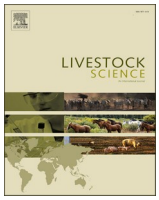
Livestock Science 276 (2023) 105310

Contents lists available at ScienceDirect

Livestock Science

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



Identification of genomic regions associated with reproductive longevity in the Rubia Gallega beef cattle breed using a censored threshold model

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HIGHLIGHTS

- Reproductive longevity in the Rubia Gallega breed has a relevant heritability.
- Herd is the main source of variation in reproductive longevity.
- Additive genetic variation of reproductive longevity is heterogeneously distributed along the genome.
- Myostatin plays an important role in reproductive longevity in the Rubia Gallega breed.
- Genes related with reproduction may also affect reproductive longevity.

ARTICLE INFO

Keywords:
GWAS
Survival
Beef cattle
QTL

ABSTRACT

In beef cattle, the economic viability of farms is heavily influenced by the cow's ability to survive subsequent pregnancies. To understand the genetic basis of reproductive longevity in Rubia Gallega beef cattle breed, a ssGWAS was performed by back-solving the output of ssGBLUP under a censored threshold model. It considers the number of parities each cow reaches during its productive life as a phenotypic trait. The results of the study showed that the main source of variation of reproductive longevity is the herd. However, the posterior mean of the heritability of reproductive longevity was 0.173, indicating the potential for an appropriate genetic response to selection. Furthermore, it is shown that four genomic regions in chromosomes 2, 11 and 29 explain a large proportion of the additive genetic variance. The most important signal was detected on chromosome 2 in the vicinity of the MSTN (*myostatin*) gene that is associated with double muscling, and that it is segregating in the Rubia Gallega population. Some other interesting genes located within these regions encoded for several PAGs (*Pregnancy-associated glycoprotein*), LHCGR (*luteinizing hormone/choriogonadotropin receptor*), FSHR (*follicle stimulating hormone receptor*), PROKR1 (*prokineticin receptor 1*) and EHD3 (*Eps15 homology domain-containing protein 3*). This confirms the relevance of the reproductive performance in the reproductive longevity of cows. These findings provide valuable insights for the Rubia Gallega breeding program, as they can be used to define future selection strategies to improve reproductive longevity of the breed.

1. Introduction

The Rubia Gallega beef cattle breed is one of the most important in Spain, primary found within the Autonomous Region of Galicia. It is predominantly raised in very small herds and specialized in extensive meat production. The breeding plan for the Rubia Gallega population focuses on traits related with calving ease, reproduction, growth, carcass quality and longevity.

Longevity is included in the selection objectives since cows need to remain in production for several years to generate an economic profit (Snelling et al., 1995). Additionally, better longevity reduces the number of new heifers to raise and the cost associated (Roberts et al., 2015). Generally, two measures of longevity have been used for breeding purposes: a) the length of the productive life measured as the period between the first and the last calving (Forabosco, 2005), and b) the probability of a cow to survive to a given age or stayability (Maiwashe

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https://doi.org/10.1016/j.livsci.2023.105310
Received 20 April 2023; Received in revised form 18 July 2023; Accepted 27 July 2023
Available online 28 July 2023

et al., 2009). Alternatively, the breeding program of the Rubia Gallega uses the number of parities that a cow delivers along her productive life as a measure of reproductive longevity, understood as longevity corrected by culling due to reproductive failure. Nevertheless, using this option has two challenges: a) it is a categorical trait, and b) it is censored, as it is lower bound of the number of parities in alive cows. To take these characteristics into account, a censored threshold mixed model (Heringstad et al., 2006) is implemented in this study.

Successful genetic improvement of longevity is difficult because it is expressed late in life increasing the generation interval and therefore, genomic information may provide a relevant increase of prediction accuracy. The ssGBLUP approach (Legarra et al., 2014) assumes the same prior weight for all SNP markers, but some studies have proved that giving different priors for each SNP can improve the accuracy of prediction (Tiezzi and Maltecca, 2015; Zhang et al., 2016). This study aims to identify genomic regions associated with additive genetic variance for longevity in the Rubia Gallega breed from the predictions obtained by ssGBLUP (Wang et al., 2012).

