UDC 575.630.6
https://doi.org/10.2298/GENSR1803885K
*Original scientific paper*

# THE POLYMORPHISM OF *CAST* GENE IN RAMS POPULATIONS OF TUVAN BREED

Ksenia A. KULIKOVA[1], Yusupjan A.YULDASHBAEV[1], Milan P.PETROVIC[2], Violeta CARO PETROVIC[2], Dragana RUZIC MUSLIC[2], Nevena MAKSIMOVIC[2], Dusica OSTOJIC ANDRIC[2]

[1]Federal State Budgetary Educational Institution of Higher Education "Russian Timiryazev State Agrarian University", Faculty of Animal Science and Biology, Moscow, Russian Federation
[2] Institute for Animal Husbandry, Belgrade, Republic of Serbia

Kulikova K.A., Y.A. Yuldashbaev, M. P. Petrovic, V. Caro Petrovic, D. Ruzic Muslic, N. Maksimovic, D. Ostojic Andric (2018): *The polymorphism of CAST gene in rams populations of Tuvan breed*.- Genetika, Vol. 50, No.3, 885-893.

The Tuva Republic is one of the main sheep breeding centers in the Russian Federation. The Tuvan short-fat-tailed sheep is a unique local breed of the Tuva Republic. The studied breed has sheep of steppe type and mountain type. Each type of sheep was having formed according to relief of the ground and environment features. The breed originated in different parts from steppe and mountains. This study aim was to show the genetic polymorphism of calpastatin gene (*CAST*) in ram populations of two main breeding centers for tuvan short-fat-tailed sheep. Genomic DNA was isolated from 51 animals from Municipal unitary enterprise "Despen" (steppe type of sheep), and 100 animals from State unitary enterprise "Malchyn" (mountain type of sheep). Genomic DNA was extracted using the commercial kits. The detection was done using the restriction fragment length polymorphism of the polymerase chain reaction products (PCR-RFLPs). We used a pair of primers: F: 5'-TGGGGCC CAATGACGCCATCGATG-3' and R: 5'-GGTGGAGCAGCACTTCTGATCACC-3' to get 622 b.p. fragment of CAST gene. The PCR products are having

*Corresponding author:* Ksenia A. Kulikova, Federal State Budgetary Educational Institution of Higher Education "Russian Timiryazev State Agrarian University", Faculty of Animal Science and Biology, 49, Timiryazevskaya st., Building 5, Moscow, 127550, Russian Federation. Tel: +(499) 976-0236, Fax: +(499) 976-1447, e-mail: dvulunie@yandex.ru

digested with MspI restriction enzyme. Calpastatin locus digested with MspI has shown three genotypes: MM, MN and NN. The frequency of MM, MN and NN genotypes were 80.39%, 17.65% and 1.96% in steppe type of breed and 77.00%, 22.00% and 1.00% in the mountain type of breed. The highest allelic frequency was for allele M (0.89 in MUE "Despen" rams and 0.88 in SUE "Malchyn" rams). Allelic frequency of allele N had lower values of 0.11 in steppe type and 0.12 in mountain type.

*Keywords:* Sheep, Calpastatin gene, CAST, PCR-RFLP**,** genetic polymorphism

## INTRODUCTION

The Tuva Republic situated in the geographical center of Asia, in southern Siberia. It's the mountainous region, with alternation of mountain ranges and intermontane basins. Mountains and hills join over 80% of the Republic's territory. Intermontane basins presented by steppe landscape. A northern and eastern part of Tuva has the Sayan Mountains, and the Academic Obruchev Mountain range connects with Eastern Sayan Mountains in the middle part of Republic. West and west-south regions of the Tuva Republic situated on the Altai Mountains. The Tuva Republic borders with Mongolia on the south, and has the landscape characterized by steppe and depressions (lowland).

