Polymorphism of some Genes Associated with Meat-Related Traits in Egyptian Sheep Breeds

K.F. Mahrous1, M.S. Hassanane1, M. Abdel Mordy2, H.I. Shafey1* and H.E. Rushdi3

1 Department of Cell Biology, National Research Center, Dokki, Giza, Egypt
2 Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt
3 Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt

Received on: 25 May 2014
Revised on: 6 Sep 2014
Accepted on: 15 Nov 2014
Online Published on: Sep 2015

*Correspondence E-mail: poohdode@yahoo.com

© 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran
Online version is available on: www.ijas.ir

ABSTRACT

The genetic polymorphism of some genes related to meat production in three Egyptian sheep breeds (Barki, Rahmani and Osseimi) was studied. The candidate genes were: Calpastatin, Myostatin, Diacylglycerol-acyltransferase 1, Insulin-like growth factor binding protein-3 and Booroola fecundity gene. The technique applied was the restriction fragment length polymorphism for the polymerase chain reaction products. Polymorphism was found in the genes: Calpastatin, Myostatin and Diacylglycerol-acyltransferase 1, while no polymorphism was exhibited by the other two genes, Insulin-like growth factor binding protein-3 and the Booroola fecundity in the three breeds understudy. Calpastatin locus digested with MspI had two genotypes MM and MN. The highest allelic frequency was for allele M. The same locus Calpastatin digested with NcoI also exhibited two genotypes MM and MN. The NN genotype was absent with both the MspI and the NcoI enzymes in all breeds. Myostatin digested with DraI had two genotypes AB and BB. The AA genotype cannot be detected. The highest allelic frequency was for allele B. Diacylglycerol-acyltransferase 1 digested with AluI showed two genotypes CC and CT. The highest allelic frequency was for allele C. The detected CT genotype might explain the moderate intramuscular fat content and muscle marbling in the Egyptian sheep breeds. Each of the remaining two loci (Insulin-like growth factor binding protein-3 and the Booroola fecundity) had only one genotype, BB genotype for Insulin-like growth factor binding protein-3 digested with HaeIII and ++ genotype for the Booroola fecundity digested with AvaII enzyme, therefore they are not recommended in the selection program. The result of Chi-square analysis indicated that the three Egyptian sheep breeds were in Hardy-Weinberg equilibrium.

KEY WORDS genetic polymorphism, PCR-RFLP, sheep.

INTRODUCTION

Sheep is one of the most important domestic animals raised in Egypt. Sheep population in Egypt increased by 66.7% from 1961 to 2005 (FAO, 2007). There are almost 5.5 million head of sheep (FAOSTAT, 2011). Sheep are raised mainly for meat production with carpet sheep wool as a secondary product (Galal et al. 2002). The three major Egyptian sheep breeds are: Barki, Rahmani and Osseimi.

They represent about 65% of the total sheep population in Egypt (Elshennawy, 1995). The Egyptian sheep breeds are characterized as small-to medium-sized body, fat tailed and fleece is coarse wool (Aboul-Ela et al. 1987). Meat is an integral part of a healthy diet and meat consumption continues to increase steadily, while the world’s producers of livestock for red meat production are finding it difficult to cope with the increase in demand (FAO, 2007). Sheep contribute 6% of the total red meat produced and are con-
sidered the second source of red meat in Egypt (Galal et al. 2005). At the present time, the consumers demand for sheep meat is not focused on quantitative traits only, but also qualitative traits of meat. Animal breeders could accelerate the rate of genetic improvement attained in carcass composition and meat quality traits through the application of gene-assisted selection (GAS), which is simply based on incorporating favorable candidate genes in traditional breeding programs (Andersson, 2001). The allele variation within candidate genes between breeds is warranted to differentiate them on genetic basis. The result of this variation may assist in choosing the best strategy of improvement options for these genetic resources (Tohidi et al. 2013). There are many of published articles on different genes associated with meat-related traits in varied sheep breeds, among these genes are CAST, MSTN, DGAT1, IGFBP3 and FecB.

