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Effects of the FecB Allele on Body Weights of Naeimi and Naeimi Crossbred Lambs

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Abstract: This study aims to investigate the effect of carrying one copy of the FecB allele on body weight of Naeimi and Naeimi crossbred lambs at birth, weaning, 75 and 100 days of age. A total of 107 lambs representing 24 purebred Naeimi (NN) and 83 Naeimi-Booroola carrier crossbred (NB) were examined. The results showed that 43 lambs out of the 83 NB lambs were heterozygous carrier of the FecB allele whereas the other NB lambs were homozygous non-carrier of the wild allele. The frequency of the B^+ genotype was 0.52 among all born NB lambs. The results also revealed that mean body weights of NN lambs were heavier ($p<0.05$) than that of NB lambs at all studied ages. Body weights of NB^{++} were heavier ($p<0.05$) than NB^{B+} lambs at birth, weaning and 75 days of age, the differences in weight between NB^{++} and NB^{B+} at 100 days of age was not significant ($p>0.05$). The influence of type of birth on live body weight at birth and weaning age was significant ($p<0.05$) but these differences between single and twin born lambs and between twin and triplet born lambs disappeared ($p>0.05$) at 75 and 100 days of age. No differences ($p>0.05$) were noted in birth and weaning weights of male and female lambs, however, the male lambs being 7.2% heavier ($p<0.05$) than the female lambs at 75 and 100 days of age.

Key words: Naeimi lambs, Booroola gene, body weight, FecB allele, crossbred

INTRODUCTION

Lamb production and sheep breeding efficiencies in Naeimi can be improved if it is possible to increase the prolificacy, the same output of lambs can be derived from fewer parent ewes thus will decrease production costs. In addition, breeding organizations will benefit from the potential to screen more offspring for those with improved genetics. Yet, prolificacy of Naeimi sheep is low and thus a major constraint to increase lamb production (Swelum *et al*., 2014). A single gene mutation on chromosome 6 is responsible for the high prolificacy in Booroola Merino sheep (Mulsant *et al*., 2001; Wilson *et al*., 2001). This mutation is located in the kinase highly conserved domain of the Bone Morphogenetic Protein Receptor 1B (BMPR-1B), leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles (Souza *et al*., 2003).

The significantly high ovulation rate of Booroola sheep has been shown to increase number of lambs born per ewe, therefore, genetic progress of such trait can be rapid if it is possible to identify and select for the beneficial allele. This mutation can be selected directly by forced PCR Restriction Fragment Length Polymorphism (RFLP) approach based on the reports described by Souza *et al*. (2003) and Davis *et al*. (2002).

There are conflicting reports about the effect of the presence of one or two copies of the *FecB* gene on birth weight and growth rate of lambs. Some researchers concluded that *FecB* gene was not associated with changes of birth weight (Abella *et al*., 2005). By contrast, Smith *et al*. (1993) and McNatty *et al*. (1995) have suggested that one copy of the *FecB* gene was associated with negative effects on fetal growth, development and body mass during gestation. Moreover, the presence of the *FecB* gene has no negative effects on the lamb growth traits (Abella *et al*., 2005; Guan *et al*., 2007). The aim of this study was to investigate the effect of carrying one copy of the FecB allele on live body weights at birth, weaning, 75 and 100 days of age in Naeimi and Naeimi crossbred lambs.

MATERIALS AND METHODS

Experimental sheep flocks: A total of 107 newborn individuals, representing 24 purebred Naeimi lambs (NN) and 83 Naeimi x Booroola carrier crossbred lambs (NB; F1) were included in this study. All lambs were born during September to June period, 2012 to 2013 and maintained at the University of King Saud Experimental Livestock Farm, Riyadh, Saudi Arabia. The dams in this study were all of Naeimi breed in their second parity and weighing 50-55 kg.

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To produce the NN lambs, dams were mated to Naeimi rams whereas the NB F1 lambs were the product of mating Naeimi dams to Booroola carrier rams. The imported Booroola carrier rams were a backcross Awassi with Indian sheep breeds, namely, Deccani and Garole, carrying one copy of the FecB allele. At lambing, sex of lambs and their type of birth were recorded; thereafter each lamb was identified with a plastic ear tag. The weights of lambs were recorded to the nearest 50 g at birth, weaning (56 days of age), 75 and 100 days of age. Lambs were allowed to suckled freely and creep fed up to the weaning age, thereafter, lambs were weaned and raised similarly during the post-weaning period up to 100 days of age. During post-weaning, lambs were given *ad libitum* access to a commercial fattening pellets (DM basis; 14.53% CP, 1.16% EE, 24.91% NDF, 14.22% ADF, 0.54% Ca, 0.31% P, 7.46% ashes and 2.78 Mcal ME kg^{-1} DM). Fresh drinking water and mineralized salt blocks were freely available.

