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Application of PCR-RFLP technique to determine Booroola gene polymorphism in the Sangsari sheep breed of Iran

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Abstract: Phenotypic evaluation and the culling of candidate animals for traits by applying traditional animal breeding are usually costly tasks, which need to be carried out over a considerable span of time. The use of molecular genetics as an alternative method enables animal breeders to select eligible animals for the desirable trait, or traits, at earlier ages. Selection based upon markers could result in increased accuracy as well as a better selection response of animals. The Booroola gene has long been recognized as a candidate gene responsible for a higher rate of ewe ovulation, which results in increased litter size. In this study, blood samples were taken from 150 Sangsari sheep breed (140 ewes and 10 rams) in the Damghan animal breeding center using Venojects treated with an anticlot substance (EDTA). The sample DNA contents were salted out and extracted. After extraction, and after undertaking quantitative and qualitative tests (including spectrophotometry and gel agarose, 8%), the required amounts of DNA for polymerase chain reaction (PCR) were determined. Using the relevant primer, the related part was reproduced (190 bp) and then the PCR products were cut by AvaΠ enzymes for the gene, and 2 parts (30 bp and 160 bp) were produced for the target site. The results obtained in this study revealed that only the wild-type allele (+) was observed in the samples, indicating that, most likely, only genotype ++ was present in the population under consideration.

Key words: Sangsari sheep, PCR-RFLP, FecB gene

1. Introduction

The Sangsari breed is a light-weight and fat-tailed sheep. The meat production of this breed is considered to be of major economic importance. Generally, its weight trait can be considered as a composite trait influenced by the reproductive and growth performance of the animal. The reproduction performance of an animal is of particular economic importance (1,2). Detection of genetic markers, along with mutants of the genes associated with economically important traits, could assist the breeders in designing practical animal breeding plans (1,3).

Reproduction is a quantitative trait with polygenetic inheritance, meaning that the sheep's reproductive performance is controlled by many genes. Recently, a new strain of Merino sheep breed called Booroola has been introduced in Australia and New Zealand with high fecundity due to major gene effects that are responsible for multiple births. Therefore, the sheep's reproductive performance is influenced by both minor and major gene effects. Thus far, the effect on litter size of a number of genes, localized on sexual (X) and autosomal chromosomes (pair 11), have been reported (3–6). The Booroola sheep are carriers of an autosomal gene named FecB (4,6–8).

Although 2 decades have passed since this gene was first found, its biological mechanism has not been fully understood (9–11). The Booroola gene is located between the secretory phosphoprotein 1 locus (spp1) and alcohol dehydrogenase 2 locus (ADH2) on chromosome number 6 (4,12).

New findings indicated that multiple births in sheep bearing the Booroola gene (FecB) are a result of chromosome 6 mutation in a part comprising bone morphogenic protein receptor gene IB (BMPR-IB) (1,4,13–15). The BMPR-IB gene encodes the morphogenic growth factor βs. It is also known that a displacement (Q249R) in the BMPR-IB coding locus is closely related to higher fecundity in Booroola sheep (9). This point mutation happens to the kinase receptor (2,9), which in turn results in a change to nucleotide 746 in coding locus (A→G), causing glutamine conversion to arginine (2). The letters F and + show fecundity and the wild type. It has recently been renamed FecB by the Committee on Genetic Nomenclature of Sheep and Goats (4). The most important physiological effects of the FecB locus are on ovulation

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rate, as well as on the size and number of ovulatory follicles in the ovary. As compared to ++ ewes, significantly smaller follicles are matured and released in ewes that are of genotypes B+ and BB. The smaller follicles for genotype BB consist of fewer granulosa cells in comparison with the follicles in genotype ++ (4,5,12,16).

FecB mutation results in an increased follicular cell sensitivity to gonadotropic stimulation (1,4,5,17–21). Each mutant gene results in an increase to the ovulation rate of 1.6 times (4). It has also been recognized that the Booroola phenotype is correlated to the bone morphogenic receptor-IB (BMPR-IB) (2,3). To identify this point mutation, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using a short-acting enzyme of AvaΠ that cuts the 6/6 ACC locus, was utilized and the results indicated that noncarrier animals are free of this locus (22–26). In some countries, such as New Zealand, India, the Philippines, Indonesia, Poland, Iceland, France, and Ireland, mutation of the FecB gene has been studied in sheep of high prolificacy (4). The FecB mutation was reported in Garol and Javanese sheep breeds, while no mutation was found in Thoka, Woodlands, Olkuska, Lacaune, Belclare, or Cambridge sheep breeds. These findings finally generate the conclusion that, based upon previous theories, the Booroola gene was, in fact, originally transmitted from the Garol sheep breed to Australian flocks (3).

The main objective of the present research was to apply the PCR-RFLP technique for determining Booroola gene polymorphism to the Sangsari sheep breed of Iran.