Calpastatin (CAST) gene is an endogenous inhibitor of non-lysosomal proteases-calpains, present in cytosol, mapped to ovine chromosome 5 (OAR 5). It is essential for the formation of skeletal muscles during the postnatal period and proteolysis after slaughter, having therefore an effect on traits of particular importance to consumers such as meat tenderness or water binding (Padma et al. 2004). It was also proved that there is a relationship between polymorphism in the calpastatin gene in sheep and slaughter traits such as lamb’s body weight at birth and its growth rate until weaning (Byun et al. 2008). Associations between variation in CAST gene with carcass and meat quality traits in sheep have been reported by several authors (Palmer et al. 1998; Zhou et al. 2007; Sumantri et al. 2008; Gabor et al. 2009).

Using molecular genetics approach to study meat quality in sheep, Palmer et al. (1998) have suggested CAST gene as a candidate gene for meat quality in ovine species. Post-slaughter tenderization depends on the balance between the level of activity and inactivation of proteolytic enzymes (Warner et al. 2010). A mutation in CAST gene can influence on calpastatin activity followed by a level of proteolytic enzymes at slaughter. Myostatin (MSTN) gene, (also known as growth and differentiation factor 8, GDF8) is the major regulator of myogenesis and it functions as a negative regulator of muscle growth in mammals. Mutations in MSTN are associated with increased skeletal muscle mass (double muscling) in sheep (Clop et al. 2006; Kijas et al. 2007). The regulation of muscularity, adiposity and tendon structure potentially has important implications for sheep meat production. Ovine MSTN is located on OAR 2. Myostatin is known to directly influence muscular hypertrophy and carcass conformation (Nakev et al. 2013). Diacylglycerol-acyltransferase1 (DGAT1) is a gene that encodes one of the key enzymes controlling the rate of triglyceride synthesis in adipocytes (Winter et al. 2002). It plays a central role in the metabolism of cellular glycerolipids (Cases et al. 1998) and also acts as a key modulator in fat deposition of livestock breeds (Cui et al. 2011; Patel et al. 2009). Ovine DGAT1 has been localized to OAR 14. Over expression of DGAT1 leads to increased cellular triacylglyceride (TG) storage in white adipose tissue, skeletal muscle, and liver in a tissue specific manner and thereby limits the amounts of intracellular diacylglycerol (DAG) and free fatty acids (Roorda et al. 2005; Liu et al. 2007; Liu et al. 2009; Kamisaka et al. 2010).

Insulin-like growth factor binding protein-3 (IGFBP3) is a specific carrier protein that binds non-covalently to IGFs in circulation (Bale and Conover, 1992). It acts as a marker for different body functions such as growth, metabolism, reproduction, in controlling body weight, immunity and energy balance (Sharma et al. 2012). IGFBP3 has been localized to OAR21 (Hastie et al. 2004). Polymorphism in IGFBP3 has been studied in different live stocks. There are a few reports in cattle (Shukla, 2001), buffalo (Padma et al. 2004) and sheep (Kumar et al. 2006), which suggest the polymorphic/non-polymorphic nature of IGFBP3 gene.

The Booroola fecundity gene (FecB) is a single autosomal gene, which increases ovulation rate and litter size in sheep (Davis, 2004). The FecB locus is located in the region of ovine chromosome 6. In recent years, many aspects of the FecB gene are reported including reproductive endocrinology (Zhong et al. 2006), ovary development (Yao et al. 2006), litter size (Chu et al. 2007; Guan et al. 2006), organ development and body measurements (Guan et al. 2006; Yu et al. 2008). Litter size and lamb growth are important economic values in sheep breeding and reproduction.

The aim of the present study is to identify the genetic polymorphism for five genes correlated with meat gain and traits (CAST, DGAT1, MSTN, IGFBP3 and FecB) in three Egyptian sheep breeds (Barki, Rahmani and Osseimi). Application of Polymerase Chain Reaction-Restriction Fragment Length polymorphisms (PCR-RFLPs) was carried out.

**MATERIALS AND METHODS**

**Animals and blood collection**

A total of 60 animals representing the three Egyptian sheep breeds under study (Osseimi, Barki and Rahmani) were randomly sampled (20 samples per breed). All animals were born and reared in the Agriculture Research Station, belonging to the Faculty of Agriculture, Cairo University. Blood samples were collected in tubes containing EDTA as anticoagulant and transported to the laboratory under cooled conditions.

---

DNA isolation
DNA was extracted and purified from blood samples using the whole blood salting out technique described by Miller et al. (1988). DNA concentration and purity were determined using an UV spectrophotometer at optical density of 260 and 280 nm.