The climate of region is sharply continental. Summer signalize by moderately warm temperature in the mountains (+25-30°C), very hot weather can be observed in the depressions (+30-40°C). The temperature drops to – 30-40°C in the winter. Average annual precipitation is from 200 millimeters on the plains to 1000 millimeters in the mountains. Vegetation period endures 150-160 days. Some parts of the Tuva territory are affected by permafrost.

Tuvan short-fat-tailed sheep is indigenous sheep breed in this region. This breed has a local character of spread. Tuvan sheep are adapted for specific climate environment. Animals have a high immunity and stamina. In terms of productivity, the Tuvan breed is similar to the other local sheep breeds from the Russian Federation and countries of Asia. Level and quality of production depend on environment in each year and season. Minimum grades of productive traits are differing in breed types. The minimal live weight of adult rams of steppe type is 78 kg, of ewes – 56kg. The natural wool is strong, 12-14 cm length. The sheep wool clip from a ram is 1.5-2.0 kg, from an ewe – 1.2-1.7 kg. The level of traits from mountain type sheep is lower. Adult rams characterized by 55 kg of live weight, ewes – 42 kg. Length of wool is 10-12 cm. The sheep wool clip is 1.2-1.8 kg (from a ram), and 1.0-1.2 kg (from an ewe).

The birth rate of the Tuvan sheep is 100–110 lambs per 100 ewes. The wool of Tuvan sheep breed is rough; the color of the coat from each breed type is white. Dark spots can be placed on the head. Wool grease is light, but it is not very pronounced. The clean equivalent weight of wool is 50-60%.

In the Republic of Tuva, sheep are reared by smallholder farmers, municipal unitary enterprises, and individual farmers and are grazed in flocks of different size on open natural pastures. The fundamental building has not use for keeping of sheep.

The global aim of sheep husbandry as an agricultural branch is supplying for people high-quality meat, development of the ways of more efficient use of the gene pool of available sheep breeds in order to increase the level and the quality of meat.

The one of way for selection enhancement in sheep breeding can be using DNA markers of useful productive traits for organization marker-assisted selection. Marker-assisted selection (MAS) is applying of DNA markers to improve response to selection in a population of animals

(ZAID *et al.*, 2008).The markers should be closely linked to one or more target loci, which may often be quantitative trait loci. It is one of new DNA-based methods that improve accuracy and progress in sheep selection programs.

The other advantage of using genetic analyzing is possibility to control of the selection process, which can improve the economic performance of the industry. The great perspective can be present use marker-associated selection for forming high-productive flocks including sheep of indigenous breed (PETROVIC *et al.*, 2017).

Meat quality is one of the important economic marks in modern sheep breeding. Search of candidate genes of meat quality traits is very important in conditions of competition in meat product markets. One of the potential candidate gene for growth, carcass traits and to improving slaughter lamb production is ovine calpastatin gene (SHIROKOVA et al., 2015; DEYKIN et al., 2016). Calpastatin gene is of 100 kb length, includes four exons and is located on the fifth sheep chromosome (PALMER et al., 1998; GÁBOR et al. 2009).

Calpastatin activity is characterized high heritability ($h^2$=0.65 ± 0.19), which makes possible to achieve rapid genetic response in selecting activity against calpastatin. This selection can improve livestock meat tenderness (SHACKELFORD et al., 1994).

The aim of our study was to identify the genetic polymorphism of calpastatin gene (CAST) in ram populations of tuvan short-fat-tailed sheep at two main breeding centers.

## MATERIALS AND METHODS

### *Blood samples*

The Blood samples have collected from 151 animals' belong to 2 different types of Tuvan short fat-tailed sheep's breed. A 51 steppe type sheep's originated from municipal unitary enterprise "Despen" and 100 animals of the mountain type of sheep's from State unitary enterprise "Malchyn" are having presented for investigation. An approximate of 9.0 mL blood samples of each have collected from the jugular vein in sterile tubes. K3-EDTA tubes have used for superior safety of samples. The collected bloods in tubes were having refrigerated in a –20ºC and have transported in frozen condition.

