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THE APPLICATION OF MODERN MOLECULAR TECHNIQUES IN ANIMAL SELECTION

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Review paper

Abstract: Some problems of modern analysis of the genome of domestic animals and the possibility of applying the results of the research in the selection have considered. Molecular genome analysis has progressed rapidly. Today, using modern DNA analysis, the domestic animals breeding value can be safely foreseen. Based on the scientist's intuition of early-20th century, after half a century, genetic markers had discovered. Thus, the traditional selection method has integrated with molecular techniques of selection by applying marker-assisted selection (MAS). Microsatellites have attracted great attention from scientists because they are widespread in the genome and have a high level of polymorphism. Thousands of SNPs have discovered with their exact position in the genome. This very reliable method occurred for analyzing the genome which shows the change of only one nucleotide base into the DNA molecule. All modern achievements of molecular genetics have opened the way for practical application in the selection of all kinds of domestic animals. Today, a combination of molecular or genomic selection combined with a traditional is very reliable method for a faster, more accurate assessment of the breeding value of animals.

Key words: domestic animals, selection, molecular techniques, genetic markers

Introduction

The primary goal of modern selection is the association of all known sources of animal information (phenotype, origin and genetic markers) in order to obtain the highest reliability of the estimated breeding value (EBV) and ensure

genetic improvement *(Petrovic 2000; Petrovic et al., 2001; Petrović and Pantelić, 2015; Petrovic et al., 2017a; Petrovic et al., 2017b).* Genomics offers ample opportunities; it's not only for genetic improvement of animals but can also be used as an important tool for the assessment of genetic diversity of local sheep and goat breeds (*Rupp et al., 2016).* The genome of domestic animals contains 20,000-35,000 useful-useful genes. Generally, selection by using of molecular techniques should involve the process of DNA molecular analysis in order to search for markers or individual nucleotide polymorphisms (SNPs) to obtain information on the genetic potential of the product's characteristics of the individual, which will serve as a selection criterion. From the first ideas of DNA molecular analysis for this purpose, many procedures have applied of such; a number of genetic markers, methods and in practice is necessary to accept a common platform, the name of which this would be a genomic selection. The term "genomic selection" was first introduced by *Haley and Visscher (1998). Meuwissen (2001)* developed and presented a methodology for the analytical estimation of the value of breeding by using a map of markers covering the whole genome.

Genomic selection (GS) is at the present level of science, a method by which high density markers cover the entire genome. This leads to individual genetic evaluation - genomic estimated breeding values (GEBV). Genomic selection can increase the precision of selection, shorten the generation interval by selecting individuals at an early stage of life, and accelerate genetic progress *(Christensen et al., 2014).*

Practical work in genomic selection involves taking a sample of biological material (blood, seed, hair) from which the DNA is isolated in the laboratory. The procedure for isolating the pure DNA is very complex and includes cutting restriction endonucleases, radioactive or non-radioactive labeling, ligation, use of special detergents, bases, organic solvents and enzymes. Following the isolation of the DNA, a genotyping procedure is performed using the Illumina SNP50K chip. Genotyping is a method by which genetic variations are determined without the sequencing of the whole genome. The result of genotyping is the signals for each of the 50,000 SNPs that are calculated in the SNP genotype (AA, AB or BB). Thus, for the genotyping individual, the result is (more precisely, the genotype) for each of the 50,000 SNPs. The fact that we have come to know about the genotype of a large number of SNPs does not give a complete answer to the breeding value of the animal. In order to solve this, it is necessary to evaluate the influence of the individual SNP by using the SNP equation. Thus, the genomic breeding value (GBV) of a genotyped animal is the sum of the effects of all SNPs, obtained using the SNP equation *(Špehar, 2013).*

In many countries, genomic selection becomes the leading method of genetic evaluation (*Hayes et al., 2009, 2012*). Especially for GS cows GS can soon replace the traditional system of genetic evaluation of animals.

Development of molecular techniques

Genomic technologies allow deciphering the genotype of the animal at birth. This can be done at the same time by selecting breeding animals. SNP as the latest technology has been designed to further improve the accuracy and reliability of selection and assessment of the breeding value of animals. The SNP genome predecessors are selective markers, which make it possible to determine the presence or absence of certain genes (alleles) of the carrier of quantitative traits in the animal genome.