Polymerase chain reactions (PCR)
Reactions were performed using specific primers for each gene under study. Details of the primer sequences are listed in Table 1. Amplification reaction was carried out in a 25 µL volume containing 100 ng genomic DNA, forward and reverse primer (both at concentration 10 pmol/µL), 1U Taq polymerase, 2.5 µL Taq polymerase buffer, four dNTPs (each at final concentration of 2.5 mM/µL) and de-ionized double distilled H2O up to a total volume of 25 µL. The general PCR program for the amplification of the genes included in the current study was: initial denaturation: 95 °C for 3 min., 95 °C for 15 sec. (denaturation), 58-63 °C for 30-60 sec. (annealing depending on the gene) and 72 °C for 30 sec. up to 35 cycles, then final extension at 72 °C for 5 min. and finally storage at 15 °C. For PCR optimization the temperature and the time of the annealing temperature were changed. The success of PCR was tested after running some of the products on 2% horizontal agarose gel electrophoresis, and staining with ethidium bromide.

Restriction fragment length polymorphism (RFLP)
Ten µL from the PCR products were digested with 5 units of the fast restriction enzyme including specific buffer (Fermentas, Germany) specific for each gene (Table 1) in a final reaction volume of 15 µL. The reaction mixture was incubated at 37 °C in water bath for 30 minutes. After restriction digestion, the restricted fragments were visualized after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide, except after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide, except after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide, except after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide, except after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide, except after running in horizontal gel electrophoresis (2-3% agarose) and staining with ethidium bromide.

RESULTS AND DISCUSSION

Genetic polymorphism of CAST gene digested with MspI
Digestion of 622 bp PCR product of CAST gene with restriction endonuclease (MspI) differentiated alleles N and M. The MspI digestion of the PCR products produced fragments of 336 bp and 286 bp for allele M, but the allele N was not digested (Figure 1). The homozygous genotype NN (622 bp) was not observed in the three sheep breeds studied. The heterozygous genotype NM (336 bp and 286 bp), both were detected in all breeds involved. Polymorphism of CAST gene has been reported in Kurdi sheep in Iran (Nassiry et al. 2006), Karakul sheep in Iran (Shahroudi et al. 2006), Merino, Corriedale, Romney, Poll Dorset, and crossbred NZ sheep in New Zealand (Zhou et al. 2007).

Our results are in agreement with the polymorphism detected in CAST gene that was previously observed by Palmer et al. (1998) in Dorset sheep, Chung et al. (2001) in mixed population sheep of Tsigai, Improved Valachian, East Friesian, Lacaune and Lacaune × Tsigai breed and Gabor et al. (2009) in local sheep raised in Slovakia. They found only two genotypes MM and NM. Palmer et al. (1998) detected 3 genotypes MM, MN, NN of CAST gene in Corridale rams; also Shahroudi et al. (2006) found the same three genotypes in Iran Karakul sheep, while Sumantri et al. (2008) found only NN and NM genotypes in Indonesian Jonggol sheep.

There was a tendency of the three Egyptian sheep breeds to have higher frequencies of M allele than the N allele. Other studies also showed similar high frequency of allele M and low frequency of allele N (Table 2). Shahroudi et al. (2006) reported that the frequencies for M and N alleles of CAST gene digested with MspI in Karakul sheep were 0.79 and 0.21 respectively. Gabor et al. (2009) also reported that the genotyping results of CAST gene locus MspI in Tsigai sheep, Improved Valachian, Lacaune, East Friesian, and Tsigai sheep × Lacaune sheep had allele frequencies of M 0.94 and N 0.06. In addition, Suleman et al. (2012) found that the frequency of M allele was higher than that of N allele in Thalli, Lohi and Kazhi Pakistan sheep. In contrast, the result of the current study differs from that reported by Sumantri et al. (2008) who reported that the allele N frequency was higher than allele M in Indonesian Jonggol sheep breed. The highest genotypic frequency NM was found in Barki breed and the highest genotypic frequency MM was found in Osseimi breed.

There are limited studies for association between polymorphism of CAST gene and growth and meat characteristics in sheep.
However, Diyono et al. (2007) demonstrated an association between MN genotype with live body weight. They reported that male sheep with MN genotype had higher body weight in comparison to NN genotype.

