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Assessment of genetic diversity using microsatellite markers and milk productivity of Mugalzhar horses

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Abstract

Background and Aim. At "Chromtau Beef" LLP in Aktobe region, Mugalzhar horse breed is primarily used for meat and milk production. This largely depends on improving breeding techniques through introduction genetic achievements in farm and identifying and realizing genetic potential for productivity and breeding quality in production. The purpose this research was to study the zootechnical and genetic characteristics of modern population horses Mugalzhar breed using DNA markers.

Materials and Methods. In this study, we studied polymorphism microsatellite markers to assess genetic differentiation sample Mugalzhar horses. Data on 17 microsatellite loci 20 heads horses from Aktobe population were used. The results were compared using GenAlEx 6.503 program: FST was calculated using matrix distances between alleles; according to matrix distances between genotypes.

Results. The study zootechnical characteristics revealed that horses did not differ from breed standard in measurements. The milk production linebred mares varied considerably during 105 days lactation (average milk production Bekzat, Bau and Paluantora mares was 1701.0, 1492.05 and 1431.15 litres, respectively). Higher productivity was observed at 2-3 months lactation, after which milk yields gradually decreased and more sharply towards end lactation. Estimates genetic differentiation breed were obtained: FST = 0.028, matrix distances between genotypes = 0.015. A high correlation of FST and GD with Nei polocus differentiation scores was found (100). The inbreeding coefficient Fis for studied loci was found to have negative value (-0.014), which shows predominance heterozygous genotypes in Mugalzhar breed horse population.

Conclusion. The coefficients variability (Cv) for both size and live weight are low, indicating uniformity horses. Our research has shown that Mugalzhar mares from different lines have different levels milk production. Simulation Mugalzhar breed with GD close to zero resulted in increase in number alleles per locus by 40%. It is advisable to use results obtained in development breeding measures to preserve diversity of breed.

Key words: DNA marker; horse breeding; Mugalzhar horse breed; selection; STR.

Introduction

Horse breeding in Kazakhstan has always held a special place among other branches of animal husbandry. Productive horse breeding is essential for the population of the Aktobe region, where three local breeds are raised: Kushum, Mugalzhar, and Kazakh horse Zhabe type [1]. All three breeds are highly valued for their adaptability to the steppe, desert, and semi-desert conditions of Kazakhstan. These hardy breeds have excellent meat and dairy qualities [1].

At the LLP "Chromtau Beef" in the Aktobe region, the Mugalzhar breed is primarily used for meat and milk production. To increase productivity, it is necessary to constantly improve these horses. This largely depends on enhancing breeding techniques through the introduction of genetic achievements in the farm and identifying and realizing the genetic potential for productivity and breeding quality in production [2]. DNA analysis using microsatellite markers has been widely used in animal species to identify individuals, verify paternity, and preserve endangered animals. It has also been used to study the genetic diversity and origin of local animals and promote their conservation [2-3].

Countries around the world have used microsatellite markers since the mid-1990s to track the origin and hereditary characteristics of local animals. This information is important for improving production on farms and promoting the conservation of these animals [4, 5].

An important aspect of increasing production on farms is improving the reproduction of horses. In 2020-2023, the output of foals per 100 mares was 79-81 heads.

In the future, "Chromtau Beef" LLP plans to increase the number of Mugalzhar horses and improve their quality through targeted breeding work.

The dietary value, nutritional benefits of horse meat and koumiss compared to other types of agricultural products, the high profitability of their production, and the low cost contribute to an increase in production of these valuable food products in Kazakhstan [6].

Mares of different types and lines have different levels of productivity, both in terms of quantity and quality, under the same conditions of feeding and care. A comparative study of their economically useful characteristics has contributed to the selection of mares suitable for specific conditions, opening up additional opportunities for increasing dairy productivity [1-3].

The aim of these studies was to investigate the zootechnical and genetic characteristics of modern livestock from the domestic horse population of the Mugalzhar breed, using DNA markers. This allowed us to identify polymorphisms, as well as identify typical alleles present in representatives of this breed, in order to select a sample of horses for future genome-wide research.

