal reproduction and lactation periods, belongs to a growth hormone - somatotropin. Somatotropin (growth hormone, GH) is an important regulator having lactogenic and fat-mobilizing effect, so the study of polymorphism of this gene is important in the analysis of genetic determinism of productive qualities of animals. The growth hormone gene in cattle is localized on the 19thchromosome and consists of five exons and four introns.

Along with alleles of genes of somatotropin (GH), leptin (LEP) is considered as a potential marker of cattle milk productivity. Leptin is a polypeptide hormone synthesized and secreted primarily in fat cells. In cattle LEP gene is located on chromosome 4. It consists of 3

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exons and 2 introns.

In addition, scientists consider diacylglycerol *O*-acyltransferase (DGAT)gene as a positional gene, a candidate of fat content in milk. This marker causes encoding a key enzyme in the synthesis of milk fat. The fat content in milk, as well as the protein content it is an important technological characteristics of this product. DGAT gene was mapped to chromosome 14 in Bostaurus genome as a marker influencing milk quality. Analysis of the nucleotide sequence makeit possible to identify the sequence as structural DH-encoded region of diacyl-glyceraldehyde-acetyl transferase gene. This Enzyme Is Involved In Biosynthesis Of Lipids.

The search of candidate genes of lipid metabolism of animals, development of test systems analysis and study of their expression, effect of polymorphic variants of these genes on lipid metabolism of animals is a challenge of modern animal production science [1,2].

In connection with this, the objective of our research was to study the expression of genes in determining polymorphism of the genes of somatotropin, leptin, and diacylglycerol *O*-acyltransferase in studying the relationship of investigated genotypes with milk production and processing properties of milk from Simmental cows of LLP "Galitskoe" of Pavlodar oblast.

The research was performed as part of the state budget program of grant funding of the Committee of Science of Ministry of Education and Science of the Republic of Kazakhstan, state registration number 0115RK01287 on: "The study of expression of candidate genes of protein and lipid metabolism in milk cattle".

Objectives and methods

Work on allocation of genes was performed in 2015 in a certified laboratory of DNA technologies "Biotechnology of Animals" on the basis of Pavlodar State University named after S.Toraigyrov. The laboratory is certified by the National Center of Expertise and Certification, certificate number 370.

The object of research was the purebred Simmental cows. Studies on identifying the relationship of genotypes with milk productivity were carried out in conditions of LLP "Galitskoe" of Pavlodar oblast. The animals were kept in loose housing in a newly built dairy unit for 1000 animals with the flow-shop system of milk production using a German technology. Feeding was conducted by conventional diets according to the productivity and physiological condition of cows. The average yield on the herd is 5023 kg.

In carrying out production tests the following criteria of zootechnic performance were examined: milk yield, fat content in milk, milk fat yield for 305 days.

For carrying out the DNA diagnosis in animals in the amount of 123 animals blood samples were selected. Blood was obtained from the jugular vein of the animals and put to tubes containing 100 mMof EDTA to a final concentration of 10 mM.

The research was conducted in the following order:

Isolation of DNA from the blood of cattle. 300 l of lysis solution and 100 l of sample were put to labeled tubes. The samples were mixed thoroughly by vortexing and heated for 5 min at 65° C. The tubes were centrifuged at five thousand rpm inamicro centrifuge.

The sorbent was carefully resuspended by vortexing. 25 l of the resuspended sorbent was separately added to each vial. Then it was vortexed, placed in a rack for 2 min, stirred again and allowed to stand for 5 min. The sorbent was pelleted in tubes by centrifugation at five thousand rpm for 30 s, then the supernatant was removed.

500 l of wash solution were added to samples, vortexed until complete sorbent resuspension, centrifuged for 30 s at 10 thousand rpm inamicro centrifuge, then the supernatant was removed.

The procedure of washing was repeated for removing the supernatant.

The tubes were placed in a thermostat at 65° C for 5–10 min of drying the sorbent. With that, tube caps must be open. 50 l of TE buffer were added to the tube for DNA elution. They were mixed and placed in an incubator at 65° C for 5 min and vortexed periodically.

