

Valorization of extensive breeding of the Spanish Merino Sheep through the quality wool improvement: Preliminary results of GWAS of fiber diameter from Whole Genome Sequences

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The autochthonous Spanish Merino is one of the most emblematic breeds in Spain for its productive importance and history. This breed was created and improved to become the sheep with the highest wool quality in the world. During the last forty years, due to the wool market crisis, the breeders crossed the Spanish Natives Merino with other merino breeds focused in meat production, which caused a decrease in the wool quality. Not often, there are around 130,000 Spanish pure merino that still have the high-quality wool inherited from the historical lines. Currently its production system continues to have a high level of extensification, so the reevaluation of its breeding has a high value in the maintenance of the pasture and/or mountain ecosystems where it is managed. For the present study, wool quality from a population of 6,200 purebred Spanish Merino (registered in the flock-book) were analysed using the Australian OFDA 2000 to determine classical wool quality criteria such as average fiber diameter and its coefficient of variation, in addition to other very important criteria for the textile industry such the comfort factor, staple length, fibre curvature or spin Fineness. The evaluation of these animals determined that the Merino breed still has a high potential to produce fine quality wool but is currently highly heterogeneous. For this reason, in addition to the classical genetic evaluations for the selection of breeding animals, the aim is to search for molecular markers that improve the reliability of these evaluations and make possible an early selection of these breeding animals. In this first approach, the most extreme animals in terms of fiber fineness were selected (44 animals with the highest fineness and 31 animals with the highest diameter). The Whole Genome Sequences of animals were obtained at an average coverage of 4.5x (Neogen, Ayr, Scotland), mapped against the Rambouillet 2.0 reference genome. Subsequently, sequences with high quality levels were used for variant calling. The GWAS performed determined the presence of 17 regions associated with fiber diameter distributed in 8 chromosomes. After the ontological assay, 39 genes were detected. Of them, the 92 % were related with protein coding and the rest with the synthesis of different types of RNA. These preliminary results require further studies using a larger population to validate them.

Abstract

Keywords: Spanish Merino, GWAS, NGS, fiber diameter, wool.

Introduction

The native Spanish Merino is one of the most emblematic breeds in Spain, not only for its economic importance but also for its impact on our history since its origin. It is believed that Romans started to improve this breed in the Iberian Peninsula looking for white fleece and fine wool 2,000 years ago. Thus, we could say that the origin of the Merino was focused on the wool quality of the animals (Sánchez 1970). Exportation of animals out of the Peninsula were forbidden until the XVII-XVIII centuries when animals were exported to other countries where the animals were used to improve local breeds (Laguna 1986). This is the reason because the Spanish Merino is considering the origin of the worldwide Merino and Merino-derived breeds (Ciani *et al.*, 2015; Granero *et al.*, 2022).

During the 1960s decade, due to the international wool market crisis, the Spanish merino census decrease drastically, and the breeders looked to change the selection objective to animals with mixed meat-wool aptitude. To achieve this objective, many breeders were crossed its sheep with foreign breeds with greater meat aptitude, such as the Merino Precoz, Fleischschaf, Landschaf, Île de France, among others. Those crossings, carried out on the Spanish Merino Type, caused the improvement of its meat production but also caused a decrease in its wool quality. Even most pure breeders stopped including wool quality as a selection objective and only several flock, characterized as historical lines, keep the morphotype, genetic and phenotype of the original animals with the exceptional wool quality (Granero *et al.*, 2022). Due to this, currently, although there are more than 3,2 million of sheep in Spain with a racial pattern closely to the Spanish Merino (Spanish Merino Type), only approximately 130.000 are actually pure Spanish Merino and are registered in the flock-book as pure by the National Association of Merino Sheep Breeders (ACME).

In Spain, it is common practice that high quality wool from purebred Merino sheep is mixed with normal quality wool from crossbred sheep, penalizing these elite fleeces, and causing a depreciation in the price of the wool. Besides, the Spanish Merino production system continues to have a high level of extensification. The animals are bred in mountain pastures or in the especial Iberian habitat called “Dehesa” in semi-liberty conditions. So, the revaluation of the wool quality as an elite product are closely linked to the maintenance of the pasture and/or mountain ecosystems where it is managed.

Currently, ACME together with SAT Merino Breeders and the textile company group Holistex are working under the “Merino Genuino®” project to revalorize the Spanish Merino wool. In that sense, ACME recently implement a breeding program aimed at improving wool characteristics typical of the original Merino.

In this sense, a wool quality control nucleus has been organized, in which around 7000 fleeces a year are analysed using the ODA-2000 machine, having already carried out three genetic evaluations for the characteristics of the fleece. A further step is the search for genetic markers associated to these demanded phenotypes to improve the reliability of these valuations. New molecular tools such as Next Generation Sequency have allowed to deep in the knowledge of whole sheep Genome. Although genomic tools have been previously used to looking for genes related to wool quality, this is to our knowledge the first work carried out on the genuine Spanish Merino breed.

In the present study, 6,200 purebred Spanish Merino (all registered in the flock-book) belonging to 24 herds were used. Wool of all the sheep were collected from the same costal area. The samples were sent to the Centre of Selection and Reproduction Animals (CENSYRA) in Badajoz where were analysed with Australian OFDA 2000. Fiber Diameter (FD), Standard Deviation (SD), Coefficient of Variation (CV%), Comfort Factor (CF%) and Staple Length (SL) were measured.

Material and methods

Animal Selection and Phenotype characterization

In this first approach, the most extreme animals according to the Breeding FD values were selected (44 animals with the highest fineness and 31 animals with the highest diameter). DNA were purified from blood samples. After the quality control made with the Thermo Scientific™ NanoDrop™ One (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), the DNA were sequenced using the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, California, USA) in NEOGEN Genomics (Lincoln, NE, USA).

