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Evaluation of the genetic structure and main productive traits of Lebedin cattle based on genetic markers polymorphism

Ocena struktury genetycznej i głównych cech produkcyjności bydła
rasy lebedyńskiej na podstawie polimorfizmu markerów genetycznych

Summary. The article highlights the results of the Lebedin cattle genetic structure evaluation by 12 microsatellite *loci*, kappa-casein and growth hormone genes polymorphism studies. Seventy allelic variants of microsatellite *loci* were identified and the largest number of alleles was observed for TGLA53 *locus* (10 alleles). All microsatellite DNA markers, except BM1818, showed a high degree of polymorphism. QTL gene allelic variants association with the cattle productive traits was investigated. A sufficiently high B-allele frequency (0.323) of kappa-casein gene was detected and a large number of animals (0.645) have the heterozygous AB genotype variant. The L-allele variant predominance of the growth hormone gene was fixed (0.903). The complex genotypes affecting the value of such traits as milk yield, milk fat and protein content were identified.

Keywords: microsatellite DNA *loci*, kappa-casein gene, growth hormone gene, polymorphism, productive traits, Lebedin cattle

INTRODUCTION

A Lebedin cattle is a dairy-beef productivity breed. The breed was created and approved in 1950 by the local Ukrainian gray cattle crossbreeding with Swiss bulls and the follow breeding of hybrids “in itself”. The milk yield at the Lebedin cattle breeding farms in the various years were 3500–5500 kg, milk fat 3.8–4.0%, (occasionally 4.5% and more), the adult cows body weight is 530–560 kg, bulls-sires 850–1000 kg and more.

The spread of specialized cattle breeds has led to the reduction of the some local breeds, including the Lebedin breed. Therefore, the Lebedin breeding area in Ukraine – is only one breeding farm “Mykhailivka” of the Sumy region (379 head of cows) and three reproduction farms: PAF “Bulat” of the Belopolsky region 100 cows, PAF “Komyshanska” 305 heads and “Garden” of the Akhtyrsky region 100 heads.

Lebedin breed has an unique economically valuable traits, such as hardiness, adaptability to extreme growing conditions, resistance to diseases, rapid response to better feeding, productivity and longevity. Considering the good commercial traits of the Lebedin cattle, there was a need of its preservation and breeds gene pool conservation events implementation. The average productivity of Lebedin breeding stock on farms (3592 kg of milk) does not fully meet the genetic potential of the breed (5000–5500 kg of milk), that the gene pool formation work, cows milking, purposeful growing of young animals on farms were provide.

Genomic selection with the single nucleotide polymorphisms (SNPs) study is a new widely used powerful tool in agricultural science. The new molecular-genetics analyzing methods using at the genes (*loci*) level in a breeding work and associated economically useful traits (QTL) evaluation have several advantages over traditional breeding methods. Especially for the combined dairy-beef productivity breeds the some genes of milk proteins (eg. caseins) and growth hormone genes polymorphism identified will assist to objectively assess the true genetic potential of animals, increasing the efficiency of selection and breeding work in general [Ardak 2005, Curi *et al.* 2005, Ernst and Zinovieva 2008]. The using of the microsatellite DNA markers for animal~~s~~ individual identification and parentage control has been intensively investigated, but only a few studies have explored their practical applications for meat production at the breed level [Negrini *et al.* 2008].

The aim of this work is the molecular-genetic characteristics of the gene pool of “Mykhailivka” Lebedin herd based on microsatellite and kappa-casein (*CSN3*) and growth hormone (*GH*) genes and study of relationships between genotypes and the main productive traits of studied animals identify.

MATERIAL AND METHODS

DNA was isolated from the blood of unrelated animals with proteinase K (Kawasaki’s method with modifications). Polymorphism of microsatellite DNA *loci* were estimated based on 12 markers recommended by ISAG – *BM1818*, *BM1824*, *BM2113*, *INRA023*, *SPS115*, *TGLA53*, *TGLA122*, *TGLA126*, *TGLA227*, *ETH3*, *ETH10*, *ETH225*. Microsatellite *loci* were amplified by polymerase chain reaction (PCR) using standard reagents “Bovine PCR Typing Kit II”, Applied Biosystems. Analysis of amplification was performed by capillary electrophoresis method on ABI PRISM 3110 DNA analyzer (Applied Biosystems).

