A Study on Effect of Carrying *FecB* Gene on Body Weight in Garole and Garole×Malpura Sheep

A. P. Kolte, A. K. Mishra*, S. Kumar, A. L. Arora and V. K. Singh
Animal Biotechnology Section and Division of Animal Genetics and Breeding
Central Sheep and Wool Research Institute, Avikanagar (Via: Jaipur), Rajasthan, 304 501, India

ABSTRACT : High prolificacy in Garole sheep is due to existence of *FecB* mutation in an autosomal gene, bone morphogenetic protein receptor. The mutation enhances ovulation rate and in turn litter size in Garole sheep. Garole sires were crossed with non-prolific Malpura ewes with the aim to introduce prolificacy into Garole×Malpura (G×M) crosses through *FecB* introgression programme. In the present study, the effect of carrying booroola allele on litter size and live body weight was analyzed. The average litter size at birth was found to be 1.87 and 1.48 in the Garole and the G×M crosses, respectively. At weaning, 6-month, 9-month and 12-month of age, body weights were not affected by the presence of booroola allele ($p>0.05$); however, a significant effect ($p<0.05$) was found on body weight at birth in G×M crosses. In Garole sheep, no significant effect of *FecB* was observed on live weights in any age group. The interaction between the genetic group and the *FecB* genotype was also found to be non-significant. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1379-1382)***

Key Words : Garole, Garole×Malpura, Litter Size, Body Weight, *FecB* Gene

INTRODUCTION

Garole is a small sized highly prolific sheep breed from hot and humid costal region of West Bengal. The average adult body weight of this breed ranges between 10-14 kg with mean litter size 1.74 in the native tract, and about 14 to 14.5 kg body weight with 1.87 mean litter size in the semi-arid region of Rajasthan (Ghalsasi and Nimbkar, 1993; Bose and Moitra, 1995; Bose et al., 1999; Sharma et al., 1999; Sharma et al., 2001). The Malpura is a medium to heavy sized breed and on an average its adult body weight is 40 kg in males and 30 kg in females (Mishra et al., 2005). The hyper prolificacy of Booroola ewes is due to the presence of the $FecB^B$ allele, recently identified as a single nucleotide substitution (translates into Q249R in amino acid sequence) in the bone morphogenetic protein receptor (Wilson et al., 2001; Fabre et al., 2003). The *FecB* mutation was found in Garole and Javanese sheep but could not be detected in other sheep breeds (Davis et al., 2002). The mutated allele ($FecB^B$) existed in a high frequency in Garole sheep and hypothesized to be the original genotype (Davis et al., 2002). Each copy of $FecB^B$ allele has an additive effect and increases 1.6 ovulations per cycle or one to two extra lambs. The effects of *FecB* gene on reproductive characters are well documented (Elsen et al., 1990; Montgomery et al., 1992) but little is known about its effect on body weights at different stages of growth. Walling et al. (2000) reported that animals carrying a booroola allele to be lighter than non-carrier, and booroola gene is closely linked to a locus affecting early growth. This study investigates the effect of carrying $FecB^B$ allele on live body weight at different ages and litter size in G×M half-breds.

* Corresponding Author: A. K. Mishra. Tel: +91-1437-220137, Fax: +91-1437-20163, E-mail: anilmishra65@yahoo.co.in
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MATERIALS AND METHODS

Location

The study was conducted at the Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India located at 75°-22'E longitude and 27°-17'N latitude and an altitude of 320 m above mean sea level. The climate of the location is typically hot semi-arid with yearly minimum and maximum temperatures 4°C and 46°C, respectively. The Garole sheep was purchased from the native tract i.e. hot humid region of West Bengal in 1997. Malpura, native mutton type sheep breed usually gave single birth and it was used as dam breed whereas Garole was used as sire breed in developing Garole×Malpura half breds that was supposed to segregate *FecB* gene in crosses. The G×M sheep included in the study were obtained by either crossing Malpura ewes with Garole rams or from interbreeding among G×M half breds.

Blood sampling and DNA extraction

Blood samples of thirty-five Garole and fourty-nine G×M sheep of both sexes were collected randomly from Institute flocks by venipuncture in ACD (Citric acid, Sodium citrate, D-Glucose). The DNA was isolated by standard proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation.