### Table 1

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Primer sequences</th>
<th>Restriction enzyme</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAST</td>
<td>TGGGGCCCAATGGAGCCTGATG</td>
<td>MspI</td>
<td>Shahroudi et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>GGTTGAGCAGCTTCTGATGCC</td>
<td>NcoI</td>
<td></td>
</tr>
<tr>
<td>MSTN</td>
<td>TGGCGTTACTCAAAGGCAA</td>
<td>Dra I</td>
<td>Xianglong et al. (2008)</td>
</tr>
<tr>
<td>DGAT1</td>
<td>GCTTTCGCTGCCCTGCTG</td>
<td>Alu I</td>
<td>Xu et al. (2008)</td>
</tr>
<tr>
<td>IGF3P3</td>
<td>CCAGGAGAACGAGAACAGAT</td>
<td>HaeIII</td>
<td>Ali et al. (2009)</td>
</tr>
<tr>
<td>FecB</td>
<td>CAAGATTTTCATGCCCTCAACAGG</td>
<td>AvaII</td>
<td>Guan et al. (2006)</td>
</tr>
</tbody>
</table>

### Figure 1

DNA electrophoretic pattern of CAST amplicon after digestion with MspI endonuclease. Lane M, 100 bp DNA ladder, lanes genotype NM (1, 4, 5, 6, 9); (622, 336 and 286 bp) and lanes (2, 3, 7, 8, 10) genotype MM (336 and 286 bp).

### Table 2

Genotypic frequency, allelic frequency, observed heterozygosity (Ho), expected heterozygosity (He) and \(\chi^2\) estimates of CAST gene digested with MspI and NcoI.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Restriction Enzyme</th>
<th>Genotypic Frequency</th>
<th>Allelic Frequency</th>
<th>Ho</th>
<th>He</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barki</td>
<td>MspI</td>
<td>0.65 0.35</td>
<td>0.32 0.68</td>
<td>0.65</td>
<td>0.45</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td>NcoI</td>
<td>0.50 0.50</td>
<td>0.75 0.25</td>
<td>0.50</td>
<td>0.38</td>
<td>1.96</td>
</tr>
<tr>
<td>Rahmani</td>
<td>MspI</td>
<td>0.40 0.60</td>
<td>0.20 0.80</td>
<td>0.40</td>
<td>0.32</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>NcoI</td>
<td>0.55 0.45</td>
<td>0.77 0.23</td>
<td>0.45</td>
<td>0.35</td>
<td>1.47</td>
</tr>
<tr>
<td>Osseimi</td>
<td>MspI</td>
<td>0.30 0.70</td>
<td>0.15 0.85</td>
<td>0.30</td>
<td>0.26</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td>NcoI</td>
<td>1.00 0.00</td>
<td>0.00 1.00</td>
<td>0.30</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### Genetic polymorphism of CAST gene digested with NcoI

The digestion of 622 bp PCR product of CAST gene with restriction endonuclease NcoI differentiated alleles N and M. The NcoI digestion of the PCR products produced digestion fragments of 374 and 248 bp for allele N but the allele M was not digested. The size of fragment for allele M was 622 bp (Figure 2).

### Figure 2

DNA electrophoretic pattern of CAST amplicon after digestion with NcoI endonuclease. Lane M, 100 bp DNA ladder, lanes genotype MM (1, 2, 3, 4, 6, 9, 10, 11, 12, 13, 17, 18, 19) 622 bp and lanes (5, 14, 15, 16) genotype NM (336 and 286 bp).

Regarding the heterozygosity values for this gene in the breeds under study, in general, allelic frequency of the observed heterozygosity was higher than the expected heterozygosity in all breeds. The highest (0.65) was in Barki, while the lowest value (0.30) was in Osseimi (Table 2). The CAST locus MspI had \(\chi^2\) value of 4.22, 1.07 and 0.508 for Barki, Rahmani and Osseimi breeds respectively (Table 2). These values shown are non-significant deviation from HWE (P>0.05).
This result is similar to that obtained by Sutikno et al. (2011) where genotypes NN was absent in population of local sheep in JASTRU. On the contrary, Palmer et al. (1998) identified three genotypes MM, MN and NN in Dorset Down, Dorset Down × Coopworth, and Corriedale sheep.