The growth hormone (*GH*) and kapa-casein (*CSN3*) gene polymorphism were identified by PCR-RFLP method. *GH* gene polymorphism was detected using the primers [Gordon *et al.* 1983, Zhou *et al.* 2005, Bochkov *et al.* 2009] – F: 5'-GCTGCTCCTGAGGGCCCTTC-3' and R: 5'-GCGGCGGCACTTCATGACCC-3'. PCR temperature conditions: DNA denaturation at 95°C – 1 min, annealing of primers at 62°C – 1 min, and DNA synthesis at 72°C – 1 min, then 40 cycles by the following scheme: 94°C – 30 sec, 60°C – 60 s, 72°C – 120 s (in the last cycle – 10 min). For *locus*-specific amplification of the *CSN3* gene the primers [Contreras *et al.* 2011] – F: 5'-GAAATCCCTACCATCAATACC-3' and R: 5'-CCATCTACCTAGTTTAGATG-3' – were used at the temperature: 94°C – 4 min, 94°C – 30 s, 58°C – 30 s, 72°C – 30 s – 35 cycles, 72°C – 5 min. The PCR products were processed with specific endonuclease restriction (Fermentas, Lithuania): HindIII for kappa-casein gene and AluI for growth hormone gene, according to the scheme: H_2O – 3.5 µl, 10 × buffer for enzyme – 1.0 µl, the restriction enzyme – 0 5 µl and 5.0 µl of the amplification [Lara *et al.* 2002, Biase *et al.* 2005, Curi *et al.* 2005]. Visualization of the restriction results were performed by electrophoresis separation of DNA fragments in a 2% agarose gel in 1 × TBE buffer by staining with ethidium bromide.

The results were statistically analysed using MS Excel program and Tfpga 2000 version 1.3 (Tools for Population Genetic Analyses).

RESULTS AND DISCUSSION

As a result of the studied Lebedin cattle testing by 12 microsatellite DNA markers the 70 alleles were identified. The largest number of alleles was observed for *TGLA53 loci* (10 alleles), also a large number of alleles per *loci* were identified for *TGLA227* and *TGLA122*. Even distribution of alleles for other *loci* was shown, and by the lowest number of which the *INRA023 locus* (only three alleles) was characterized. The frequency of alleles at specific *loci* was calculated, the values of the analyzed parameters are summarized in table 1.

Analysis of the data showed, that the most polymorphic *locus* was *TGLA53*, with the highest frequency of allele 162 bp (0.414) mostly in heterozygous state (only three animals were characterized by homozygous genotypes by this allele), while our previous researches of microsatellite *loci* polymorphism of Gray Ukrainian cattle [Shkavro *et al.* 2010], as a parental in Lebedin breed creation, were showed, that the 162 bp allele frequency was much lower (0.040) and with highest frequency for its was 168 bp allele (0.380). The lowest frequency of *TGLA53 locus* for studied Lebedin cattle were characterized alleles 154 and 166 bp (per 0.017). For *TGLA227* locus 9 allelic variants were identified from 77 to 97 bp, among which with the highest frequency was represented alleles 83 bp (0.345) and 81 bp (0.224). Also, for the studied early Gray Ukrainian cattle the *TGLA227 locus* was also very polymorphic (8 allelic variants) with highest frequency of allele 93 bp (0.222).

