Small Ruminant Research 139 (2016) 15–19

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Single marker assisted selection in Brazilian Morada Nova hair sheep community-based breeding program

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ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form 29 March 2016
Accepted 21 April 2016
Available online 23 April 2016

Keywords:
Animal genetic resources
GDF9
Ovis aries
Performance test
Morada nova sheep

ABSTRACT

Morada Nova hair sheep show traits desirable for lamb production especially in extensive production systems in Northeastern Brazil, representing an important genetic resource for producing lamb in semi-arid climates in Brazil and elsewhere. Performance testing has been carried out annually with this breed since 2008. In the present study, Morada Nova sheep from two Brazilian states: Ceará (140 animals) and São Paulo (112 animals) were genotyped for a SNP associated with litter size, which is almost only found in Brazilian locally adapted sheep breeds (*FecG^E*). The total observed frequency of *FecG^E* was 0.65, while an increased number of observed heterozygotes was also observed ($\chi^2 = 7.274$, $p < 0.01$). No significant *FecG^E* allele frequency differences were observed ($p = 0.3708$) in 139 performance-tested rams classified as Elite/Superior or Regular/Inferior in the states of Ceará and São Paulo. Considering that litter size has been shown to positively affect farm profitability in medium to high input systems, we suggest that inclusion of *FecG^E* genotyping information in future selection indexes estimated with basis on performance test data, fine-tuned to regional production systems may contribute to increase profitability gains observed in the Morada Nova community-based breeding program.

1. Introduction

The use of locally adapted sheep breeds for meat production has been increasing in Brazil as farmers look for animals that are productive in stressful environments, especially in the Northeast region, where soils are shallow, poor and climates range from tropical semi-humid to semi-arid, with irregularly distributed rainfall rates ranging from 250 to 700 mm/year. Purebred and crossbred hair sheep breeds with high heat tolerance (Castanheira et al., 2010) and parasite resistance (McManus et al., 2009) are frequently raised in these areas (McManus et al., 2014), and account for most of the regional lamb production.

The Morada Nova breed was originally described by Domingues (1954) and is one of the most important hair sheep used for lamb production in the aforementioned regions. The breed shows good production traits, such as high rusticity and average growth in pasture-based systems (Facó et al., 2008). Ewes are sexually precocious, highly fertile and prolific, and show good maternal ability, which added to the small average adult size contribute to the profitability of local lamb production in low input systems (Facó et al., 2008; Lôbo et al., 2011).

Recent studies in animal nutrition, reproduction, genetics and breeding, combined with restructuring of the Morada Nova Breed Association in 2008, provided the basis for the establishment of a local community-based breeding program coordinated by Embrapa Sheep and Goat Research Centre (CNPCO). The program is responsible for overlooking production and pedigree data collection and

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http://dx.doi.org/10.1016/j.smallrumres.2016.04.009
0921-4488/

analysis, and for centralizing performance testing to identify young superior rams for use as sires in associated flocks (Facó et al., 2009).

Profitability studies showed that prolificacy (number of lambs born per adult ewe) is a major determining factor affecting production efficiency in local systems (Paim et al., 2011). Lôbo et al. (2011) and McManus et al. (2011) showed that prolificacy is directly linked to the economic viability of using Morada Nova as well as other hair sheep breeds for lamb production in Brazil, respectively. According to Rao and Notter (2000), litter size can be easily measured and can respond to directional selection besides its low estimated heritability (Fernandes, 1992). Genetic gains of 1% in the litter size have been estimated to result in profit increments of US$ 0.781 ewe/year (4% of the total profit), considering a pasture-based production system with Morada Nova sheep in Brazilís semi-arid region (Lôbo et al., 2011).

Sheep have provided a valuable model to study ovulation rate in mammals, as several genes/mutations have been identified in parallel studies in multiple breeds (e.g., Otsuka et al., 2011; Juengel et al., 2013). Polymorphisms on ovine *GDF9* (Growth and Differentiation Factor 9) exon 2 have been shown to cause ovulation rate differences. At least three distinct mutations have been described: $FecG^H$ (Hanrahan et al., 2004), $FecG^T$ (Nicol et al., 2009) and $FecG^E$ (Silva et al., 2011). Ewes homozygous for the GDF9-S77F (Hanrahan et al., 2004) and GDF9-S109R (Nicol et al., 2009) mutations have been shown to be infertile, while heterozygotes show increased ovulation rates.