Materials and Methods

Scientific and economic experiments were conducted at the seasonal kumis farm of "Chromtau Beef" LLP in the Aktobe region in 2023.

There were three groups of mares involved in the experiment, each with 5 members. Each mare was measured and weighed, and four measurements were taken: height at the withers, oblique body length, chest circumference, and metacarpal circumference [7].

Milking was done manually, five times a day with breaks of 2-2.5 hours between each milking. Mares were only milked during the day and kept together with their foals at night in the pasture. Commercial milk production was determined during lactation using the method of controlling milk yields twice a month for two days.

The milk productivity was calculated by taking into account the milk sucked by the foal at night, according to the formula proposed by I.A. Saigin [8]:

$$Yc = Yd / t \times 24, \quad (1)$$

where, Yc – estimated amount of milk in 24 hours, kg; Yd – the actual amount of milk received per day, kg; t – the time period from the beginning to the end of milking mares during the day, hours; “24” – hours per day

All experimental data were analyzed using biometric methods according to N.A. Plokhinsky [9].

Microsatellite markers and DNA genotyping data from 20 Mugalzhar horse heads were used for scientific research. The STR - genotyping process was carried out in the Genetics laboratory of the Kostanay Regional University named after Akhmet Baitursynov, using the ABI 3100 genetic analyzer from Applied Biosystems. Genotyping was performed at 17 DNA loci, including VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, and HMS1.

PCR products were analyzed using an automatic gene analyzer (ABI 3100 Genetic Analyzer, USA), and subsequent electrophoresis was conducted on POP 7 polymers (Applied Biosystems, USA). Based on the data from a series of peaks, the sizes of alleles (in base pairs) were determined for each marker, using the results of an equine test conducted by the International Society of Animal Genetics (ISAG), and the GeneMapper software from Applied Biosystems (USA). The study of the genetic characteristics of the Mugalzhar horse breed was conducted using population-genetic analysis based on the frequency of types and alleles for 17 microsatellite loci. The level of polymorphism, effective number of alleles at each locus, and subpopulation inbreeding coefficient (Fis) were calculated. Allele frequencies and number of alleles per locus were estimated through direct calculation based on observed genotypes. Observed heterozygosity (Hobs), expected heterozygosity (He), number and frequency of allelic variants, and polymorphism information content (PIC) values for the breed were also calculated using GenAlEx 6.503 [10].

Results

In "Chromtau Beef" LLP, foaling of mares took place in mid-March and early April. Milking of the mares began in early May, which is a month after foaling.

For a complete zootechnical characterization of the development and body type of the mare, measurements and weights were taken. The data from these measurements and live weights are presented in Table 1.

Table 1- Measurements and live weight of mares from different lines

Indicators	Measurement parameters, centimetre (cm)				Live weight, kg
	Height at the withers	Oblique body length	Chest circumference	Metacarpal circumference	
Paluantora line 136-91					
M+m	144.6±0.37	151.8±0.43	184.3±0.68	19.3±0.12	493.5±2.36
lim	143-145	149-152	183-185	19-20	470-500
σ	0.84	1.12	1.58	0.27	7.85
C_v	0.58	0.74	0.86	1.40	1.59
Bau line 208-96					
M+m	145.2±0.40	152.5±0.49	183.8±0.71	19.0±0.10	490.2±2.12
lim	144-146	148-152	181-184	18.5-19.5	460-495
σ	0.79	1.09	1.52	0.22	7.66
C_v	0.54	0.71	0.83	1.16	7.56
Bekzat line 187-91					
M+m	144.1±0,46	151.2±0.51	183.2±0.74	19.1±0.08	485.3±2.41
lim	143-145	148-152	180-183	19-20	450-490
σ	0.75	1.06	1.49	0.21	7.62
C_v	0.52	0.70	0.81	1.10	1.57

From the data in Table 1, we can see that the experimental mares, which were acquired from the Mamyr-Aktobe farm when they were 2 years old, and which had already reached the age of 5 at the farm of "Chromtau Beef" LLP, did not differ from their line representatives. Mares from the Bau 208-96 and Paluantora 136-91 lines exceeded the mares from the Bekzat 187-91 line in height by 1.1 and 0.5 cm at the withers and in oblique body length by 1.3 and 0.6 cm, respectively, and in chest circumference by 0.6 and 1.1 cm. In terms of live weight, the Paluantora mares exceeded the Bau and Bekzat mares by 3.5 and 8.2 kg.