The frequency of homozygous genotype MM had (0.50, 0.55 and 1) while heterozygous genotype MN had frequency (0.50, 0.45 and 0.00) this suggests a superiority of M allele (0.75, 0.77 and 1) than N allele (0.25, 0.23 and 0.00) in Barki, Rahmani and Osseimi, respectively (Table 2).

In general, low to moderate observed and expected heterozygosity estimates were obtained. The Barki had the highest expected heterozygosity (0.38) followed by Rahmani (0.35). No heterozygosity, either observed or expected could be noticed in Osseimi breed (Table 2). The value of the observed heterozygosity was higher than that of the expected heterozygosity in both Barki and Rahmani, this means that the inbreeding rate within each of these two breeds is low (Lewontin, 1974).

Genetic variation of breeds was evaluated by using χ²-test (Table 2). The CAST locus NcoI had χ² value of 1.96 and 1.47 for Barki and Rahmani. χ² showed non-significant deviation from HWE (P>0.05). This explains the high value of observed than expected heterozygosity in these breeds. Generally, HWE can be affected by inbreeding, assort native mating, natural selection and population subdivision (Nei and Kumar, 2000).

Genetic polymorphism of MSTN gene digested with Dral

The ovine MSTN gene produced a DNA fragment of 497 bp. Digestion of this fragment by restriction endonuclease Dral generated only two different genotypes, AB and BB, no AA genotype was found. The uncut was related to the A allele, while the cut fragment (427 and 70 bp) was corresponding to the B allele (Figure 3). In general the goat A allele was characterized by the deletion of a small DNA fragment (TTTTA). This deletion unique for goats was found in 5’UTR of goat MSTN gene and causing a significant effect on body weight and size (Xianglong et al. 2008). The present study indicates that this deletion can be also seen in different sheep breeds and not limited to goats. It was observed that the BB genotype was the more common genotype in all breeds under study; this might be due to the low frequency noticed for the allele A (Table 3).

The values of the observed heterozygosity were higher than the expected ones in all breeds (Table 4). The frequency of the B allele was significantly higher than that of A allele, especially in Osseimi breed. This may explain the lowest heterozygosity in this breed.

Absence of AA genotype and presence of AB genotype in Egyptian sheep’s may be due either to a mutation (deletion) in corresponding locus or allele A is naturally exists in the sheep genome. Further studies, especially sequencing of the heterozygous genotype (AB) is recommended.

![Figure 3](image)

DNA electrophoretic pattern of MSTN amplicon after digestion with Dral endonuclease. Lane M, 100 bp DNA ladder, lanes (1, 2, 3, 6, 10, 11, 12) genotype AB (497, 427and 70 bp) and lanes (4, 5, 7, 8, 9) genotype BB (427 and 70). It was difficult to classify the 70 bp fragment on 2.5% agarose gel since it could not be seen.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Restriction enzyme</th>
<th>Breed</th>
<th>Genotypic frequency</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSTN</td>
<td>Dral</td>
<td>Barki</td>
<td>AA 0.45 AB 0.55 BB 0.23</td>
<td>A 0.77 B 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rahmani</td>
<td>0.35 0.65 0.17 0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osseimi</td>
<td>0.15 0.85 0.07 0.93</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locus</th>
<th>Restriction enzyme</th>
<th>Breed</th>
<th>Ho</th>
<th>He</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSTN</td>
<td>Dral</td>
<td>Barki</td>
<td>0.45</td>
<td>0.36</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rahmani</td>
<td>0.35</td>
<td>0.30</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osseimi</td>
<td>0.15</td>
<td>0.14</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The highest heterozygosity either observed (0.45) or expected (0.36) was in Barki sheep. The lowest values, 0.15 and 0.14 were in Osseimi but Rahmani has moderate value of 0.35 for (Ho) and 0.30 for (He) (Table 4). HWE of breeds was determined by using χ²-test. The MSTN locus Dral had χ² value of 1.47, 0.75 and 0.085 for Barki, Rahmani and Osseimi, respectively. χ² values indicate non-significant deviation from HWE (P>0.05) (Table 4).

