



Polymorphism of *LEP* and *TNF- α* Genes in the Dairy Cattle Populations of Ukrainian Selection

Roman Kulibaba^{1*}, Yuriy Liashenko², Polina Yurko³, Mykola Sakhatskyi¹,
Yulia Osadcha¹ & Hamza Alshamaileh¹

¹ National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony st., 15, Kyiv, 03041, Ukraine ² Institute of Animal Science, the National Academy of Agrarian Sciences of Ukraine, 1-A, Tvarynykiv Str., Kharkiv, 61026, Ukraine ³ Kharkiv State Zooveterinary Academy of the Ministry of Education of Science of Ukraine, 1, Akademichna Str., Smt Mala Danylivka, 62341, Ukraine

*Corresponding author e-mail: romankx37@gmail.com

Received 29 November 2020; Accepted, 30 January 2020; Available online 23 February 2021

Abstract: Study was conducted by investigating the genetic structure of cattle populations of Ukrainian Black-and-White and Ukrainian Red-and-White dairy breeds by polymorphism of leptin gene (*LEP*) and tumor necrosis factor-alpha gene (*TNF- α*). The studies were carried out using the method of polymerase chain reaction (PCR) and restriction analysis for *LEP* and using classic PCR with subsequent SSCP analysis in case of *TNF- α* . According to the results of the study, it was shown that the *LEP* gene was polymorphic in both experimental populations by HphI-polymorphism in the third exon of the gene (A59V mutation). In the population of the Ukrainian Black-and-White dairy cattle breed, the frequency of allele C (HphI-) was 0.77; allele T (HphI+) – 0.23. Wright's fixation index was 0.23; which indicates a significant excess in the number of homozygous individuals. This population had a deviation from the Hardy-Weinberg equilibrium state. In the population of the Ukrainian Red-and-White dairy breed, the frequency of allele C was 0.72; allele T – 0.28; Wright's fixation index was -0.18. According to the SSCP-polymorphism of the *TNF- α* gene, 6 alleles with a size of 450-1200 bp were detected (alleles A, B, and F for Ukrainian Black-and-White dairy breed; A, B, C, D, E, F – for Ukrainian Red-and-White dairy breed). The frequency of allele A prevailed in the populations of both breeds (0.58 and 0.54, respectively). Alleles C, D, and E had a low frequency of occurrence (0.04-0.16) and they were found only in the population of Red-and-White dairy breed.

Keywords: alleles; genetic diversity; mastitis; PCR-RFLP; SSCP; cattle.

Introduction

For the last decades, livestock was one of the fastest-growing sectors of the world's economy. Dairy cattle breeding plays an important role in

ensuring the population's food security among the entire agro-industrial complex. However, despite all the advantages and perspectives, the

successful implementation of livestock development programs are complicated by several factors, one of which is related to the problem of animal diseases (Hogeveen & Van Der Voort, 2017).

In dairy cattle breeding, on the background of an ever-increasing level of milk production in the world, one of the most common diseases is mastitis (Mesquita *et al.*, 2012; Gomes & Henriques, 2016). Along with the positive dynamics in the productivity of cows, the level of disease with mastitis also increases. Thus, according to literary sources, the disease is diagnosed in 48% of the world, while in the European Union the number of clinically sick animals is about 30% of the total dairy herd. This problem is also typical of Ukraine (Mass *et al.*, 2016; Panevnyk & Suprovych, 2016).

Mastitis is a serious cause of economic losses in animal cattle breeding (Chopra *et al.*, 2015). This disease causes significant economic losses to dairy cattle breeding due to a decrease in milk production and the risk of increasing the sickness of young animals, deterioration in the quality of milk and dairy products (Seegers *et al.*, 2003). Accordingly, the amount of economic costs for mastitis includes the costs of treatment and the implementation of preventive measures and depends on the stage of the inflammatory process. It should be noted that in highly productive animals that have suffered mastitis, productivity does not fully recover. Moreover, this disease should be considered not only as an economic and veterinary problem but also as unsafe for human health (Hameed *et al.*, 2006). Thus, mastitis is recognized as one of the priority problems of veterinary medicine in cattle-breeding practice, due to its widespread,

significant economic losses, and the sanitary threat to humans.

The causes of mastitis are very different, so the disease is related to polyethiological ones (Litwinczuk *et al.*, 2015; Abebe *et al.*, 2016). The subclinical form of the disease is the most widespread (Zainettinova *et al.*, 2019). Along with the widely known and common methods of the prevention and control of mastitis, researches aimed at studying the genetic aspects associated with this disease has become more widespread (Mulder *et al.*, 2011; Kurtz *et al.*, 2018).