The coefficients of variability (Cv) for both size and live weight are low, indicating the uniformity of the horses. Our research has shown that the Mugalzhar mares from different lines have different levels of milk production (Table 2).

Table 2 - Milk production of mares from different lines

Live weight, kg	Actual milk yield		Milk		
	yield per day	For 105 days of lactation	Per day	For 105 days of lactation	Per 100 kg of live weight
493.5	Paluantora line 136-91				
	5.68±0.15	596.4±4.92	13.63±0.31	1431.15.15 ±20.3	290
490.2	Bau Line 208-96				
	5.92±0,17	621.6±5.92	14.21±0.35	1492.05.05±22,6	304
485.3	Bekzat Line 187-91				
		708.7±4.50	16.20±0.43	1701.0±28.3	351

From the data in Table 2, we can see that for 105 days of lactation, the average milk production of mares from the Bekzat, Bau, and Paluantora lines was 1701.0, 1492.05, and 1431.15 liters, respectively.

The commercial milk yield from mares in each line was 708.7, 621.6, and 596.4 liters for the Bekzat, Bau, and Paluvantora lines, respectively. Thus, the Bekzat mares produced 14% more milk than the Bau mares and 19% more than the Paluvantora mares.

According to the milk production index (per 100 kg of live weight), the Bekzat and Paluvantora lines showed high performance, with 351 and 290 liters per 100 kg, respectively, while the Bau line was slightly lower at 304 liters.

It is worth noting that the milk production of linear mares varied significantly during 105 days of lactation. Higher productivity was observed at 2–3 months of lactation, after which milk yield gradually decreased and more sharply towards the end of lactation (Table 3).

Table 3- Change in milk production of mares of different lines s by months of lactation, liters

Milk production indicators	Lactation month			
	May II	June III	July IV	August V
Paluantora line 136-91				
Per day	14.40±0.31	15.17±.23	14.16±0.27	13.10±0.26
Per month	432.0±5.08	470.22±7.20	438.90±5.24	170.36±3.36
Bau Line 208-96				
Per day	13.78±0.26	14.45±0.32	13.63±0.27	12.53±0.29
Per month	413.28±7.74	447.90±4.93	422.60±4.98	162.86±2.07
Bekzat Line				
187-91 Per day	16.42±0.033	14.48±0.35	16.35±0.32	14.57±0.28
Per month	492.70±4.12	542.06±5.50	506.97±5.02	189.43±3.39

From the data in Table 3, we can see that the milk production of mares from the Bekzat, Bau, and Paluantora lines at the 2nd month of lactation was 492.7, 413.28, and 432 liters, respectively. At the 5th month, these values were 189.43, 170.36, and 262.86 liters.

This suggests that with seasonal milking and the selection of dairy rather than meat mares on farms producing koumiss, it is possible to increase milk productivity significantly.

To identify the genetic diversity and relationships among individuals in the Mugalzhar horse breed, 20 horses with the desired phenotype were genotyped using 17 microsatellite markers. Microsatellites are useful in identifying the paternity of animals [11-14] and studying the structure of populations [15-17]. They have been widely used in breeding control programs for cattle, pigs, horses, and dogs. In most countries, these controls are based on microsatellite typing, standardized through regular comparative tests conducted by the International Society for Animal Genetics (ISAG).

Horses of the Mugalzhar breed stand out for their high genetic diversity in microsatellite loci. When we analyzed 17 microsatellite loci in these horses, we found 122 different alleles, with the number of alleles per locus ranging from 9 (AHT4) to 12 (ASB17) (Table 4).