A. Serebrovski, a Russian Scientist was first given the idea and the theory of the use of markers in breeding during the second decade of the 20th century. The term "marker" (then called "signal" while the English term "keeper") began to use later. According to Serebrovsky, the marker is an allele gene with pronounced phenotype, localized next to another allele responsible for an economically significant trait, but there is no clear phenotypic expression. Thus, the selection of the phenotypic expression of this allele-signal is possible by selecting related alleles that determine the manifestation of the observed trait. However, the direct marker is far more reliable than the associated marker for the prediction of phenotypic variations of the particular character of domestic animals (*Dekkers, 2004, Scherbatov et al., 2013*). Genetic markers were initially based on morphological characteristics (phenotypic markers).

However, quantitative traits are the result of a complex inheritance process. The markers used and environment conditions determined their manifestation. Therefore, its use is limited. Polymerase chain reaction (PCR) and sequencing (DNA nucleotide assay) DNA occupies a significant place in genomic animal selection *(Avise, 2004). Buisson et al. (2014*) conclude a genomic breeding scheme (GS) appears technically and economically relevant in French dairy sheep breeds. The α_{s1} casein genotype had a significant effect on milk yield, fat content and protein content (*Carillier-Jacquin et al., 2016).* In recent years, several new molecular techniques have been developed, in addition to genetic markers such as microsatellites, the leading site has taken the polymorphism of single nucleotides (SNP - Single Nucleotide Polymorphism), which allows the selection options to expand considerably and deepen.

The application of DNA technology and genetic markers in livestock breeding began in the 1980s. Molecular genetic markers have used for programs for the conservation of genes of endangered breeds of domestic animals. Also, markers have found application for solving the problem of origin and parenthood of the distribution of species, genetic causes of hereditary diseases. The application of markers was positive to accelerate the selection of individual characteristics - resistance to certain factors from the impact on productive performance.

The traditional methods of farm animals' selection can be integrated with molecular techniques of selection by applying marker-assisted selection (MAS) (*Lande and Thompson, 1990, Ollivier 1998, Caro Petrovic et al., 2017).*

Modern molecular methods in selection

Microsatellites have attracted great attention from scientists because they are widespread in the genome and have a high level of polymorphism. Microsatellites, such as SSR (Simple Sequence Repeats) or STR (Simple Tandem Repeats), repeated several times in the tandem by the genome of more than one hundred thousand loci *(Weber and May, 1989).*
Both of the mentioned about are highly polymorphic and suitable for individual DNA typing, or the production of a genetic profile of the individual. They are considered to be the most informative genetic markers.

Microspheres are non-coding parts of DNA, but some studies reveal their role in gene expression (*Moxon and Wills, 1999*). It is known that the variants of microsatellites differ in the number of repetitions of the underlying motive, which is actually the result of mutations. The structure of microsatellites can have several forms:
• filled (CACACACACACA),
• interrupted (CACACACAggggCACACA) or
• assembled (CACACACAGTGTGTGTCACA).

Their multiplication is carried out by the chain reaction of polymerase - PCR technique. Microspheres are relatively short compared to other genetic markers, as they generally have 50 and 150 pb.

Their advantage is that very small amounts of DNA are required for duplication so in similar research have been used for these reasons. It is also extensively used to examine the genetic distance between individual animal populations *(Cunningham et al., 2001; Teneva et al., 2010; 2013).*

Some countries have developed a genetic test system. In addition to a number of advantages, microsatellites insufficiently map some specific genome areas. On the other hand, the high cost of equipment and reagents and the development of automatic methods using SNP chips slowly take their place. A very practical method for analyzing the genome is the SNP polymorphism of a nucleotide, which shows the change of only one nucleotide base into the DNA molecule.

Thousands of SNPs have discovered with their exact position in the genome. Such differences in the molecule, are often encoded with letters, e.g., A and B. Possible combinations are AA (homozygote saturated A), AB (heterozygote) and BB (homozygote for allele B) for each SNP marker. However, for many SNPs, it is not known whether they cause some changes in the

manifestation of individual traits or maybe they are just near a genome *(Spehar, 2013).*

SNPs are point mutations that may occur as a result of spontaneous mutations or the action of mutagens. The difference in only one base can cause characteristic changes. SNPs are widespread in the human and animal genome. In addition, SNPs have a low rate of mutation per generation (~ 10-8) as opposed to microsatellites, making them suitable markers for population genetic analysis *(Scherbatov et al., 2013).*