2. Material and methods

The datasets utilized in this study consisted of phenotypic and pedigree information that was collected by ACRUGA (*Asociación Nacional de Criadores de Ganado Vacuno Selecto de Raza Rubia Gallega*). The phenotypic dataset included the number of calvings achieved per cow, for a total of 54,933 cows born after the 1st of January of 1980 until the end of 2017. On average, each cow had 5.28 parities, with a standard deviation of 3.38. Among the cows, 39,553 had finished their productive life, and 15,380 were still alive, and its last parity was considered a lower bound (28.52% of the total). A more comprehensive depiction of the data can be observed in Figs. 1 and 2. Fig. 1 displays the distribution of the alive and non-alive cows according to their year of birth, while Fig. 2 illustrates the distribution of alive and non-cows in relation based on the number of recorded parities. Furthermore, the pedigree used consisted of 72,238 individual sire-dam entries.

The data were collected from a total of 3872 herds, with an average of 14.2 ± 21.4 cows per herd. The distribution of herds based on the number of recorded cows is summarized in Table 1.

Additionally, we used the Axiom Bovine platform from ThermoFisher Scientific to genotype 4439 individuals. Among them, 688 were genotyped with the Axiom_BovMDv2 and 3751 with the Axiom_BovMDv3. The PLINK v1.19 (Purcell et al., 2007) software was used to merge the files. The genotyped individuals consist of 1034 sires, 1073

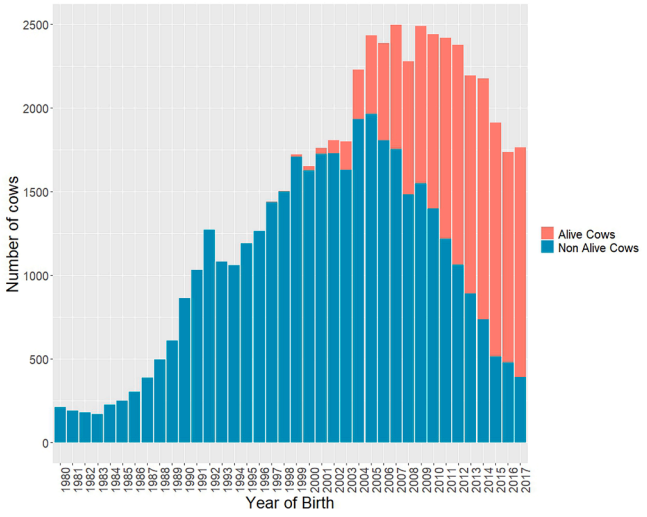

Fig. 1. Distribution of alive and non-alive recorded cows according with their year of birth.

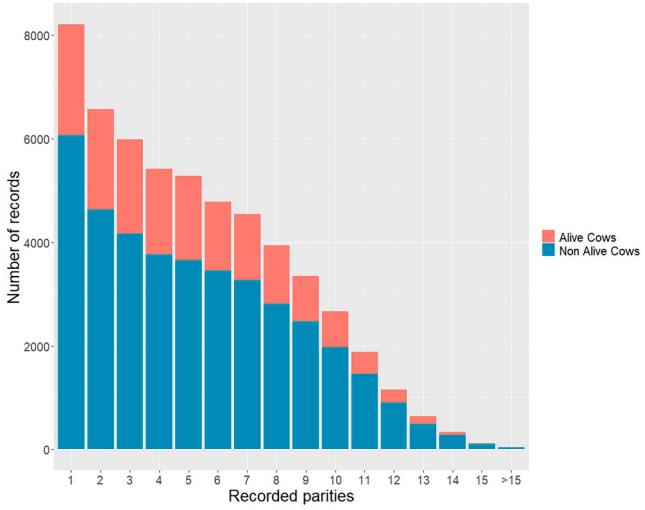

Fig. 2. Distribution of the number of recorded parities in alive and non-alive cows.