The *FecB* mutation assay was attempted by Forced RFLP-PCR technique using PCR primers F-12 (5'-GTCGCTATGGGGAAGTTTGGATG-3') and R-15 (5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3') as described by Wilson et al. (2001). These primers amplified

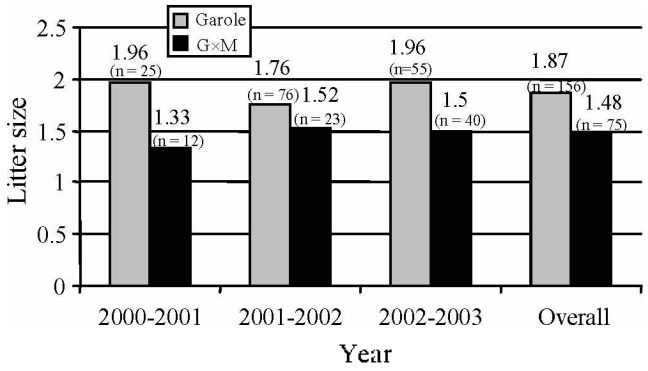

Figure 1. Litter size of Garole and G×M ewes during different years

a 140 bp region of the BMPR-1B gene. The R-15 primer introduces a point mutation in PCR product amplified from the BMPR-1B gene and the copies having Q249R thus gains *AvaII* restriction site, whereas PCR product from non-carrier ($FecB^{-}$) does not complete restriction site and fails to cut by *AvaII*. The $FecB^{BB}$ (homozygous carrier) individual showed single 110bp band, $FecB^{B+}$ (heterozygous carrier) showed 140 and 110 bp bands and the $FecB^{++}$ animals (homozygous non-carrier) revealed a 140 bp band.

The data on body weights at birth, 3, 6, 9 and 12 months of age maintained for all the animals tested for *FecB* carriers were analyzed using analysis of variance technique to test the effect of *FecB* gene on body weights. The significance of interaction between genetic group and *FecB* genotypes using litter size records of all the Garole (n = 156) and G×M ewes (n = 75) from year 2000 to 2003 were also analyzed.

RESULTS AND DISCUSSION

Litter size

In the Garole and the G×M crosses average litter size at birth was found 1.87 and 1.48, respectively (Figure 1). The litter size of Garole observed in present study was on higher side as compared to the report of Nimbkar et al. (2003), who found that the average litter size of Garole as 1.74 in Deccan plateau of Maharashtra and similar results were also reported by Bose et al. (1999) in their native tract. In a study conducted on 12 homozygous and heterozygous carriers, mean litter size in Garole and Javanese ewes was observed as 1.7 and 2.1, respectively (Davis et al., 2002). The average litter size as 1.46 was observed in G×M crosses by Sharma et al. (2004). Smith et al. (1993) suggested that the *FecB* gene influenced litter size. The increased litter size in G×M ewes revealed that *FecB* gene has been incorporated into crosses, and the change of climate has not affected the reproductive efficiency (prolificacy) of Garole sheep adversely and, increased prolificacy is attributed to genetical factor rather than environmental.

Genotype of animals

The genotypes of sheep carrying *FecB* gene are presented in Table 1, and forced EFLP-PCR of *FecB* gene in the Garole and the G×M sheep are given in Figure 2 and 3, respectively. It was observed that in the Garole 80% sheep carry *FecB* gene in homozygous state ($FecB^{BB}$), whereas in the G×M crosses it was only 6.12%. In total samples studied, carriers for *FecB* gene in the Garole were observed as 97.14% ($FecB^{BB}$ and $FecB^{B+}$), whereas in the G×M crosses it was 79.58%. The majority of the Garole animals (~97.0%) showed mutated copy of *FecB* gene, which indicates that the mutation is present at a high frequency in this climate. These results are in line with the report of Davis et al. (2002) who reported that *FecB* is present at a high frequency in Garole and has become fixed (all sheep homozygous carriers of the gene) in some Garole

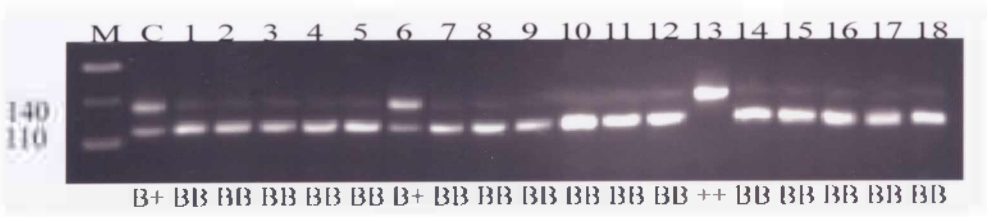

Figure 2. Forced RFLP-PCR of *FecB* gene amplified from the Garole sheep. M = Molecular weight marker (50 bp ladder), C = Control (Heterozygous carrier).

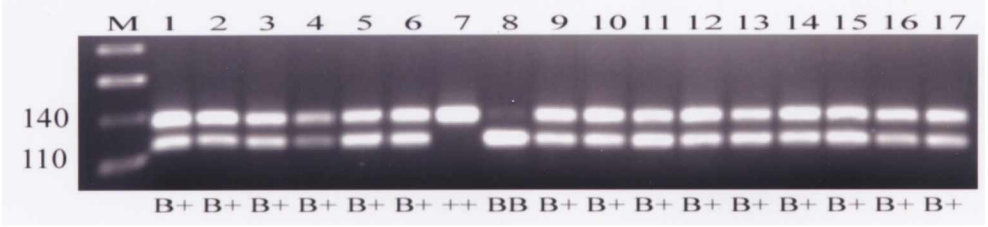

Figure 3. Forced RFLP-PCR of *FecB* gene amplified from the Garole×Malpura sheep. M = Molecular weight marker (50 bp ladder).

