USING DNA MARKERS IN SELECTIVE BREEDING WITH DIFFERENT KINDS OF UKRAINE FARM ANIMALS

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Today intensification of livestock requires further development and improvement of theoretical foundations organizational forms of breeding farm animals by attracting new methods for assessing genotypes. These methods include using of DNA markers. The value of these markers is that they do not change over the life of animals, inherited by the laws of Mendel and relatively easy to detect in the laboratory and can therefore serve as signaling genes in solving some problems of selection (**Andersson et al.**, 2003; **Bruford et al.**, 2003).

Study of genetic polymorphisms and searching of genetic markers in rabbits and cattle is one of the urgent problems of genetics this farm animals. Along with the morphological differentiation, DNA genotyping (certification) is an accurate and efficient method of reliable assessment of the breed that can be used to recognize the unique genotypes and further directional selection (**Baumung et al.**, 2004). Using of DNA technology allows to quickly and efficiently identifying genotype of animals and promotes effective genetic evaluation of population (**Fadiel et al.**, 2005).

Identification of genes and their mutations that determine the direction and extent of quantitative trait (QTL) in Europe and the U.S.A. makes it possible to obtain large profits by reducing generation interval, early introduction of breeding stock in process of reproduction and application selection using molecular genetic markers (MAS). For MAS is carried out by selecting breeding pairs and receive descendants with corresponding genetic potential for major productivity metrics (**Heyen et al.**, 1997; **Alison**, 2007; **Dekkers et al.**, 2004).

MATERIAL AND METHODS

We used blood samples from Ukrainian Black and White Dairy (125 goals), Ukrainian Red and White Dairy (90 goals), Simmental (112 goals) and Holstein cattle breed in total of 380 heads and 202 goals from 24 breeds of bulls from the National Bank of Animal Genetic Resources NAAS. Also we used blood samples from New Zealand White, Californian and Silver breed rabbits (360 goals).

Genomic DNA was isolated from peripheral blood of animals (**Sambrook et al.**, 2001) using standard commercial set «DNA-sorb B» («Amplisens», Russia) according to the manufacturer's recommendations.

We used nucleotide motifs for genotyping rabbits different breeds by ISSR-markers: $(AG)_9C$, $(GA)_9C$, $(ACC)_6G$, $(GAG)_6C$.

Mixture of ISSR-PCR incorporates contained: 2 µl DNA polymerase buffer, 1 µl mixture of triphosphates («Amplisens», Russia), 80 pmol each primer (0,8 µl /reaction), 0.83 U. 0,2 µl) DNA-polymerase («Fermentas», Lithuania). Amount of genomic DNA – 1.5 µl (25 ng). The total volume of the PCR mixture was 10 µl.

Amplification of total DNA with ISSR-primers was performed by following the following conditions: 7 minutes denaturation at 94°C («hot start»), 30 seconds of denaturation at 94°C, 30 seconds - primer annealing at 58 (60)°C, 2 minutes - elongation at 72°C, 7 minutes – synthesis at 72°C; 32 cycles of amplification.

The study of genetic structure at microsatellite markers was performed on the genetic analyzer ABI Prism 3130 Genetic Analyzer (Applied Biosystems). In this work we used following structures of microsatellites: $(GT)_n$, $(CA)_n$, $(AC)_n$, $(CA)_nTA(CA)_6$, $(AC)_n(AT)_n$, $(TG)_n$, $(AC)_n$, $(TG)_4CG(TG)(CA)_n$, $(GT)_nAC(GT)_6$.

Characteristic of candidate genes polymorphisms was determined by PCR-RFLP (**Grodziker T**., 1974). For PCR we used the reaction mixture (10 µl): dH_2O – 4.3 µl, 5-x PCR buffer – 2,0 µl; 10-x dNTP mixture (2мМ) – 0,8 µl; two primers (140 ng) – 0,8 µl; Taq-polymerase (1 M/1000 U) – 0,1 µl; DNA 50-100 ng – 2,0 µl.