Genetic polymorphism of DGTA1 gene digested with AluI

The size of PCR amplicon of DGTA1 gene was 309 bp. The amplified segment includes part of exon 16, whole intron 16 and exon 17.
Xu et al. (2008) reported presence of a point mutation in exon 17 (C→T) which creates a cut site for endonuclease Alu1 (AGCT). The present locus has two alleles; C with 309 bp, and T with 272 and 37 bp. Two genotypes were generated after its digestion with Alu1, which are CC (309 bp) and CT (309, 272 and 37 bp) (Figure 4).

Figure 4 DNA electrophoretic pattern of DGTA1 amplicon after digestion with Alu1 endonuclease. Lane M, 100 bp DNA ladder, lanes (1, 4, 5, 9, 10, 11, 14, 15) genotype CC (309 bp) and lanes (2, 3, 6, 7, 8, 12, 13) genotype CT (309, 273, 37). It was difficult to classify the 37 bp fragment on 2.5% agarose gel since it could not be seen.

This SNP is a silent mutation \( \text{GCC(Ala)} \rightarrow \text{GCT(Ala)} \), which creates a no substitution change for amino acid sequence of DGTA1 protein (Yang et al. 2011). The genotypic and allelic frequencies in exon 17 of DGTA1 gene of studied breeds are shown in Table 5.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Breed</th>
<th>Genotypic frequency</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGTA1 (Alu1)</td>
<td>Barki</td>
<td>0.75 0.25 0</td>
<td>0.88 0.12</td>
</tr>
<tr>
<td></td>
<td>Rahmani</td>
<td>0.60 0.40 0</td>
<td>0.80 0.20</td>
</tr>
<tr>
<td></td>
<td>Osseimi</td>
<td>0.50 0.50 0</td>
<td>0.75 0.25</td>
</tr>
</tbody>
</table>

In the two breeds, Barki and Rahmani, the CC genotype was higher than that of CT, while in Osseimi breed, the two genotypes CC and CT were equal. The TT genotype was not exhibited by any of the three breeds. With regard to allelic frequencies, the C allele was the predominant one in all breeds. Its frequency was 0.88, 0.8 and 0.75 in Barki, Rahmani and Osseimi, respectively. On the other hand, the highest frequency for T allele was found in Osseimi (0.25) while the lowest one was seen in Barki (0.12) (Table 5). These results are in agreement with that obtained by Yang et al. (2011) whom reported that CC genotype was higher than that of CT and TT in Tan sheep, Oula sheep, Ganjia sheep and Qiaoke sheep. In addition, the SNP (C→T) in exon 17 of Chinese indigenous sheep breeds had a significant association with intramuscular fat content (IMF), muscle marbling and meat tenderness. Xu et al. (2008) reported that the mutated T allele of DGTA1 gene had a positive effect on meat quality traits of sheep and the CT genotype had a moderate IMF content and marbling score. Detection of CT genotype and absence of TT genotype in the studied breeds may explain the moderated IMF content and muscle marbling in the Egyptian sheep breeds. The highest observed heterozygosity (0.5) was found in Osseimi breed and the lowest was (0.25) detected in Barki breed (Table 6). Regarding genetic variation, the values of Chi-square test were within HWE (P>0.05). This is similar to the result of Yang et al. (2011). The allele distribution in Tan sheep and Oula sheep were within Hardy-Weinberg equilibrium (Table 6).

Table 6 Observed heterozygosity (Ho), expected heterozygosity (He) and \( \chi^2 \) estimates of DGTA1 gene digested with Alu1

<table>
<thead>
<tr>
<th>Locus</th>
<th>Breed</th>
<th>Ho</th>
<th>He</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGTA1 (Alu1)</td>
<td>Barki</td>
<td>0.25</td>
<td>0.25</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>Rahmani</td>
<td>0.40</td>
<td>0.33</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>Osseimi</td>
<td>0.50</td>
<td>0.40</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Genetic polymorphism of IGFBP3 gene digested with HaeIII

The length of the amplified product of ovine IGFBP3 gene was 654 bp in sheep. The amplified 654 bp fragment comprises the last part of exon 2, complete intron 2, exon 3 and a part of intron 3. The exon-intron regions were assigned on the basis of the published reports of this gene in cattle (Maciulla et al. 1997). It is clear from HaeIII RFLP pattern represented in Figure 5 that, there was no polymorphism among the three Egyptian sheep breeds in respect to IGFBP3 gene.