First of all, we mean the study of genetic factors associated with the immunity (resistance) of various breeds and lines of animals (Sender *et al.*, 2013). In the context of the ongoing marker-assisted selection (MAS), various candidate genes which allelic variants are associated with animal resistance to mastitis are studied. Additionally, researches are conducted aimed at studying the productive traits of experimental animals in order to identify potential counterproductive alleles of various genes. There has been found several quantitative trait loci (QTL) associated with mastitis resistance. One of the promising candidate genes associated with mastitis resistance includes the leptin gene (LEP) and tumor necrosis factor α gene (TNF- α).

Leptin is a peptide hormone that synthesized by adipose tissue (Daix *et al.*, 2008). It takes part in the regulation of a large number of different organism's functions, primarily fat and energy metabolism, as well as in the regulation of the immune system activity and reproductive functions (Barb & Kraeling, 2004).

The cattle leptin gene is located on the fourth

chromosome and consists of three exons and two introns. As a rule, *LEP* is considered as a candidate gene for markers of meat production (primarily as an indicator of live weight), as well as the indicators of milk quality (Yang *et al.*, 2007). There are a lot of perspective mutations (in the terms of marker-assisted selection) in the leptin gene were discovered in the cattle populations of different breeds. First of all, there are many publications about associations between different allelic variants of the leptin gene and the parameters of growth, carcass, and meat quality traits of cattle (Wang *et al.*, 2020). The investigations about polymorphism of the leptin gene are not only been connected with the parameters of meat productivity but also with the studies of milk production traits. Moreover, as well as commercial cattle breeds the studies also conducted for the local breeds and the buffalo. In the recent years, the studies have been carried out to explore the relationship between milk traits and leptin gene polymorphism in Autochthonous Busha Cattle (Maletic *et al.*, 2019), Egyptian Buffalo (El-Debaky *et al.*, 2020), etc.

The problems that have been tried to be solved also consider some veterinary aspects. For example, the scientific work of M.A. Ferchichi *et al.* (2018) studied the relationship between Sau3AI-polymorphism in the leptin gene and lameness prevalence in Tunisian Holstein cows. It should be noted the studies were carried out not only on cattle but also on sheep (Saleem *et al.*, 2018), goat (Avondo *et al.*, 2019), and last but not least, on the pig (Snegin *et al.*, 2020) that indicates *LEP* as one of the most interesting targets for the tasks of marker-assisted selection in livestock. Additionally, publications of various authors

revealed an associative relation between different allelic variants of leptin and the somatic cell count in milk in different cattle breeds, which makes it a promising object in the context of researches regarding mastitis resistance (Kulig *et al.*, 2010; Kiyici *et al.*, 2019).

TNF- α , a tumor necrosis factor-alpha, is a multifunctional anti-inflammatory cytokine, related to one of the key mediators of the local immune response (Kushibiki 2011). It takes part in the regulation of the activity of a large number of different genes of the immune system, stimulates the proliferation of lymphocytes, and initiates the synthesis of interleukins.

The TNF- α gene is located on the 23rd chromosome and contains about 30 polymorphic fragments. Various allelic variants have been identified according to their different positions of the gene, as well as their relation to productivity indicators and also to resistance/sensitivity to various diseases, including mastitis (Ranjan *et al.*, 2015). There is no investigation about features of the genetic structure by TNF- α gene polymorphism in the cattle population of Ukrainian selection.

Considering all of the above, the aim of our work was the study of the polymorphism of *LEP* and TNF- α genes in the dairy cattle populations of Ukrainian Black-and-White and Ukrainian Red-and-White breeds.

Materials & Methods

Dairy cattle breeds of Ukrainian selection, Ukrainian Black-and-White dairy breed (n = 100) and Ukrainian Red-and-White dairy breed (n = 100) were used in the present study.

The study was carried out in the laboratory of molecular-genetic and physiological-biochemical research in animal science of the Institute of Animal Science of the NAAS. Experienced groups of cows were kept in the experimental farm “Hontarovka” (Vovchansk district, Kharkov region).

DNA was extracted using a commercial DNA-Sorb-B reagent kit (Amplisens, Russian Federation), according to the manufacturer's instructions. Hair follicles were used as a DNA source. The DNA extraction efficiency was determined by agarose electrophoresis (0.7%).

For each sample, the reaction was carried out using specific primers flanking the corresponding gene fragment, which potentially contains a polymorphic site.

Using the PCR-RFLP method, HphI-polymorphism in the third exon of the LEP gene was studied (C/T transition, A59V mutation). The following primers were used for amplification:

GGGAAGGGCAGAAAGATAG and
TGGCAGACTGTTGAGGATC (Haegeman *et al.*, 2000).

In both cases, amplification was performed with a DreamTaq PCR Master Mix kit (Thermo Scientific) using appropriate standard programs. The volume of the final PCR mixture was 10 μ l. The concentrations of each primer (forward and reverse) were 0.2 μ M.