The tubes were centrifuged at 12 thousand rpm for 1 min in a microcentrifuge. The supernatant contained purified DNA. Samples were prepared for PCR amplification.

Evaluation of polymorphism of the gene of somatotropin. While amplifying bGH fragment locus to identify L/V allelic variants the following pairs of oligonucleotide primers were used:

5′-CCG TGT CTA TGA GAA GC-3′
5′-GTT CTT GAG CAG CGC GT-3′

The amplification conditions for this pair of primers at concentration of 2.5 mM magnesium chloride: 94 °C - 30 s - denaturation, 60 °C - 1 min - annealing, 72° - 30 s - synthesis (the total is 30 cycles).

For RFLP identification of genotypes of bGH-gene 20 l amplification product were treated with 10 units of restriction endonuclease AluIв 1 × «Y»-buffer of SibEnzymecompany (Russia) at 37 °C overnight.

The size of the resulting fragments is determined by electrophoresis on a 2% agarose gel.

Restriction fragment of L-allele is 264, 96 and 51 bp, and V-allele is 265 and 14 bp.

Evaluation of leptin gene polymorphism. To amplify fragments of LEP-gene the following primers were used:

LEP-F1:5′- GAC-GAT-GTG-CCA-CGT-GTG-GTT-TCT-TCT-GT -3′
LEP-R1:5′- CGG-TTC-TAC-CTC-GTC-TCC-CAG-TCC-CTC-C -3′
LEP-F2:5′- TGT-CTT-ACG-TGG-AGG-CTG-TGC-CCA-GCT -3′
LEP-R2:5′- AGG-GTT-TTG-GTG-TCA-TCC-TGG-ACC-TTT-CG -3′

The reaction mixture is shown in Table 1.

To visualize the DNA fragments the samples were introduced into wells of 2.5–4.0% of agarose gel containing ethidium bromide (0.5 mg/ml), and horizontal electrophoresis was performed at 15 V/cm for 40 min in 1 × TBE buffer. After electrophoresis, the gel was viewed in UV transilluminator at 310 nm wavelength. Identification of genotypes was determined by quantitative and qualitative characteristics.

Evaluation of gene polymorphism of gene of diacylglycerol O-acyltransferase. To amplify fragments of DGAT locus gene the following primers were used:

DGAT1:5′- GCA-CCA-TCC-TCT-TCC-TCA-AG -3′
DGAT2: 5′- GGA-AGC-GCT-TTC-GGA-TG -3′

After amplification, the resulting fragment of DNA was subjected to digestion with the help of endonucleases AcoI, (SibEnzyme (Russia). Hydrolysis was carried out on a programmable thermal cycler, in accordance with the manufacturer's recommendations, in the following way: 4 incubation cycle at 37 °C, 30 min; 1 inactivation cycle: at 65 °C, 20 s.

Visualization of fragments was carried out with electrophoretic separation of restriction products in a 2% agarose gel in the presence of 5 μm of 10% ethidium bromide, then fixed and documented via GelDoc system.

Electrophore gram of the result of amplification of the genomic DNA of a cow with a pair of primers and DGAT1 DGAT2:

AK fragments correspond to 203, 208, 411 bp, fragments 203, 208bp correspond toK-allele, fragment with the length of 411 bp–A-allele.

The frequency of genotype occurrence was determined by the

Table 1
Protocol and PCR conditions for leptin gene (LEP).

Reactants	Initial concentration	Working concentration	For one sample	For 10 samples
dH_2O			13,8	138
dNTP	2,5 мМ	0,25 мМ	2	20
Buffer	10×	1×	2	20
PolymeraseTaqDNA	5U	1U	0,2	2
LEP-F1	50 μm	0,25 μm	0,1	1
LEP-R1	50 μm	0,25 μm	0,1	1
LEP-F2	50 μm	1 μm	0,4	4
LEP-R2	50 μm	1 μm	0,4	4
DNA Sample			1	
TOTAL			*20*	
Thermal process of amplification				
×1: 94 °C – 5 min				
×40: 94 °C – 10 s, 63 °C – 10 s, 72 °C – 10 s				
×1: 72 °C – 5 min				
Storage at 10 °C				
AS-PCR amplification products				
CC-genotype = 239/164 bp				
TT-genotype = 239/131 bp				
CT-genotype = 239/164/131 bp				

formula:

$$p = n / N$$

where p is the frequency of genotype determination, n is the number of individuals with a specific genotype, N is the number of individuals.

