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RELATIONSHIP WITH THE DOUBLE-MUSCLED PHENOTYPE IN "RUBIA GALLEGA" CATTLE BREED

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INTRODUCTION

The myostatin gene (MSTN) is a negative extracellular regulator that expresses during development of muscle and skeletal tissue in adult animals. Since its first appearance, bovine muscular hypertrophy has extended widely among European beef cattle. Molecular research has shown that double-muscling in European breeds is caused by seven mutations (mh mutated alleles) in MSTN exons II and III. The phenotype mainly implies an increased muscle development, responsible of the name double muscling, increased growth rate and carcass value (Ménissier 1982; Arthur 1995).

Six different disrupting mutations were known until recently: C313Y, nt821 (del11), nt419 (del7-ins10), Q204X, E226X and E291X (Cappucio et al. 1998; Grobet et al. 1998). After studying 28 European cattle breeds, two new missense mutations, known as S105C and D182N have also been described (Miranda et al., 2000).

The objective of the present study was to determine myostatin allele frequency in "Rubia Gallega" breed individuals in Galicia (Northwest Spain).

MATERIAL AND METHODS

Seven mutations of the gene of the Myostatin have been studied (D182N, nt419(del7ins10), Q204X, E226X, nt821(del11), E291X and C313Y), in a total of 385 animals of both sexes (279 females and 106 males, including the sires used for artificial insemination), the less related as possible and all them inscribed in the Genealogical Book of the "Rubia Gallega" breed.

The extraction of DNA from blood samples and seminal dose, was carried out using the protocols described by Systems, Inc it Centers. (Generation * DNA Purification System: Column™ Kit capture).

The detection of the mutations in the sequence of the gene of the Myostatin was carried out by means of the technique "primer extension analysis". They were amplified by means of PCR multiplex two located fragments one in the exon II and another in the exon III of this gene, with an extension of 390 and 401 base pairs respectively. The later dephosphorylation was carried out using ExoSAP-IT® (Exonucleasa I/Shrimp Alkaline Phosphatase with buffer of USB Corporation).

The reactions of first extension were carried out using the kit ABI Prism® SNaPshot™ Multiplex Kit of Applied Biosystems. In a first reaction the mutations D182N, nt419(del7-inslO), Q204X, E226X were analyzed, and in the second, nt821(delll), E291X and C313Y. Subsequently you proceeded to a new dephosphorylatión using SAP (Shrimp Alkaline Phosphatase of USB Corporation). Later on the results were visualized by means of capillary electroforesis in a genetic analyzer ABI Prism® 3100-Avant Genetic Analyzer and using the analysis software GeneMapper™ of Applied Biosystems.

RESULTS AND DISCUSSION

According to previous studies carried out it is known that the mutation more broadly diffused in the animals of the races of meat aptitude it is the one denominated nt821(delll), that coinciding with the present results, because of the 7 studied mutations it is the only one that we have found. In this sense, Bouzada et al. (2004) found "Rubia gallega" female in heterocigosis for the mutation Q204X.

We have found that the 55% of the samples was homozigous (mh/mh) (table 1). In Belgian Blue breed the 100% was homozigous (mh/mh) for the deletion while for the Charolaise only the 12% were homozigous (mh/mh) for the specific breed mutation and the 54% was found at the wild type status (+/+) (Pozzi et al., 2004). The results obtained confirmed the reports of other authors about the incidence in the breed population of each mutation analized (Grobet et al., 1998; Fahrenkrug et al., 1999; Smith et al., 2000, Bongioni et al., 2003).

Table 1. Allelic and genotypic frequencies of the myostatin of the seven studied mutations in "Rubia Gallega" breed.

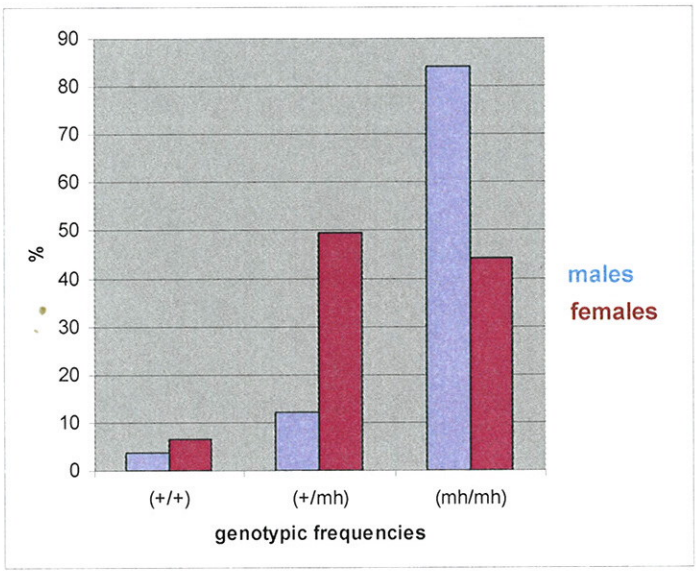
Frec.	Genotypic	N°	%	Allelic	
D182N	+/+	385	100	+	100,000%
	+/mh	0	0	mh	0,000%
	mh/mh	0	0		
		385	100		100,00%
nt419	+/+	385	100	+	100,000%
	+/mh	0	0	mh	0,000%
	mh/mh	0	0		
		385	100		100,00%
Q204X	+/+	385	100	+	100,000%
	+/mh	0	0	mh	0,000%
	mh/mh	0	0		
		385	100		100,00%
E226X	+/+	385	100	+	100,000%
	+/mh	0	0	mh	0,000%
	mh/mh	0	0		
		385	100		100,00%
nt821	+/+	22	5,714	+	25,325%
	+/mh	151	39,221	mh	74,675%
	mh/mh	212	55,065		
		385	100		100,00%
E291X	+/+	385	100	+	100,000%
	+/mh	0	0	mh	0,000%
	mh/mh	0	0		
		385	100		100,00%
C313Y	+/+	385	100	+	100,000%
	+/mh	0	0	mh	0,000%
	mh/mh	0	0		
		385	100		100,00%

An striking result when analysing the mutation nt921 (del11) (table 2; Fig 1) was its much more common appearance in adult males than in females (84% vs 44%), probably because of the farmer use of females without double-muscled phenotype while this is a preferred phenotype in males. (table 2).

Table 2. Allelic and genotypic frequencies of the myostatin nt821 (del 11) allele according to sex.

Frec.	Genotypic	N°	%	Allelic	
MACHOS	+/+	4	3,774	+	9,906%
	+/mh	13	12,264	mh	90,094%
	mh/mh	89	83,962		
		106	100		100,00%
HEMBRAS	+/+	18	6,452	+	31,183%
	+/mh	138	49,462	mh	68,817%
	mh/mh	123	44,086		
		279	100		100,00%

Figure 1. Genotypic frequencies of the myostatin nt821 (del 11) allele according to sex.



The genetic analysis in individual animals, specially in those used as parents, would be very useful as an additional information to improve the selection of the "Rubia Gallega" breed in optimizing this character through its rational use in both pubered and crossbred animals.

CONCLUSIONS

The results suggest the interest of the breeders in the character double muscling, randomly promoting the selection in favor of the mutation.

Further research is needed on this gene that includes productive data to establish the association between it and important economic parameters such as dystocial birth frequency, birth, weaning and market weights and carcass characteristics.

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