The annealing temperature is 56 °C. The size of the amplified fragment is 331 bp. The restriction was performed using restriction endonuclease HphI according to the manufacturer's (Thermo Scientific) protocol. HphI-polymorphism of the locus leads to the creation of a restriction site and, accordingly, to

the appearance of two restriction fragments of 311 and 20 bp.

Amplificated products were separated by 1.5% agarose gel (150 V for 30 min) in TBE buffer. Visualization was carried out with ethidium bromide (UV light).

The sizes of restriction fragments were determined using molecular weight markers M-50 (InterLabService, AmpliSens® Biotechnologies, Russia). We used the single 284 bp DNA-fragment as an additional molecular weight marker as well.

The TNF- α gene polymorphism was studied by the PCR-SSCP method. Amplification was performed using primers: TACTGCTTCCATCCCTTGAC and GAGAAGACAAGACCCATCAG (Ranjan *et al.*, 2015).

The amplified gene fragment contains a part of intron 1, exon 2, and a part of intron 2. The temperature of the primer annealing is 55 °C. The size of the amplified fragment is 239 bp. After amplification, electrophoresis was performed to detect SSCP polymorphism according to the appropriate technique (Barroso *et al.*, 1998).

Amplificated products were separated by 8–12% polyacrylamide gels with a different value of the glycerol component (2–10%) at 300–350 V for 180–300 min in TBE buffer. The samples were stained according to the silver staining protocol (Sambrook & Russell 2001).

The main parameters of the genetic structure of the experimental populations were determined using standard techniques and appropriate software. Allele frequencies were determined by the formulas of maximum likelihood according to E.K. Merkur'eva (1977). Basing

on the obtained frequencies of alleles and genotypes, the observed (H_o) and expected (H_e) heterozygosity, the effective number of alleles (n_e), the Wright's fixation index (F_{is}), and the correspondence to the state of genetic equilibrium according to Hardy-Weinberg (with using the χ^2 method) were determined according to standard techniques with the use of the Popgen32 software (https://sites.ualberta.ca/~fyeh/popgene_download.html).

Results & Discussion

The use of restriction analysis made it possible to identify the variability of the leptin gene in experimental cattle populations (Fig. 1).

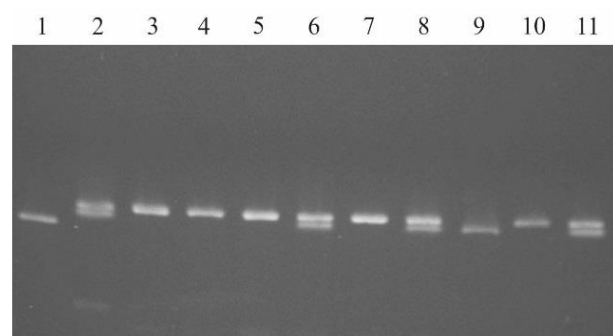


Fig. (1): LEP gene polymorphism (HphI-polymorphism in the third exon) in the dairy cattle populations. 1 – single molecular weight marker (284 bp); 2, 6, 8, 11 – genotype C/T; 3-5, 7, 10 – C/C; 9 – T/T

Allelic variants of the LEP gene resulting from HphI-polymorphism in the third exon of the gene are shown on the electrophoregram of restriction fragments.

As follows from the research results, the leptin gene is polymorphic in both experimental populations. In each population, individuals of

all possible genotypes (C/C, C/T, T/T) are identified (Fig. 1).

The genetic structure of the experimental animal populations by the LEP locus is presented in table (1).

Table (1): Genetic structure of dairy cattle populations of Ukrainian selection (mutation A59V).

Populatio n	Genotype			Allele		χ^2
	CC	CT	TT	C	T	
Black- and- White	0.6 4	0.2 7	0.0 9	0.7 7	0.2 3	4.9 7
Red-and- White	0.4 8	0.4 7	0.0 5	0.7 2	0.2 8	2.4 2

According to the ratio of the frequencies of LEP genotypes, the experimental populations differ considerably from each other.

The population of the Ukrainian Black-and-White dairy breed is characterized by a significant prevalence of the number of individuals of the CC genotype, while such a trend was not observed in the population of the Ukrainian Red-and-White dairy breed (Table 1). In the population of the Ukrainian Black-and-White dairy breed, the frequency of allele C (HphI-) was 0.77; allele T (HphI+) was 0.23. The value of the observed and expected heterozygosity was 0.27 and 0.35, respectively. Wright's fixation index was 0.23; which indicates a significant excess of homozygous individuals. In its turn, in the population of Red-and-White dairy breed, the frequency of allele C was 0.72; allele T was 0.28; the

observed and expected heterozygosity was 0.47 and 0.40; Wright's fixation index was -0.18; which indicates an expressed excess in the number of heterozygous individuals and population was in the state of genetic equilibrium. In the Black-and-White Ukrainian cow's population, a deviation from the state of genetic equilibrium was noted ($\chi^2 = 4.97$; $p < 0.05$). In terms of the effective number of alleles, the population of the Red-and-White dairy breed is characterized by a large value – 1.66 as against the value of 1.54 in the Black-and-White dairy breed.