Table 1
Distribution of herds according with the number of recorded cows per herd.

Cows	Number of Herds
>11	2443
11–20	596
21–30	320
31–50	275
51–100	192
>100	46

non-alive cows, and 2332 alive cows. We carried out a standard SNP quality control by setting the number of missing genotypes per individual to less than 5%, resulting in 4439 individuals. We then excluded SNPs with missing genotypes greater than 5% and minor allele frequency (MAF) lower than 0.05. Furthermore, only autosomal-linked SNP markers were retained, resulting in a total of 42,867 SNPs. The analysis excluded the use of the non-autosomal SNP markers to prevent potential compatibility issues with the genomic relationship matrix (VanRaden, 2008) required for the ssGBLUP approach. Further, we performed an additional filtering with the preGSf90 software (Misztal et al., 2018) to remove SNP markers with linkage disequilibrium over 0.7 within a genomic distance of 1 Mb. These filtering rendered 33,713 SNP markers.

After filtering the SNP dataset, we analyzed the data with a censored threshold model (Heringstad et al., 2006). The model describes the probability of the observed data (**y**), given the vector of Gaussian liabilities (**l**) as:

$$p(\mathbf{y}|\mathbf{l}) = \prod_{i}^{n} p(y_i|l_i)$$

where n is the number of records. In particular, the probability for the *ith* non-censored record (y_i) is:

$$p(y_i|l_i) \sim 1\left[l_i \in \left(t_{y_i-1}, t_{y_i}\right)\right] + 0\left[l_i \notin \left(t_{y_i-1}, t_{y_i}\right)\right]$$

and the probability for the *jth* censored record (y_j):

$$p(y_j|l_j) \sim 1\left[l_j \in \left(t_{y_j-1}, t_N\right)\right] + 0\left[l_j \notin \left(t_{y_j-1}, t_N\right)\right]$$

where t_j is the *jth* threshold, $t_0 = -\infty$, $t_1 = 0$, $t_N = \infty$, and N is the number of categories (16). For instance, in the case of a non-censored record with a phenotype value of 3 falling within one of the four

liability categories, the liability would lie between threshold 2 and threshold 3. Conversely, for a censored record with a value of 3, the liability would extend to every value above threshold 2.

Moreover, the conditional distribution of the liability given the systematic effects (**β**, year − season of calving with 152 levels), random herd effects (**r,** 3872 levels**)** and additive breeding values (**u**):

$$p(\mathbf{l}|\boldsymbol{\beta},\mathbf{r},\mathbf{u}) \sim N\left(\boldsymbol{X\beta}+\boldsymbol{Wr}+\boldsymbol{Zu},\boldsymbol{I}\sigma_e^2\right)$$

where σ_e^2 is the residual variance that is set to 1, and **X, W** and **Z** are the incidence matrices that connects the systematic, herd and additive genetic effects with the liability. The prior distributions for the herd and additive genetic effects are:

$$\boldsymbol{r} \sim N\left(0,\boldsymbol{I}\sigma_r^2\right)$$

$$\boldsymbol{u} \sim N\left(0,\boldsymbol{H}\sigma_u^2\right)$$

where **H** is the matrix that combines the numerator relationship matrix (**A**) and the Van Raden's (VanRaden, 2008) genomic relationship matrix (**G**), as described by Aguilar et al. (2010). The prior distributions for the variance components and the systematic effects were described to follow a uniform distribution between 0 and M and between –M and M, respectively, where M represents a large number. The model was implemented with the software GIBBSF90+, from the family of software programs BLUPF90 (Misztal et al., 2018), with a total of 525,000 chains, a burn-in of 25,000, and sampling every iteration.

