ABSTRACT
of dissertation for the degree of Doctor of Philosophy (PhD)
6D070100 – Biotechnology

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"Camel's gene diagnostics with using DNA-technology and its implementation in agricultural production"

General characteristics of the work. This work considers the study of polymorphism of microsatellite loci of camels' gene pool from different areas of Kazakhstan based on the DNA technology and introduction of the high-milk genotypes' genetic profile into production.

Actuality of the research topic. Camel husbandry is a traditional area of agricultural production and is based on the effective use of desert and semi-desert zones for sustainable and intensive development of agro-industrial complex in arid regions.

In the arid zones of Kazakhstan, which occupy 25% of the entire territory of the country, where the maintenance of other types of dairy animals is difficult, the main source of milk production is dairy camel husbandry.

In modern conditions of the camel husbandry development, the priority direction of production is dairy, which requires a transition from extensive to intensive management.

The branch products of the camel husbandry – camel milk and products of its processing – are in demand both in the domestic market and in the external market (Customs Union, the European Union and the Asia-Pacific Region). The dairy camel husbandry in the south-west region of Kazakhstan has the opportunity to occupy the premium class niche, i.e. branded camel milk and shubat differing in dietality, healing properties and environmental friendliness.

Currently, domestic agricultural producers are interested in breeding highly productive camels and high-milk camel genotypes are in unlimited demand in both domestic and foreign markets for the development of the dairy industry.

In the camel husbandry, monitoring of genetic resources is not developed, i.e. genodiagnostics of camels of different populations using modern biotechnological methods of the DNA technology in order to identify, systematize and certify high-value genotypes for effective implementation in agricultural production.

Currently, domestic camel husbandry lacks the genetic characterization of camels in various zones based on the DNA technology.

In this regard, the genotyping of the gene pool of camels from different areas of Kazakhstan using the DNA technology and its introduction into agricultural production for the development of the dairy industry in the branch is a relevant area of research in biotechnology of farm animals.

Objectives and tasks of the research

The research objective is the genetic characterization of the gene pool of camels of the dairy direction of productivity in various zones of the south-west

region of Kazakhstan using the DNA technology and the introduction of high-value genotypes into agricultural production.

To achieve this objective, the following tasks were set:

- research of the milk production of the gene pool of camels of the milk direction of productivity in different zones of Kazakhstan;
- determination of population-genetic parameters of camels of different populations by microsatellite loci;
- establishment of the genetic profile of the milk productivity direction camels with creating a database;
- determination of the economic efficiency of the research.

Objects of the research. The objects of the research were populations of camels in different areas of dislocation of productive camel husbandry in Kazakhstan: Arys-Turkestan (Peasant Farm "Ussenov N.", LLP "Syzdykbekov A."), Karatau-Moiynkum (Peasant farm "Bagdat"), Balkhash Lake region (LLP "Daulet-Beket"), Caspian Sea region (LLP "Zhana-Tan") and Mangystau (LLP "Taushyk").

Materials of the research: The material for the research was camels of Arvana and Kazakh Bactrian breeds of the dairy direction of productivity. As a source of DNA, tissue samples (earmark) were used.

Methods of the research: In the process of the work, biotechnological research methods were used – the DNA technology of microsatellites and zootechnical methods.

The DNA samples isolated from histological samples using Diatom DNA and Elxtra Gene DNA Prep kits were obtained as research material. For DNA extraction, commercial kits from leading manufacturers were used: Gene Pak PCR Core; Qiagen; Litech; DNA technology; Diatom DNA; Extra Gene DNA Prep.

Based on Stock Marks primer set, the isolated DNA samples were amplified in 2720 Thermal Cycler amplifier. All works on isolation, amplification and sequencing were performed in accordance with the manufacturer's protocols, adapted to a specific set of reagents.

The choice of microsatellite loci was made in accordance with the recommendations of the European Society of Geneticists (FAO / SAG).

The polymorphism of DNA microsatellites was determined using AbI-310 sequencer. The DNA typing panel consists of 7 and 8 microsatellites, typical for genetic analysis of the origin of camels. The interpretation of graphical profiles of the results of genotyping of samples and determination of camel genotypes was carried out taking into account the recommendations of International Camelid Institute and International Society for Animal Genetics.