### *DNA extraction and polymerase chain reaction*

Genomic DNA having isolated using the commercial kits (DNA-Extran-1, Syntol Ltd, Moscow, Russia) from the animal's blood samples after the manufacturer's instructions. The DNA amplification of the Calpastatin gene having achieved by using two primer pairs (5'-TGGGGCCCAATGACGCCATCGATG-3' (forward) and 5'-GGTGGAGCAGCACTTCTG ATCACC-3' (reverse). The PCR amplification reaction solution was having performed in a total volume of 20 µL.

Each reaction mixture was contained ddH2O (11.8 µL), PCR buffer (2.0 µL), $MgCl_2$ (2.0 µL), dNTP (2.0µL), primers (0.5µL of each), Taq DNA polymerase (0.2 µL, 5 U/µL), and DNA - template (~ 1.0 µL). The PCR was implemented by following parameters: a preliminary denaturizing at 95ºC for 3 min, followed by 1 cycle of denaturing at 95ºC for 15 sec, annealing at 60ºC for 40 sec, and extension at 72ºC for 30 sec followed by 35 cycles and a final extension by 5 min at 72ºC. Reactions were performed on a BioRad DNA Engine Tetrad 2 thermo cycler.

***Restriction fragment length polymorphism***

The PCR products are having digested with MspI restriction enzyme. The digestion mixture included (for each amplification assay) 0.2 µL MspI restriction enzyme (10 000 U/ml, SibEnzyme LLC, Moscow, Russia) 2.5 µL 10X SE-buffer B (SibEnzyme LLC, Moscow, Russia) and 7.3 µL $ddH_2O$. Restriction digestion lasted 12-16 hours in 37ºC. Results of amplification and digested by restriction enzyme are visualized on 2% agarose gel by trans illuminator under UV light after having stained by ethidium bromide. The 100 bp ladder and pUC19/MspI (SibEnzyme LLC, Moscow, Russia) have used as molecular size marker. Gels are being photographed using digital gel documentation system (Bio-Rad, USA).

***Statistical analyses***

The allelic and genotypic frequency, observed heterozygosity ($H_O$), expected heterozygosity ($H_E$) and Chi-square ($\chi^2$) values for the Hardy-Weinberg equilibrium were calculated as standard methods.

Genotype frequency was calculated by following formula:

$$P_i = \frac{n_i}{N}; \quad (1)$$

Where: $P_i$– i$^{th}$ genotype frequency;
$n_i$ – number sample of i$^{th}$ genotype;
$N$ – total sample of animals.

Allelic frequency was calculated by in the following way:

$$p_i = \frac{2n_{(homozygote)}+n_{(heterozygote)}}{2N}; \quad (2)$$

Where: $p_i$ - i$^{th}$ allele frequency;
$n$ – number sample of homozygotes and heterozygotes respectively;
$N$ – total number of animals.

Observed heterozygosity was computed by:

$$H_O = \frac{n}{N}; \quad (3)$$

Where: $H_O$ - observed heterozygosity;
$n$ – total number of heterozygote genotypes;
$N$ – total number of genotype.

The formula for calculated expected heterozygosity:

$$H_E = 2pq; \quad (4)$$

## RESULTS AND DISCUSSION

The amplified product of 622 bp fragment of CAST gene was obtained after amplification in all the analyzed samples.

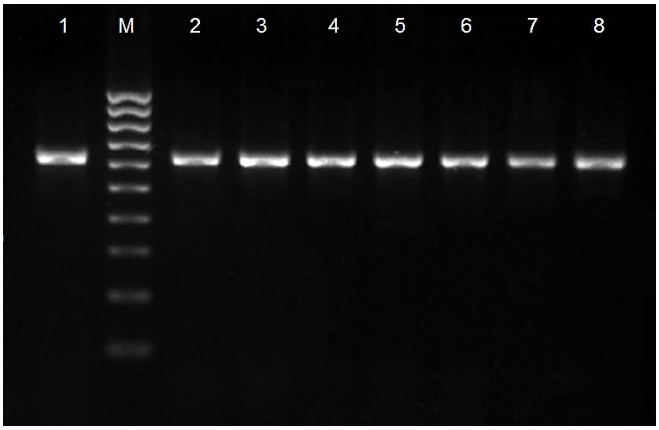


Fig.1 DNA electrophoretic pattern of CAST amplicons, lane M – 100 pb DNA ladder, lane 1-8 – 622 bp CAST amplicons (viewed on 2 % agarose gel).

