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Genetic parameters and QTL mapping of within-lactation heat stress responses in milk traits of Sarda dairy ewes

M.G. Usai[1*], S. Salaris[1], S. Miari[1], and A. Carta[1]

[1]*AGRIS Sardinia, Sassari, Italy;* [*]*Corresponding author: gmusai@agrisricerca.it*

Introduction

Heat stress (HS) represents an important challenge for dairy sheep production in Mediterranean environments, where even more frequent and intense heat events are expected to increase the risk of productivity losses and the negative effects on animal welfare. This is of particular relevance in Sardinia, where the Sarda sheep breed plays a key role in the local agricultural sector. The productive cycle of Sarda sheep is closely linked to the Mediterranean climate, characterized by mild, rainy winters and hot, dry summers. Rainfall occurs mainly in the autumn-spring period, leading to strongly seasonal pasture growth, with a rapid decline from late spring onwards due to rising temperatures and drought conditions (Carta *et al.*, 2009). Heat intensifies from late spring, reaching its peak during July and August. Historically, Sardinian sheep farming relied on natural grazing, and local shepherds developed adaptive strategies to cope with these climatic constraints and enhance productivity. These strategies included off-season lambing, primarily in autumn, despite the natural tendency to lamb in spring, the introduction of forage crops, infrastructure improvements and management practices such as shaded areas and pre-summer shearing. However, recent climatic trends suggest that these measures alone may be insufficient to mitigate the growing impact of HS. Milk volume and composition are of major importance in Sardinia, where sheep milk is mostly used for cheese production. Thus, losses in productivity due to HS, although mitigated by the seasonal production system, may have significant economic consequences. On the other hand, the effect of HS on milk production may be interpreted as a biological indicator of the physiological response to heat load, reflecting stress perception, resilience, and animal welfare rather than solely immediate economic outcomes. Within this framework, analyzing variability in individual responses to HS is essential to understand the biological processes and the genetic background. While individual variability in HS response is well documented in dairy cattle (Ravagnolo and Misztal, 2000; Aguilar *et al.*, 2009, Bernabucci *et al.*, 2014), studies in dairy sheep are sparser (Finocchiaro *et al.*, 2005, Carabaño *et al.*, 2025). Moreover, to our knowledge, genome-wide association studies on HS responses of milk traits in dairy sheep are lacking. The objective of this study was to quantify the genetic variability of within-lactation HS responses in Sarda ewes for milk production traits using a reaction-norm approach, and to identify genomic regions associated with both baseline production and sensitivity to HS.

Materials & Methods

The study meets all Italian legal requirements and ethical guidelines for animal research. The dataset comprised 179,943 test-day records for milk yield (MY) and 153,835 for milk fat (FP) and protein (PP) percentages, collected on 317 (MY) and 281 (FP and PP) test dates between 2000 and 2022. Records corresponded to 16,249 lactations of 4,940 Sarda ewes reared at an experimental farm in southern Sardinia, Italy. Animals were managed under the traditional Sardinian system, with a December-July milking period, and all ewes were genotyped using the