Population-genetic analysis was carried out according to generally accepted methods, taking into account the frequency, level of polymorphism and the degree of heterozygosity of alleles of microsatellite loci. Statistical calculations were carried out individually using Fortran Power Station statistical package and algorithmic programming. The database was created using Microsoft Office Access 2007.

Scientific novelty of the research: For the first time in Kazakhstan, the scientific and methodical foundations of the DNA technology of the dairy productivity direction camels were laid, providing a reliable assessment of the genetic profile of highly productive individuals by the microsatellite loci for the development of the dairy industry in different regions of the camel husbandry.

In the process of the research, the allelic pool of Arvana and Kazakh Bactrian camels was established in 7 and 8 microsatellite loci.

The level of genetic diversity, heterozygosity and the degree of inbreediness for each population were determined.

It was proved that the milk productivity of camels in different regions directly depends on their genotypes. The correlation coefficient between the camel genotype and milk productivity was 0.218±0.060 (P<0.001), and the relationship with milk fat content was 0.508±0.047, t_r=10.8, P<0.001. The influence of the camel genotype on milk productivity and fat content was high (η_x^2=0.370±0.041, F=9.0, P<0.001 and η_x^2=0.613±0.025, F=24.5, P<0.001). The variance analysis results confirmed that the influence of the camel genotypes in general on the indicators of dairy products is in the range of 30.0-65.6%.

Based on the carried out DNA-microsatellites, 612 heads of camels of the dairy productivity in various areas of the camel husbandry in Kazakhstan were identified and certified, including 300 heads of camel dams, 300 heads of colts, and 12 males-producers.

Practical value and implementation of the work results. For the first time, the genotyping of the milk productivity direction camels with the DNA microsatellite allowed to characterize the genetic profiles of different populations and establish genetic differences between populations.

The high efficiency of the DNA microsatellites in the identification and certification of the high-value genotype camels was proved.

The milk productivity of Arvana camel breeds bred in the area of the Caspian lowland and the Mangistau peninsula is relatively close, and the milk productivity of Arvana camel breeds bred in the Arys-Turkestan and Balkhash regions is relatively high compared to other populations (P <0.001). It was established that the level of milk productivity of species with a homogeneous pair of alleles found in the entire population of camels is 18.7% higher than that of heterogeneous peers, the net income from 1 kg of milk is 61.2 tenge with the profitability of milk production is 24.2% higher.

The electronic database of the high-milk camels' genetic resources was created based on the DNA technology in the amount of 612 head.

The main provisions for the defense:

- Dairy productivity in camels of different zones of Kazakhstan;
- Population-genetic parameters in camels of different populations by the microsatellite loci;
- Genetic profile of camels of milk productivity;
- Economic efficiency of the research.

Main research results and conclusions:

Based on the results of the work, the following conclusions were made:

- milk productivity of camels in the studied populations depends on their breed and regional characteristics. The average daily milk yield in camels of Arvana breed is 9.3 ± 0.06 kg, and of Bactrian breed – 6.5 ± 0.04 kg. The static difference between the compared groups of camels in terms of milk yield is highly reliable ($P<0.001$). The milk fat content in camels of Bactrian breed was high ($P<0.01$).

- no significant difference was found between different populations of camels in allelic frequencies. Moreover, 87 alleles were identified in the group of Arvana breed camels and 90 alleles in Bactrian population, i.e. 10.87 ± 1.26 and 11.25 ± 1.30 alleles per locus, respectively.

- it was revealed that the genetic diversity of the allelic profile of the camel population in Arys-Turkestan region is lower than in the group of camels from other regions. The number of alleles in the camel population in this region by 1.7 times ($P<0.01$), informational alleles by 1.3 times ($P<0.05$), effective alleles by 1.4 times ($P<0.05$) compared with the group of camels in other regions, and private alleles – 7.5 times less ($P<0.001$).

- it was found that there was no significant difference in the observed heterozygosity in the interregional population of camels. The actual level of heterozygosity in the population of camels of Caspian-Mangistau region was 0.689 ± 0.014%, in Balkhash-Karatau-Moinkum group – 0.717 ± 0.012% and in the populations of Arys-Turkestan – 0.691 ± 0.015%. However, in the group of camels in all regions, the difference between the actual and expected levels of heterozygosity was highly reliable ($P<0.001$), which means that the gene balance in the studied camel populations is disturbed.