Afterwards, we used the posterior mean estimates of the breeding values of genotyped individuals ($\mathbf{u_g}$) to estimate the SNP effects (**g**) as proposed by Wang et al. (2012):

$$\widehat{\boldsymbol{g}} = \frac{\boldsymbol{W_g'G^{-1}u_g}}{\sum_{i=1}^{Nsnp} 2\widehat{p}_i(1-\widehat{p}_i)}$$

Where $\mathbf{W_g}$ is the matrix which contains the gene content adjusted for the estimated allelic frequencies of each SNP of the population $\widehat{p}_i$ Later, we used the SNP effects obtained to estimate the variance explained by each SNP effect as $\widehat{\sigma}_i^2 = 2\widehat{p}_i(1-\widehat{p}_i)\widehat{g}_i^2$ and the additive variance explained by a segment of a set of SNPs by:

$$\widehat{\sigma}_S^2 = \sum_{i=1}^{Ns} \widehat{\sigma}_i^2$$

where *Ns* is the number of SNP included within the genomic segment. These calculations were performed with the POSTGSF90 software program, also from the family of software programs BLUPF90, where we used the options "windows variance 25″ and "windows variance 50″ to estimate the amount of additive genetic variances explained by genomic regions of 25 and 50 consecutive SNP markers. The choice of segments defined by a number of SNPs instead of genomic distance was done to avoid the potential presence of spurious associations due to the heterogeneous SNP marker density (Li et al., 2021). However, determining the number of SNPs is a subjective decision. To mitigate the impact of this choice, we have opted to use two different options.

Finally, we selected the genome regions that explained a significant proportion of the additive genetic variance using the BiomartTool (www.ensembl.org) (Cunningham et al., 2022), which contains the latest version of the bovine genome, *Bos taurus* (ARS-UCD1.2), to mine for genes present in those windows.

3. Results and discussion

The genetic and genomic evaluation of longevity traits faces limitations due to the unavailability of phenotypic information for living individuals. However, it is evident that the expected longevity of a cow that has completed ten parities surpasses that of a young cow with only one or two parities. Data pertaining to living individuals are often replaced by a pre-calculated prediction of their projected lifespan (VanRaden and Klaaskate, 1993). To avoid this approach, the most widely employed method for longevity analysis is survival analysis (Ducrocq, 1994; Ducrocq and Casella, 1996), which utilizes a hazard function to model the risk of culling. However, survival analysis entails the use of a non-linear model. In contrast, the censored threshold model offers an advantage by automatically estimating the projected lifespan, and it can be implemented using standard mixed animal model approaches, incorporating genomic data. Furthermore, its categorization aligns with the characteristics of the hazard function developed in conventional survival analysis.

The results of the posterior mean and standard deviation of the thresholds are presented in Table 2.

The first threshold which separates the end of the first parity and the beginning of the second parity is set to zero. Hence, it is important to note that the liabilities associated with cows culled after their first parities are negative. Additionally, the thresholds determine the lower and upper bounds of liabilities for each parity of culled cows, whereas only the lower bound is applicable to alive cows. For example, the liability range for a non-alive cow reaching 4 parities was found to be between 1.534 (threshold 4) and 1.894 (threshold 5). Conversely, a cow still alive with 4 parities would have a liability exceeding 1.534, but without an upper limit.

The Bayesian implementation of the censored threshold model with a Gibbs Sampler approach provides estimates of the variances of the random effects (herd and additive genetic), that were transformed into the ratio of herd variance and heritability. Figs. 3 and 4 illustrate their posterior distributions.

The study found that the reproductive longevity of Rubia Gallega cows is mainly influenced by herd management practices, as indicated by the posterior mean estimate of the ratio of herd variance of 0.344. However, it is crucial to exercise caution when interpreting this estimate, as the proposed model was limited by the small size of the Rubia Gallega herds, which precludes consideration of potential herd-by-year interactions.

The estimate of the heritability (posterior mean estimate of 0.173) was higher than previous estimates of longevity in other beef cattle populations (Hamidi Hay and Roberts, 2017; Jamrozik et al., 2013; Van Melis et al., 2010; Varona et al., 2012). This suggest that including predicted breeding values in the selection criteria for the Rubia Gallega population could increase the average reproductive longevity of the population.

