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Phenotype and Genotype Differences of Meat Type “Tselinny” within Kalmyk Horse Breed

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Abstract

In the Republic of Kalmykia the horse breeding segment is reaching up to 6 % of the whole agricultural industry. The number of horses has the tendency to increase. This is determined by the low cost, as well as by environmental friendliness of keeping horses, and horses' adaptability to harsh conditions. There are four breeding plants on the territory of Kalmykia, which are actively involved into Kalmyk horses breed rearing. Recently a distinct meat type was isolated within the Kalmyk breed, which was named ``Tselinny'' meat type (further Tselinny). The goal of the presented paper is to compare purebred horses with pool of horses belonging to newly isolated type Tselinny. Genotype of both populations consists of 14 and 15 alleles, as 84.4 % and 78.8 % of horses have these alleles (purebred and Tselinny type respectively). Animal of Tselinny meat type do not have 16 and 17 alleles, while more than 10 % of purebred horses of Kalmyk breed have these two alleles in their genotype. The difference in genotype is confirmed by the difference in phenotype: these are difference in size and meat productivity of horses. Both stallions and mares of Tsenilly meat type are bigger, and have higher weight. When we come to the meat productivity, the weight of the meat from young animals of Tselinny type was by 21 % (45 kg) higher. Horses belonging to the Tselinny type differ from purebred animals of Kalmyk breed both in genotype and phenotype. At the same time, exemplar of both groups keep features of Kalmyk horse breed. It is important to develop different types within the breed, based on the intended goal of breeding.

Keywords: Kalmyk horse breed, intrabreed type, genotype, phenotype

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1. Introduction

During last centuries horse breeding is traditional activity of kalmyk people. If before horses were most widely used in army, nowadays horses are breeded because of all the advantages of herd animal husbandary. The main advantage of herd husbandary is the low cost per kg of meat of animals. If we suppose that the production cost of 1 kg of beef meat is at 100 %, then the production cost of 1 kg of lamb meat would be 74 %, and the production cost of 1 kg of horsemeat would be the lowest and will make 50 % [3].

One of the conditions of the herd horse breeding to be profitable is to breed those horse breeds, which are most adapted to the local live conditions. To all the conditions of the are of Republic of Kalmykia is most adapted horses of Kalmyk breed. This horses are tough, proper body type, they can use local grazing lands during the harsh winter and summer drought [1].

Currently, simultaneously purebred animals as well as mixed blood horses are breeded. Sevaral techniques are used to make new specialyzed breed types with a higher meat mass, for example, Russian sturdy plow horses and Soviet sturdy plow horses are mixed with the purebred animals of different breeds. As a result of many years long selection process, it was meat type Tselinny isolated within the Kalmyk breed. The type is characterised by the higher meat productivity, and at the same time, the horses belongin to the type keep the unique adaptative ability of the Kalmyk breed.

In the Russian Federation and abroad, DNA information is widely used for animals breeding [2, 4], as well for sheep breeding, pig breeding [6--8], camel breeding [9] and horse breeing [10, 11]. One of the most important points is to keep aborigen breeds of animals, as the global community associates with such breeds the keeping of cultural traditions.

Any breeding process starts with defining of the gene pool of the breed. Thus, one of the main goals of our research was to reveal the main features of the genetic profile of purebred horses of Kalmyk breed, and comparison of the obtained genetic profile with the created new meat intrabreed type within Kalmyk horse breed, which was named ``Tselinny'' (further we refer it as Tselinny type). As well, one of the goals of the investigation was to compare the productivity of the Kalmyk breed with the new type within it.

2. Methods and Equipment

The object of the investagation is group of horses of Kalmyk breed from the Breeding Stud named after 28th Army, breeding reproducing ``Kirovsky'' of Yashkul district and horses of the new meat type Tselinny from ``Angai'' farm of Tselinny district Republic of Kalmykia. Stallions, mares and young animals at the age 2.5 years were included into the analysis.