The SNP great advantage also allows the ability to use automatic detection methods, for example, using DNA strings. In the world, databases are already being formed, testing a large number of animals to reveal the connections between known point mutations and productivity. Massive numbers of polymorphic variants of the gene are currently defined, as well as their impact on animal production characteristics. Some genetic tests that use markers that define production qualities are already publicly available for use in breeding. We'll list some of these markers (*Shcherbatov et al., 2013*):

• Meat and meat production efficiency markers - MC4R, HMGA1, CCKAR, POU1F1

• Fertility markers: ESR - estrogen receptor gene, EPOR - erythropoietin receptor gene

• Markers of disease resistance - ECR F18 receptor gene

Through the results of markers, can estimate the frequency of desired and undesired alleles in the population and continue the selection in the future, so that all animals get only desirable gene alleles.

Genomic selection - is a powerful tool for future use. Currently, the effectiveness of genomic selection is limited in various interactions between the locus of quantitative traits, the variability of quantitative properties, and the influence of the environmental factors. However, the results of the research in many countries have confirmed that the use of standard statistical methods together with genomic selection increases reliability in predicting breeding values of animals.

That in today's conditions of science development, the goal of genomic selection of domestic animals is to connect all known sources of information (phenotype, origin and genetic markers) to obtain the highest accuracy of the assessed breeding value (EBV) and ensure genetic improvement.

Animal Breeding Assessment is a very complex process. It supports the fact that the selection with statistical methods in some areas such as resistance to disease, meat quality, fertility, is characterized in low efficiency due to the following factors (*Petrovic and Pantelic, 2015*):

• the low heritability of traits

• significant influence of environmental factors

• sex restrictions
• the manifestations of properties only under the influence of certain factors
• when the manifestation of the characteristics is relatively late
• the fact that the trait is difficult to measure (for example, health specifics)
• the presence of hidden carriers of traits

In the above cases and mentioned limitations, in the application of the standard statistical procedure and for a more accurate assessment it is necessary to analyze the offspring, which is necessary to wait until breeding maturity. However, the use of DNA markers makes it possible to analyze the genotype immediately after birth, without waiting for the manifestation of the trait or appearance of the progeny, which greatly accelerates the selection. On the other hand, if the selection was direct to properties with a high degree of inheritance, then the genomic selection will not bring significant benefits.

It has concluded that genomic selection should not negate the traditional approaches to the assessment of the nursing value of the animal. Statistical analysis and genomic selection technology complement each other. The use of genetic markers makes it possible to accelerate the animal selection process, and standard methods assess the effectiveness of this selection.

In practice, genomic selection will be a reliable "prognosis" for livestock production and will accelerate the process of selecting the most valuable animals. The main advantages of genomic selection are:

1. High accuracy of testing
2. New evidence and assessment characteristics
3. Very quick selection
4. Accelerated genetic improvement of animal husbandry

The advancement of molecular genetics has enabled the sequencing of the genome of several species of domestic animals in the last few years, partly or completely. Information on the whole animal genome becomes more interesting for researchers and breeders because they give the opportunity to identify genetic variations that produce different production performances *(Bai Y. et al.,2012).* This could also increase the chances of resistance to pathogens that slow down the production of animals.

These findings can also provide useful information in the production of healthy food for human consumption *(Bai X. et al., 2012).* The first genome sequencing has done in livestock *(Burt, 2005)* followed by pigs *Archibald et al. (2010)* cattle *Zimin et al. (2009)* horses *Wade et al. (2009*) and sheep (*International sheep Genomics 2010; Bai et al. 2012*). In the study of *Suárez-Vega et al. (2015*) the quality scores of the extracted RNA samples for each breed from four animals were sequenced for time points D10, D50 and D150,and three biological replicates

were sequenced for D120 have acquired a total of 1,116 million paired-end reads from the transcriptome sequencing of the 30 milk samples analyzed. The alignment of the reads to the Ovis aries genome Oar_v3.1 genome build have yielded a mean of 985.05 million reads (88.10%) that aligned to unique locations in the ovine genome per RNA-seq sample; a mean of 1.47 million reads (4.01%) per sample that aligned to multiple locations in the genome; however, a mean of 2.84 million reads (7.65%) per sample that did not align to any genome location.

