



Short Communication

Determination of *CAST/MspI* Gene Polymorphism and its Effects on 180-Day Weight in Meat Production Line of Kazakh Saryarka Lambs

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ABSTRACT

The *CAST* gene has been extensively studied in livestock for meat quality and growth traits. A fat-tailed Kazakh sheep, the Saryarka is characterized by a hardy constitution that allows for year-round grazing. In 2016, two factory lines (for meat and wool production) were created from the Zhanaarka type of the Saryarka sheep breed. The aim of this study was to investigate the polymorphism of the *CAST* gene in lambs of the Saryarka meat production line and to evaluate associations between the *CAST* genotype and live weight in 180-day-old lambs. A total of 60 lambs were genotyped by the PCR-RFLP method. Two genotypes, MM and MN, were identified with frequencies of 0.59 and 0.41, respectively. According to the association analysis, lambs with the MN genotype produced significantly ($P < 0.01$) more live weight (4.48 kg) than lambs with the MM genotype. The study was the first to demonstrate that the Saryarka breed has a genetic variation of the *CAST* gene and its effects on lamb live weight. Consequently, it can be said that the *CAST* gene could potentially be a molecular marker for the Saryarka breed to increase lamb production and be integrated into the current selection program for the breed.

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Authors' Contribution

BS and KK designed the study. BS performed a laboratory analysis and collected the data. BS and KK analyzed the data. All authors wrote the article and approved the final version.

Key words

Saryarka, Lamb weight, *CAST* gene, PCR-RFLP, *MspI*, Polymorphism

In Kazakhstan, the breeding of fat-tailed sheep, which accounts for about 70% of the Kazakh sheep population, has traditionally been a leading branch of livestock farming. The Saryarka breed, one of the seven available fat-tailed sheep in the country, is notable for the transferability of its useful traits to its own offspring in pure breeding, and therefore it is used in breeding studies (Sadykulov *et al.*, 2014). This breed was created in the period between 1970 and 1998 by breeding the local Kazakh fat-tailed breed, which is considered the descendant of ancient sheep varieties in Kazakhstan (Pozharskiy *et al.*, 2020).

Sheep meat production is one of the most important activities. There are two important factors affecting the profitability of sheep; multiple births and live weight. Saryarka sheep show a high variation in phenotype in relation to live weight from a young age. Despite this variation, there is less information at the DNA level. In addition, breeding studies on this breed have been made based on traditional methods. It is well known that the traditional methods are not enough due to the low heritability of economic traits like weaning age (Gregula-Kania, 2012). However, in recent years, advances in molecular genetics have enabled the identification of candidate genes for important traits in livestock (Andersson, 2001). This advance has enabled scientists to use genetic improvement programs through marker-assisted selection and introgression (Dekkers and Hospital, 2002). In general, more meaningful results are obtained in genetic improvement studies when molecular information is used together with traditional breeding methods (Andersson, 2001).

Calpastain is one of the candidate genes affecting growth traits and meat quality. The gene, which is an

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inhibitor of calpains, plays an important role in meat quality and growth traits due to its role in myogenesis and meat tenderization (Jawasreh and Ismail, 2019). In general, molecular studies on Kazakh sheep breeds are lacking apart from some SNP and SSR studies (Dossybayev *et al.*, 2019; Pozharskiy *et al.*, 2020). Although there is much research on the *CAST* gene in the literature, no data availability from research subjects has been observed in the Saryarka breed (Bayram *et al.*, 2019; Jawasreh and Ismail, 2019; Bozhilova-Sakova *et al.*, 2020; Kırıkçı *et al.*, 2021; Kırıkçı, 2022).

Identifying the most profitable genotypes is one of the major difficulties in selecting (breeding) and improving livestock, including sheep. Although some attempts have been made to address selection in seep farming in Kazakhstan (Islamov *et al.*, 2021), molecular research for application in selection programs, particularly in native Khazak sheep breeds, have not been encountered. Therefore, we wanted to study the polymorphism of the *CAST* gene and its effects on live weight in Saryarka meat production line lambs born in the fourth generation and selected for live weight at day 180.