Phenotype indicators included live weight, body measurements, as well as some numbers were obtained by weighting the meat obtained from horses. Genotyping experiments to distinguish alleles of horses were done using PCR. PCR was proceed by means of PCR-machine «Terzik», using GenePakTMPCRCore kit (IsoGene, Moscow)

with PCR-primers: (GA)9C and (AG)9C. After analyzing, we found out 30 alleles, the frequency of alleles was calculated, as well as its frequency and number in genotype.

Live weight of the adult stallions and mares was defined while weghting in the mornig before giving them water and food with the accuracy up to 1 kg (number of animal measured is: 36 and 12, 754 and 66 respectively). At the age of 2.5 years to control the meat weight, control slaughtery of young stallions was performed (n=7 and 16). Live weight before slaughtery (after 24 hours without food), weight of meat and offals in kg and percentage from the whole weight, as well as amount of protein and fat in muscles were analyzed for all animals.

Exterior was defined using following indexes: height at the withers, torso length, chest girth, metacarpal circumference with the accuracy up to 0.1 sm.

Laboratory investigations were performed at the ``Biovet'' laboratory within Kalmyk State University after B.B. Gorodovikov.

For the analysis 10 ml of blood from jugular vein was gathered into the tubes with Trilon B (etrasodium salt of ethylenediaminetetraacetic acid (Na4EDTA)). DNA was isolated from the gathered blood. Then, for molecular biology anaysis, ISSR technique was used together with PCR technique.

For PCR analysis it was amplification machine «Terzik» used, for PCR master mix we used GenePakTMPCRCore (IsoGene, Moscow) together with PCR primers (GA)9Si(AG)9C. The amplification scheme was: 94-95℃ (denaturation step) for 120', then 35-37 PCR cycles. Each cycles contained 30' denaturation step (94-95℃), annealing for 30' (at 55℃), and elongation stage: 120' at 72℃. Final elongation was at 72℃ during 10 min.

Mixture of PCR products was divided using 1,5 % agarose gel electrophoresis. After colouring the bands of DNA using ethidium bromide, they were visualazed under UF-light. Electrophoresis was run during 30 min under 120 V. The electrophoregramm has revealed different heterogenity as well as polymorphic differentiation.

Allels of 30 loci were revealed, and their frequency was analyzed. Mathematical analysis was applied, and breed-specific allels were indicated, as well as genotypes of horess belonging to new meat type and horses belonging to purebred pool of Kalmyk breed.

3. Results

3.1. Results of study of genotype of two pools of Kalmyk breed horses: purebred horses and intrabreed meat type Tselinny.

After the analysis of genotype of two different pools (new meat type and purebred horses of Kalmyk breed) it was found, that horses from 2 pools are different in their allele composition (Table 1).

The analysis of data from Table 1 has shown, that there both mutual alleles and difference in alleles frequency presented. Most frequent mutual alleles are A21 (0.948 at Tselinny type pool and 0.912 at purebred pool); A23 (0.879 & 0.912 respectively); A18 (both 0.845). Alleles A30 and A3 are quite common. It is supposed, that these alleles mark the Kalmyk breed in general.

Another alleles are both rare met in both populations. These are following alleles: A29 (0.017 & 0.027); A28 (0.017 & 0.041; A32 (0.034 & 0.054); A25 (0.034 & 0.048).

Significant difference observed while analysing allele A22, which is not presented at the population of Tselinny type, but was detected at the genotye of 49 purebred horses (0.333).

Purebred horses are characterized by significantly lower frequency of alleles A5 (0.088 & 0.379); A10 (0.204 & 0.603); A12 (0.116 & 0.448; A26 (0.061 & 0.638), at the same time they have higher frequency rate for following alleles: A11, A14, A17, A27 and A33 (0.721 & 0,345; 0.252 and 0.121; 0.340 and 0.155; 0.966 and 0.397; 0.639 and 0.345 -- these are frequency rates for purebred horses and horses of Tselinny type for listed alleles accordingly). Allele A26 is supposed to be special marker, which can be used for determination of Tselinny type. It has frequency rate at Tsenilly type 0.638, which is 10 times higher than it's frequency rate at purebred population.

Thus, mutual alleles for all horses of Kalmyk breed, including rare and frequent alleles were described. As well, differences between their frequency in the populations of ``Tselinny'' type, as well as in Kalmyk horse breed.