Statistical calculations were performed using the “Pastprogram” computer program.

Results and analysis

The conducted research has discovered a polymorphism of the genes examined (Table 2).

According to the results of research on GH-gene locus it was found that of the 123 cows 19% had VV genotype; 77% of the cows – VL-genotype; 4% of the cows – LL-genotype. The frequency of allele was V – 58% and the allele L – 42%, the frequency of heterozygous VL-genotype was much higher than the frequency of alleles of VV and LL-genotypes.

The research conducted on Lep- gene locus showed the following results: from 123 cows the genotype distribution was as follows: CC – 42%; CT – 48% and TT-10%. In our studies, frequency of alleles C and T was 66 and 34%.

Analysis of cows on DGAT-gene locus showed that 25 %had KK-genotype; 75% - AA-genotype; no animals have been identified with AA-genotype. K-genotype allele frequency was 63%, and A-genotype - 37%.

Table 3 shows the results of analysis of milk productivity of cows of different genotypes on lipid metabolism.

By GH-gene cows with the VV-genotype with a yield of 5490 kg, with a fat content of milk and milk fat yield of 4.21% and 231.2 kg, surpassed the cows with LL and VL-genotype in all investigated productivity indicators. Study of the genotype expression showed that the minimum and maximum milk yield indicator varied from the test animals from 3089 to 8017 kg, fat content ranged from 2.20 to 6.20%. Thus, according to the lactation research for 305 days on GH-gene cows with VV-genotype on investigated indicators showed a higher level of productivity.

Results of the research of milk productivity depending on Leptin gene (Lep) have shown that high milk yield results were received from cows with TT-genotype - 5757,4 kg, the difference between cows' yields with CC and CT-genotypes was 578.8 kg and 134.2in favor of cows with TT-genotype. The best indicator for milk fat of 4.08% and milk fat production of 229.4 kg belonged to cows with CT-genotype. The study of the Lep-gene expression showed that the maximum milk yield was received from cows with CT-genotype (9056 kg) and a minimum in the CC-genotype (7407 kg), for a maximum fat content was observed in cows with CT-genotype (6,1%), and the minimum in CC-genotype (2,2%). The variation of the studied milk yield indicators ranged from 2544 to 9056 kg of milk fat content, in the milk fat content - from 2.2 to 6.1%.

Analysis of milk productivity of cows on DGAT-gene showed the effect of genotypes on cows' milk productivity. The greatest amount of milk with a high fat content was obtained from cows with CC-genotype: 5385.8 kg and 3.85%. Study of DGAT gene expression showed fluctuation level of milk production of 3377 kg–7985 kg of milk. The maximum yield of high fat content was obtained from cows with KK-genotype (7985 kg - 5.9%), the minimum result on the milk yield was received from cows with KK-genotype (3377 kg) and on fat – cows with AK-genotype (2.2%).

Introduction of DNA technologies in animal husbandry allows to monitor and forecast economic traits of animals, which is extremely important to determine the future use of each animal [3].

Table 2
Frequency of alleles and genetic structure of Kazakhstani simmentals by genes of candidates of lipid metabolism.

Candidate-genes	allele frequency, %		Genetic structure, %		
Somatotropin (GH)	V - 58	L - 42	VV - 19	VL - 77	LL – 4
Leptin (Lep)	C - 66	T – 34	CC - 42	CT - 48	TT– 10
Diacylglycerol Acetyl Transferase (DGAT)	K - 63	A - 37	KK - 25	AK - 75	AA - 0

Table 3
Effect of candidate genes of lipid metabolism in milk production of cows for 305 days of lactation.