The results obtained in this research on the frequency values of the alleles of the leptin gene (mutation A59V) are consistent with the results of other authors. Similar gene frequencies were obtained by Liefers *et al.* (2003) in Holstein-Friesian cattle, Clempson *et al.* (2011) in Holstein cattle, Yazdani *et al.* (2010) in Iranian cattle and Silva *et al.* (2012) – in Indian cattle. Abbas *et al.* (2019) and Maletic *et al.* (2019) also noted the predominance of the C allele, up to a monomorphic state, in Pakistan and Balkan cattle. The observed pattern might be explained by the influence of the ongoing selection program in the direction of increasing milk production, as well as by the stochastic genetic factors (genetic drift).

Work has been done on optimizing the technique of SSCP analysis for genotyping by the locus of tumor necrosis factor-alpha (*TNF- α*). According to the research results it was shown that for effective genotyping the 12% polyacrylamide gels (acrylamide/bisacrylamide ratio 100/1) with a glycerol content of 5% should be used, the temperature of the buffer used during electrophoresis should be 4-12 °C;

electric field parameters – 300-350V during 4 hours. Silver staining was used to visualize amplified *TNF- α* fragments (Sambrook & Russell, 2001).

The Use of the optimized technique of SSCP analysis made it possible to separate single and double-stranded DNA fragments efficiently (Fig. 2).

The electrophoregram (silver staining) of the amplification products of the second exon of the *TNF- α* gene is presented in fig. (3).

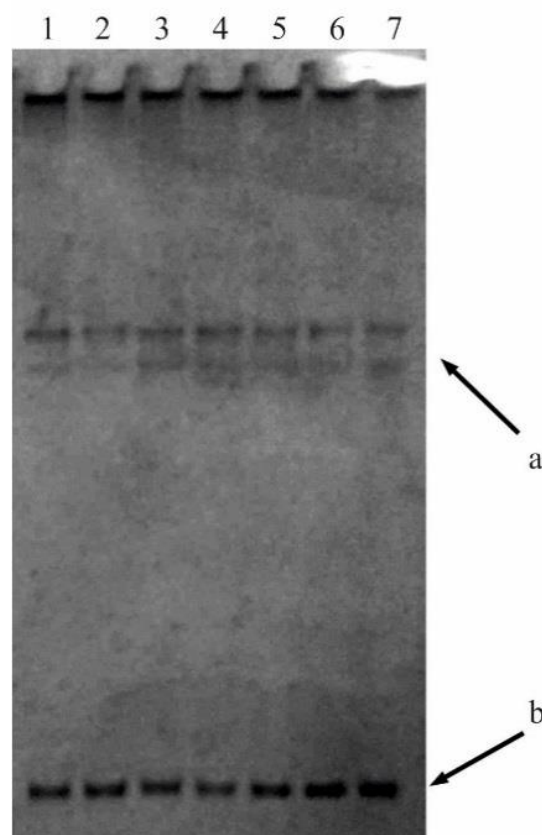


Fig. (2): SSCP-polymorphism in the *TNF- α* gene in the dairy cattle populations. a – single stranded DNA fragments; b – double-stranded DNA fragments.

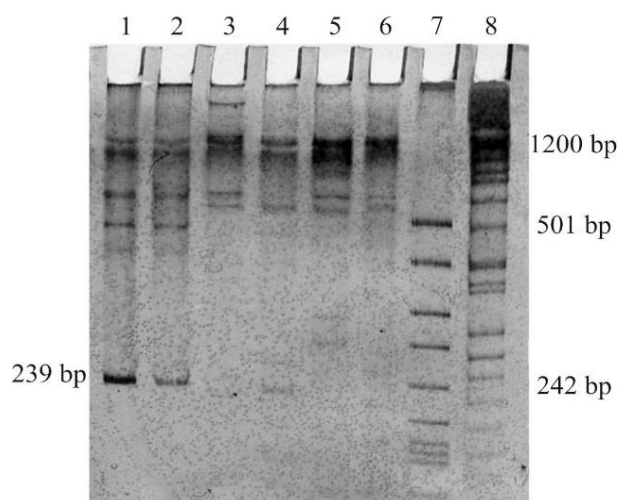


Fig. (3): Electrophoregram of the SSCP-polymorphism in the second exon of the *TNF-α* gene in the dairy cattle populations. 1 – AE; 2 – AF; 3 – AB; 4 – AC; 5 – AD; 6 – AB; 7 – Molecular ladder 1; 8 – Molecular ladder 2.