Illumina OvineSNP50 BeadChip. Daily climatic records were obtained from meteorological stations located on the farm or in nearby areas. These data were used to compute the temperature humidity index (THI). HS was quantified using the average THI over the four days preceding each test date (THI_{4d}). HS was modeled as proposed by Ravagnolo and Misztal (2000), by using a piecewise linear covariate defined as $f_H = \max(0, THI_{4d} - THI_{Hthr})$. Trait-specific HS thresholds (THI_{Hthr}), derived from preliminary THI_{4d} class estimates, were set at 20, 22, and 15 for MY, FP, and PP, respectively. HS effects were assessed through a random regression animal model fitting a reaction norm to f_H defined as:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Xb} + \mathbf{Wd} + \mathbf{f}_H h + (\mathbf{Z}_l\mathbf{l}_a + \mathbf{Z}^*_l\mathbf{l}_b) + (\mathbf{Z}_p\mathbf{p}_a + \mathbf{Z}^*_p\mathbf{p}_b) + (\mathbf{Z}_g\mathbf{g}_a + \mathbf{Z}^*_g\mathbf{g}_b) + \mathbf{Qt} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the vector of the test-day records; μ is the overall mean; $\mathbf{b}$ is the vector of environmental fixed effects; $\mathbf{d}$ is the vector the Days in Milk (DIM) by parity classes fixed effect, with DIM defined as 1 class every 10 days from lambing; h is the population-wide fixed slope describing the average phenotypic change per unit of f_H; $\mathbf{l}_a$, $\mathbf{p}_a$, and $\mathbf{g}_a$ are the individual intercepts representing the baseline production level for the ewe-within-parity, permanent environmental, and genomic components, respectively; $\mathbf{l}_b$, $\mathbf{p}_b$, and $\mathbf{g}_b$ are the corresponding slopes describing individual HS responses; $\mathbf{t}$ is the vector of the test-date random effect; $\mathbf{e}$ is the vector of random residuals; $\mathbf{1}$ is a vector of 1s; $\mathbf{f}_H$ is the vector of the linear HS covariate; $\mathbf{X}$, $\mathbf{W}$, $\mathbf{Z}_l$, $\mathbf{Z}_p$, $\mathbf{Z}_g$, and $\mathbf{Q}$ are incidence matrices and $\mathbf{Z}^*_l$, $\mathbf{Z}^*_p$, and $\mathbf{Z}^*_g$ are design matrices including f_H values pertaining each observation and specific individual levels. Random effects $\mathbf{t}$ and $\mathbf{e}$ were assumed to be distributed as $\mathbf{t} \sim N(0, \mathbf{I}\sigma_t^2)$ and $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where σ_t^2 and σ_e^2 are the test-date and residual variance, respectively. For individual random effects, intercept-slope pairs were assumed jointly distributed as $[\mathbf{l}_a, \mathbf{l}_b] \sim N(0, \mathbf{I} \otimes \mathbf{\Sigma}_l)$; $[\mathbf{p}_a, \mathbf{p}_b] \sim N(0, \mathbf{I} \otimes \mathbf{\Sigma}_p)$; $[\mathbf{g}_a, \mathbf{g}_b] \sim N(0, \mathbf{G} \otimes \mathbf{\Sigma}_g)$, where $\mathbf{I}$ are specific identity matrices, $\mathbf{G}$ is a genomic relationship matrix, and $\mathbf{\Sigma}_l$, $\mathbf{\Sigma}_p$ and $\mathbf{\Sigma}_g$ are 2×2 (co)variance matrices including the intercept variances σ_{ka}^2, the slope variances σ_{kb}^2, and their covariances σ_{kab}, with $k \in \{l, p, g\}$. Variance component estimation and model solution was carried out by BLUPf90+ software (Lourenco *et al.*, 2022). For each trait, two individual phenotypes were derived from the predictions of the random regression model described above and used for QTL mapping. The intercept phenotype represents individual baseline production and combines the average ewe-within-parity, permanent environmental and genomic intercepts, and the average residuals. The slope phenotype represents individual HS response and combines the corresponding average ewe-within-parity, permanent environmental, and genomic slopes, along with the slope of the regression of individual residuals on f_H. QTL mapping was performed following the approach of Usai *et al*. (2019), using the principal components to summarize identity-by-descent (IBD) probabilities between gametes of genotyped individuals.IBD probabilities were estimated at each OvineSNP50 marker position (assembly 4.0) by combining linkage disequilibrium and linkage analyses. The QTL detection model included a genome-wide IBD matrix to account for polygenic effects. Genome-wide 5% significance was assessed using a Bonferroni correction for 43,724 tests, corresponding to a threshold of 10^{-6}.