Materials and methods

The study was conducted at the Zhenis breeding facility in the Zhanaarka district of the Ulytau (former Karaganda) region of Khazakistan. The test material consisted of a total of 60 male lambs of the Zhanaarka type Saryarka sheep breed.

Lambs raised by their mothers until weaned. The lambs were weighed on day 180 after birth, and blood samples were taken under veterinary supervision in 15 ml tubes containing an anticoagulant (EDTA). DNA was obtained from whole blood using DNA-sorb-B extraction kit (REF K1-2-100-CE, AMPLISENS, Moscow, Russia).

A 622 bp fragment of the *CAST* gene was amplified with the primer pairs; 5-TGGGGCCCAATGACGCCATCGATG-3 and reverse 5-GTGGAGCAGCACTTCTGATCACC-3 using a SimpliAmp thermal cycler (Applied Biosystem). The PCR cycle was performed: an incubation step at 95 °C for 12 mins, followed by 35 cycle of 95 °C for 1 min, annealing at 64 °C for 1 min and an extraction step at 72 °C for 1 min, and 10 mins at 72 °C as final extraction.

PCR products (622 bp) were digested using the restriction endonuclease enzyme *MspI* (EURX) to genotype the animals for the *CAST* gene. The PCR products were incubated for 1 h at 37°C in a final volume of 50 µl, the 10 µl PCR product, 5 µl 10x One buffer, 0.5 µl BSA [100x], 0.3 µl *MspI* enzyme and finally ultrapure contained water brought to final volume and after digestion heated to 65°C for 20 min for inactivation. A 50 bp DNA ladder was used

to determine fragment sizes of PCR-RFLP results.

Allele and genotype frequencies for the *CAST* gene were calculated using PopGene 32 software (Yeh *et al.*, 1999). Chi-square analyzes were performed to determine whether the studied population was in Hardy-Weinberg equilibrium. The general linear model (GLM) was used to demonstrate the effects of *CAST* gene genotypes on live weight at day 180 using SPSS 22 (IBM SPSS Inc., Chicago, IL, USA). The effect of farm, feeding and age factors etc. was not used in the GLM analysis as all lambs were reared on the same farm.

Results and discussion

The 622 bp fragment of the *CAST* gene was successfully amplified by PCR and all individuals were genotyped by digestion of the *MspI* enzyme. In the population of Saryarka sheep from Khazakistan, PCR-RFLP results showed two genotypes, heterozygous MN and homozygous MM. In the study, NN genotypes were not observed in the examined samples. Animals with MN genotypes showed three fragments of 622 bp, 336 bp and 286 bp, while homozygous animals MM showed two fragments of 336 bp and 286 bp on 3 % agarose gel. The most of the studied animals were of heterozygous genotype. In the study, the calculated allele and genotype frequencies were given in Table 1.

Table 1. Allele and genotype frequencies and heterozygosity values for the *CAST* gene.

Gene	Allele frequency		Genotype frequency		Ho	He	x ²	P
	M	N	MM	MN				
<i>CAST</i>	0.80	0.20	0.59	0.41	0.4107	0.3293	3.5534	NS

NS, not significant; Ho, observed heterozygosity; He, expected heterozygosity; x², Chi-square.

The M and N allele frequencies for the *CAST* gene in Saryarka sheep were 0.80 and 0.20, respectively, while the genotype frequencies were 0.59 for MM and 0.41 for MN. The result of the Chi-square test showed that the examined Saryarka sheep was in HWE (P>0.05).

Lamb live weight at day 180 in the study was calculated as 48.01±3.03 kg for MM genotypes and 52.49±2.96 kg for MN genotypes, indicating that MN genotype lambs gained an average of 4.48 kg more weight than lambs with MM genotype. As a result, live weight at day 180 was statistically (P<0.01) influenced by the genotype. The values of observed (Ho) and expected (He) heterozygosity were estimated as 0.4107 and 0.3293, respectively. The chi-square analysis showed that the studied population was in Hardy-Weinberg equilibrium.