The achievements of molecular techniques in practice

Xu et al. (2017) noted that selection intensity can be heightened significantly through improvements in the scale and precision of genotyping and phenotyping, that the breeding cycle time could shorten by accelerating breeding procedures through integrated breeding approaches such as marker-assisted selection and doubled haploid development. Genomic resources also provide a basis for genetic improvements of economically important traits in sheep with the identification of functional genes and variants associated with these traits helps facilitate traditional breeding techniques using quantitative approaches and molecular breeding techniques are cost-effective and time-saving compared to conventional crossbreeding schemes (*Song-song and Meng-hua 2017)* . In sheep and goat farming, traditional methods of selection are increasingly complemented by the modern DNA analysis, to detect the gene that affects the expression of particular production traits or are located near the site responsible for the given property *(Carillier et al., 2013, 2014).* As is well known, the use of genetic markers enabled the detection of genes responsible for expressing significant properties or determining their approximate location in the genome. Therefore, genetic markers are not genes that define production or other properties, but rather indicate a specific site in the genome that potentially finds these genes. In recent years, *Kijas et al. (2012); Zhang et al. (2013)* identified the genes that are responsible for some of the economically important traits of sheep and goats. Since the eighteenth decade of the twentieth century, the development of molecular genetics methods, sheep breeding has been among the first to meet modern selection procedures. Experimentally and in practice, all known genetic markers were then used. A special echo in the selection of sheep and goats had the application of microsatellites to characterize individual breeds and determine their genetic distances, or the population structure. These researches are actual today *(Kijas et al., 2009;Jevšinek Skok et al., 2015; Murital et al., 2015; Yilmaz et al.,2015; Zinovieva et al., 2015; Ocampo et al., 2016; Seilsuth et al., 2016; Souheil Gaouar and Samiakdidi 2016; Edea et al., 2017; Deniskova et al.,2018; Selepe et al., 2018).* Likewise the contribution of microsatellites markers in the clarification of the origin, genetic risk factors, and implications for conservation of

sheep breed has studied *(Sassi-Zaidy et al.2016).* Since 2010, the international consortium for the exploration of the genome of 23 sheep breeds developed Illumina Ovine SNP50K chip, a new phase of genomic selection of sheep begins. In recent years, more than 50,000 SNPs have tested to see their connection with individual production characteristics. Otherwise, the Illumina SNP50K chip is a small glass tile that has 12 panels where 50,000 SNPs have tested for each animal. This reveals which nucleotide was present in a particular SNP position. The signals received for each of the 50,000 SNP markers have calculated as "SNP marker genotype" (AA, AB or BB). In this way, the final result is in the form of a genotype for 50,000 SNP markers for each genotyped sheep. Since 2011, chips have used in copiers, and the Illumina SNP50K chip has been developed using the genome of 25 goats. For a relatively short period since finding this method to date, thousands of SNPs for which the position of the genome is accurately known and the consequence of the change in the nucleotide base have discovered. For example, some studies have shown that sheep with a heavy run in a particular SNP have a base of adenine (A), where the animals with a lighter run in the same SNP have a guanine base (G) *(Petrovic and Pantelic, 2015).* The application of genomic selection in sheep farming is increasingly taking place. Among the leading countries are Australia, New Zealand, Russia, China, and more precisely the countries with the most developed sheep breeding. When it comes to European countries, France is most prominent, especially in the Lacone breed. The SNP s58995.1 (rs420767326 A>G) in MEF2B gene and OAR3_115712045.1 (rs401775061 A>C) in TRHDE gene, which significantly associated with the post-weaning gain in sheep have discovered (*Zhang et al. 2013;2016)*. The MEF2B gene encodes the protein from the MEF2 family of which the interaction of proteins from the MEF2 family with the promoter myostatin gene of sheep has a stimulating effect on the expression of myostatin; a protein that limits muscle growth in mammals *(Du et al., 2007).* Therefore, the mutation of the MEF2B gene can have a significant effect on the production of sheep meat through the change in the production of myostatin which was confirmed in some other studies *(Chen et al., 2015).*The MEF2B is a new promising candidate gene defining the productive qualities of sheep *Trukhachev et al. (2016).*

The production and properties of milk in the Spanish breed of sheep Churra by *Garcia-Gamez et al. (2012)* and found a link to certain genes. Similar research was carried out by *Gras et al. (2016)* found a positive association between LGB and milk yield and composition which recommend this candidate gene like the marker for a future MAS program. Diacylglycerol O-Acyltransferase 1 (DGAT1) a gene turned out to be a functional and positional candidate gene for a major region on chromosome 14 closely associated with fat content *(Martin et al., 2017).*

Carillier et al. (2015) stated that the availability of SNP54k chips for goats has made it possible to genotype alpine and saanen goats in France. In both breeds,

genomic selection can improve annual genetic improvement by reducing the length of the father-son interval. The quality of predicting the value of an individual in this way is sensitive to the size of the reference population, which can affect the accuracy of genomic indicators.