The distribution of the additive variance along the autosomal genome was calculated using the procedure proposed by Wang et al. (2012). The amount of additive genetic variances associated for genomic regions of 25 and 50 SNP are presented in Fig. 5.

The findings indicated that the distribution of the additive genetic variance was uneven across the autosomal genome. Specifically, four

Table 2
Posterior mean and standard deviation of the thresholds.

Threshold	Posterior Mean	Posterior Stan. Dev.
1	0	–
2	0.665	0.224
3	1.170	0.317
4	1.534	0.313
5	1.894	0.280
6	2.122	0.224
7	2.311	0.195
8	2.514	0.177
9	2.737	0.174
10	2.990	0.191
11	3.287	0.229
12	3.649	0.280
13	3.929	0.311
14	4.203	0.357
15	4.618	0.446

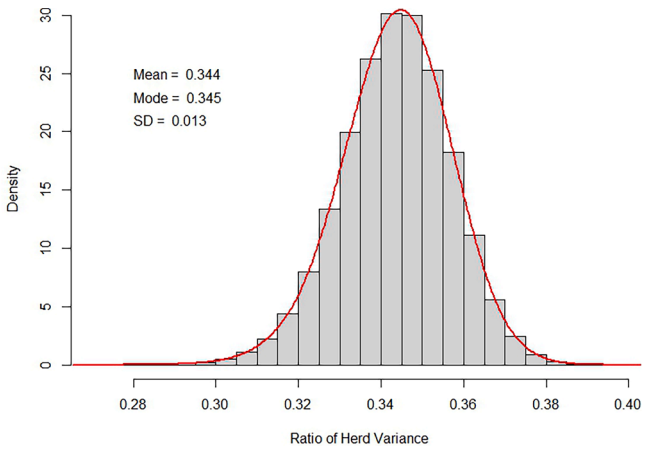

Fig. 3. Posterior Distribution of the ratio of herd variance of Reproductive Longevity measured as the number of parities delivered per cow.

genomic regions comprising 25 (or 50, in parenthesis) SNPs were linked to over 0.5% (0.75%) of the additive genetic variation. The genomic region associated with the highest proportion of the additive genetic variation was situated on BTA2, between bp 1467,475 and 11,605,232. The MSTN (*myostatin*) gene is located within this region. *Myostatin* is a growth differentiation factor associated with double muscling (Grobet et al., 1997), and it is known that some allelic variants linked to double muscling are segregating in the Rubia Gallega population (Dunner et al., 2003; González-Rodríguez et al., 2017; Martinez-Castillero et al., 2021). Double muscling is strongly associated with calving difficulty (Bellinge et al., 2005), which may explain why individuals with MSTN mutations related to double muscling may reduce reproductive longevity, as farmers tend to avoid double muscling females.

The second genomic regions that explained a higher proportion of the additive genetic variation is located on BTA29 between bp 36,926,628 and 38,194,335. This genomic region contains several PAG (*Pregnancy-associated glycoprotein*) genes (PAG10, PAG2, PAG12, PAG5, PAG18, PAG7 and PAG15). These genes are highly expressed in the ruminant placenta, and recent studies have shown that the level of circulating PAG proteins is associated with embryonic mortality in both beef and dairy cattle (Reese et al., 2019).

Finally, there are two other genomic regions identified as relevant on BTA 11. The first one is located between bp 2,9046,346 and 32,426,665 and contains interesting genes such the LHCGR (*luteinizing hormone/choriogonadotropin receptor*) and the FSHR (*follicle stimulating hormone receptor*). The FSH (*follicle stimulating hormone*) stimulates the growth of the small follicles, and LH (*luteinizing hormone*) induces the final maturation of follicles enabling ovulation. Both hormones are segregated after the stimulus of *gonadotropin-releasing hormone* (GnRH). The association of these two genes with the reproductive performance in cattle has been reported in previous studies (Widmer et al., 2021).