According to the results of the analysis of single-chain patterns of the *TNF-α* gene region, 239 bp (second exon), 6 alleles were detected with a size of 450-1200 bp. Alleles A, B, and F were found in the population of the Ukrainian Black-and-White dairy breed, and alleles A, B, C, D, E, F were found in the population of the Ukrainian Red-and-White dairy breed. The genotypes of both breeds the frequency of allele A was 0.58 and 0.54, respectively. Alleles C, D, and E had a low frequency of occurrence (0.04-0.16) and were detected only in the Black-and-White breed. Homozygous genotypes in both populations were only for the A allele.

The frequencies of genotypes and alleles by SSCP polymorphism of the second exon of the *TNF-α* gene in both experimental cattle populations are presented in table (2).

The Ukrainian Red-and-White dairy breed, in the present study, show a significantly higher level of polymorphism of the *TNF-α* locus in terms of the number of identified alleles and the corresponding genotypes. However, despite the differences in the number of alleles and genotypes, the frequencies of the most prevailing allele in both populations practically coincide – 0.58 versus 0.54, respectively, which may be a consequence of the general orientation of the ongoing selection program toward the increasing of milk yield.

Table (2): Genotype and allele frequencies in the dairy cattle populations by SSCP-polymorphism in the *TNF-α*.

Population	
Ukrainian Black-and-White dairy breed	Ukrainian Red-and-White dairy breed
Genotype	
AA – 0.16; AB – 0.32; AF – 0.52	AA – 0.08; AB – 0.12; AC – 0.2; AD – 0.32; AE – 0.08; AF – 0.2
Allele	
A – 0.58; B – 0.16; F – 0.26	A – 0.54; B – 0.06; C – 0.1; D – 0.16; E – 0.04; F – 0.1

As it was already noted, the *TNF-α* locus under study contains the second exon (49 bp, <http://www.ensembl.org>) and adjacent sections of 1 and 2 introns (190 bp). According to the Ensembl database in the second exon of *TNF-α*, 4 SNPs were identified: rs451672471 (A/G,

synonymous variant), rs456866435 (C/T, synonymous variant), rs110320728 (T/C, synonymous variant), and rs469370538 (G/C, synonymous variant). Therefore, the present results fully agree with the likelihood of the correspondence of different polymorphic variants to the different SSCP patterns revealed. In addition, the intron region of the locus, on which there is no information about SNP, can add additional patterns (variants). In general, we found 6 different SSCP patterns – 3 in the Ukrainian Black-and-White population and 6 in the Red-and-White dairy breed (two of which were found at a low frequency (0.04-0.06). For comparison, 2 SSCP patterns, alleles A and B with frequencies of 0.73 and 0.27 (Ranjan *et al.*, 2015), were revealed in a crossbreeding Indian cattle population (Holstein-Frisian × brown Swiss × jersey). Based on an example of a study of two groups of cows with different infectious loads, the authors suggest that the A allele is mainly found among individuals sensitive to mastitis. In addition, the expression of mRNA of *TNF-α* is significantly higher in cows with the AA genotype compared to AB and BB, which suggests the use of the obtained SSCP-patterns as possible markers of mastitis resistance in dairy cattle. Unfortunately, the authors of this article do not provide data on the size of the alleles; therefore, we can only assume that the more widespread and slowly migrating DNA strands (allele A) in the population correspond to the SSCP pattern A that we found (1200 bp) in Ukrainian cattle populations.

Conclusion

The genetic structure specificities of cattle populations of Ukrainian Black-and-White and Red-and-White dairy breeds by leptin gene and

tumor necrosis factor-alpha gene were studied. According to the research results, the frequency of occurrence of the allele C significantly prevails at the leptin locus in both populations. Moreover, a deviation from the equilibrium state according to Hardy-Weinberg was noted in the population of the Black-and-White dairy breed. The *TNF-α* locus in both populations also showed a predominance of the frequency of occurrence of the allele A. Considering the high level of polymorphism of the studied locus, as well as its potential in regulating the body's immune system, further studies are promising and will be carried out in the direction of studying the relation of various allelic variants of *TNF-α* with economically desirable traits of cattle populations. Also it is planned to analyze the nucleotide sequences of the identified allelic variants of *TNF-α*.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval: all applicable national and international guidelines for the care and use of animals were followed.

Acknowledgements

The authors thank to the Laboratory of molecular-genetic and physiological-biochemical research in animal science staff, Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine.