The study showed that the Saryarka breed has *CAST/MspI* gene polymorphism, and the gene has a significant effect on live weight at day 180. This polymorphism has also been reported in many sheep breeds from different part of the world such as Bulgarian (Bozhilova-Sakova *et al.*, 2020), Brazil (Santos *et al.*, 2016), New Zealand (Byun *et al.*, 2008), Russian (Kolosoov *et al.*, 2021), Turkey (Kırıkçı *et al.*, 2021; Kırıkçı, 2022) and Iranian (Eftekhari Shahroudi *et al.*, 2006). The M allele frequency in the Saryarka sheep was higher than the N allele frequency. Heterozygous MN genotype frequency value for the Saryarka breed were higher than the some previous studies reported for Awassi-0.35 (Jawasreh and Ismail, 2019), Prydniprovskaya -0.13 (Pomitun *et al.*, 2019), Colombian Creole hair sheep-15.5 (Vergara *et al.*, 2019) and West Siberian mutton breed – 0.23 (Afanasyeva *et al.*, 2019). The present study could not detect the NN genotype in the lambs examined. This result was consistent with the study by Suleman *et al.* (2012) who examined three Pakistani sheep breeds– Thali, Lohi and Kajli. Similar results were also observed for the Turkish Akkaraman and Karayaka sheep breeds (Kırıkçı *et al.*, 2021; Kırıkçı, 2022).

No study has been found in the literature that demonstrates the genetic structure for the CAST gene in the Saryarka breed and its effects on body weight. To the best of our knowledge, the current study was the first to examine the CAST gene and its impact on day 180 live weight in Saryarka lambs. Therefore, the results obtained were limited to comparison with other Kazakh breeds. In the study, the frequency percentage (0.41) of the heterozygous genotype was quite high. It can be said that this is mainly caused by the selection of the studied animals in terms of 180th day live weight for many years. Afanasyeva *et al.* (2019) investigated the CAST gene polymorphism and its impact on live weight in the West Siberian sheep breed at various ages. According to the findings, heterozygous lambs produced 3.2 kg more live weight than homozygous animals. This finding was comparable to the current study, which showed that heterozygous Saryarka lambs produced 4.48 kg more live weight than homozygous lambs, which was a desired result. On the 180th day, heterozygous Saryarka lambs averaged 52,49±2.96 whereas homozygous lambs averaged 48,01±3.03. There were clearly significant differences ($P<0.01$) between genotypes for live weight at day 180. Likewise, heterozygous Volgograd sheep have been reported to have 3.7 kg more live weight before slaughter than homozygous ones (Kolosoov *et al.*, 2021).

Bayraktar and Shoshin (2022) found an association between CAST genotypes and body weight at one year of age in Awassi sheep. According to their findings, the body weight difference between MN and MM genotypes was

0.54 kg. Another study found no association between the CAST gene and some live weight elements (Bayram *et al.*, 2019). Therefore, it is worth noting that the live weight differences obtained for Saryarka lambs between MN and MM genotypes were quite higher than in most of the studies mentioned above. The only limitation of the current study may be the sample size. Despite this limitation, the results of this study are important as they provide the polymorphism of the related gene and an association with live weight.

Conclusions

This study was the first to show the genetic variation of Saryarka lambs for the CAST gene and provided evidence for the relationship between genotype and phenotype. The CAST gene MN genotype significantly affected the live weight of lambs at 180 days. Consequently, this gene may be used as a potential molecular marker to increase meat production and integrated into the current selection program in the Saryarka breed meat production line. However, given the lack of molecular studies and sample size in the present study, further investigations should be done in the Saryarka and other Kazakh sheep breeds.

DECLARATIONS

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Statement of conflict of interest

The authors have declared no conflict of interest.

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