Using number of alleles which were encountered, it was defined the number of markers for each genotype (Table 2).

The analysis of alleles frequency rate shows, that the genotype, associated with 12 and 13 alleles is very rare in both populations, and significant differences in 14 allele. Frequency rate of 14 allele is 0.810 at the population of Tselinny type, and two times lower at the purebred population. In case of allele 15, the frequency rate is 4 times higher in the population of purebred horses. Alleles 16 and 17 are extremely rare in

Table 1: Alleles frequency of purebred horses of Kalmyk breed and horses of new meat type Tselinny.

№	Alleles	Intrabreed new meat type Tselinny		Purebred horses of Kalmyk breed	
		Number of horses	Frequency	Number of horses	Frequency
1	A5	22	0.379	13	0.088
2	A6	8	0.138	28	0.190
3	A7	26	0.448	93	0.633
4	A8	34	0.586	98	0.673
5	A9	11	0.190	53	0.361
6	A10	35	0.603	30	0.204
7	A 11	20	0.345	106	0.721
8	A 12	26	0.448	17	0.116
9	A 13	25	0.431	88	0.599
10	A 14	7	0.121	37	0.252
11	A 15	39	0.672	127	0.864
12	A 16	40	0.690	88	0.599
13	A 17	9	0.155	50	0.340
14	A 18	49	0.845	124	0.844
15	A 19	4	0.069	15	0.102
16	A 20	53	0.914	136	0.925
17	A 21	55	0.948	134	0.912
18	A 22	0	0	49	0.333
19	A 23	51	0.879	135	0.918
20	A 24	27	0.466	90	0.612
21	A 25	2	0.034	7	0.048
22	A 26	37	0.638	9	0.061
23	A 27	23	0.397	142	0.966
24	A 28	1	0.017	6	0.041
25	A 29	1	0.017	4	0.027
26	A 30	40	0.690	130	0.884
27	A 31	42	0.724	130	0.884
28	A 32	2	0.034	8	0.054
29	A 33	20	0.345	94	0.639
30	A 34	42	0.724	99	0.673

purebred population; meanwhile these alleles are not met at the population of Tselinny type.

Table 2: Comparison of genotypes of two populations of horses.

№	Number of alleles	Intrabreed meat type «Tselinny»		Purebred horses of Kalmyk breed	
		Number of horses	Frequency	Number of horses	Frequency
1	12	3	0.051	3	0.020
2	13	6	0.103	11	0.075
3	14	47	0.810	57	0.388
4	15	2	0.034	59	0.401
5	16	0	0	15	0.102
6	17	0	0	2	0.014

Consequently, main population of Tselinny type has in its genotype 14 alleles while purebred population have 14 and 15 alleles, and 10 % of horses from this population characterized by 16 alleles.

The differences in allele composition are confirmed by differences in phenotype (Table 3).

Table 3: Comparison of characteristics of body's measurements and live weight of stallions and mares belonging to different pools within Kalmyk horse breed: purebred animals and type Tselinny.

Indicators	Stallions		Mares	
	Purebred pool of Kalmyk breed (n=36)	Pool of intrabreed Tselinny type (n=12)	Purebred pool of Kalmyk breed (n=36)	Pool from intrabreed Tselinny type (n=12)
Height at the withers, sm	150.8	156.2	147.1	150.7
Body length, sm 2.2	156.1	163.5	152.2	158.5
Chest circumference, sm 2.8	180.8	192	176.1	183
Metacarpal circumference, sm 2.6	20.4	22	19.1	21
Live weight, kg	466	532	435	473

From the Table 3 we conlcude, that stallions of Tselinny type are bigger than purebred stallions: difference in height at the withers is 5.4 sm (3.4 %), diference in body length is 7.4 sm (4.7 %), diference in chest circumference is 11.2 (6.2 %), and difference in metacarpal circumference is 1.6 sm (7.8 %).

Average live weight of stallions of Tselinny type is 532 kg, which is 66 kg (12.5 %) heavier than average weight of purebred stallions. Similar situation was observed for mares: mares of Tselinny type are bigger and heavier than purebred mares. Horses of Tselinny type outperform purebred animals not only in live weight, but also in the amount of meat, which can be obtained after horses slaughtery (Table 4).