- the level of heterozygosity by loci (F_{IS} and F_{IT} indices) ranged from 0.8 to 34.4%. Interpopulation genetic differences (F_{ST} index) for microsatellite loci averaged 4.6%. I.e. 95.4% of genetic changes in the studied populations of camels are intrabreed, and 4.6% are interbreed. This is a very high intrabreed diversity, therefore, it is necessary to restore the polymorphism in the populations through breeding work.

- the greatest similarity of microsatellite alleles was observed in Taushyk - Bagdat population, and genetic differences - in Taushyk - Daulet-Beket camel group.

- it was proven that milk yield in camels of different ecotypes directly depends on their genotypes. The correlation coefficient between the camel genotype and milk yield was 0.218 ± 0.060 ($P<0.001$), and the correlation with the fat content in milk was 0.508 ± 0.047 ($t_r = 10.8$, $P<0.001$). The influence of the camel genotype on milk yield and fat content was high = 0.370 ± 0.041, $F = 9.0$, $P<0.001$ and = 0.613 ± 0.025, $F = 24.5$, $P<0.001$). The results of analysis of variance confirmed that the influence of common camel genotypes on dairy products is in the range of 30.0-65.6%.

- the milk productivity of Arvana camels grown in the Caspian region and on the Mangistau peninsula is relatively close, while Arvana camels grown in Arys-Turkestan and Balkhash regions are relatively high compared to other populations

(P<0.001). It was revealed that milk productivity in an individual with homozygous allele types is 18.7% higher than in their peers. At the same time, the net income from 1 kg of milk is 61.2 KZT, and the profitability of milk production is higher by 24.2%.

- as a result of genetic monitoring of camel populations bred in different regions, a genetic information and computer base was created, and 300 heads of highly productive camels were certified.

Personal contribution of the author. All the results of the dissertation work were obtained in the presence and personal participation of the author. The author independently analyzed the literature data on the research topic, experimental research, processing and analysis of the research results, writing and formatting the dissertation manuscript.

Connection with the plan of the main scientific works. The dissertation work was carried out within the framework of the budgetary scientific and technical program "Sustainable management of genetic selection processes in animal husbandry", projects "Development of a system for managing genetic processes and its intensification in a camel farm", "Identification, systematization, documentation of camel genetic resources for milk production" (State registration number 0115PK02579).

The main results of the dissertation work: The main provisions of the dissertation research were reported and discussed in the following international scientific practical conferences:

- International scientific and practical conference "Fundamental and applied scientific research" (2018, Berlin, Germany);
- Proceedings of V International conference "Industrial technologies and engenering", M. Auezov South Kazakhstan State University (ICITE-2018, Shymkent, Kazakhstan);
- Collection of materials of XXXIII international scientific and practical conference "International scientific discoveries 2018" (2018, Moscow);
- XXXI international scientific and practical conference "Actual problems of modern science" (2018, Moscow – Astana – Kharkov – Vienna);
- Materials of scientific and theoretical international conference "Innovative technologies and prospects for the development of professional education in the conditions of globalization". Tadzhik Pedagogical Institute in the city of Panjakent (2018, Panchakent);
- International science. Chemistry, physics, biology, mathematics: theoretical and applied research. Collection of articles by materials of XXIII international scientific and practical conference (2019, Moscow).
- International scientific practical conference. IX Global Science and Innovations 2020: Central Asia (2020, Nur-Sultan, Kazakhstan).
- Collection of articles on the materials of LXX international scientific and practical conference ("Innovative approaches in modern science" 2020, Moscow).

Publication of the research results. According to the main scientific results of the dissertation, 16 articles were published, 5 of them in scientific editions recommended by the Committee for Control in the Sphere of Education and

Science of the Republic of Kazakhstan, 2 articles in scientific editions included in the international database Scopus, Web of Science, 1 thesis and 7 articles in materials of international conferences and 1 abstract in the edition of foreign conference.

The structure and scope of the dissertation. The dissertation consists of 112 pages, including normative references, definitions and abbreviations, literature review, research objects, methods and results of the research and their analysis, conclusion, list of references. The amount of the used literature consists of 171 titles, 42 tables, 10 images and 8 appendixes.