Blood sampling and DNA extraction: Approximately 10 mL blood was collected aseptically from the jugular vein of each lamb in EDTA. All blood samples were taken back to the laboratory under low temperature. Genomic DNA was extracted from blood using QIAamp DNA extraction kit according to the manufacturer's instructions. The quality of DNA was checked by taking ratio of optical density value at 260 and 280 nm by spectrophotometer. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further research. The DNA samples were dissolved in TE buffer (pH 8.0) and stored at -20°C pending analysis.

PCR-forced RFLP of *FecB* gene: A region of *FecB* gene (190 bp) was amplified by using a set of forward and reverse primers (Wilson *et al*., 2001): F, 5'-CCAGAGGAC AATAGCAAAGCAAA-3' and R, 5'-CAAGATGTTTTC ATGCCTCATCAACAGGTC-3'. The reverse primer was deliberately introduced a point mutation resulting in PCR products with FecB carrier sheep containing an AvaII restriction site (G/GACC) whereas products from non-carrier lacking this site. For amplication, 25 μL of PCR reaction was prepared by adding 10 pM of each primer, 100 μM of each dNTPs, 1.5 mM $MgCl_2$, 1X PCR assay buffer, 100 ng DNA template and 1 unit Taq DNA polymerase. The amplification was carried out using a pre-programmed thermal cycler 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 sec 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 h at 37°C with 10 units of AvaII restriction enzyme. The restriction enzyme digested PCR products were separated by 4% agarose gel and stained with ethidium bromide. The forced PCR of the *FecB* gene produced a 190 bp band. After digestion with AvaII enzyme, the *FecB* gene homozygous carriers had a 160 bp band ($FecB^{BB}$), the non-carrier had a 190 bp band ($FecB^{++}$), whereas heterozygous had both 160 and 190 bp bands ($FecB^{B+}$).

Statistical analysis: At lambing time, type of birth and gender of each lamb were recorded. To overcome the difficulty of disproportionate subclass numbers and non-orthogonality, estimates of effects of gender of lamb, type of birth and genotype were analyzed by least-square procedures (SAS Version 9.2, SAS Inst., Inc., Cary, NC). It was assumed that interactions were non-existent and all effects were fixed. The following statistical model was used:

$$Y_{ijkl} = \mu + T_i + S_j + G_k + e_{ijkl}$$

Where:

Y_{ijkl} = Individual value
μ = Expected general mean
T_i = Effect of type of birth
S_j = Effect of gender
G_k = Effect of lamb's genotype
e_{ijkl} = Residual error

Duncan's new multiple-range test was used to make pair-wise comparison among the means for significant effects, the level for statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The *BMPR-1B* gene has two alleles, A nucleotide (wild type) and the G nucleotide (mutant Booroola carrier), the presence of the A nucleotide in wild type codes for glutamine amino acid but presence of G nucleotide replaces this amino acid with an arginine (CAG/CGG-transition mutation) at codon 746 (Souza *et al*., 2001). The forced PCR of the *BMPR-1B* gene produced an amplicon of 190 bp representing the wild type and after digestion with AvaII for FecB loci, two bands of 160 and 190 bp were detected representing the heterozygous carrier genotype (Fig. 1). Accordingly, the results revealed that 43 lambs out of the 83 Naeimi crossbred born lambs were heterozygous carrier of the FecB allele ($FecB^{B}/FecB^{+}$) whereas the other crossbred lambs were all of the homozygous non-carrier of the wild allele ($FecB^{+}/FecB^{+}$). The frequency of the B^{+} genotype was 0.52 among all Naeimi crossbred born lambs.