ORCID

R. Kulibaba: 0000-0003-1776-7147

Yu. Liashenko: 0000-0003-2747-476X

P. Yurko: 0000-0003-4870-1570

M. Sakhatskyi: 0000-0002-6113-0226

Yu. Osadcha: 0000-0003-4126-2456

H. Alshamaileh: 0000-0002-4757-8585

References

- Abbas, N., Suleman, M., Zahur, A. B., Ghafoor, A., Rashid, F., Jan, A. U., Akbar, F., Ali, S., Aziz, A., Islam, Z., & Shah, A. (2019). Molecular Analysis of Leptin Gene Polymorphism in Achai, Sahiwal Cattle and Nili-ravi Buffalo Breeds of Pakistan. *International Journal of Genetics and Genomics*, 7, 75-79. <http://doi:10.11648/j.ijgg.20190703.17>
- Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K. (2016). Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, 12, 270. <http://doi:10.1186/s12917-016-0905-3>
- Avondo, M., Di Trana, A., Valenti, B., Criscione, C., Bordonaro, S., De Angelis, A., Giorgio, D., & Di Gregorio, P. (2019). Leptin gene polymorphism in goats fed with diet at different energy level: Effects on feed intake, milk traits, milk fatty acids composition, and metabolic state. *Animals*, 9, 424. <http://doi:10.3390/ani9070424>
- Barb, C. R., & Kraeling, R. R. (2004). Role of leptin in the regulation of gonadotropin secretion in farm animals. *Animal Reproduction Science*, 82-83, 155-167. <http://doi:10.1016/j.anireprosci.2004.04.032>
- Barroso, A., Dunner, S., & Canon, J. (1998). Technical note: Detection of bovine kappa-casein variants A, B, C, and E by means of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). *Journal of Animal Science*, 76, 1535-1538. <http://doi:10.2527/1998.7661535x>
- Chopra, A., Gupta, I. D., Verma, A., Chakravarty, A. K., & Vohr, V. (2015). Lactoferrin gene promoter variants and their association with clinical and subclinical mastitis in indigenous and crossbred cattle. *Polish Journal of Veterinary Sciences*, 18, 465-471. <http://doi:10.1515/pjvs-2015-0061>
- Clempson, A. M., Pollott, G. E., Brickell, J. S., Bourne, N. E., Munce, N., & Wathes, D. C. (2011). Evidence that leptin genotype is associated with fertility, growth, and milk production in Holstein cows. *Journal of Dairy Science*, 94, 3618-3628. <http://doi:10.3168/jds.2010-3626>
- Daix, M., Pirotte, C., Bister, J. L., Wergifosse, F., Cuvelier, C., Cabaraux, J. F., Kirschvink, N., Istasse, L., & Paquay, R. (2008). Relationship between leptin content, metabolic hormones and fat deposition in three beef cattle breeds. *The Veterinary Journal*, 177, 273-268. <http://doi:10.1016/j.tvjl.2007.04.004>
- El-Debaky, H., Mahmoud, K. M., Abd El-Razik, K. A., Sosa, A. S. A., Kandiel, M. M. M., & Ahmed, Y. F. (2020). PCR-SSCP and sequencing analysis for studying Leptin gene polymorphism and its association with reproductive status of Egyptian Buffalo. *Egyptian Journal of Veterinary Sciences*, 51, 11-21. <http://doi:10.21608/ejvs.2019.16438.1094>
- Ferchichi, M. A., Jemmali, B., Amiri, S., Gara, A. B., & Rekik, B. (2018). Effect of leptin genetic polymorphism on lameness prevalence in Tunisian Holstein cows. *Archives Animal Breeding*, 61, 305-310. <http://doi:10.5194/aab-61-305-2018>
- Gomes, F., & Henriques, M. (2016). Control of Bovine Mastitis: Old and recent therapeutic approaches. *Current Microbiology*, 72, 377-382. <http://doi:10.1007/s00284-015-0958-8>
- Haegeman, A., Van Zeveren, A., & Peelman, L. J. (2000). New mutation in exon 2 of the bovine leptin gene. *Animal Genetics*, 31, 79. <http://doi:10.1111/j.1365-2052.2000.579-14.x>
- Hameed, K. G. A., Sender, G., & Korwin-Kossakowska, A. (2006). Public health hazard due to mastitis in dairy cows. *Animal Science Papers and Reports*, 25, 73-85.
- Hogeveen, H., & Van Der Voort, M. (2017). Assessing the economic impact of an endemic disease: the case of mastitis. *Revue scientifique et technique (International Office of Epizootics)*, 36, 217-226. <http://doi:10.20506/rst.36.1.2623>
- Kiyici, J. M., Akyüz, B., Kaliber, M., Arslan, K., Aksel, E. A., & Çınar, M. U. (2019). LEP and SCD polymorphisms are associated with milk somatic cell count, electrical conductivity and pH values in Holstein cows. *Animal Biotechnology*, 31, 498-503. <http://doi:10.1080/10495398.2019.1628767>