The molecular genetic study by restriction enzyme of the Tuvan short-fat-tailed sheep identified the allelic variant soft the CAST gene (Fig.2) and established the genotypes presented by fragments of different size.

Digestion of the 622 bp PCR product of CAST gene with restriction enzyme MspI enable to differentiate the alleles N and M. The MspI digestion of the PCR products produced fragments of 336 bp and 286 bp for allele M. The allele N was not digested in the of the earlier studies (PALMER et al., 1998; ALAKILLI 2015; DINÇEL et al., 2015)**.** In the present study, the homozygous genotypes MM (2 bands: 336 bp and 286 bp), NN (622 bp) and the heterozygous genotype MN (3 bands: 622 bp; 336 bp and 286 bp) were having detected in two breed types.

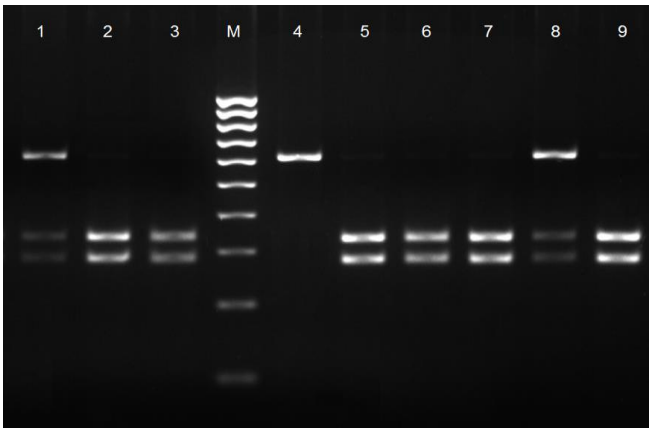


Fig.2 DNA electrophoretic pattern of CAST amplicons after digestion with MspI endonuclease, lane M – 100 pb DNA ladder, lane 2,3,5,6,7,9- genotype MM, lane 1,8 - genotype MN, lane 4 – genotype NN (viewed on 2 % agarose gel).

These results of genotyping are compatible with the polymorphism detected in ovine CAST gene previously observed in investigates (YILMAZ et.al., 2014; TOHIDI et al., 2013; DINÇEL et al., 2015; SZKUDLAREK-KOWALCZYK et al., 2011; GÁBOR *et al.*2009; SHIROKOVA *et al.*, 2015)**.**

The following frequency of different genotypes in population of steppe type of Tuvan sheep breed was observed after investigation.

*Table 1. The alleles and genotypes frequency for the CAST gene in different types of Tuvan short-fat-tailed sheep breed*.

| Type of sheep breed | Genotype | The number of animals | Observed frequency (%) | Allele frequency | |
|---|---|---|---|---|---|
| | | | | M | N |
| Steppe type (rams from municipal unitary enterprise "Despen") | MM | 41 | 80.39 | 0.89 | 0.11 |
| | MN | 9 | 17.65 | | |
| | NN | 1 | 1.96 | | |
| Mountain type (rams from State unitary enterprise "Malchyn") | MM | 77 | 77.00 | 0.88 | 0.12 |
| | MN | 22 | 22.00 | | |
| | NN | 1 | 1.00 | | |

The highest level of frequency was observed for genotype MM (80.39%), which was detected in 41 animals. 9 animals from investigation group had genotype MN, its frequency level is 17.65%. The smallest level of genotype frequency (1.96%) was for genotype NN. Only one animal had that genotype in group of steppe breed type.