Figure 5 Polyacrylamide gel electrophoresis for DNA of IGFBP3 ampli- cion after digestion with HaeIII endonuclease. Lane M, 50 bp DNA ladder, all lanes (1—14) genotype BB (201, 201, 87, 76 and 57 bp). It was difficult to classify the (19, 16 and 8 bp) fragments on polyacrylamide gel.
Digestion of the PCR product of IGFBP3 gene with HaeIII revealed only one type of restriction pattern including fragments of sizes 201, 201, 87, 67, 57, 19, 16 and 7 bp (the first five fragments were visible) which represent the allele B only. This result indicates the homozygosity of this gene in the three sheep breeds studied. The present findings are similar to those reported by Kumar et al. (2006) who found no polymorphism in IGFBP3 gene in five Indian sheep breeds. However, they obtained HaeIII restriction pattern of eight fragments of sizes 201, 201, 87, 67, 57, 19, 16 and 7 bp in all animals studied revealing absence of polymorphism in these Indian sheep breeds.

Also, the present results showed only one genotype (BB) in extra the three Egyptian sheep breeds. Ali et al. (2009) reported the same result in the four Egyptian sheep breeds (Rahmani, Osseimi, Barki and Awassi) indicating the homozygosity of this gene in the four breeds studied. They also reported that all the sheep have intact HaeIII restriction site (GG↓CC) indicating the absence of polymorphism at this site.

Genetic polymorphism of FecB gene digested with AvaII
The PCR products of the FecB gene from the three Egyptian breeds produced a single band of 190 bp. The primer used in the present study was that used by Guan et al. (2006). It was specific for detection of the restriction site, G/GACC when the FecB gene products are digested with AvaII enzyme.

Digestion of the amplified products of the FecB gene revealed only the same band (190 bp). No differences in the band patterns of digested products were detected. Only the wild type alleles (++) were recognized and all breeds for this locus were monomorphic (Figure 6). Similar results were reported by El-Hanafy and El-Saadani (2009). They found the 190 bp band in five Egyptian sheep breeds, among them were Rahmani, Barki and Osseimi.

Guan et al. (2006) studied polymorphism of FecB gene in nine sheep breeds. Seven out of the nine breeds had only the wild type (+++) genotype, i.e. they have only the 190 bp band. One breed (the Hu sheep) was all homozygous for FecB gene (BB), the mutant form. In the remaining breed which is the Chinese Merino prolific meat strain, the three genotypes BB, B+ and ++ were found. In the present work, only the wild type (++) was detected in the three Egyptian breeds. The present findings and those reported by El-Hanafy and El-Saadani (2009) confirm the absence of the restriction site, G/GACC in the Egyptian breeds. Similar results were published by Abouheif et al. (2011) in Najdi and Naeimi sheep breeds of Saudi Arabia and Al-Barzinji and Othman (2013) in five Iraqi sheep breeds (Hamdani, Karadi, Arabi, Naeimi and Awassi). The mean litter size of ewes with genotype ++ was less than that of those having BB and B+ genotypes.

The body weights of ++ genotype lambs were lower than that of BB/B+ genotype lambs. In addition, the heart girth and chest width of ++ genotype lambs were shorter than those of the BB/B+ genotype lambs (Mulsant et al. 2001; Guan et al. 2006).

It was reported that the FecB gene has negative effects on fetal body weight, body size and development during pregnancy (Smith et al. 1996). The prevalence of the ++ genotype in the three Egyptian breeds under study may explain the low litter size of ewes, the low body weights and the short heart girth and chest width of lambs in these breeds. In this respect Galal et al. (1996) reported that Egyptian sheep breeds are of medium size and low growth rate.

CONCLUSION
The three genes: CAST (MpsI, NcoI), MSTN (DraI) and DGTA1(AluI) were polymorphic in all breeds examined. Barki sheep breed showed the highest observed heterozygosity for both CAST and MSTN genes. The same breed also showed the highest genotypic frequency (AB) for MSTN gene, followed by Rahmani then Osseimi. Osseimi sheep showed the highest genotypic frequency (CT) and the highest observed heterozygosity for DGTA1 gene, followed by Rahmani and Barki. The IGFBP3 and FecB genes were monomorphic, therefore they are not recommended in the selection program. In order to have sharp molecular markers especially for economically important traits such as gain and meat traits in the Egyptian sheep breeds, studies must be carried out on the candidate genes on a large sample size from each breed.

REFERENCES