Table 4 - Genetic characteristics of horse polymorphism of the Mugalzhar breed based on 17 DNA microsatellites

Locus	N	Na	Ne	I	Ho	He	uHe	Fis
AHT4	20	9.000	5.594	1.915	0.800	0.821	0.842	0.026
AHT5	20	7.000	5.442	1.777	0.750	0.816	0.837	0.081
ASB17	20	12.000	6.897	2.163	0.900	0.855	0.877	-0.053
ASB2	20	9.000	5.594	1.892	0.850	0.821	0.842	-0.035
ASB23	20	7.000	4.188	1.651	0.600	0.761	0.781	0.212
CA425	20	7.000	2.909	1.330	JUST 0.700	0.656	0.673	-0.067
HMS1	20	6.000	2.712	1.286	0.500	0.631	0.647	0.208
HMS2	20	6.000	5.031	1.684	0.650	0.801	0.822	0.189
HMS3	20	6.000	3.865	1.512	0.800	0.741	0.760	-0.079
HMS6	20	7.000	4.848	1.733	0.900	0.794	0.814	-0.134
HMS7	20	5.000	2.974	1.297	0.750	0.664	0.681	-0.130
HTG10	20	6.000	2.532	1.141	0.600	0.605	0.621	0.008
HTG4	20	6.000	3.636	1.507	0.800	0.725	0.744	-0.103
HTG6	20	5.000	3.042	1.322	0.600	0.671	0.688	0.106
HTG7	20	6.000	3.636	1.450	0.750	0.725	0.744	-0.034
LEX3	20	9.000	6.061	1.982	0.500	0.835	0.856	0.401
VHL20	20	9.000	6.015	1.924	0.900	0.834	0.855	-0.079
N - sample size, Na- number of alleles, Ne - the number of effective alleles, I-information index, Ho - observed heterozygosity, He - expected and uHe -unbiased expected heterozygosity and Fis the coefficient of inbreeding.								

The genetic structure of the Mugalzhar breed is characterized by a relatively high variability in the frequency of occurrence of alleles (Fig.1)

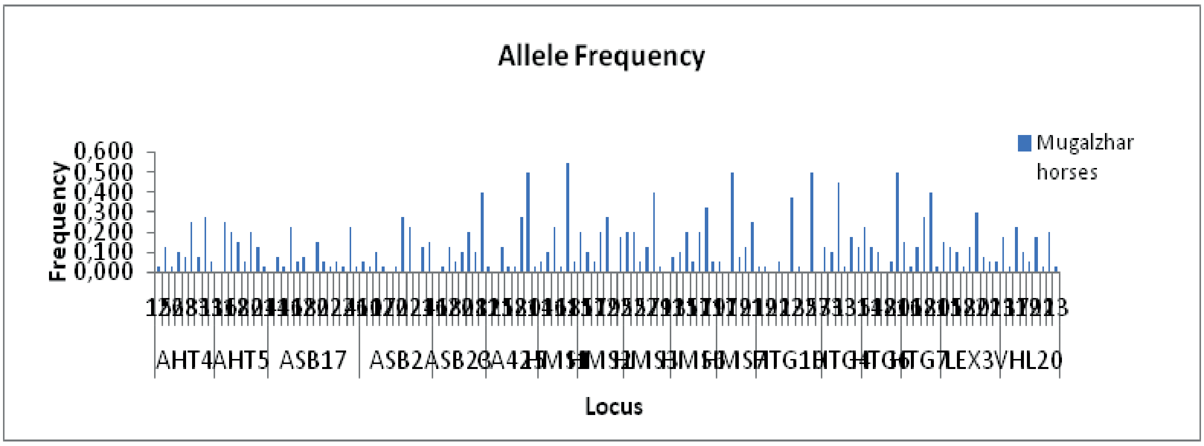

Figure 1- Allele frequencies for a population of Mugalzhar horses (n=20) with a plot by loci for codominant data

The average number of effective alleles across all studied loci, or the average level of polymorphism (Ne), is of interest in order to preserve genetic intra-breed diversity. In the Mugalzhar horse breed, the average number of effective alleles per locus was found to be 4,410. The polymorphism level of the tested loci ranged from 1,286 to 1,982, with the minimum and maximum observed heterozygosity values being 0.500 (HMS1) and 0.900 (ASB17) (Fig. 2). The average inbreeding coefficient (Fis) for the studied loci was found to have a negative value of -0.014, indicating the predominance of heterozygous genotypes within the population of Mugalzhar horses.