The second genomic region of the BTA 11 ranges between 66,341,589 and 70,065,583 bp, where a run of homozygosity island was identified in an original Brown Swiss population (Moscarelli et al., 2021) and a signature of selection in the Original Braunvieh population (Rothammer et al., 2013), suggesting that the region is under selective or adaptative processes. Moreover, these genomic regions have been associated with bovine temperament in Nellore cattle (Valente et al., 2016), and docility may play a role in the stayability of cows in a herd. In addition, PROKR1 (*prokineticin receptor 1*) is located on the same region there. PROKR1 is a receptor of the PROK1 (*prokinecticin 1*), that has been associated with proliferation and survival of luteal endothelial cells (Kisliouk et al., 2005) and with the corpus luteum regression (Kisliouk et al., 2007). Finally, EHD3 (*Eps15 homology domain-containing protein 3*), located on the same region, has been associated with follicle development (Zielak et al., 2007).

As previously mentioned, it is important to emphasize that the analysis is limited to autosomal chromosomes due to the requirements of the ssGBLUP approach for the censored threshold model. This approach relies on the genomic relationship matrix derived from the autosomal SNP markers. However, future studies can explore the approach proposed by Druet and Legarra (2020) to include in genomic matrices markers on the X chromosome.

4. Conclusion

Based on the findings of this study, it can be concluded that there is a significant amount of genetic variation available for reproductive longevity in the Rubia Gallega population, indicating the potential for an appropriate genetic response through selective breeding. The identification of genomic regions that explain a large proportion of the additive genetic variance underscores the crucial role of reproduction and fertility in the farmer's culling decisions. Despite the limitations of the dataset due to the small herd size, these findings provide valuable insights for the Rubia Gallega breeding program, as they can be used to inform future selection strategies to improve reproductive longevity and ultimately enhance the sustainability of the breed by the increase of the economic return for each reproductive cow.

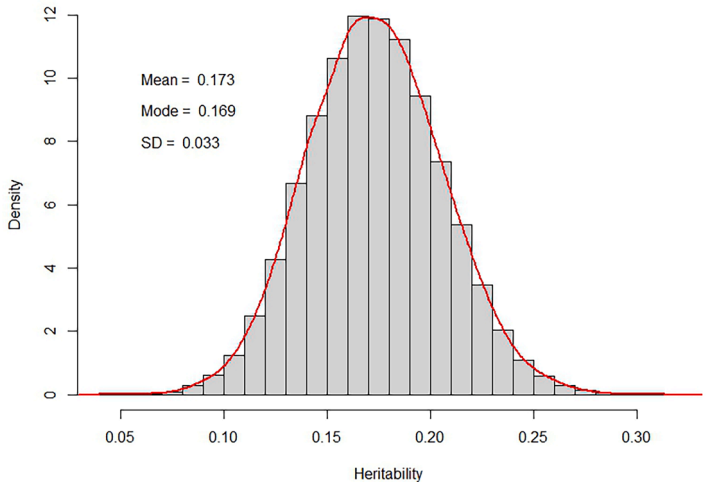

Fig. 4. Posterior Distribution of the Heritability of Reproductive Longevity measured as the number of parities delivered per cow.