A similar picture was observed in ram's population of mountain type of Tuvan sheep. Frequency of MM, MN and NN genotype was 77.00%, 22.00% and 1.00% respectively.

The highest allelic frequency was for allele M. It was meant 0.89 in MUE "Despen" rams and 0.88 in SUE "Malchyn" rams. Allelic frequency for allele N had a lower value. The value of that parameter was 0.11 in steppe type of sheep and 0.12 in mountain type.

The lowest frequency of genotype NN was also observed in previously studied. In other investigation of three groups of local Pakistan sheep breeds values of NN genotype frequency was 3.00%, 6.00% or was not observed. At the same time frequency of MM genotype was from 68% to 80% (SULEMAN *et al.*, 2012).

The similar relation between observed genotypes and superiority of MM genotype was described in investigation of Europe breeds of sheep. Studying of united population of sheep breeds used in Slovak Republic revealed two genotype (MM and MN) and values of frequency was 87% and 13% respectively (GÁBOR *et al.*, 2009). The investigated native breeds in turkey (Kangal, Awassi, Güney, Karaman, Akkaraman, Morkaraman, Karayaka, and Karakas), the frequencies of M and N alleles of CAST gene have determined as 0.92-0.08, 0.59-0.41, 0.67-0.33, 0.69-0.31, 0.87-0.13, 0.86-0.14, and 0.89-0.11 (BALCIOĞLU *et al*., 2014). Results of molecular analysis of polymorphism CAST gene in flocks of Bulgarian milk sheep observed

showed the availability of genotypes MM, MN and NN and its frequencies was 84%, 15% and 1% (GEORGIEVA *et al., 2015*). In the South European part of Russia, for the Soviet Merino breed have obtained MM, MN, and NN genotypes with a frequency of 0.82, 0.12, and 0.06 while for Salsk sheep breeds only genotypes MM and MN with a frequency of 0.78 and 0.22 (GORLOV *et al*., 2016).

The values of the observed and expected heterozygosity were 0.176 and 0.213 for steppe type of breed. The significant values were 0.220 and 0.211 for mountain sheep type.

*Table 2. Observed heterozygosity ($H_O$), expected heterozygosity ($H_E$) of alleles and $\chi^2$ estimates of ovine CAST gene digested with MspI.*

| Type of sheep | Observed heterozygosity ($H_O$) | Expected heterozygosity ($H_E$) | $\chi^2$ |
|---|---|---|---|
| Steppe type (from municipal unitary enterprise "Despen") | 0.176 | 0.213 | 0.344 |
| Mountain type (from State unitary enterprise "Malchyn") | 0.220 | 0.211 | 0.173 |

At one degree of freedom ($P< 0.01$) the calculated $\chi^2$ values were less than the tabulated values, so all the $\chi^2$ values for studied types of breed were non-significant. The chi-square test showed that the two populations of short-fat-tailed sheep used in this study were in Hardy-Weinberg equilibrium for the investigated *CAST* locus. Our result is partly in consonance to the chi-square test of the other population tested by (BALCIOĞLU *et al*., 2014) which consistent with Hardy-Weinberg equilibrium.

## CONCLUSION

The performed genotyping of the resource population of Tuvan sheep breed for the CAST gene is one of the steps in the implementation of the candidate gene approach in sheep breeding. In the present study, the investigated types of Tuvan sheep breed showing polymorphism for CAST gene. With regards to the previewed results of the investigation, the existence of polymorphism in the Calpastatin gene locus of these populations revealing that the quality of meat in these breeds possibly improved by selection programs

However, an additional need to investigate the meat quality of Tuvan Sheep for each genotype groups for ascertainment the fact of associate genotype with the level of meat quality in the local breed. The good practice can be additionally investigations association between this polymorphism and growth characteristics in sheep breeds. Evaluation of this SNP effect is necessary to find significantly favorable allele for improvement of production traits in Tuvan sheep.