Temperature and number of cycles of PCR - amplification for each gene were individually. Amplification fragments of the studied genes was performed using the following primers: for the kappa-casein locus (cattle), κ-Cn: (5`-GAAATCCCTACCATCAATACC-3` and 5`CCATCTAC CTAGTTTAGATG-3`) (**Pinder D**., 1991); beta-lactoglobulin (cattle), βLG: (5`-GTG CTGGACACCGACTACAAAAAG-3` and 5`-GCTCCCGGTATATGACCACCCTCT-3` (**Medrano D**., 1990); growth hormone (cattle), GH: (5`-GCTGCTCCTGAGGGCCCTTC-3` and 5`-GCGGCGGCACTTCATGACCC-3`) (**Sorensen P**., 2002); leptin(cattle),LEP:(5`-GTCACCAGGATCAATGACAT-3`; and 5`-AGCCCAGGAATGAAGTCCAA-3`) (**Pomp D**., 1997); pituitary-specific transcription factor (cattle), PIT-1: (5`-CAAT GAGAAAGTTGGTGC-3` and

5`TCTGCATTCGAGATGCTC-3`) (**Moody J**., 1995); myostatin (cattle), MSTN: (5`-TCTAGGAGAGATTTTGGGCTT-3` and 5`-TGGGTATGAGGATACTTTTGC-3`) (**Grobet L**., 1997), myostatin (rabbits), MSTN: (5`-TAACTGAAAAGAACCCTCTAGTAGC-3` and 5`- TCGGTAGTTGTTTCCCACTTT -3`) (**Fontanessi L**., 2008), progesterone receptor (rabbits), PGR (5`- GAAGCAGGTCATGTCGATTGGAG -3` and 5`- CGC CTCTGGTGCCAAGTCTC -3`) (**Peiro M**., 2008) To analyze structural polymorphism loci κ-Cn, βLG, GH, PIT-1, LEP, PGR, we used restriction, selected for each locus, except MSTN, which typed immediately after PCR - analysis. The mixture for restriction: H_2O – 3.0 µl, buffer (10 mM $MgCl_2$,100 mM KCl, 0,1 mg/ml BSA) – 1.5 µl, endonuclease – 0.5 µl, PCR - product – 10 µl.

Restriction products we separated by electrophoresis in 2% agarose gel followed by staining with a solution of ethidium bromide. Visualization was performed under UV light, followed by digital camera photography elektroforehram. Differentiation amplicon size was performed using molecular weight marker GeneRuler TM 50 bp DNA Ladder, # SM0378, («Fermentas», Lithuania).

Population genetic and biometric analysis of the results was performed using the methods of mathematical statistics (χ^2, Student and Fisher test) and using standard computer programs «GenAlex6», «BIOSIS-1», «Statistica».

RESULTS AND DISCUSSION

As a result of genetic certification of New Zealand White, Californian and Silver breed rabbits and their hybrids with $(AG)_9C$, $(GA)_9C$, $(ACC)_6G$ primers on average received 11 polymorphic fragments which accounted for 68% of the total amplicon. All primers except $(GAG)_6C$ were effective for detecting polymorphisms.

Using analysis of molecular variance (AMOVA) we found genetic differences between species and within three breeds of rabbits (table. 1).

It should be noted that evaluation index of genetic differentiation R_{st} had divergence with the index of polylocus differentiation between populations G_{st} (29.3%) that perhaps a consequence of limited number of loci in a population-genetic analysis.

Also cluster analysis was conducted (UPGMA) representatives of three breeds of rabbits and their hybrids using ISSR-PCR method by Jaccard similarity matrix. It was found that the range of genetic distances between breeds of rabbits varied within from 0.0229 to 0.4216. It should be noted that rabbits of Silver and Californian breed have formed a mate branch while to New Zealand White breed – proved to be far removed from the values of genetic distance (0.0229).

In New Zealand White rabbits breed by myostatin locus, frequency of the homozygous genotype for the C allele had the lowest. The highest incidence was observed in homozygous animals by T allele (1.4 greater than the average). In turn, the number of heterozygote's was 10.8% higher than the average.

Quantitative distribution of genotypes rabbits by PGR gene was characterized by AG heterozygote advantage. Number of homozygous animals (A allele) was less than 27.5% heterozygote's, and homozygous (G allele) - by 22.5%. In animals frequency of G allele was higher than allele A at 33.4%. Found that the range of fluctuations in the effective number of alleles for myostatin and progesterone receptor gene was negligible (6%). The value of Shannon information index for both genes differed from each other by 29%. Wright's fixation index F_{IS}, for myostatin locus was negative (-0.11) and locus of progesterone-receptor was positive (0.24).