Sequenciation

The quality control of the raw data was conducted with fastqc V0.11.9 software. The remaining high-quality sequences were aligned with the GCA_016772045.1_ARS-UI_Ramb_v2.0 sheep genome using the Burrows-Wheeler Aligner (BWA) software (Li and Durbin 2009). Duplicates were removed with Picard software (<http://broadinstitute.github.io/picard/>). Finally, only the quality sequences were retained with Samtools v1.12 software. Finally, the genotypes were called with bcf tools to generate the vcf file (Li 2011).

Sequences quality control, alignment, and variant calling

To the GWAS, the Multivariate Linear Mixed Model of Gemma software were used correcting by the genomic matrix (Zhou and Stephens 2012, 2014).

Genome wide association study

Statistical data of the wool parameters studied in the present work are shown in Table 1. The FD ranged between 15.2 μ to 28.5 μ with an average of 22.2 μ which means that the breed has animals with extra fine wool highly appreciated in the market, but also animals have a fine wool and even of low quality. Not often, the CV% of 12% indicate the relatively high variability within the breed. A previous characterization made by Arrebola (2002) in this same population showed a FD value of 21.31 μ (max 25 μ and min 18 μ) and a CV% of 7%. The evolution in this two decades showed a little increase of the FD and its CV%.

Results and discussion

The animals showed elevated CF% values with an average of 95.7 which implies that approximately the 85% of the Spanish Merino Sheep have CF% values over 90. The SL showed very differences within the population ranging from 15 to 85 mm (average of 42 mm). These results are significantly different to those obtained at the beginning of the century, when the SL averaged in 69.2 mm (Arrebola 2002). So, SL is one of the main traits that should be solved to the wool revalorization. The evaluation of these animals determined that the Merino breed still has a high potential to produce fine quality wool but is currently highly heterogeneous.

Table 1. Descriptive statistical of the wool parameters related to Fiber Diameter (FD) and Staple Length analysed (SD), obtained from the Spanish Merino population analysed.

Sample	Min	Max	Mean
Fiber Diameter (μ)	15.2	28.5	22.2
Standard Deviation FD (μ)	2.3	6.6	3.8
Coefficient of Variation FD (%)	12	29	17.48
Comfort Factor (%)	70.8	100	95.7
Staple Length (mm)	15	85	42

In this preliminary GWAS analysis, focused on the fiber diameter, our results show the presence of 17 regions associated with FD, distributed in eight chromosomes (1, 2, 3, 4, 10, 11, 19 and 22; figure 1). The chromosome 1 accumulated 9 of these 17 regions. After the ontological assay, 39 genes were detected. Of them, the 92 % were related with protein coding and the rest with the synthesis of different types of RNA. These preliminary results require using a larger population to validate them and further studies going deeper into the implicated genes.

Conclusions

The revaluation of this natural fiber will allow, on the one hand, the protection of the natural areas where the animals are raised and, on the other hand, the creation of a quality product that competes with the best wools in the world. The Spanish Merino Type has lost much of its wool quality due to crossbreeding for meat production purposes. However, the pure Spanish Merino was left out of these practices and has proven to have a high potential to produce quality wool, although it has also lost some wool quality and, above all, its variability has increased in recent decades. The search for markers related to wool parameters will help in the selection of animals with this elite wool.

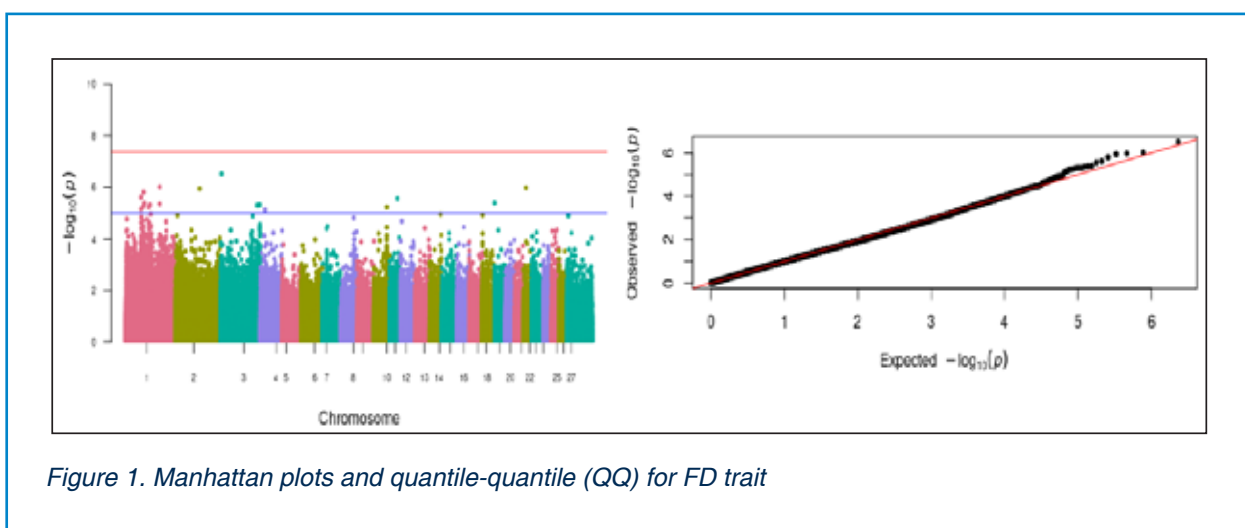


Figure 1. Manhattan plots and quantile-quantile (QQ) for FD trait

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