Received, December 13st, 2017
Accepted, June 18th, 2018

REFERENCES

ALAKILLI, S.Y.M. (2015): Analysis of Polymorphism of Caplstatin and Callipyge Genes in Saudi Sheep Breeds Using PCR-RFLP Technique. Int. J. Pharm. Sci. Rev. Res. *30*(1): 340-344.

BALCIOĞLU, M.S., T. KARSLI, E. ŞAHIN, Z. ULUTAŞ, Y. AKSOY (2014): Determination of Calpastatin (CAST) Gene Polimorphism in Some Native Sheep Breeds Reared in Turkey by PCR-RFLP Method. Tarım Bilimleri Dergisi-Journal of Agricultural Sciences *20*: 427-433.

DEYKIN, A.V., M.I. SELIONOVA, A.Y.U. KRIVORUCHKO,D.V. KOVALENKO, V.I. TRUHACHEV (2016): Genetic markers in sheep meat breeding. Vavilov Journal of Genetics and Breeding *20*(5): 576-583.

DINÇEL, D., S. SARDIÇLI, B. SOYUDAL, M. ER, F. ALPAY, H. ŞAMLI, F. BALCI (2015): Analysis of FecB, BMP15 and CAST gene mutations in Sakiz Sheep. Kafkas Univ. Vet. Fak. Derg. *21*(4): 483-488.

GÁBOR, M., A. TRAKOVICKÁ, M. MILUCHOVÁ (2009): Analysis of polymorphism of CAST gene and CLPG gene in sheep by PCR-RFLP method. Sci. Pap. Anim. Sci. Biotechnol. *42*(2): 470-476.

GEORGIEVA, S., D. HRISTOVA, I. DIMITROVA, N. STANCHEVA, M. BOZHILOVA-SAKOVA (2015): Molecular analysis of ovine calpastatin (CAST) and myostatin (MSTN) genes in Synthetic Population Bulgarian Milk sheep using PCR-RFLP. J. BioSci. Biotechnol. *4*(1): 95-99.

GORLOV, I.F., N.V. SHIROKOVA, A.V. RANDELIN, V.N. VORONKOVA, N.I. MOSOLOVA, E.Y. ZLOBINA, Y.A. KOLOSOV, N.F. BAKOEV, M.A. LEONOVA, S.Y. BAKOEV, A. YUREVICH, Y. KOLOSOVA, L.V. GETMANTSEVA (2016): CAST/MspI gene polymorphism and its impact on growth traits of Soviet Merino and Salsk sheep breeds in the South European part of Russia. Turk J Vet Anim Sci. *40*: 399-405.

PALMER, B.R., N. ROBERTS, J.G. HICKFORD, R. BICKERSTAFFE (1998): PCR-RFLP for MspI and NcoI in the ovine calpastatingene. J. Anim. Sci. *76:*1499-1500.

PETROVIC, P.M**.,** D. RUŽIC MUSLIC, V. CARO PETROVIC, N. MAKSIMOVIC, B. CEKIC, A.Y. YULDASHBAEV, I.M. SELIONOVA (2017).Trends and challenges in the genetic improvement of farm animals. 11- th International Symposium Modern trend in livestock production, Belgrade, 11-13 October. Proceedings: 1-14.

SHACKELFORD S.D., M. KOOHMARAIE, L.V. CUNDIFF, K.E. GREGORY, G.A. ROHRER, J.W. SAVELL (1994): Heritabilities and phenotypic and genetic correlation for bovine post rigor calpastatin activity, intramuscular fat content, warner-bratzler shear force, retail product yield and growth rate. J. Anim. Sci. *74*: 857-863

SHIROKOVA, N.V., Y.A. KOLOSOV, L.V. GETMANTSEVA, A.V. RADUK, N.F. BAKOEV (2015): Optimization techniques for PCR-RFLP for genotyping sheep. Scientific Journal of Kub SAU *113*(09): 1-9. From http://ej.kubagro.ru/2015/09/pdf/102.pdf.