Two groups of young mares stallions slaughtered at the control point of 1,5 years: there were 16 stallions of Tselinny type, as well 7 purebred stallions. It was found, that average mass fraction of meat is 56 % for young animals of Tselinny type and 54 % for purebred stallions.

Average live weight of 2.5 years stallions belonged to Tselinny meat type is 462 kg, weight of meat (1 category) 258.7 kg, for 2.5 years stallions of purebred pool this numbers are 396 and 213,8 kg respectively. Weight of subproducts was higher in kg, but calculated in percnets from the whole weight it was lower: 11.1 kg and 12.9 kg or 5.2 % and 5.0 %.

Table 4: Weight of meat after 2.5 years stallions slaughter.

Indicators	Purebred pool of Kalmyk breed (n=7)	Pool of intrabreed ``Tselinny'' type (n=16)
Average live weight, kg	396	462
Meat weight, kg	213.8	258.7
Meat, %	54 %	56 %
Subproducts weight, kg	11.1	12.9
Subproducts, %	5.2 %	5 %
Protein in muscle's sample, %	20.2	22.6
Intramuscular fat, %	9.3	6.7

After chemical analysis of the samples of longest back muscle it was found, that mass fraction of protein is 22.6 % (Tselinny type) comparing to 20.2 % (purebred), at the same time mass fraction of fat was higher in samples from purebred horses: 6.7 % (Tselinny type) and 9.3 % (purebred). It is possible to confirm, that horses of two observed populations differ from each other in terms of exterior and productivity.

4. Discussion

The comparative analysis of purebred horses of Kalmyk breed from breeding farms of Republic of Kalmykia and horses of intrabreed type Tselinny resulted in defining serie of mutual alleles with the sames frequency rate. Horses of both populations are characterized with following allels: A20, A 21, A23, A18, with the frequency rate more than 0.845, and A29, A28, A32 & A25 with the frequency rate less than 0.054. We suppose that these alleles characterize all the horses of the Kalmyk breed.

When we come to the main differences between population of Tselinny type and purebred horses, its possible to list between them absence of allele A22 in population

of meat type, more than 10 times difference in the frequency rate of allele A26, and increase in the frequency rate of A5, A10, A12 allels. Purebred horses also have higher frequency rate of following alleles: A11, A14, A16, A27.

Genotype of both populations consist of 14 and 15 alleles, which characterize 84.4 % and 78.8 % of all described horses. 16 and 17 alleles are not within alleles of horses of Tselinny type, meanwhiles they were found in more than 10 % of purebred horses. Differences in genotype was confirmed by the phenotype characteristics of horses (weight, height) and their productivity. Both stallions and mares have higher numbers of phenotype characteristics, as well as higher weight. As a result of slaughter 2.5 years animals, it was found, that horses of Tselinny type were heavier in live weight for 2.5 kg (per 66 kg), and it was possible to obtain for 45 kg more meat (21 % increase comparing to purebred horses).

5. Conclusion

The results of investigation have shown, that horses exemplars within Kalmyk breed and within type Tselinny have series of mutual alleles with the same frequency. In both populations horses have alleles A20, A21, A23, A18 with a frequency more than 0.845 and A29, A28, A32 and A25 with a frequency less than 0.054. We guess, that this features are mutual for all the animals of the Kalmyk breed, and they can be used as a characteristic of the breed in the future.

At the same time, several differences between meat type Tselinny and purebred horses of Kalmyk breed of the breeding plants were observed: lacking allele A22, the 10 times difference in the frequency of allele A26, as well as abundant increasing of frequency of A5,A10,A12. Also we can mention, that purebred horses of Kalmyk breed have higher frequency of A11, A14, A16, A27 alleles.

Thus, horses of intrabreed type Tselinny have several genotypic and phenotypic features, which differ from purebred horses of Kalmyk breed. However at the same time both populations keep the main features of the Kalmyk horse breed.

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Conflict of Interest

The authors have no conflict of interest to declare.

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