Table 1. The microsatellite DNA *loci* alleles frequency, polymorphic information content (PIC), degree of heterozygosity (Ĥ), number of effective alleles (E) in Lebedin cattle
Tabela 1. Frekwencja alleli w *loci* mikrosatelitarnego DNA, współczynnik polimorfizmu (PIC), współczynnik hetrozygotyczności (Ĥ) i efektywna liczba alleli (E) u bydła rasy Lebedin

Locus *Locus*	No. of alleles Liczba alleli	Alleles Allele	Alleles frequency Frekwencja alleli	Range of allele sizes (bp) Zakres wielkości alleli (pz)	PIC	Ĥ	E
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
TGLA 227	9	77 79 81 83 87 89 93 95 97	0.117 0.034 0.224 0.345 0.138 0.052 0.103 0.017 0.069	74–104	0.766	0.792	4.81
BM 2113	7	125 127 133 135 137 139 141	0.155 0.155 0.241 0.138 0.086 0.121 0.103	125–143	0.822	0.842	6.32
TGLA 53	10	154 160 162 166 168 170 172 174 176 184	0.017 0.052 0.414 0.017 0.172 0.103 0.086 0.034 0.052 0.052	144–190	0.749	0.771	4.37
ETH 10	4	217 219 221 223	0.293 0.310 0.190 0.207	210–226	0.691	0.739	3.83
SPS 115	6	248 250 252 254 256 258	0.293 0.052 0.259 0.138 0.121 0.138	240–262	0.761	0.792	4.81

continue table 1
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1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
TGLA 126	4	115	0.552	109–127	0.536	0.599	2.49
		117	0.259				
		121	0.017				
		123	0.172				
TGLA 122	8	141	0.034	130–164	0.588	0.651	2.87
		143	0.431				
		147	0.017				
		149	0.017				
		151	0.397				
		153	0.017				
		155	0.034				
		161	0.052				
INRA 23	3	206	0.552	197–223	0.525	0.593	2.46
		208	0.190				
		214	0.259				
ETH 3	4	117	0.621	117–129	0.524	0.564	2.29
		119	0.155				
		125	0.138				
		127	0.086				
ETH 225	6	140	0.207	140–156	0.787	0.813	5.34
		144	0.138				
		146	0.103				
		148	0.155				
		150	0.276				
		156	0.121				
BM 1824	4	178	0.362	178–196	0.673	0.724	3.62
		180	0.276				
		182	0.138				
		188	0.224				
BM 1818	5	258	0.017	258–268	0.460	0.492	1.97
		260	0.103				
		262	0.138				
		264	0.052				
		266	0.690				

The significant number of alleles (8 allelic variants) was identified for *TGLA* 122 *locus*. For other *loci* was showed a sufficiently even distribution of allele variants from 4 to 7 with the highest frequency over other of 266 bp (0.690) for the *BM1818 locus*, 117 bp (0.621) for the *ETH3 locus*, 115 bp (0.552) for the *TGLA126 locus* and 248 bp (0.293) of the *SPS115 locus*, with lowest allele number only for *INRA23 locus* (3 alleles) with the greatest frequency of 206 bp allele (0.552).

The *BM2113 locus* was represented by the 7 alleles with highest level of 133 bp allele (0.241), that also characteristically for Gray Ukrainian cattle 133 bp allele with max frequency (0.630). A similar pattern for locus *ETH225* i.e. max frequencies of 140 bp and 150 bp alleles for both breeds (0.207, 0.276 and 0.333, 0.278, respectively) was observed. So, based on the obtained data of alleles frequency at specific microsatellite DNA *loci*, it will be possible, with high probability, to verify the origin (paternity) of individuals belonging to specific species, and study and supply the information about breed saturation by specific DNA markers.

On the base of identified alleles number for each locus and determined allele frequencies the basic population-genetic parameters, such as the heterozygosity degree (Ĥ), the number of effective alleles per locus (E) and the polymorphic information content (PIC) were estimated (tab. 1). The parameters-calculated in this study showed that for the studied cows population the selected microsatellite *loci* have a high polymorphism degree. Thus, the heterozygosity degree (Ĥ) for each marker, besides *BM1818 locus*, exceeded 56% (an average (Ĥ) = 0,698, or ~70%), and, in some cases, even 85% (by *BM2113 locus*). The level of polymorphism is an important integral index, which characterized the number of effective alleles in the population (E). The *BM2113 locus* was characterized to have the highest level of estimated parameters (H = 0.842, E = 6.32). For the *TGLA53 locus* with the highest detected number of alleles per locus (n = 10), the number of effective alleles among them was much lower (E = 4.37). The significant value of studied parameters characterized *ETH225 locus* (0.813 and 5.34). Similar pattern were observed for loci *SPS115* and *TGLA227*. For other *loci* the number of effective alleles ranged from 1.97 (*BM1818* locus) to 3.83 (*ETH10* locus). The PIC and (Ĥ) parameters showed that the investigated microsatellite DNA sequences were characterized by high levels of polymorphism. The PIC, calculated for each locus were at over 0.500 to 0.822, excluding the *BM1818 locus* (0.460).