SULEMAN, M., S.U. KHAN, M.N. RIAZ, M. YOUSAF, A.S.R. ISHAQ, A. GHAFOOR (2012): Calpastatin (CAST) gene polymorphism in Kajli, Lohi and Thalli sheep breeds. Afr. J. Biotechnol. *11*(47):10655-10660.

SZKUDLAREK-KOWALCZYK, M., E. WIŚNIEWSKA, S. MROCZKOWSKI (2011): Polymorphisms of calpastatin gene in sheep. Journal of Central European Agriculture, *12*(3): 425-432.

TOHIDI, R., G. ELYASI, A. JAVANMARD, J. SHOJA, R. REZAEI, O. PIRAHARY (2013): Molecular analysis of ovine calpastatin gene in six iranian sheep breeds using PCR-RFLP. J. Anim. Prod. Adv. *3*(9): 271-277.

YILMAZ, O., T. SEZENLER, N. ATA, Y. YAMAN, I. CEMAL, O. KARACA (2014): Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds. Turk. J. Vet. Anim. Sci. *38*: 354-357.

ZAID, A., H.G. HUGHES, E. PORCEDDU, F. NICHOLAS (2008): Glossary of Biotechnology for Food and Agriculture. A Revised and Augmented Edition of the Glossary of Biotechnology and Genetic Engineering. From http://www.fao.org/docrep/004/y2775e/y2775e00.htm.

# POLIMORFIZAM *CAST* GENA U POPULACIJAMA OVNOVA TUVANA RASE

Ksenia A.KULIKOVA[1], Yusupjan A.YULDASHBAEV[1], Milan P. PETROVIC[2], Violeta CARO PETROVIC[2], Dragana RUZIC MUSLIC[2], Nevena MAKSIMOVIC[2], Dusica OSTOJIC ANDRIC [2]

[1]Federalna državna budžetska obrazovna ustanova visokog obrazovanja "Državni agrarni univerzitet "Timirjazev", Fakultet za zootehniku i biologiju, Moskva, Ruska Federacija
[2]Institut za stočarstvo, Beograd-Zemun, Republika Srbija

Izvod

Tuva je jedan od glavnih centara za razmnožavanje ovaca u Ruskoj Federaciji. Tuvanska rasa ovaca je jedinstvena lokalna populacija ove Republike. Ispitivana rasa se javlja u dva tipa koji su posledica uslova gajenja. To su stepski tip i planinski tipu ovaca, aoba su potpuno prilagođena na spoljne faktore koji karakterišu stepe i planine. Ciljovogistraživanja bio je da se utvrdi genetski polimorfizam kalpastatinskog gena (CAST) u populaciji ovnova Tuva rase kod oba tipa i to u dva glavna centra u kojima se ona gaji. Genomska DNK je izolovana od 51 životinje stepskog tipaovaca i 100 životinja koje pripadaju planinskom tipu ovaca. Genomska DNK je izolovana pomoću komercijalnih kitova. Detekcija je izvršena pomoću PCR-RFLP. Korišćen je par prajmera: F: 5'-TGGGGCC CAATGACGCCATCGATG-3 'i R: 5'-GGTGGAGCAGCACTTCT GATCACC-3' da bi dobili 622 b.p. fragmenta CAST gena. Produkti PCR-a su rastvarani sa MspI restrikcionim enzimom. Kalpastatin lokus rastvoren sa MspI, je pokazao tri genotipa: MM, MN i NN. Učestalost genotipova MM, MN i NN bila je 80,39%, 17,65% i 1,96% u stepskom tipu i 77,00%, 22,00% i 1,00% u planinskom tipu ove rase. Najveća alelna frekvencija bila je za alele M 0,89 u MUE kod stepskog tipa i 0,88 u SUE kod ovnova planinskog tipa. Frekvencija alela N kod stepskog tipa imala je nižu vrednost 0,11, dok je u populacijiplaninskog tipa iznosila 0,12.

Primljeno 13.XII.2017.
Odobreno 18. VI. 2018.