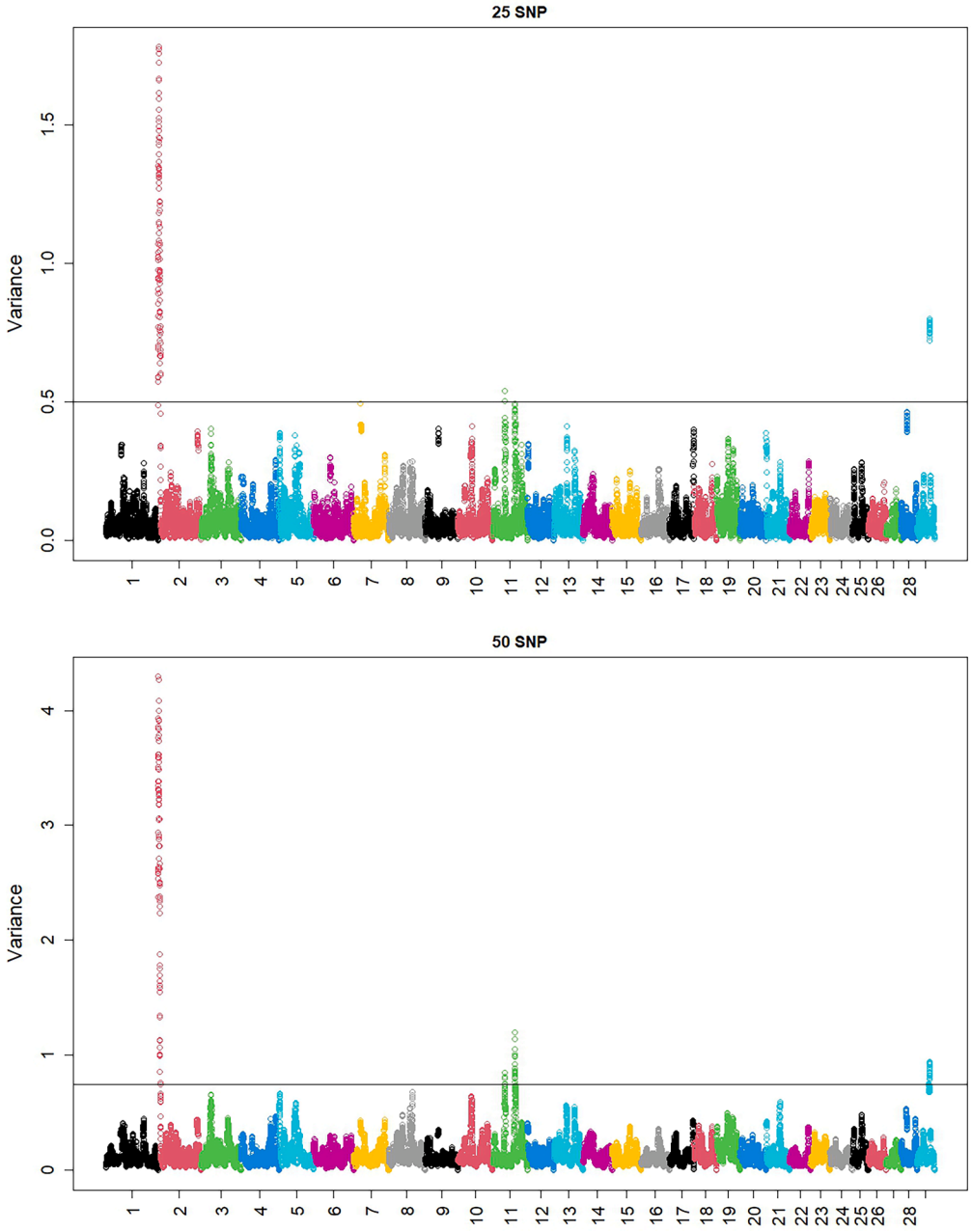

Fig. 5. Manhattan plot of the standardized additive genetic variance (y axis) for reproductive longevity explained by windows of 25 and 50 SNPs along the autosomal genome.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (openai.com) in order to check grammar and spelling. After using this tool/service, the authors reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

María Martínez-Castillero: Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **David López-Carbonell:** Software, Investigation, Visualization. **Houssemeddine Srihi:** Software, Investigation, Visualization. **Carlos Hervás-Rivero:** Software, Investigation, Visualization. **Juan Altarriba:** Resources, Data curation, Writing – review & editing, Funding acquisition. **Paulino Martínez:** Resources, Data curation, Writing – review & editing, Funding acquisition. **Miguel Hermida:** Resources, Data curation, Writing – review & editing, Funding acquisition. **Luis Varona:** Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Acknowledgements

The authors are grateful to ACRUGA (Asociación de Criadores de Ganado Vacuno Selecto de Raza Rubia Gallega) for providing the records, pedigree and genotypic information. This research was funded by

FEADER, grant number 2019/053A.

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