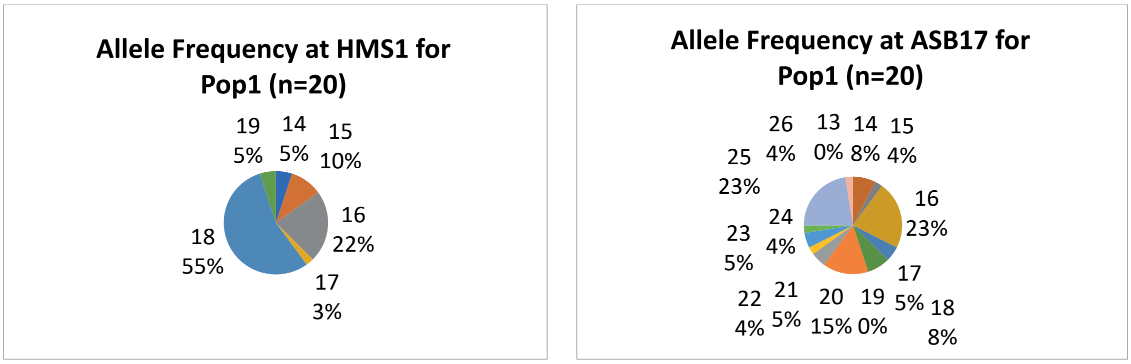

Figure 2- Frequency of alleles by population for HMS 1 and ASB 17

Discussion and Conclusion

122 alleles of STR loci were identified in the tested horses of the Mugalzhar breed, which indicates a significantly high level of polymorphism in microsatellite DNA. Within the sample, the observed heterozygosity (Ho) exceeds the expected heterozygosity (He), indicating the preservation of genetic diversity. The inbreeding coefficient (Fis) showed negative values, indicating a low level of inbreeding.

Other researchers [18] in the genetic monitoring of the Mugalzhar horse breed of two inbred types (Kozhamberdi and Kulandi), the allele pool of horses was investigated for 16 DNA microsatellites and from 5 to 13 alleles were identified. The data obtained characterised the polymorphism of each of the markers. The highest level of polymorphism in Mugalzhar horses was observed in the ASB17 locus, which also corresponds with our results.

Evaluation of selection and genetic parameters of the main economic and biological traits of horses of the Mugalzhar breed, gives the opportunity to obtain a complete productive and breed characteristic of the horse stock, both zootechnical and genotypic analysis by DNA-technology, which in modern conditions are included in the organisation of centralised breed registration.

Selection work in productive horse breeding is a continuous process. The practical significance of this work is to confirm the origin of animals by 17 STR loci and to identify unrelated individuals in the sample of horses of the Mugalzhar breed of the Aktobe population, which includes representatives of outstanding lines of this horse breed. Further, whole genome sequencing (WGS) of the obtained DNA samples will allow conducting research and searching for breed-specific SNP polymorphisms.

The Mugalzhar breed is characterized by a high level of genetic diversity, as evidenced by the high observed heterozygosity and low inbreeding values. This indicates that the breed has the potential for further improvement in terms of productivity and can be used as a source of genetic diversity for other breeds. Breeding should continue along the most promising lines, such as the Bekzat and Bau lines, to maximize the benefits of heterosis on a molecular genetic level. The data obtained will be used for future comparisons with the allele pool of other local breeds in order to update the genetic database.

Authors' Contributions

ShK, KI and SR: Conceptualized and designed the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. ZhB, DK and ST: Conducted the final revision and proofreading of the manuscript. All authors have read, reviewed, and approved the final manuscrip.

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