- Kulig, H., Kmiec, M., & Wojdak-Maksymiec, K. (2010). Associations between Leptin Gene Polymorphisms and Somatic Cell Count in Milk of Jersey Cows. *Acta Veterinaria Brno*, 79, 237-242. <http://doi:10.2754/avb201079020237>
- Kurtz, J. P., Yang, Z., Weiss, R. B., Wilson, D. J., Rood, K. A., Liu, G. E., & Wang, Z. (2018). A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. *Immunogenetics*, 71, 35-47. <http://doi:10.1007/s00251-018-1088-9>
- Kushibiki, S. (2011). Tumor necrosis factor- α -induced inflammatory responses in cattle. *Animal Science Journal*, 82, 504-511. <http://doi:10.1111/j.1740-0929.2011.00931.x>
- Liefers, S. C., Pas, M. F. W., Veerkamp, R. F., Chilliard, Y., Delavaud, C., Gerritsen, R., & Lende, T. (2003). Association of leptin gene polymorphisms with serum leptin concentration in dairy cows. *Mammalian Genome*, 14, 657-663. <http://doi:10.1007/s00335-003-2275-y>
- Litwinczuk, Z., Krol, J., & Brodziak, A. (2015). Factors determining the susceptibility of cows to mastitis and losses incurred by producers due to the disease – a review. *Annals of Animal Science*, 15, 819-831. <http://doi:10.1515/aoas-2015-0035>
- Maletic, M., Paprikic, N., Lazarevic, M., Hodzic, A., Davidovic, V., Stanisic, L., & Stanimirovic, Z. (2019). Insight in leptin gene polymorphism and impact on milk traits in autochthonous Busha cattle. *Acta Veterinaria-Beograd*, 69, 153-163. <http://doi:10.2478/acve-2019-0012>
- Mass, A., Ovcarenko, G., & Vaseska, A. (2016). The effectiveness of diagnosis and therapy cow's with mastitis. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj*, 18, 101-104.
- Merkur'eva, E. K., (1977). Geneticheskie osnovy selektsii v skotovodstve [*Genetic Basis of Breeding in stock raising*]. Moscow]: Kolos. 240 pp. (In Russian).
- Mesquita, A. Q., Rezende, C. S. F., Jose de Mesquita, A., Garcia da Veiga Jardim, E. A., & Kipnis A. P. J. (2012). Association of TLR4 polymorphisms with subclinical mastitis in Brazilian Holsteins. *Brazilian Journal of Microbiology*, 43, 692-697. <http://doi:10.1590/S1517-83822012000200034>
- Mulder, H. A., Lidauer, M. H., Vilkki, J. H., Strandén, I., & Veerkamp, R. F. (2011). Marker-assisted breeding value estimation for mastitis resistance in Finnish Ayrshire cattle. *Journal of Dairy Science*, 94, 4164-4173. <http://doi:10.3168/jds.2010-4112>
- Panevnyk, V., & Suprovych, T. (2016). Etiological factors mastitis cows Ukrainian black-pied dairy breed. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S.Z. Gzhytskyj*, 18, 191-195. <http://doi:10.15421/nvlvet7046>
- Ranjan, S., Bhushan, B., Panigrahi, M., Kumar, A., Deb, R., Kumar, P., & Sharma, D. (2015). Association and Expression Analysis of Single Nucleotide Polymorphisms of Partial Tumor Necrosis Factor Alpha Gene with Mastitis in Crossbred Cattle. *Animal Biotechnology*, 26, 98-104. <http://doi:10.1080/10495398.2014.929582>
- Saleem, A.H., Javed, K., Babar, M.E., Hussain, T., Ali, A., Ali, A., Ahmad, N., Farooq, M.Z., & Dawood, M. (2018). Association of Leptin Gene Polymorphism with Growth Rate in Lohi Sheep. *Pakistan Journal of Zoology*, 50, 1029-1033. <http://doi:10.17582/journal.pjz/2018.50.3.1029.1033>
- Sambrook, J., & Russell, R. W. (2001). *Molecular cloning: A laboratory manual*, 3rd ed. Cold spring harbor laboratory press, cold spring harbor, N.Y. 2344pp.
- Seegers, H., Fourichon, C., & Beaudeau, F. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary Research*, 34, 475-491. <http://doi:10.1051/vetres:2003027>
- Sender, G., Korwin-Kossakowska, A., Pawlik, A., Hameed, K. G. A., & Oprzade J. (2013). Genetic basis of mastitis resistance in dairy cattle – A review. *Annals of Animal Science*, 13, 663-673. <http://doi:10.2478/aoas-2013-0043>
- Silva, R. C. G., Ferraz, J. B. S., Meirelles, F. V., Eler, J. P., Balieiro, J. C. C., Cucco, D. C., Mattos, E. C.,