The results of DNA testing by the kappa-casein and growth hormone genes showed the presence of too alleles per gene A and B and L and V, respectively (tab. 2). The kappa-casein gene allele distribution showed the highest frequency for the studied animal A-allele (q = 0.677 ±0.073) associated with increased total yields, but B allelic variant was associated with increased content of casein proteins in milk, and therefore with better coagulation properties of milk, which is a desired indicator of cheese production presented with a frequency only 0.323 ±0.073. Among the studied cattle the vast majority (64.5%)

Table 2. Lebedin cattle genetic structure by *CSN3* and *GH* genes polymorphism evaluation
Tabela 2. Ocena struktury genetycznej bydła rasy lebedyńskiej na podstawie polimorfizmu genów *CSN3* i *GH*

QTL	Alleles frequency Frekwencja alleli		Genotype frequency Frekwencja genotypów		
CSN3	A	B	AA	AB	BB
	0.677 ±0.073	0.323 ±0.073	0.355	0.645	–
GH	L	V	LL	LV	VV
	0.903 ±0.053	0.097 ±0.053	0.806	0.194	–

animals have heterozygous variant of genotype AB, and others have an AA genotype's variant, animal with desired for good quality cheese production homozygous genotype BB were not found.

For the growth hormone gene polymorphism study a significant advantage of the L allele frequency (0.903 ±0.053) was established. Most studies animals (80%) were homozygous with LL genotype variant, the frequency of heterozygous LV genotype variant's was 0.194. Animals with homozygous VV genotype in the researched herds were not detected. V allelic variants are associated with increased milk yield and, according to various researchers data, and the animals with VV genotype's variant have less rate of growth compared with LL and LV genotype variants.

Results of cows milk production study depending on the genotype by kappa-casein and growth hormone genes are shown in table 3. The analysis of the distribution of cow productive parameters by genotype variants for studied genes have not found the significant deviations from the average, and, consequently, was not informative for the studied sample. While, the best results by the milk yield and milk fat content, that does not much, but higher than the average index, characterized animals with homozygous LL-genotype's variant in the growth hormone gene (4630.8 kg/lactation). The milk fat and protein content almost at average level were observed for animals with homozygous AA, as well as with heterozygous AB genotype variants in the kappa-casein gene; the studied animal with heterozygous LV genotype in the growth hormone gene were inferior for all parameters others and the average of the sample indicator. The total protein content for animals with heterozygous AB genotype variants in the kappa-casein gene was lower than the average of the sample, although, for today, in genomic selection concerning this gene, the B allele is considered as a desirable and BB genotype is characterized to be associated with much higher protein content in milk, increasing to 5–10% of cheese yield, compared to other genotypes.

Table 3. Studied Lebedin cows productivity depending on *GH* and *CSN3* genes genotype
Tabela 3. Badania produkcyjności krów rasy Lebedin w zależności od genotypu genów *GH* i *CSN3*

Genotype Genotyp		Milk yield (kg/lactation) Wydajność mleka (kg/laktacja)	Fat (%) Tłuszcz (%)	Protein (%) Białko (%)
By the kappa-casein gene (*CSN3*) W zależności od genu kappa-kazeiny (*CSN3*)	AA	4462.2	3.9	3.3
	AB	4583.9	4.0	3.2
By the growth hormone gene (*GH*) W zależności od genu hormonu wzrostu (*GH*)	LL	4630.8	4.0	3.3
	LV	4165.1	4.0	3.2
On average by studied animals Średnio u badanych zwierząt		4540.7	4.0	3.3

We consider that our results are caused by the fact that the total protein content determination without the casein fraction consideration leads to inadequate data interpretation, and exactly quantitative composition of casein fractions affects to the aforementioned desirable features manifestation.