Kim et al. (2016) had use caprine and ovine 50K SNP BeadChips from Barki goats that have identified several candidate regions under selection that spanned 119 genes. A majority of the genes were involved in multiple signaling and signal transduction pathways in a wide variety of cellular and biochemical processes. In particular, selection signatures spanning several genes that directly or indirectly influenced traits for adaptation to hot arid environments, such as thermo-tolerance (melanogenesis) (*FGF2*, *GNAI3*, *PLCB1*), body size and development (*BMP2*, *BMP4*, *GJA3*, *GJB2*), energy and digestive metabolism (*MYH*, *TRHDE*, *ALDH1A3*), and nervous and autoimmune response (*GRIA1*, *IL2*, *IL7*, *IL21*, *IL1R1*) were also identified. Genomic selection in sheep concentrates on all genetic aspects and production directions. However, it should point out that genomic selection in sheep farming, and especially in the case of chewing, is taking place slower in comparison with cattle breeding. However, this stated conditionally, since each of these animal husbandry branches has own significance, objectives and selection requirements depending on the different natural and social influences. In the study of *Nan et al.(2014*) have revealed thousands of differentially expressed genes, of which most were possibly associated with wool growth and several potential gene families might participate in hair growth regulation.

Results of SNP genotypes can be applied to determine the history and diversity of the population of sheep and goats in the framework of our country and the world. It is also possible to determine the origin of individuals, genetic variations associated with certain diseases, with some properties of carcasses, etc.
In the end, we can summarize how the genomic selection of sheep and goats has the following benefits:

• More precisely, predicting the genetic value for the desired breeding goal
• With properties that are usually difficult to improve
• For features that are difficult or expensive to measure
• For features that cannot be measured early
• Characteristics with low heritability, of such as yield and quality characteristics of meat, production of wool, reproductive rate, resistance to parasites.

Conclusion

Selection as the principle of improving animal production characteristics is a permanent process. By this developing science, the techniques of selecting animals are changing and improving. In addition to traditional selection, the development of molecular genetics has opened up a new era. Today, animal

science has gained a powerful tool called genomic selection. Combined with the traditional assessment of the breeding value of domestic animals through molecular indicators at the level of the genome, is the path to faster and more efficient genetic progress in the future.

Primena savremenih molekularnih tehnika u selekciji životinja

Milan P. Petrović, Marina I. Selionova, Violeta Caro Petrović, Dragana Ružić Muslić, Nevena Maksimović, Zoran Z. Ilić, Ivan Pavlović

Rezime

Razmotreni su i neki problemi savremene analize genoma domaćih životinja i mogućnosti primene rezultata istraživanja u selekciji. Analiza molekularne osnove domaćih životinja napredovala je brzo. Danas, koristeći savremenu analizu DNK, može se sa značajnom pouzdanošću predvideti odgajivačka vrednost domaćih životinja. Na osnovu intuicije naučnika početkom 20. veka, a posle pola veka otkriveni su genetski markeri. Na taj način je, tradicionalni metod selekcije integrisan sa molekularnim tehnikama i počelo se sa primenom selekcije pomoću markera (MAS). Mikrosateliti su privukli veliku pažnju naučnika jer su rasprostranjeni u genomu i imaju visok nivo polimorfizma. Nakon toga, hiljade SNP-a su otkriveni sa svojom tačnom pozicijom u genomu. Ovaj vrlo pouzdan metod za analizu genoma pokazuje promenu samo jedne nukleotidne baze u molekulu DNK. Savremena dostignuća molekularne genetike otvorila su put za praktičnu primenu u selekciji svih vrsta domaćih životinja. Danas je kombinacija molekularne ili genomske selekcije kombinovana sa tradicionalnom veoma pouzdanom metodom za bržu i preciznu procenu odgajivačke vrednosti životinja.

Ključne reči: domaće životinje, selekcija, molekularna tehnika, genetski markeri

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