The *GDF9* $FecG^E$ allele was identified in the locally adapted Brazilian Santa Ines breed (Silva et al., 2011). The mutation results in a change of a phenylalanine to a cysteine in the mature peptide, and causes an increase in ovulation rate only in homozygotes. The aim of this work was to estimate $FecG^E$ allele frequencies in Morada Nova flocks in Brazil and to elaborate a strategy and to estimate potential results from implementing marker assisted selection for this SNP as a regular procedure in the breedís community-based breeding program.

2. Material and methods

DNA was extracted from blood from 252 Morada Nova sheep with a protocol modified from Miller et al. (1988). Samples included 122 young rams from four distinct Performance Tests (PT) (between 2008–2010) originated from 20 production farms and 18 animals from Embrapa Sheep and Goats (Sobral, CE, Brazil) genetic conservation nucleus. These animals were all from Ceará State (Northeast region), Brazil. Additional samples from 112 sheep derived from five farms in São Paulo State (Southeast region) were also used (17 of which were derived from a single PT in São Paulo State). Samples were derived from major rams for each farm included in the study to reduce within-farm relationships when pedigree data was not available and avoid sampling of animals with common grandparents on farms where pedigree data was available.

Performance testing of young rams was carried out according to Facó et al. (2009). Rams received a final score based on daily weight gain (DWG), rib eye area (REAp = REA/$final\ weight^{0.75}$) and scrotal circumference (SCp = SC/$final\ weight^{0.75}$) weighted by metabolic weight, as well as fat thickness (FT) and visual scores (VS) (Facó et al., 2009) where: Final Index (FI) = 0.40(DWG) + 0.15(REAp) + 0.10(SCp) + 0.10(FT) + 0.25(VS). Animals were then classified as Elite (FI > mean + 1.0 standard deviation (SD), Superior (FI between mean and 1 SD), Regular (FI between mean and −1 SD) and Inferior (FI < mean − 1SD). The number of observed $FecG^E$ alleles ($FecG^E$/$FecG^E$ (1), $FecG^E$/$FecG^+$ (2) and $FecG^+$/$FecG^+$ (3)) in rams from the Ceará PT was regressed on final category (Elite/Superior (1) or Regular/Inferior (0)) in a logistic regression using SAS v. 9.3 (SAS Institute, Cary, North Carolina). The model is stated in terms of the probability that Final Category (Y) = 1 (Elite/Superior), referred to as $\hat{p}$.

The probability that Y is 0 (Regular/Inferior) is

$$ln\left(\frac{\hat{p}}{1-\hat{p}}\right) = \beta_0 + \beta_1 X$$

where X is the number of $FecG^E$ alleles; ln is the natural log; β_0 and β_1 are the regression coefficients and the odds ratios were calculated as $Odds = e^{\beta_0+\beta_1 X}$.

The GDF9$FecG^E$ mutation was genotyped by direct Sanger sequencing of PCR-amplified fragments. Two pairs of primers were used: one pair for amplifying a larger fragment of ~900 bp (5′-GAGAAAAGGGACAGAAGC-3′ and 5′-ACGACAGGTACACTTAGT-3′, from Silva et al., 2011), and one internal pair for amplifying a smaller fragment of ~400 bp (5′-CCTCCACCCTAAAAGGAAGC-3′ and 5′-GGTCTTGGCACTGAGGAGTC-3′). PCR was carried out with annealing temperature of 60 °C for 30 cycles, in a final volume of 10 μl containing 6 ng genomic DNA, 1X QUIAGEN Multiplex PCR Master Mix (Qiagen Inc., Valencia, CA, USA), 0.5x Q-Solution, 0.1 μM of each primer and RNase-Free Water to complete the final reaction volume. PCR products were purified with an EXOSAP-IT and used for sequencing, following BigDye terminator v.3 (Applied Biosystems) manufacturer instructions. Electrophoresis was performed in an ABI3100 automated sequencer (Applied Biosystems) and electropherograms were analysed by SeqScape v2.5 Software (Applied Biosystems). GENES version 2009.7.0 (Cruz, 1998) and Arlequin (Excoffier and Lischer 2010) was used to obtain allelic and genotypic frequency and Hardy-Weinberg Equilibrium (HWE) estimates, and to perform chi-square tests of frequency comparisons. To reduce the bias from unbalanced sample size among locations, samples were grouped by State (Ceará vs São Paulo) and performance test.