Table 1. Genotype of Garole and Garole×Malpura sheep for *FecB* gene (in numbers)

Particulars	Garole			G×M crosses		
	M	F	Total	M	F	Total
Homozygous non-carrier ($FecB^{++}$)	1	0	1 (02.86)	5	5	10 (20.40)
Heterozygous carrier ($FecB^{B+}$)	1	5	6 (17.14)	22	14	36 (73.46)
Homozygous carrier ($FecB^{BB}$)	17	11	28 (80.00)	2	1	3 (06.12)
Total	19	16	35 (100.00)	29	20	49 (100.00)

Figures in parentheses are percentage to total.

Table 2. Average body weight of carriers ($FecB^{BB}$ and $FecB^{B-}$) and non-carriers ($FecB^{++}$) lambs at different age in Garole and G×M sheep

Genetic group/genotypes	No.	Birth weight	3-M wt	6- M wt	9-M wt	12-M wt
Garole						
FecB genotype		NS	NS	NS	NS	NS
$FecB^{++}$	1	1.50	4.5	9.40	14.80	12.60
$FecB^{B+}$	6	1.23±0.13	6.58±0.78	10.13±1.24	11.53±1.86	12.20±1.61
$FecB^{BB}$	28	1.18±0.05	6.33±0.27	9.61±0.39	11.65±0.52	13.15±0.66
Overall	35	1.20±0.04	6.32±0.26	9.69±0.38	11.72±0.51	12.97±0.59
Garole×Malpura						
FecB genotype		p<0.05	NS	NS	NS	NS
$FecB^{++}$	10	2.71^{a}±0.16	12.90±0.77	19.61±1.25	24.31±1.94	30.07±3.74
$FecB^{B+}$	36	2.39^{ab}±0.08	11.89±0.40	18.60±0.85	21.64±0.80	26.85±0.63
$FecB^{BB}$	3	1.92^{b}±0.29	11.67±0.69	19.66±1.62	22.87±1.92	23.10±3.70
Overall	49	2.34±0.11	12.09±0.33	18.87±0.67	22.26±0.72	26.94±0.74

NS = Non-significant, figures under same superscripts did not differ significantly.

populations.

Effect of *FecB* gene on body weight

The average body weight of carriers ($FecB^{BB}$ and $FecB^{B+}$) and non-carrier ($FecB^{++}$) sheep from birth to 12-months of age for the Garole and the G×M crosses are summarized in Table 2. In the Garole sheep, the non-significant effect of *FecB* was observed from birth to 12-months of age. The result of insignificancy of carrying *FecB* mutant gene in the Garole used in this study might be partially due to the genetic characteristics of the Garole population used in this study. In addition, the number of the non-carrier of the Garole was merely one. The average body weight of carriers was found lighter than non-carrier at birth only, after that an erratic trend was observed. This is in accordance with the study of Willingham et al. (2002) who reported that genotype of lamb had no effect on birth weight, adjusted 91 d weaning weight and adjusted 203 d weaning weight. Kleemann et al. (1985) also suggested that the effect of the *FecB* gene on production traits is negligible.

In the Garole×Malpura crosses, non-significant effect of *FecB* gene was observed from weaning to 12 month of age, whereas at birth it was found significant (p<0.05) and the carriers (homozygous and heterozygous) were lighter than non-carriers at all the ages. Though, the results are based on less number of observations (the number of G×M sheep carrying homozygous mutant *FecB* gene is merely three), further study should be needed to reach on definite conclusion. At six month of age in the G×M half-breds heterozygous carrier was 1.01 kg lighter than non-carriers, however at 12-month of age difference was 3.22 kg. Walling et al. (2000) hypothesized that 80% of animals inheriting the Booroola allele also inherit the low growth allele. However, Visscher et al. (2000) in his study reported that carriers and non-carriers had same initial and end weights.

The interaction between the genetic group and the *FecB* genotype was found non-significant. The result suggested that *FecB* gene had no significant effect on body weights from weaning to 12-month of age in the G×M crosses, but carriers are lighter than non-carriers. The result clearly indicates that the G×M crosses may be propagated as new crossbred sheep having *FecB* gene towards evolving new prolific strain for enhancing overall productivity. Since the effect of *FecB* gene on the body weight from weaning to one year of age is non-significant in the G×M crossbred in this study, the useful character can be introduced into other breeds without compromising body weight. The mutation-screening test can be used as molecular marker for early selection of the animals and that will be helpful for designing breeding strategies. The effect of this prolificacy allele on other production parameters needs to be investigated.

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