It was found that proportion of MSTN gene influence the level of average daily increments was $\eta^2 = 0.45$ ($P < 0.05$) on carcass weight $\eta^2 = 0.35$ ($P < 0.05$). Share impact factor of genotype MSTN of the level feed consumption per 1 kg increase (60-120 days) proved to be unreliable ($\eta^2 = 0.24$, $P > 0.05$). In terms of average daily increments of New Zealand White rabbits breed with genotype TT ahead the animals with genotype CC by 15%, and animals with genotype CT - by 18% (4.9 g i 6.2 g respectively).

We obtained results for establishment genetic structure features for loci: κ-Cn, βLG, GH, LEP, PIT-1 and MSTN four breeds of cattle allowed a comparative analysis of their gene pools for the studied genes. For kappa-casein locus genotype frequency AA amounted to 0.664; 0.778 and 0.793 for animals Ukrainian Black And White Dairy, Ukrainian Red And White Dairy and Holstein breeds and homozygous animals for the A allele animals occurred at a frequency of 0.467 in Simmental breed.

In Holstein and Ukrainian Black And White Dairy, Ukrainian Red and White Dairy breeds of animals homozy-

Table 1**. The rezults of analysis molecular variation (AMOVA) among different breeds of rabbits which genotyped by ISSR-markers**

Differentiation	*SS*	*MS*	*EMS*	R_{st}	*P*
Between populations	166.167	83.37	97.31	0.311	0.01
Within populations	683.134	341.56	300.01	-	-
Total	849.301	424.93	397.32	-	-

Note: *SS* - sum of squares, *MS* – mean squares; *EMS* – hypothetical mean squares; *Rst* – genetic differentiation

gous genotype BB were detected, in Ukrainian Black And White Dairy breed homozygous animals occurred with a frequency of 0.024, which is 4.5 times lower than in animals combined direct productivity Simmental breed which the figure was 0.108. According to the distribution of alleles were most similar Holstein and Ukrainian Black And White Dairy, Ukrainian Red and White Dairy breeds which the frequency of allele A was 0.820; 0.888; 0.896 respectively. Significantly lower frequency of this allele was observed in animals of Simmental breed – 0,679.

For κ-Cn locus animal dairy productivity is set regularity to reduce the frequency of allele B with increased milk as the frequency of allelic variant in animals Ukrainian Black And White Dairy breed was 0.180, Ukrainian Red and White Dairy breed– 0.112 and in the Holstein breed – 0.104, against 0,320 in Simmental breed ($P < 0.01$).

For βLG locus in the studied species revealed an overwhelming number of animals with genotype AB. Chance of deviation of the actual distribution of genotypes of the theoretically expected for this locus in animals Ukrainian Black And White Dairy, Ukrainian Red and White Dairy breeds was statistically significant ($P < 0.01$). The frequency of this genotype in animals Ukrainian Black And White Dairy, Ukrainian Red and White Dairy breeds almost identical: in accordance 0.576 and 0.578 and close to the frequency of this genotype in animals of Holstein breed 0.566, in Simmental breed the number of heterozygous animals somewhat less – 0.489. The frequency of homozygous animals with the same genotype BB in Ukrainian Black And White Dairy, Ukrainian Red and White Dairy breeds was 0.344 in animals of Holstein breed – 0.302 and Simmental breed – 0.359.

In populations of Ukrainian Red And White Dairy and Holstein breeds homozygous animals for the allelic variant V of growth hormone locus not found. Proportion of homozygous animals for V allelic variant of growth hormone locus in population of Ukrainian Black And White Dairy breed was not significant – 0.056, and in the Simmental breed individuals of the desired genotype were 0.207. The frequency of heterozygous genotype LV was as follows: Ukrainian Black And White Dairy breed – 0.328, Ukrainian red And White Dairy breed – 0.167, Holstein breed – 0.207 and Simmental breed – 0.391. For animals of dairy performance was characterized by a high frequency of homozygote's LL compared with animals of Simmental breed which the frequency of this genotype was 0.402 and in animals of Ukrainian Black And White Dairy breed, Ukrainian Red And White Dairy breed and Holstein breed – 0.616, 0.833, 0.793 respectively. The frequency of L allele was lowest in animals of Simmental breed – 0.598.