3. Results

Table 1 shows $FecG^E$ allelic and genotypic frequencies for individual farms. Overall observed $FecG^E$ frequency was 0.65. HWE deviations were observed in samples from Ceará and São Paulo, with an increase number of observed heterozygotes ($\chi^2 = 7.274$, $P < 0.01$). The same was true for an AMOVA test were 7% ($p < 0.001$) of genetic difference was observed between both States. $FecG^E$ genotypic and allelic frequencies for performance-tested rams classified according to performance test classification are shown in Table 2. Observed $FecG^E$ allelic frequencies in samples from Ceará, São Paulo and overall were 0.76, 0.70 and 0.75, respectively and they do not departed from HWE. In addition, significant differences in $FecG^E$ allelic frequencies between Elite/Superior (0.76) and Regular/Inferior (0.70) groups were not observed ($\chi^2 = 1.98$, $P = 0.3708$). Logistic regression with Ceara PT samples showed a moderate association of homozygous $FecG^E$ (geno = 1- Fig. 1) for animals classified as Regular/Inferior (class 0- Fig. 1) with an odds ratio of 0.463.

4. Discussion

Previous *GDF9* studies in sheep (Mullen and Hanrahan, 2014) and goat breeds (Ahlawat et al., 2013) with a high frequency of multiple ovulations, did not observe the $FecG^E$ allele after its discovery by Silva et al. (2011). In addition, analyses of whole genome shotgun NGS data generated by the International Sheep Genome Consortium from 74 animals from 44 different breeds and two wild species (*Ovis canadensis* and *Ovis dalli*) did not reveal any $FecG^E$ alleles in any of the sequenced animals (data not shown), corroborating claims from Silva et al. (2011) that this mutation was first identified in Brazilian hair sheep. Nevertheless, additional ovulation studies are required to corroborate the present hypothesis.

Table 1
FecG^{E} allele and genotype frequencies by farm for Morada Nova sheep from Ceará (N = 140) and São Paulo (N = 112) States, Brazil.

Ceará																						São Paulo					
Farms		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	***Total***	A	B	C	D	E	***Total***
Genotypic	*FecG*$^{E/E}$	0.3	0.4	0.4	0.4	0.6	0.7	0.6	0.8	0.8	1	0.8	0.6	0.7	0.3	0	1	1	0.3	0.7	0.5	0.3	***0.53***	0.6	0.1	0	0.3	0.1	***0.2***
Frequency*	*FecG*$^{E/+}$	0.7	0.6	0.5	0.6	0.3	0.3	0.3	0.3	0.2	0	0.2	0.2	0.3	0.7	1	0	0	0.7	0.2	0.5	0.7	***0.42***	0.4	0.7	1	0.7	0.8	***0.67***
	FecG$^{+/+}$	0	0	0.1	0	0.1	0	0.1	0	0	0	0	0.2	0	0	0	0	0	0	0.1	0	0.1	***0.05***	0.1	0.2	0	0	0.2	***0.13***
Number Animals		7	7	17	5	8	9	10	3	13	1	5	5	10	3	1	1	1	3	9	4	18	***140***	20	32	1	23	36	***112***
Allele	*FecG*$^{+}$	0.4	0.3	0.4	0.3	0.3	0.2	0.3	0.2	0.1	0	0.1	0.3	0.2	0.3	0.5	0	0	0.3	0.2	0.3	0.4	***0.26***	0.3	0.5	0.5	0.4	0.6	***0.46***
Frequency*	*FecG*^{E}	0.7	0.7	0.6	0.7	0.8	0.8	0.8	0.8	0.9	1	0.9	0.7	0.9	0.7	0.5	1	1	0.7	0.8	0.8	0.6	***0.74***	0.8	0.5	0.5	0.6	0.4	***0.54***

* Difference between states was significant p < 0.01.

Table 2
FecG^{E} genotypic and allelic frequencies in Morada Nova rams in Ceará (N = 122) and São Paulo (N = 17) States according to performance test classification.

