SMARTER

SMAll RuminanTs breeding for Efficiency and Resilience

Research and Innovation action: H2020 – 772787
Call: H2020-SFS-2017-2
Type of action: Research and Innovation Action (RIA)
Work programme topic: SFS-15-2016-2017
Duration of the project: 01 November 2018 – 30 June 2023

Genome-wide association studies for resilience traits in small ruminant populations in Europe and Uruguay

Hazard* D[1], Ciappesoni G[2], Palhiere I[1], Kaseja K[3], Triantafyllidis A[4], Arsenos G[5], Banos G[3], Chassier M[6], Wicki M[1], Rupp R[1], Tsartsianidou V[4] and Conington J[3]

[1] **INRAE** GENPHYSE, Université de Toulouse, INRAE, ENVT, 31326 Castanet-Tolosan, France;

[2] **INIA-UY** National Research Program on Meat and Wool Production, Instituto Nacional de Investigacion Agropecuaria, INIA Las Brujas, Ruta 48 Km 10, Canelones 90100, Uruguay;

[3] **SRUC**, Easter Bush, Roslin Institute Building, Edinburgh EH25 9RG, United Kingdom

[4] **AUTH** Department of Genetics, Development & Molecular Biology, School of Biology, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece;

[5] **AUTH** Laboratory of Animal Husbandry, School of Veterinary Medicine, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

[6] **IDELE** French Livestock Institute, 149 Rue de Bercy, 75595 Paris Cedex 12, France

*Deliverable leader: Dominique Hazard (INRAE) – dominique.hazard@inrae.fr

DELIVERABLE D2.5

Work package N°2
Due date: M56
Actual date: 22/08/2023
Dissemination level: Public

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 772787

About the H2020 SMARTER research project

SMARTER will develop and deploy innovative strategies to improve Resilience and Efficiency (R&E) related traits in sheep and goats.

SMARTER will find these strategies by: i) generating and validating novel R&E related traits at a phenotypic and genetic level ii) improving and developing new genome-based solutions and tools relevant for the data structure and size of small ruminant populations, iii) establishing new breeding and selection strategies for various breeds and environments that consider R&E traits . SMARTER with help from stakeholders chose several key R&E traits including feed efficiency, health (resistance to disease, survival) and welfare. Experimental populations will be used to identify and dissect new predictors of these R&E traits and the trade-off between animal ability to overcome external challenges. SMARTER will estimate the underlying genetic and genomic variability governing these R&E related traits. This variability will be related to performance in different environments including genotype-by-environment interactions (conventional, agro-ecological and organic systems) in commercial populations. The outcome will be accurate genomic predictions for R&E traits in different environments across different breeds and populations.

SMARTER will also create a new cooperative European and international initiative that will use genomic selection across countries. This initiative will make selection for R&E traits faster and more efficient. SMARTER will also characterize the phenotype and genome of traditional and underutilized breeds. Finally, SMARTER will propose new breeding strategies that utilise R&E traits and trade-offs and balance economic, social and environmental challenges. The overall impact of the multi-actor SMARTER project will be ready-to-use effective and efficient tools to make small ruminant production resilient through improved profitability and efficiency.

SMARTER assembles 27 partners from 13 different countries and has received a funding from the European Commission (through the H2020-SFS-15-2016-2017)) for a project period of 4 years (2018-2022).

Table of contents

About the H2020 SMARTER research project ... 2
Table of contents ... 3
Preamble ... 4
Abstract ... 4
1. Introduction ... 5
2. