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FINGERPRINTING OF *FECB* GENE IN FIVE EGYPTIAN SHEEP BREEDS

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Abstract: Recently, many aspects of *FecB* gene, including reproductive endocrinology, organs development and body mass have been studied. *FecB* has an additive effect on litter size and ovulation. The present investigation was carried out to study polymorphism by forced PCR-RFLP of *FecB* gene in five Egyptian local sheep breeds and its comparison with other foreign sheep breeds. Genomic DNA was isolated from a total of 100 animals of Egyptian sheep breeds namely Rahmani, Ossimi, Awassi, Barki and Awassi x Barki crossbred. Forced PCR of the *FecB* gene 190 base pair (bp) was amplified using specific primer designed to introduc a point mutation in the resulting PCR products with *FecB* carrier sheep containing an *Ava*II restriction site (G|GACC), whereas products from noncarriers lacked (of) this site. .Digestion of *FecB* gene 190 base pair with *Ava*II restriction enzyme resulted in non carrier 190 bp band (wild type) in all the animals belonging to the five Egyptian breeds studied revealing absence of this restriction site in those five breeds.

Key words: *FecB* gene; Forced PCR-RFLP; Sheep; Polymorphism

Introduction

Sheep contribute 6% of the total red meet produced in Egypt. The total sheep population in Egypt is 4,200,000 heads. Rahmani, Ossimi, and Barki, are of the main sheep breeds in Egypt with a population of 990,000, 514,000 , and 470,000 respectively (*Galal et al., 2005*).Regarding the economic importance of sheep in meat production in Egypt , it becomes essential to make fingerprinting of some genes related to economic traits such as litter size and growth rate in order to determine the polymorphism pattern of these genes in the Egyptian sheep breeds.

Determination of the genetic diversity of indigenous sheep in Egypt in respect to these important economic genes has not been sufficiently studied. Genetic characterization and determination of genetic differences between sheep breeds will help in the genetic improvement programs. In Egypt, most commercial sheep are raised in very small flocks in low-input systems. Genetic improvement is largely accomplished through government-owned flocks, with progeny from these flocks distributed to producers. Breeding objectives are needed to develop selection programs for these breeds (*Almahdy et al.,2000*[b])

Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objective (*Crawford and Littlejohn, 1998; Kumar et al., 2006; EL Hanafy and Salem,2009*).

In recent years, many aspects of the *FecB* gene, including reproductive endocrinology (*Smith et al., 1993*), ovary development (Cognie *et al*., 1998), litter size, organ development and body mass (*Smith et al., 1996*) have been studied. This gene has an additive effect on litter size and ovulation rate, but has negative effects on fetal growth and development and body mass during gestation. For example, body weights were lighter ($P < 0.05$) at most gestational ages in BB/B+ than in ++ fetuses (*Smith et al., 1993*), and crown-rump length was shorter in BB/B+ than in ++ fetuses (*McNatty et al., 1995*).

Litter size and lamb growth are important economic values in sheep breeding and reproduction. Recently, Mulsant et al. (2001), *Souza et al. (2001)* and *Wilson et al. (2001)* reported that bone morphogenetic protein receptor IB (BMPR-IB) gene mutation was responsible for the high prolificacy associated with the *FecB* gene in Booroola Merino sheep. This mutation is located in the kinase highly conserved domain of the bone morphogenetic protein receptor IB, and is characterized by 'precocious' differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles (*Souza et al., 2003*). This mutation can be detected directly by forced PCR restriction fragment length polymorphism (RFLP) approach based on the reports described by Souza *et al*. (2001) and *Davis et al. (2002)*.

Due to the importance of fingerprinting of economic genes in the Egyptian local sheep breeds, the present study was undertaken to find out polymorphism pattern of the Egyptian sheep *FecB* gene and compare it with those of foreign sheep breeds in order to identify the genotype of this gene in these breeds which will help in genetic improvement of these breeds via determination of required crossbreeding ratio with foreign breeds carry the favorite genotypes of this gene which will lead to increase of expression of these gene in the local Egyptian breed without affect on the acclimatization traits of these breeds to the environmental conditions in Egypt.

Materials and Methods

Animal materials and DNA extraction:

The present study was conducted on a total of 100 animals belonging to five Egyptian sheep breeds viz. Rahmani (20), Ossimi (20), Awassi (20) , Barki (20) and Awassi x Barki crossbred (20) maintained at Animal Production Research Station, Borg EL-Arab, Alexandria, Egypt. Approximately, 10ml venous blood was collected from each animal using 0.5 ml of 2.7% EDTA as an anticoagulant. Genomic DNA was isolated from blood using QIAamp DNA extraction kit (QIAGEN GmbH, Hilden Germany) according to the manufacturer's instructions. The quality of DNA was checked by spectro photometry taking ratio of optical density (OD) value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further work. The poor quality DNA was re-extracted with phenol–chloroform.

PCR- Forced RFLP of FecB gene

A region of *FecB* gene (190 bp) was amplified by using a set of forward (5-`-CCAGAGGACAATAGCAAAGCAAA -3`) and reverse (5`-CAAGATGTTTTCATGCCTCATCAACAGGTC -3`) primers (*Wilson et al., 2001*). The reverse primer was deliberately introduced a point mutation resulting in PCR products with FecB carrier sheep containing an *Ava*II restriction site (G|GACC), whereas products from noncarriers lacked (of) this site. For amplification, 25 ul of PCR reaction was prepared by adding 10pM of each primer, 100µM of each dNTPs, 1.5mM MgCl2, 10× PCR assay buffer, 100 ng DNA template and 1 Unit *Taq* DNA polymerase. The amplification was carried out using a pre-programmed thermal cycler (Eppendorf Mastercycler) with the following conditions: initial denaturation of 5 min at 94 ◦C followed by 30 cycles of denaturation at 94 °C, annealing at 60 °C and extension at 72°C each of 30 s and lastly the final extension of 5 min at 72 °C. DNA tests were carried out using forced PCR RFLP based on the method described by Davis *et al*. (2002). An aliquot of 10 µl of PCR product was digested for 6 hours at 37 °C with 10 Units of *Ava* II restriction enzyme (Fermentas). The restriction enzyme digested PCR products were separated by 4 % agarose gel and stained with ethidium bromide. The digested products were visualized and documented under gel documentation system (Syngene).

Genotype analysis

The forced PCR of the *FecB* gene produced a 190 base pair (bp) band. After digestion with *Ava*II (Fermentas), the *FecB* gene homozygous carriers had a 160 bp band (BB), the noncarrier had a 190 bp band (++), whereas heterozygotes had both 160 and 190 bp bands (B+).

Results and discussion

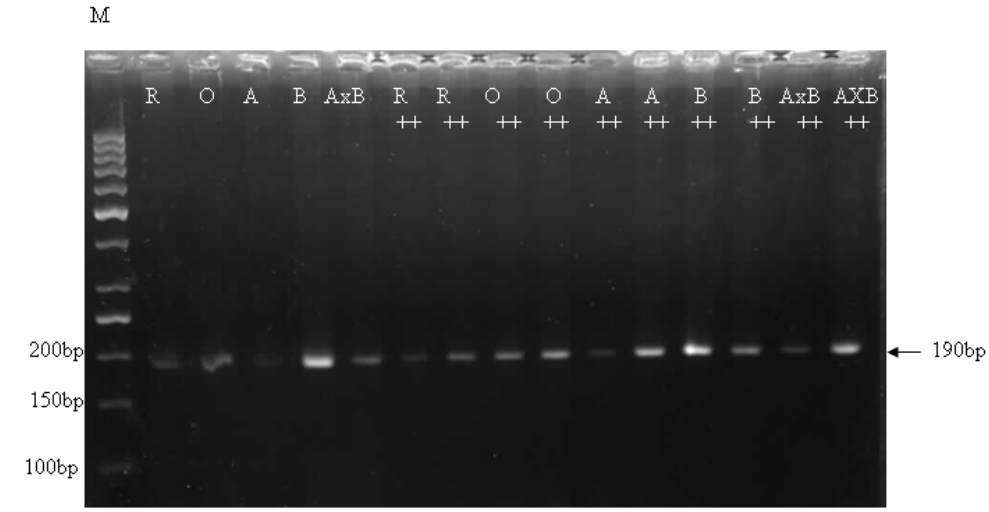
Figure (1) represent agarose gel electrophoresis of PCR amplified *FecB* gene (190 pb) and *Ava*II digested *FecB* gene product of Rahmani (R) , Ossimi (O), Awassi (A) , Barki (B) and Awassi x Barki (A x B) breeds.

It is clear from *Ava*II RFLP pattern represented in figure (1) that Digestion of *FecB* gene 190 base pair with *Ava* II restriction enzyme resulted in non carrier (++) 190 bp band (wild type) in all the animals belonging to the five Egyptian breeds studied revealing absence of this restriction site of *Ava* II in those five Egyptian sheep breeds. *Guan et al.(2007)* reported that 7 of 9 sheep breeds studied were found to be wild type (190 bp ++) in respect to restriction pattern of *FecB* gene.

On the other hand, they reported that Chinese Merino prolific meat strain had the three different Booroola genotypes (BB, B+ and ++), while Hu were all homozygous for FecB (BB). In addition, they found that Hu-sheep crossbred progeny had B+ genotype, which exhibited a simple Mendelian pattern of segregation when they were backcrossed. They reported positive relationship between mutation of FecB gene and litter size in Chinese Merino prolific meat strain., where the litter sizes of ewes with BB genotype averaged 2.84±0.74, which was significantly greater than that (1.23±0.68) of ewes with ++ genotype ($P < 0.01$,). The ewes with BB genotype also produced 0.5 lambs more than ewes with B+ genotype, although the difference was not statistically significant.

In addition, they found that at 90 days after birth, the body weights of BB/B+ lambs were higher than that of ++ lambs (18.6±3.70 kg, 18.0±3.71 kg versus 15.6± 2.22 kg, $P < 0.05$). In addition, the heart girth and chest width of BB/B+ lambs were significantly longer than ++ lambs ($P < 0.05$). No significant differences were observed in either body weight or body size at day 120.

Figure 1. PCR amplification of *FecB* gene gene (190 bp,Lanes 2-6) in Rahmani (R), Ossimi (O), Awassi (A), Barki (B) and Awassi x Barki (AxB) Egyptian sheep breeds and digesation product of samples with *Ava* II restriction enzyme (lanes 7-16) with genotype ++ . Lane M, molecular size marker (50 bp DNA ladder).



They explained the positive relationship between mutation of FecB gene and litter size on the basis that the effect of *FecB* gene was additive for litter size. on and ovulation rate, increasing corpora lutea by about 1.65 per copy and litter size by 0.9 for one copy and a further 0.4 for two copies (*Piper et al., 1985*). On the other hand, it is reported that the *FecB* gene has negative effects on fetal body weight, body size and development during pregnancy (*Smith et al., 1993*). Body weights were lighter at most gestational ages in BB/B+ than in ++ fetuses, and the BB/B+ grew slowly and body lengths were shorter than ++ fetuses. In contrast, when measuring postnatal growth and development our results showed that by day 90 after birth, the body weight, heart girth, and body height were significantly increased in BB/B+ compared with ++ lambs. Although of similar birth weight the BB and B+ lambs grew more in the first 90 days after birth. The differences probably result from the effects of different breeds and different phases of development, environmental conditions, different lambing and nutrition, or effects altogether, and the mechanism requires advanced research.

In respect to the results of the present study, the presence of non carrier 190 bp band pattern (wild type) in all the animals belonging to the five Egyptian breeds studied could be explained on the basis of low litter size in these breeds, since the presence of ++ wild type is significantly correlated with low litter size (*Guan et al.,2007*). In this respect *Galal et al. (1996*) reported that Egyptian sheep

breeds are of medium size, low growth rate, breed all year round and have small litter size ranging from 1.03 to 1.40. They mentioned that There was an attempt to increase meat production from local Egyptian breeds by introducing of Finn sheep genetics in 1970 on an experimental basis; field tests were started in 1987, as explained below, in 80 farms and four successive batches of *rams and ewes were imported later. In addition, Almahdy et al(2000)*[a] reported that Egyptian sheep breeds are characterized by extended breeding seasons, high fertility, and low prolificacy, they added that Currently in Egypt efforts are being made to intensify production systems, primarily through changing reproductive management and crossing native breeds with introduced breeds.

Conclusion

It can be concluded that Digestion of *FecB* gene 190 base pair with *Ava* II restriction enzyme resulted in non carrier 190 bp band (wild type) in all the animals belonging to the five Egyptian breeds studied revealing absence of this restriction site in those five Egyptian sheep breed. the presence of non carrier 190 bp band pattern (wild type) in all the animals belonging to the five Egyptian breeds studied could be explained on the basis of low litter size in these breeds, since the presence of ++ wild type is significantly correlated with low litter size. Further studies should be made to determine required crossbreeding ratio with foreign breeds carry the favorite genotypes of this gene which will lead to increase of expression of these gene in the local Egyptian breed without affect on the acclimatization traits of these breeds to the environmental conditions in Egypt.

Fingerprinting *fecb* gena kod pet egipatskih rasa ovaca

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Rezime

Određivanje genetskog diverziteta autohtonih rasa ovaca u Egiptu u vezi sa ekonomski važnim genima nije doboljno proučavano. Genetska krakterizacija i određivanje genetskih rzalika između rasa ovaca će nam pomoći u programima genetskog unapređenja. Ovo istraživanje je realizovano sa ciljem ispitivanja polimorfizma forsiranom PCR-RFLP *FecB* gena kod pet egipatskih lokalnih rasa

ovaca i njihovo poređenje sa drugim inostranim rasama ovaca. Genomska DNK je izolovana iz ukupno 100 grla ovaca pet egipatskih rasa - rahmani, osimi, avasi, barki i meleza dobijenih ukrštanjem avasi x barki rasa. Forsirani PCR 190 baznih parova (bp) *FecB* gena je pojačan korišćenjem specifičnih prajmera koji su namenjeni za uvođenje tačkastih mutacija u rezltirajućim PCR proizvodima sa FecB nosiocem, ovcom koja sadrži *Ava*II restrikciono mesto (G|GACC), dok su proizvodi od ne-nosilaca nedostajali na ovom mestu. Digestija 190 baznih parova *FecB* gena sa *Ava*II restriktivnim enzimom je rezultirala u ne-nosiocu 190 bp (divlji tip) kod svih grla koja pripradaju ispitvanim (5) egipatskim rasama otkrivajući odstustvo restrikcionog mesta u ovim egipatskim rasama ovaca.

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