Performance test	Genotypes	Frequency (N)	Alleles	Frequency	Elite/Superior	Regular/Inferior
2008	+/+	0.03 (1)	*FecG*$^{+}$	0.20	0.10 (1)	0.00 (0)
(CE)	+/E	0.33 (9)	*FecG*^{E}	0.80	0.10 (1)	0.47 (8)
	E/E	0.64 (18)	–		0.80 (9)	0.53 (9)
2009-	+/+	0.08 (2)	*FecG*$^{+}$	0.32	0.17 (2)	0.00 (0)
1	+/E	0.48 (12)	*FecG*^{E}	0.68	0.33 (4)	0.62 (8)
(CE)	E/E	0.44 (11)	–		0.50 (6)	0.38 (5)
2009–2	+/+	0.03 (1)	*FecG*$^{+}$	0.23	0.00 (0)	0.05 (1)
(CE)	+/E	0.41(14)	*FecG*^{E}	0.77	0.37 (6)	0.45 (8)
	E/E	0.56 (19)	–		0.63 (10)	0.50 (9)
2010	+/+	0.06 (2)	*FecG*$^{+}$	0.23	0.06 (1)	0.05 (1)
(CE)	+/E	0.34 (12)	*FecG*^{E}	0.77	0.44 (7)	0.27 (5)
	E/E	0.60 (21)	–		0.50 (8)	0.68 (13)
CE	+/+	0.05 (6)	*FecG*$^{+}$	0.24	0.07 (4)	0.03 (2)
(Total)*	+/E	0.39 (47)	*FecG*^{E}	0.76	0.33 (18)	0.43 (29)
	E/E	0.56 (69)	–		0.60 (33)	0.54 (36)
SP*	+/+	0.00 (0)	*FecG*$^{+}$	0.30	0.00 (0)	0.00 (0)
	+/E	0.59 (10)	*FecG*^{E}	0.70	0.55 (5)	0.63 (5)
	E/E	0.41 (7)	–		0.45 (4)	0.37 (3)
Total**	+/+	0.04 (6)	*FecG*$^{+}$	0.25	0.06 (4)	0.03 (2)
	+/E	0.41 (57)	*FecG*^{E}	0.75	0.36 (23)	0.44 (34)
	E/E	0.55 (76)	–		0.58 (37)	0.52 (39)

* Difference between States was non-significant p < 0.01.
** Difference between Performance Test Classification was non-significant p < 0.01.

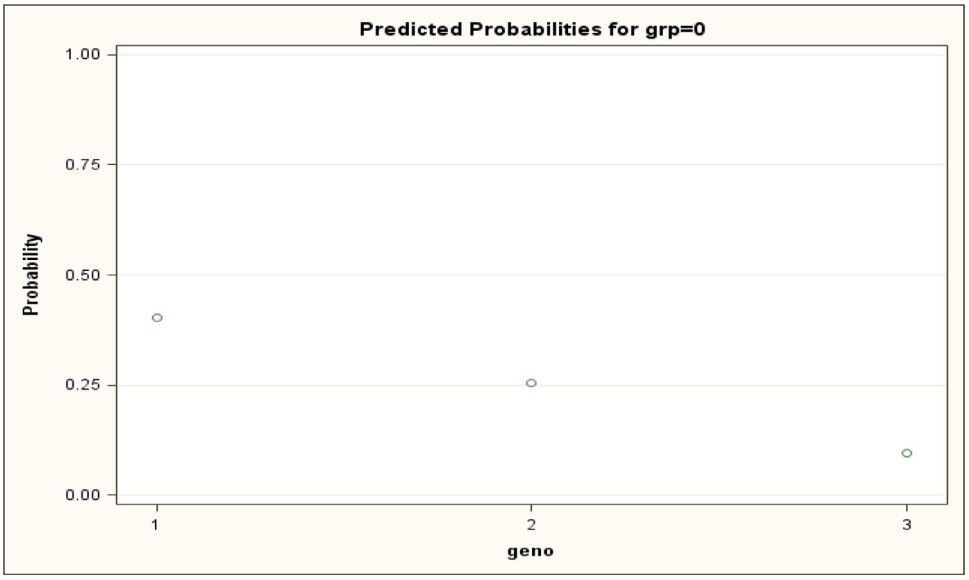

Fig. 1. Logistic regression of genotypes *FecG*^{E}/*FecG*^{E} (1), *FecG*^{E}/*FecG*$^{+}$ (2) and *FecG*$^{+}$/*FecG*$^{+}$ (3) for Morada Nova animals classified as Regular/inferior in performance test (grp=0).