Similar results about the superiority of animals with AA genotype over the animals with AB and BB genotypes referring to the milk protein content are presented in many latest papers [Dogru and Ozdemir 2009, Bovenhuis *et al.* 1992, Zinovieva *et al.* 2004, Fontanesi *et al.* 2007, Lvina 2011]. Table 4 shows the data about milk production of studied Lebedinsky cows, based on the complex genotype in kappa-casein and growth hormone genes.

Table 4. Cows productivity by the *CSN3* and *GH* genes complex genotype
Tabela 4. Analiza produkcyjności krów w zależności od układu genotypów genów *CSN3* i *GH*

Genotype Genotyp	Frequency Frekwencja	Milk yield, (kg/lactation) Wydajność mleka (kg/laktacja)	Fat (%) Tłuszcz (%)	Protein (%) Białko (%)
AALL	0.290	4633.4	3.9	3.3
AALV	0.065	3691.5	4.0	–
ABLL	0.516	4629.4	4.0	3.2
ABLV	0.129	4401.9	3.9	–
On average by studied animals Średnio u badanych zwierząt		4540.7	4.0	3.3

Thus, the highest level of milk yield (4633.4 kg/lactation) and fat content in milk (3.9%) characterized the animals with AALL complex genotype, almost similar rates, slightly higher than the average by sample, and the animals with ABLL complex genotype (4629.4 kg/lactation and 4.0, respectively), while the largest number of Lebedinsky cows among studied animals have this variant of genotype (51.6%). The lowest level of milk yield characterized cows with AALV-genotype's variant (3691.5 kg/lactation).

CONCLUSIONS

1. The investigated microsatellite DNA *loci* were characterized by high polymorphism level – the heterozygosity degree ($\hat{H}$) on average was 0.698 for all studied *loci*.

2. The *TGLA53* locus was identified as the *locus* with max number of alleles per *loci* – 10, but *BM2113 locus* was identified as the most polymorphic microsatellite *locus* for studied Lebedin cattle ($\hat{H}$ = 0.842, E = 6.32, PIC = 0.822). *BM1818 locus* was characterized by the lowest heterozygosity degree ($\hat{H}$ = 0.492).

3. The sufficiently high A-allele frequency (0.677) of kappa-casein gene was detected and a large number of animals (64.5%) have the heterozygous AB genotype's variant. Significant frequency of L-allele (0.903) of growth hormone gene was identified.

4. Animals with ABLL-complex genotype were prevailed (51.6%), the highest level of milk yield and fat content in milk characterized the animals with AALL- and ABLL-complex genotypes (4633.4 kg/lactation and 3.9%; 4629.4 kg/lactation and 4.0% respectively).

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Streszczenie. Wyniki oceny struktury genetycznej bydła rasy lebedyńskiej uzyskano na podstawie polimorfizmu 12 mikrosatelitarnych *loci*, genów kappa-kazeiny i hormonu wzrostu. Zidentyfikowano 70 alleli *loci* mikrosatelitarnych. Największą liczbę alleli stwierdzono w *locus* TGLA53 (10 alleli). Wszystkie markery mikrosatelitarnego DNA, z wyjątkiem BM1818, wykazały wysoki stopień polimorfizmu. Analizując genotypy genów QTL związanych z cechami produkcyjnymi bydła stwierdzono wysoką frekwencję allelu B (0,323) genu kappa-kazeiny, ponadto dużo zwierząt posiadało heterozygotyczny genotyp AB (0,645). Stwierdzono dominację allelu L genu hormonu wzrostu (0,903). Zidentyfikowano układ genotypów wpływających na wartość takich cech, jak wydajność mleka, zawartość tłuszczu i białka w mleku.

Słowa kluczowe: mikrosatelitarne *loci* DNA, gen kappa-kazeiny, gen hormonu wzrostu, polimorfizm, cechy produkcyjne, bydło rasy Lebedin

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