- Rezende, F. M., & Silva, S. L. (2012). Association of leptin and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle. *Genetics and Molecular Research, 11*, 3721-3728. <http://doi:10.4238/2012.August.17.10>
- Snegin, E. A., Artemchuk, O. Yu., Sychev, A. A., Barkhatov, A. S., Yusupov, S. R., Snegina, E. A., & Tishchenko, A. Yu. (2020). Polymorphism in the leptin gene (T3469C) of Different Pig Breeds of the Belgorod Region of Russia. *EurAsian Journal of BioSciences, 14*, 3125-3129.
- Wang, L., Raza, S. H. A., Gui, L., Li, S., Liu, X., Yang, X., Wang, S., Zan, L., & Zhao, C. (2020). Associations between UASMS2 polymorphism in leptin gene and growth, carcass and meat quality traits of cattle: A meta-analysis. *Animal Biotechnology*, <http://doi:10.1080/10495398.2020.1805327>
- Yang, D., Chen, H., Wang, X., Tian, Z., Tang, L., Zhang, Z., Lei, C., Zhang, L., & Wang, Y. (2007). Association of polymorphisms of leptin gene with body weight and body sizes indexes in Chinese indigenous cattle. *Journal of Genetics and single nucleotide polymorphisms in the bovine Genomics, 34*, 400-405. [http://doi:10.1016/S1673-8527\(07\)60043-5](http://doi:10.1016/S1673-8527(07)60043-5)
- Yazdani, H., Rahmani, H. R., Edris, M. A., & Dirandeh, E. (2010). Association between A59V polymorphism in exon 3 of leptin gene and reproduction traits in cows of Iranian Holstein. *African Journal of Biotechnology, 9*, 5997-6000. <http://doi:10.5897/AJB10.126>
- Zainettinova, D. B., Muhamadieva, N. N., Julanov, M. N., Stefanyk, V. Yu., Zaviruha, V. V., & Sarhambaeva, I. A. (2019). The effectiveness of the treatment of cows with mastitis. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences.*, *21*, 78-81. <http://doi:10.32718/nvlvet9414>

تعدد الطرز الشكلية لجينات LEP و TNF- α في مجتمعات ماشية الحليب الأوكرانية

رومان كوليبابا^{1*} ، يوري لياشينكو² ، بولينا يوركو³ ، ميكولا ساكاتسكي¹ ،

يوليا أسدشا¹ وحمزة الشمايله¹

¹الجامعة الوطنية للحياة والعلوم البيئية في أوكرانيا ، شارع هيروييف أوبوروني، 15، كييف، أوكرانيا

²معهد علوم الحيوان، الأكاديمية الوطنية للعلوم الزراعية في أوكرانيا، A 1-، شارع Tvarynnykiv ، خاركييف، أوكرانيا

³أكاديمية خاركييف الحكومية للحيوانات البيطرية التابعة لوزارة تعليم العلوم في أوكرانيا

المستخلص: نفذت الدراسة الحالية لتحديد التركيب الجيني في ماشية الحليب الأوكرانية من الهجائن الأبيض-و الأسود و الأحمر و الأبيض لماشية الحليب لجين (LEP) و leptin gene (TNF- α) and tumor necrosis factor-alpha gene . نفذت الدراسة اعتماداً على تفاعل التسلسل البلمري PCR والتحليل بالهضم للجين LEP، فضلاً عن استخدام التقانة ذاتها مع الجين TNF- α مع تحليل SSCP. طبقاً لنتائج الدراسة الحالية وجد إن الجين LEP متعدد الطرز الشكلية في كلا مجتمعي هجائن الماشية قيد البحث من خلال HphI في الامتداد الثالث للأكسون المتعلق بالجين (A59V mutation). ما يخص مجتمع الهجين من النوع الأبيض و الأسود لماشية الحليب الأوكرانية كان تكرار الأليل من نوع C (HphI-) بقيمة 0.77، والأليل T (HphI+) بقيمة 0.23. كما وجد إن قيمة معامل Wrights fixation كانت 0.23، والتي أشارت إلى زيادة في عدد الأفراد المتناظرة. ووجد انحرافاً في حالة Hardy-Weinberg equilibrium. أما عن مجتمع هجين ماشية الحليب الأحمر و الأبيض وجد أن تكرار الأليل C بقيمة بلغت 0.72، والأليل T كان 0.28، وقيمة معامل Wrights fixation كانت بمعدل 0.18. وفيما يتعلق بالتحليل SSCP ، بينت النتائج مع الجين TNF- α وجود ستة اليلات بأحجام تراوحت بين 450-1200 زوج قاعدة، وهي: A و B و C و D و E و F للهجين الأحمر و الأبيض. وكان تكرار الأليل A سائداً في كلا هجيني ماشية الحليب الأوكرانية بقيمة بلغت 0.58 و 0.54، على التوالي. أما فيما يخص اليلات C و D و E كانت بتكرارات واطئة بمدى بلغ 0.04-0.16 ووجدت فقط في هجين الماشية الأحمر و الأبيض.

الكلمات المفتاحية: اليلات ، التنوع الجيني، التهاب الضرع ، PCR-RFLP ، SSCP ، الماشية.