2. Materials and methods

A total of 3445 phenotypic records (representative of 350 ewes and 50 rams) collected between 1994 and 2007 in a Sangsari breeding center were used. A multiple-traits animal model was utilized to predict the breeding value (based upon the best linear unbiased prediction [BLUP] statistical method) of individual animals for the traits of the number of lambs born per lambing and mating. A derivative free restricted maximum likelihood [DFREML] algorithm was applied to estimate genetic and environmental variance and covariance components.

$y_i = X_i b_i + Z_i a_i + W_i p_{ei} + e_i$,

where:

y_i is a vector of observations for the i*th* trait,

b_i is a vector of fixed factors for the i*th* trait,

a_i is a vector of random additive genetic effect of sheep for the i*th* trait,

p_{ei} is a vector of random effect of permanent environment of sheep for the i*th* trait,

e_i is a vector of random residual effect for the i*th* trait, and

X_i, Z_i, and W_i are design matrices for fixed and random effects in the model.

In the next stage, blood samples were taken from 150 sheep (including 140 ewes and 10 rams). Each sample consisted of 5 mL of blood collected in a Venoject tube containing EDTA. The DNA contents of the samples were extracted by the salting-out method. A PCR technique was performed in a total volume of 25 µL including 1.5 µL of DNA, 1 unit of Taq polymerase (palm-cycler, Corbett, research version 2.2), 0.2 µL of dNTP, 2.5 µL of 10X PCR buffer, 0.25 µL each of forward and reverse primers, 0.75 µL $MgCl_2$(50 µg/µL), and distilled water (2,3). The following primers were used:

Forward: 5'-CCAGAGGAGAATAGCAAAGCAAA-3'

Backward: 5'-CAAGATGTTTTCATGCCTCATCAAC ACGGTC-3'

The PCR was repeated for 35 cycles with a thermocycler instrument as follows: 5 min at 94 °C, 15 s at 94 °C, 30 s at 60 °C, and 30 s at 70 °C for 35 cycles, and a final stage that included 5 min at 72 °C and 15 min at 99 °C. An aliquot of each PCR product was subjected to electrophoresis on 1.5% agarose gel and also a size marker and staining with ethidium bromide (0.5 µg/mL).

Size bands were found to be about 190 bp and the remaining PCR products were treated with narrow-spectrum AvaΠ enzyme. Final products were subjected to electrophoresis in 3% agarose gel and stained with ethidium bromide. Animals carrying the FecB mutation produce segments of 30 and 160 bp, while animals with the wild genotype show a 190-bp segment.

3. Results

According to the results of the present study, the average number of lambs were 0.94 and 0.90 per mated and pregnant ewe, respectively. The PCR results for the FecB gene are shown in Figure 1. As expected, the 190-bp segment of the FecB for this breed was produced. To approve the accuracy of the segment, marker size was used. The proliferated segment was digested by the short-acting enzyme AvaΠ and the existence of the wild monomorphic genotype (++) of the Booroola gene in the flock is shown in Figure 2, indicating a lack of mutation in the examined samples.

4. Discussion

The results of the present research are in accordance with those obtained for Madras Red, Deccani, and Bunnur breeds (1–3,20,21). Research in India has shown that the Garol breed was the only breed carrying the FecB allele.

In fact, due to the long geographic distance between rearing environments for these 3 breeds, the mutant allele might have spontaneously occurred and subsequently been imported to Bunnur and Deccani crossbreds following breeding programs (1,3,20,21,26,27). Geographically, the Sangsari's natural breeding environment is located

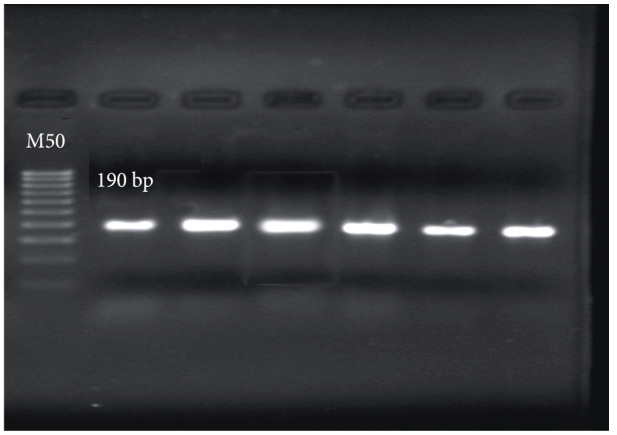

Figure 1. PCR products of FecB gene fragment of size marker: 500 – 450 – 400 – 350 – 250 – 200 – 150 – 100 – 50.