The electrophoretograms of forced PCR-RFLP revealed only one band of 190 bp product in all tested

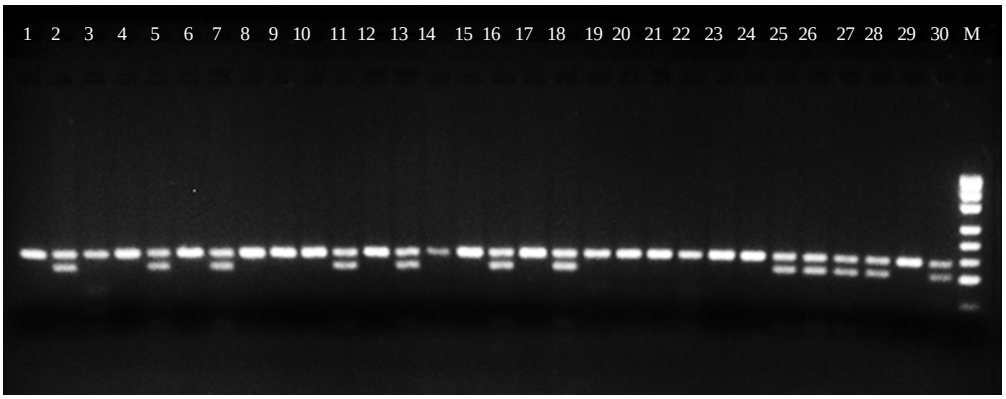

Fig. 1: A PAGE electrophoretogram for FecB loci product digested by AvaII showing genotypes. Lane M: Molecular size marker (100 bp DNA ladder); Lanes 1-30: Naeimi crossbred lambs (NB); carrier lambs ($FecB^{B}/FecB^{+}$) have 190 and 160 bp bands; non-carrier lambs ($FecB^{+}/FecB^{+}$) remained uncut at 190 bp

purebred Naeimi lambs. This result indicated that none of the purebred Naeimi lambs carried the FecB mutation in the *BMPR-1B* gene and that the examined lambs are wild homozygous ($FecB^{+}/FecB^{+}$) non-carrier sheep. This finding is in line with those of (Abouheif *et al*., 2011; Elkorshy *et al*., 2013) who reported that indigenous Najdi, Naeimi and Hiri sheep breeds of Saudi Arabia were wild homozygous non-carrier sheep in respect to restriction pattern of FecB allele. Therefore, this evidence cleared the doubts on probable presence of the gene in Naeimi sheep, since there is a chance for Naeimi to receive the FecB allele through crossing with Imported Australian Merino in the early 1980's. Similar results were also reported by Amiri *et al*. (2007) and Ghaffari *et al*. (2007) who found that digestion of *FecB* gene 190 bp with AvaII restriction enzyme resulted in non-carrier wild type in Iranian sheep breeds. The absent of FecB mutation in *BMPR-1B* gene in Egyptian sheep was also reported by El-Hanafy and El-Saadani (2009) and Elkorshy *et al*. (2013).

The results showed that the mean body weights of the purebred Naeimi (NN) lambs were significantly ($p<0.05$) heavier than that of the Naeimi x Booroola crossbred lambs (NB^{++} and NB^{B+}) at birth, weaning, 75 and 100 days of age (Table 1). Several studies have reported that Booroola carrier x local breed crosses were lighter in weights than local breed contemporaries (Davis *et al*., 1991; Schulze *et al*., 2003). These differences were generally a function of the variations between the Booroola carrier background breed and the genotype of the local breed (Schulze *et al*., 2003). In this study, the Booroola carrier rams utilized in the mating with Naeimi ewes were a composited genotype of several breeds including Awassi and other Indian small sized breeds. Accordingly (Southey *et al*., 2002), concluded that lighter body weights of the Booroola Merino crosses compared to Rambouillet ewes appeared to be due to loci other than the FecB locus because ++ Booroola-cross ewes were lighter than Rambouillet ewes.