Simmental breed by PIT-1 locus against other breeds characterized by a high frequency of homozygote's BB which was 0.719, homozygous animals by the A allele were not detected at all, frequency of heterozygote's AB was at 0.281. Fro the animals of Ukrainian Black And White Dairy breed, Ukrainian Red And White Dairy and Holstein breed distribution as the frequency of genotypes and allelic variants was similar.

The frequency of genotype AA in the animals of Ukrainian Black And White Dairy breed, Ukrainian Red And White Dairy breed and Holstein breed was 0.192, 0.155 and 0.208 respectively. Homozygote's for the B allele was distributed as follows: Ukrainian Black And White Dairy breed – 0.320 Ukrainian Red And White Dairy breed – 0.278, Holstein breed – 0.375. Simmental breed by PIT-1 locus unlike other breeds was a high frequency of homozygote's BB which was 0.719. Homozygous animals by A allele are not detected. The frequency of allele A in the animals of Ukrainian Black And White Dairy breed, Ukrainian Red And White Dairy breed and Holstein breed was 0.436, 0.438, 0.416 respectively which is associated with both the direction of productivity and the history of the creation breeds.

In the animals Ukrainian Black And White Dairy breed by leptin locus revealed such distribution of genotypes: AA – 0.616, AB – 0.344, BB – 0.04, and in the Simmental breed: AA – 0.598, AB – 0.402. Homozygous animals with the BB genotype were found. The frequency of allele A in Ukrainian Black And White Dairy breed was 0.788 and in the Simmental breed – 0.799.

Overall, population-genetic parameters for investigated QTL loci found insignificant level of heterogeneity of the compared populations. Thus, the rate of homozygosity (Ca) ranged from 0.508 in Ukrainian Black And White Dairy breed at locus PIT-1 to 0.848 in Ukrainian Red and White Dairy breed by the locus GH (table 2). The effective number of alleles (Ne), which reflects the level of polymorphism each biallelic locus, fluctuated within a small: from 1.179 in Ukrainian Red And White Dairy breed to 1.969 in the population of cows Ukrainian Black And White Dairy breed. Quite a considerable range of variation was observed for animals of Ukrainian Red And White Dairy breed, because the level of average heterozygosity (H_o) ranged from 0.167 by growth hormone gene to 0.578 for locus βLG, which may indicate the presence of genetic and automatic processes, associated with «flow» blood for the animals of Holstein breed. In general, all studied species was characterized by the predominance of the expected heterozygosity of the average heterozygosity.

Accumulation in populations of heterozygous genotypes may be due to use of sires with a corresponding set of genes. No exception mechanism of selection in the breeding of heterozygous individuals, because they usually are more adapted to the technological conditions and enables increased productivity indices. Note separately the value of fixed rate (F) within different species. Thus, the rate -0,003 in Ukrainian Black And White Dairy breed may indicate selective neutrality of this locus, scilicet distribution of genotypes at this locus does not deviate from the theoretically expected under Hardy-Weinberg (χ^2 – 0.405). On the con-

trary, for the animals of Simmental breed, value of the fixed rate for the growth hormone gene was (+0.395), which may be due to homogeneous intra-population selection, which increases the number of homozygous genotypes. By GH locus, this leads to an increase in the concentration of genotypes VV, associated with increased of fatty milk production, that is characteristic only for the animals of the Simmental breed.

Installation differences between animals based on expectations and certain genotype for locus κ-Cn made it possible to identify different associations such as « genotype - a sign» depending on species. Thus, for cows of Ukrainian Black And White Dairy breed animals with genotype AB exceeded AA upon 18.9% ($P < 0.1$), AB > BB upon 24.9% ($P < 0.05$); for cows of Ukrainian Red And White Dairy breed AA > BB – upon 8%; in the animals of Simmental breed BB > AA upon 4.09%, BB > AB upon 14.7 % ($P < 0.1$).