Studied genes	Genotype	n	Yield for 305 days, kg			Fat, %			Milk fat, kg
			max	min	M ± m	max	min	M ± m	
Somatotropin (GH)	LL	3	5852	3585	4748,3 ± 655,10	4,9	3,1	4,16 ± 0,34	197,5
Leptin (Lep)	VV	15	7729	3745	5490,8 ± 286,76	5,3	3,2	4,21 ± 0,35	231,2
	VL	59	8017	3089	5456,7 ± 194,65	6,2	2,2	3,99 ± 0,17	217,7
Somatotropin (GH)	CC	46	7407	3089	5178,6 ± 212,60	5,3	2,2	3,90 ± 0,12	201,9
Leptin (Lep)	CT	52	9056	2938	5623,2 ± 199,48	6,1	2,4	4,08 ± 0,23	229,4
	TT	11	8598	2544	5757,4 ± 609,69	5,1	2,9	3,91 ± 0,12	225,1
Somatotropin (GH)	KK	22	7336	3377	5385,8 ± 259,33	5,8	2,9	3,85 ± 0,18	207,3
	AK	64	7985	5267	5267,2 ± 186,55	5,9	2,2	3,96 ± 0,18	208,6

The greatest development of DNA-marker technology was gained at complex evaluation of milk productivity.

In studies of E. Collis, M.R. Fortes it was noted that functional candidate genes for assessing milk productivity of cows (level milking, milk fat and protein content) are considered kappa casein genes (CSN3), growth hormone (GH), diacylglycerol *O*-acyltransferase (DGAT1) and thyroglobulin (TG5) [4].

A modern trend in animal breeding is the genotyping of polymorphic variants of growth hormone genes, allowing together with the selection on a phenotype to lead selection identifying the preferred embodiments of the genes of economic traits. Of particular interest is such a gene as somatotropin.

In our studies, high milk yield on GH-gene was obtained from cows with VV-genotype with a yield of 5490 kg, with a fat content of milk and milk fat yield of 4.21% and 231.2 kg. They surpassed the cows with LL and VL-genotype in all indicators of productivity. In studies of A.V.Perchun, I.V.Lazebnaya, etc. in Kostroma breed other results were obtained, they found that the highest rates of milk yield (6456 kg) and the amount of milk fat (208.5) belonged to animals with the LL-genotype [5].

Leptin is a hormone that is responsible for the regulation of fat metabolism.

In our studies on Lep-gene locus from 123 cows the genotype distribution was as follows: CC - 42%; CT - 48% and TT - 10%. The frequency of allele C and T was 66 and 34%, our received data are consistent with studies of S.V. Tyulkin.

According to the research of S.V. Tyulkin 70 Holstein bulls had the following distribution of genotypes of leptin gene: CC - 32,9%, CT -52,8% and TT -14,3% [6].

Forhead A.J., Fowden A. L. investigated LEP-gene polymorphism at 296 cows of the Slovak motley cattle and conducted a distribution of genotypes: CC -70%, CT -27%, TT -3%, and 85 Pinzgau cows – 92% AA, AB – 8% [7].

It was found that there is the largest number of animals of the red cattle with TT-genotype - 56% in Turkey [8].

In the breeding work with dairy cattle breeds more and more attention has been given to such a gene as diacetyl glycerol-*O*-acetyl transferase (DGAT). This marker causes encoding of a key enzyme in the synthesis of milk fat. The fat content in milk, as well as the protein content is an important technological characteristics of the product [9].

Our studies of DGAT-gene discovered the effect of a studied gene on cows' milk yield productivity. The greatest quantity of milk with a high fat content was obtained from cows with CC-genotype: 5385.8 kg and 2.9%.

Thus, on the basis of the conducted research, we concluded that in order to increase milk productivity and butterfat content of milk cows it is necessary to maintain the required number of animals in the herd carrying in their genome the desired alleles of genes.

Conclusion

According to the results of our research, it was found that a more objective assessment of the genetic situation and accumulating in herds desirable genotypes, allowing to enhance milk abundance and improve the quality of milk, farms in Kazakhstan should be encouraged to conduct molecular genetic testing of dairy cattle. Carrying out assessment of genetic potential of milk productivity of cattle on genetic markers will allow to start the introduction of methods of genetic analysis in practical breeding and significantly increase the production of milk and its derivative products.

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