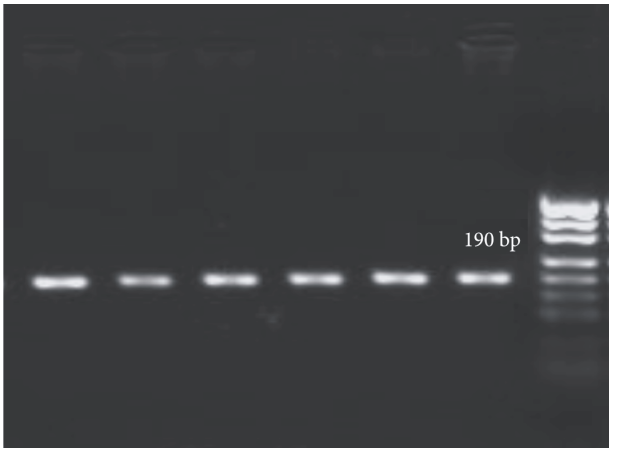

Figure 2. Enzyme digestion product of FecB gene fragment of size marker: 500 – 450 – 400 – 350 – 250 – 200 – 150 – 100 – 50.

between Mazandaran and Isfahan provinces, indicating that this allele could not have been imported to the local breeds through commercial routes. In addition, this breed actually has a high level of resistance developed against the harsh environment and diseases, leading to the failure of the mutant allele to get imported into Sangsari flocks over time.

The mutant allele (FecB) was not found in Tooka, Woodlens, Olcusa, Lacan, Belklir, and Cambridge breeds reared in 8 countries (New Zealand, India, the Philippines, Indonesia, Poland, Iceland, France, and Ireland), while the gene was reported in the Garol (Bangladesh) and Javanez (Indonesia) breeds (1,3).

A number of researchers believe that Javanez sheep originated from a region located between India and Bangladesh, while some other researchers believe that Javanez sheep received the Booroola gene from Australian Merino rams in the 1860s, as at that time they were used to improve local breeds for meat and wool purposes.

The fact is that no association was found between those breeds, suggesting that other major gene(s) effects, other than the FecB gene, may be responsible for the high prolificacy of those groups of sheep breeds (1,3,9,25,28,29).

A study undertaken on 21 sheep breeds of high prolificacy in 13 different countries detected the FecB gene in Hu and Han breeds of China, indicating that the mutant gene was stabilized in the Garol and Hu breeds but segregated in Javanez, Booroola Merino, and Han breeds (1). Recent findings confirm previous theories regarding the mutant gene transmission from the Garol to the Australian (Booroola) and Indonesian (Javanez) sheep populations (1,3).

Garol (from India) and Hu (from China) sheep breeds have been stocked along the commercial silk route, through which the mutant allele may have been transferred by transient merchants (1,3,8,10,11,14,16). Although Iran was also on the silk route, there was, in fact, little chance for the Sangsari breed to receive the FecB allele due to the fact that their rearing environment was far away from the route. Furthermore, at phenotypic level there is no resemblance between the Sangsari and the mutant-carrier breeds, suggesting that there is no common ancestor for these breeds. Hu and Garol breeds may be possibly derived from a common ancestor.

This mutant gene has been transmitted to other breeds via crossbreeding in a number of countries. The Awassi breed, for example, has a low lambing rate and the Booroola gene has been transmitted and fixed in it since 1986 (1,3,8,10,11,14). The results of these experiments indicated that the fertility rate of this breed increased from 1.2 to 2 lambs per lambing without any significant decrease in milk production (20). On the other hand, twining is under the control of genetic and environmental factors and their natural environment is against this trait. Therefore, nutrition could greatly influence the expression of any major gene effect on the reproductive performance of the animals.

There are reports indicating that the ovulation rate in the Javanez breed carrying FecB is half that of the Merino breed, due to inappropriate environmental conditions such as low quality of feeds (1–3,7,9). Although there is the same mutant allele for Booroola and Garol breeds, ovulation and lambing rates for the Booroola is higher than that of the Garol breed. This could be due to differences of breeds, nutrition, and any other genetic factors, such as modifier genes (1,3,7,9).

In connection to the Sangsari breed of Iran, the low rate of lambing may be explained by the harsh mountain environment where this breed is reared. This could lead to a low rate of lambs per individual ewe. With respect to the phenotypic observations and also molecular experiments, it seems that the occurrence of the phenomenon of

spontaneous mutation for this breed needs to be rejected. This may not be the case, because there has not been any planned selection program and there has been natural selection pressure against this trait, and so the mutant allele may have been removed from the population gradually. Moreover, the particular mountain environmental conditions for this breed have caused a selection trend against this trait.

In addition, the importation of the mutant allele from exotic breeds to the Sangsari breed is unlikely, due to the closed environment and the fact the breed's rearing grounds are in a region that is inaccessible via commercial routes. On the other hand, due to the small size of the sample studied in this research, there is a probability that the mutant allele was not available in the sample. Therefore, there is a need to undertake further research on a relatively larger sample size for the population. A number of other mutant genes affecting lambing rate have been also detected, for which the Sangsari breed may be studied.

In conclusion, with respect to the positive effect of increased lambing rate on meat production and decrease in the number of breeder ewes on the pasture, finding major genes affecting the twining trait is of great importance from an economic point of view. Therefore, establishing a well-planned program to import the mutant alleles into the local Iranian sheep breeds could result in a significant increase in production levels, leading to a higher level of breeders' income.

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