As a result of comparative analysis cows milk production different breeds of cattle, depending on the genotype at locus βLG following results were obtained: for cows Ukrainian Black And White Dairy breed for this indicator, as the protein content in milk, animals with genotype AB > BB upon 0.13%, AB > AA upon 1.18 % ($P < 0.01$); in cows of Ukrainian Black And White Dairy breed yield, animals with genotype AA > AB upon 8.5% ($P < 0.05$), AA > BB upon 9.5% ($P < 0.1$), for this indicator, as the fat content in the milk, animals with genotype AB > AA upon 0.16% and AB > BB upon 0.08%; in the Simmental breed for fat in milk animals with genotype BB > AB upon 0.04%.

For locus of growth hormone (yield of milk) animals of Ukrainian Black And White dairy breed with genotype LV > LL upon 10.9% ($P < 0.1$), LV > VV upon 7.3%, by content of fat in milk LL > LV upon 0.038%, VV > LL upon 0.037%, VV > LV upon 0.07%; in animals Ukrainian Red And White dairy breed animals with genotype LL > LV upon 0.001%, by content of fat in milk animals with genotype LV > LL upon 0.099%; by milk yield the animals of Simmental breed with genotype LL > LV upon 2.7% and LL > VV upon 10.4%, by fat content in the milk animals with genotype LV > LL upon 0.23% and LV > VV upon 0.24%. Increased concentrations of L-allele among populations indicating its selective advantage over V- variant because of its greater lactogenic actions.

By PIT-1 locus on the following parameters, as milk yield, cows Ukrainian Black And White Dairy breed with genotype AA > BB upon 8.9%, AA > AB upon 6.1%; in Ukrainian Red And White Dairy milk yield was higher in animals with genotype AB > AA upon 4.8% and AB > BB upon 4.3%, protein content in milk in animals with genotype AB > AA upon 0.021%, and fat content in milk AB > AA upon 0.064% and AB > BB upon 0.002%.

For leptin gene animals of Ukrainian Black And White Dairy breed (fat content in milk) had a highly significant difference BB > AB upon 0.06% ($P < 0.01$) and BB > AA upon 0.5% ($P < 0.01$), AB > AA upon 0.4% ($P < 0.01$); for the genotype of animals Simmental breed yield of milk was higher AB > AA upon 0.31%, and for protein content in milk from animals with genotype AB > BB на 0.05%. Noteworthy diverse nature of correlations between genotype for gene LEP and belonging of pedigree animals. Thus,

Table 2. **Genetic variability of populations Ukrainian Black And White Dairy, Ukrainian Red And White Dairy, Holstein and Simmental breeds by κ-Cn, βLG, GH, PIT-1 loci**

Breed	Locus	*n*	Ca	Ne	Ho	He	F
Ukrainian Black And White Dairy	κ-Cn	125	0.705	1.418	0.296	0.295	-0.003
	βLG		0.534	1.873	0.408	0.466	+0.124
	GH		0.656	1.524	0.216	0.344	+0.372
	PIT-1		0.508	1.969	0.505	0.492	-0.026
Ukrainian Red And White Dairy	κ-Cn	90	0.802	1.247	0.223	0.198	-0.126
	βLG		0.535	1.869	0.578	0.465	-0.243
	GH		0.848	1.179	0.167	0.152	-0.099
	PIT-1		0.511	1.957	0.567	0.489	-0.160
Holstein	κ-Cn	53	0.814	1.229	0.207	0.186	-0.113
	βLG		0.515	1.942	0.566	0.485	-0.167
	GH		0.814	1.229	0.207	0.186	-0.113
	PIT-1		0.514	1.852	0.417	0.486	+0.142
Simmental	κ-Cn	92	0.563	1.776	0.425	0.437	+0.027
	βLG		0.522	1.916	0.489	0.478	-0.023
	GH		0.519	1.927	0.291	0.481	+0.395
	PIT-1		0.758	1.319	0.281	0.242	-0.307

the high fat content in milk from cows of Ukrainian Black And White Dairy breed determined by the presence of individual's BB and AB genotypes.

We have tested and modified the method to determine the genotype of the animals for 10 microsatellite loci.

Overall, by microsatellite locus TGLA126 in the animals of studied cattle breeds were found 9 allelic variants, the size of which ranged from 114 to 130 bp. The largest number of alleles (9) we observed in animals of Holstein breed and least (2) had species of animals, as Gascony, Shvitska, Pintshau and Limousine. Allele size in 114 and 130 kb are typical only for the animals of Holstein breed.