Table 1: Least squares means for body weights at various ages as affected by genotype, type of birth and gender of lambs

		Lamb's weight (kg)			
Traits	No. of lambs	Birth	Weaning (56 days)	75 days	100 days
Genotype[1]					
NN	24	4.280[a]	16.35[a]	20.78[a]	29.16[a]
NB^{++}	40	3.820[b]	13.24[b]	18.53[b]	27.09[b]
NB^{B+}	43	3.490[c]	12.23[c]	17.29[c]	25.32[b]
Type of birth					
Single	55	4.670[a]	15.64[a]	19.41[a]	28.17[a]
Twin	46	3.960[b]	13.94[b]	18.85[ab]	27.15[ab]
Triplet	6	2.950[c]	12.22[c]	18.35[b]	26.28[b]
Gender					
Male	55	3.830	13.94	19.52[a]	28.15[a]
Female	52	3.890	13.88	18.21[b]	26.24[b]
Average	107	3.860	13.93	18.87	27.19
±SEM	-	0.142	0.457	0.633	1.024

[1]NN = purebred Naeimi lambs; NB^{++} = The F1 non-FecB carrier of Naeimi x Booroola ram crosses; NB^{B+} = The F1 of Naeimi x Booroola rams crosses that carrying FecB allele; [a-c]Means in the same columnwithin each effect carrying different superscripts differ ($p<0.05$)

The mean body weights of the NB^{++} lambs were significantly ($p<0.05$) heavier than the NB^{B+} lambs at birth, weaning and 75 days of age, however, the difference between NB^{++} and NB^{B+} in body weight at 100 days of age was not significant. Similarly, Meyer *et al*. (1994) reported that B^{+} lambs were 0.4 kg lighter than ++ lambs at weaning within similar genotypes but found FecB genotype had no influence on body weight of lambs at later ages. On the other hand, Schulze *et al*. (2003) reported that body weight at 18 months of age in Rambouillet crosses suggested that the FecB allele might have an effect on body weight. It is reported that the *FecB* gene has negative effects on foetal body weight, body size and development during

pregnancy (Smith *et al*., 1993). Preliminary data involving a local Najdi sheep breed in Saudi Arabia (personal communication) showed that F1 ram and ewe lambs carrying the B^+ genotype weighed 0.13 kg ($p<0.01$) less and 0.06 kg more ($p>0.01$) at birth and 0.79 and 0.48 kg less ($p<0.01$) at weaning (60 days), respectively than the comparable ++ genotype sheep. Walling *et al*. (2004) concluded that the *FecB* gene is closely linked to a locus affecting early growth, the low early growth allele hitchhikes with the Booroola allele during crossing programs. In contrast, Abella *et al*. (2005) found that the presence of one copy of the *FecB* gene was not associated with changes of birth weight. Similar data have been reported by Isaacs *et al*. (1995). Nevertheless, Guan *et al*. (2007) reported that mean body weights of B^+ lambs were heavier ($p<0.05$) than those of ++ lambs at 90 days of age and these differences disappeared by day 120 of age. However, the discrepancies in results across studies that estimated the differences between B+ and ++ lambs of similar background genotype probably due to the differences instages of development, nutrition during foetal, pre-weaning and post-weaning periods and breed or sire effects.

The influence of type of birth on live body weight at birth and weaning age was significant ($p<0.05$) but these differences between single and twin born lambs and between twin and triplet born lambs disappeared ($p>0.05$) at 75 and 100 days of age. On the average, single lambs were 17.9, 12.2, 3 and 3.8% heavier at birth, weaning, 75 and 100 days of age than the twin born lambs, respectively. Also, twin lambs were 34.2, 14, 2.7 and 3.3% heavier in weights than the triplet born lambs at birth, weaning, 75 and 100 days of age, respectively. As in all placental mammals, the maternal uterine space in Naeimi ewes has a finite capacity to gestate offspring and as litter size increases, individual birth weights decline. Similar results were reported by Abella *et al*. (2005), Gardner *et al*. (2007) and Baneh and Hafezian (2009).

No differences ($p>0.05$) were noted in birth and weaning weights of male and female lambs, however, the male lambs being 7.2% heavier ($p<0.05$) than the female lambs at 75 and 100 days of age. This may be due to genetic differences, the males probably beginning to liberate and rogenic substances earlier and hence growing and developing faster than the female lambs. In relation to endocrinal system, estrogen hormone has a limited effect on the growth of long bones in females. That could be one of the reason in which females have lighter weight against males (Rashidi *et al*., 2008). The results were confirmed by prior reports (Abegaz *et al*., 2005; Rashidi *et al*., 2008; Baneh and Hafezian, 2009).

CONCLUSION

The data of this study showed that the frequency of born lambs carrying one copy of the FecB allele was 0.52 of all born Naeimi x Booroola gene carrier crossbred lambs. The presence of $FecB^B/FecB^+$ genotype was associated with lighter body weights than those carrying $FecB^+/FecB^+$ genotype.

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