Results & Discussion

The average THI_{4d} observed across the entire period of the study was 18.7 (±4.8) and ranged from 9 to 30. Population-wide HS slopes were −0.20, +0.14, and −0.03 phenotypic standard deviation units for MY, FP, and PP, respectively, indicating a marked sensitivity of MY to increasing heat load. The favorable response of FP to HS may partly reflect a dilution effect driven by the concurrent reduction in milk volume, rather than a direct enhancement of fat synthesis. In contrast, the absence of a similar pattern for PP suggests the presence of more

specific regulatory mechanisms limiting the influence of dilution effects on protein concentration. Heritability estimates for the intercept were 0.25, 0.26, and 0.38 for MY, FP, and PP, respectively, in agreement with previous studies on production traits in dairy sheep. Corresponding heritabilities for HS slopes were 0.29, 0.16, and 0.36, revealing substantial genetic variability in individual HS sensitivity. Genetic correlations between intercept and slope were significant for MY (−0.72 ± 0.03) and FP (0.44 ± 0.04), while that for PP (−0.08 ± 0.04) did not significantly deviate from zero. The pronounced antagonism observed for MY is consistent with results reported by Finocchiaro *et al.* (2005) in a Sicilian dairy sheep population using a similar reaction norm framework, and provides clear evidence of a genetic trade-off between baseline production and heat tolerance. For milk components, the trait-specific patterns of intercept-slope correlations indicate a complex genotype-by-environment interaction, likely reflecting the combined effect of milk volume changes and component-specific regulatory mechanisms. Correlations among genomic slope predictions further highlighted the trait-specific nature of HS responses. A negative correlation was observed between MY and PP (−0.38), a positive correlation between FP and PP (0.17), and a near independence between MY and FP (0.06). The first two correlations are consistent with both the intercept correlations and the genetic correlations reported in the literature. In contrast, the near-zero MY-FP slope correlation differs from the corresponding intercept correlation, which was negative and in line with published estimates (Carta *et al.*, 2009). These results suggest that partly distinct genetic mechanisms govern baseline production and HS responses.