For microsatellite locus TGLA122 we obtained 22 allelic variants ranging from 142 to 190 bp. The largest number of alleles of different lengths observed in animals of Holstein breed (18), and the smallest (1) – in the animals Shvitska breed and Maine-Anjou breed. In the animals of Brown Carpathian breed detected allele size 164 and 172 bp, which in other species have not met. Only in the animals of Ukrainian Gray breed we found allele in 186bp, in the animals of Volyn and Southern Beef breeds – allelic variant in size 184 bp.

For locus INRA023 we found 14 allelic variants ranging from 208 to 234 bp. Allelic variant in size 234 bp we observed only in animals of Pintshau breed.

Among the studied species at locus ETH3 size alleles ranged from 108 to 134 bp. In the animals of Holstein breed we observed allelic variants of size 134 bp, and in animals of Ukrainian Red Dairy breed – 108 bp, which is characteristic only for this species. Allelic variant in 116 bp was present in bulls of Limousine and Gray Ukrainian breed and variant size 112 bp – in animals of Kian and Limousine breed. Overall, this locus revealed 13 different size allelic variants.

For locus ETH225 we found 13 allelic variants of size 140 – 164 bp. Sires of Angler and Ukrainian Red Dairy breed are characterized by allele in 158 bp, allelic variants of size 160 bp – in animals of Lebedynska breed, 162 bp – Holstein and 164 bp – animals of Brown Carpathian breed.

Allelic variant in size 198 bp by locus BM1824 observed only in animals of Angler and Pintshau breed. Generally we found 14 allelic variants in the range 176 – 202 bp.

Microsatellite locus TGLA227 submitted 14 different length of allelic variants ranging from 80 до 104 bp. Allelic variant in size 72 bp we found in the sires of Ukrainian Meat bree, and the size of 78 bp – in the animals of Simmental and Ukrainian Meat breed.

Locus BM2113 had 11 different allelic variants of size 114 – 142 bp. Allele in size 114 bp characterized the animals of Holstein breed, allelic variant in size 140 п.н. – for sires such breeds as meat Volyn, Ukrainian Meat and Kian.

Ten allelic variants ranging from 212 to 232 п.н. we revealed by microsatellite locus ETH10. Allele in size 232 bp we observed in animals Ukrainian Red and white dairy breed, 212 bp – in the bulls of breeds such as Ukrainian White Head and Pintshau.

For microsatellite locus SPS115 we found 11 alleles in a range of lengths 244 – 264 bp. Variant in 264 bp characteristic the animals of Ukrainian Red and white dairy breed.

CONCLUSION

By results of genotyping rabbits for ISSR-markers, information on the distribution of species on the basis of genetic affinity can be used in the selection of pairs for hybridization with the aim of obtaining the maximum range of variation in offspring which will increase the effectiveness of the selection process.

The results of genetic and population studies of three breeds of dairy cattle and one combined directly demonstrate promising performance evaluation of heterogeneous populations for the studied loci QTL in some herds in determining degree of selective pressure.

Frequency distribution of alleles and genotypes of rabbits and cattle, their formation is determined by the characteristics of selective breeding, which conducted with each species separately under the direction performance of its membership and is not associated with using of related crosses for breeding animals and their association with one or more lines. This information is in addition to the classical methods of selection and breeding enables creation of animal populations through targeted genetic selection and recruitment breeding pairs of corresponding genetic potential for particularly milk production.

On the basis of research analysis the characteristics of genetic structure at microsatellite loci, derived genotypes bulls of different cattle breeds. The results give reason to recommend the use of an appropriate panel STR-markers д to determine the genotypes and the control accuracy origin cattle according to international of ISAG recommendations.

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We conducted genetic analysis of Ukraine farm animals by different types of molecular genetic markers. Present data of frequency occurrence allelic variants of the kappa-casein, beta-lactoglobulin, growth hormone, pituitary-specific transcription factor, leptin, progesterone receptor and myostatin gene among different breeds of dairy catlle, meat cattle, rabbits and their impact on the performance of dairy productivity, meat productivity and reproductive ability. Shown shows the informativeness of using microsatellite DNA sequences and genome region between microsatellite loci to establish genetic differentiation, assessment of genetic diversity different cattle and rabbits breeds.

Key words: *cattle, rabbits, candidate genes, ISSR-markers, STR-markers, milk, meat productivity, performance ability*

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