The results represent the first *FecG*^{E} frequency estimates reported for Morada Nova sheep and show that this *GDF9* mutation is not restricted to Santa Inês hair sheep, as previously reported (Silva et al., 2011). In fact, observed *FecG*^{E} frequencies in Morada Nova (0.74) were significantly higher than reported frequencies for Santa Inês (0.52, Silva et al., 2011). Considering the effect of *FecG*^{E}, these results are corroborated by average litter size comparisons between the two breeds. While mean litter sizes of 1.25 have been reported in Santa Ines (Mexia et al., 2004), litter sizes ranging from 1.37 to 1.48 have been observed in Morada Nova

(Machado, 1999; Selaive-Villarroel and Fernandes, 2000; Quesada et al., 2002). Analyses of the genetic relationship between the two breeds suggest that Santa Inês was probably derived by alternate crossbreeding between Morada Nova, Brazilian Bergamasca and other locally adapted sheep breeds from Brazil (McManus et al., 2010).

The high number of Morada Nova animals homozygote and heterozygote for *FecG*^{E} observed in farms in Ceará and São Paulo States, and previous studies with Santa Ines, suggest that this allele may be found in most Brazilian locally adapted sheep breeds. In addition, low levels of introgression of *FecG*^{E} have also been observed in commercial or specialized breeds in Brazil (data not shown). These observations reveal a new possible approach for conservation and breeding of Brazilian sheep breeds as genotyping and selection for this *GDF9* variant may be done at low costs with great potential impacts to of local production systems. Farmers with extensive production systems in extreme environments may select against *FecG*^{E} while those with semi-intensive systems and better pastures can select in favor of this allele to increase twin birth rates in their flocks.

The significant difference of *FecG*^{E} allelic frequencies observed between samples from Ceará and São Paulo States suggests distinct selection practices may be in place in different regions. Litter size has been shown to significantly affect farm profitability (Lôbo et al., 2011), but this may be highly influenced by production system and region. Energy requirements from ewes with multiple lambs may not be fully met in low input systems frequently observed in Brazilís semi-arid region, therefore resulting in higher lamb mortality rates and lower profits as a consequence. Selection for multiple lambing may therefore lead to higher profits only in production systems using higher inputs usually seen in Southeastern Brazil. Conversely, founder effects and drift may represent significant factors to explain observed *FecG*^{E} allelic frequencies differences as studied flocks have small effective numbers.

Litter size was identified as one of the most important traits for determining overall profitability of lamb production with Morada Nova sheep in Brazilian production systems (Lôbo et al., 2011), but current performance tests do not consider this trait for computing final scores and ranking. Rao and Notter (2000) did not find any strong genetic correlations between litter size and different growth traits. Therefore, current performance tests based solely on growth and carcass traits are not likely to directly or indirectly contribute to increase prolificacy in Morada Nova sheep. The overall observed frequency of *FecG*^{E} was 0.75 and differences in homozygote frequencies in the Elite/Superior and Regular/Inferior groups were not significant (Table 2, Fig. 1). These results indicate that *FecG*^{E} allele is widespread in Morada Nova sheep and that inclusion of genotyping results in final performance test scores would contribute to increase allele frequencies and therefore, litter size and overall profitability of lamb production with this breed. Future studies to derive distinct indexes, fine-tuned to the production system used (intensive and extensive), may greatly improve genetic gains obtained in line with selection objectives based on regional farm profitability. Moreover, this study is important to connect the community-based breeding program with the National conservation program. There is urgency in adding germplasm both with *FecG*^{E} and wild type alleles in gene banks to assure food security and phenotypic plasticity to the activities performed by private sector.

5. Conclusion

Sheep on the community-based breeding program for Morada Nova hair sheep showed a high allelic diversity for the *FecG*^{E} allele (*GDF9* gene), which has been shown to increase litter size. Genetic characterization for this allele in young rams on performance tests may contribute to increase litter size in the breed, a trait shown to affect farm profitability. Genetic monitoring for this SNP mutation in the *GDF9* gene may be useful in the breeding program even if prolificacy is an inherently female characteristic. This allele had previously been described only in Brazilian Santa Ines sheep and results from this study suggests that this mutation might be associated with Brazilian locally adapted sheep breeds and can be used to improve sheep production in the country.

Acknowledgements

CNPq, FAPDF, CAPES and Embrapa provided for scholarships and funding for performing sample and data collection and analysis. The authors thank farmers form Ceará and São Paulo States who kindly donated samples for this study and the anonymous reviewers who provided useful comments to improve the article.

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