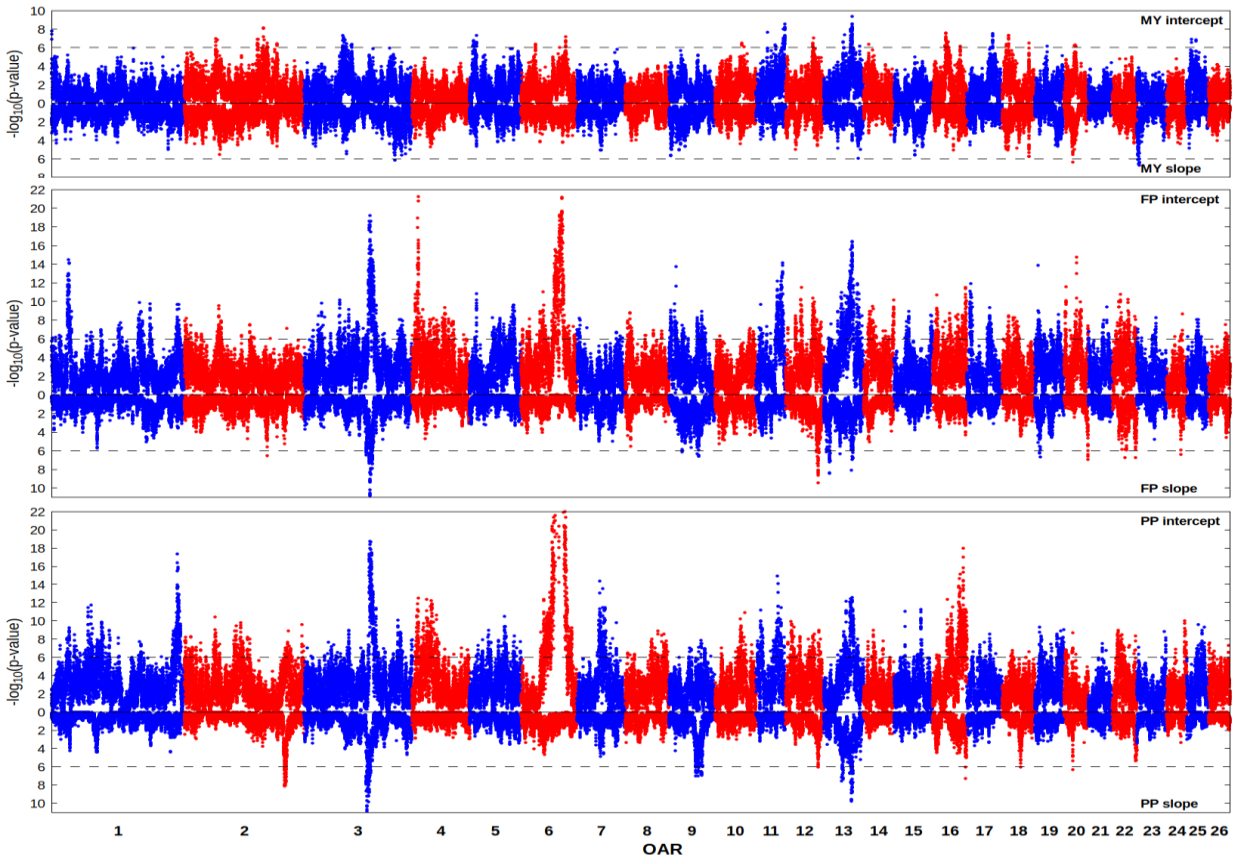

Figure 1. Miami plots showing QTL mapping for baseline production (intercept) and heat stress response (slope) for milk yield (MY), fat percentage (FP), and protein percentage (PP).

QTL mapping results are presented as trait-specific Miami plots in Fig. 1, revealing multiple significant genomic regions. A total of 84 intercept-QTL regions were identified, largely overlapping with regions previously reported for production traits in the same population (Usai *et al.*, 2019). For slope traits, 18 regions distributed across 12 autosomes were significant for at least one trait: 2 for MY, 8 for FP, and 5 for PP, with additional regions affecting both MY and PP (1) or FP and PP (2). Notably, five of these slope-QTL overlapped with intercept-QTL. Interestingly, despite MY being the trait most affected by HS at the population level, only weak slope-QTL were detected, located on OAR3, OAR20, and OAR23. This is consistent with the relatively limited number of intercept-QTL identified for this trait that might reflect the polygenic genetic architecture of MY. The most significant slope-QTL ($p<10^{-10}$) were detected on OAR3 for PP at 130 Mb and FP at 137 Mb. The latter region also exhibited a strong association with FP and PP intercepts ($p<10^{-18}$) and this is of particular interest as it harbors the LALBA gene, a key regulator of milk protein synthesis. Further major slope-QTL ($p<10^{-10}$) were identified on OAR13 for FP at 12 Mb and PP at 57 Mb, near intercept-QTL positions (58-59 Mb) for all three traits, encompassing the TFAP2C gene, which is involved in mammary gland development. Further highly significant slope associations were detected on OAR12 for FP and on OAR2 for PP, representing additional major loci underlying variation in HS sensitivity. Conversely, several major intercept-QTL, including those on OAR6 for PP ($p<10^{-94}$), OAR4 for FP ($p<10^{-21}$), and OAR11 for MY ($p<10^{-9}$), showed no association with the corresponding slopes. Collectively, the QTL results reveal a partly independent genetic architecture for baseline production and HS sensitivity, suggesting that different sets of loci contribute to trait variability under non-stress and HS conditions.

Conclusions

Heat stress affects milk traits in a trait-specific manner in Sarda ewes, with MY and PP showing a within-lactation negative response to increasing HS, whereas FP displays an opposite and counterintuitive trend. Moderate to high heritability estimates for HS slopes indicate substantial genetic variability in individual sensitivity. Genetic correlations and association analyses suggest a complex genotype-by-heat interaction and a partly independent genetic architecture for baseline production and HS response.

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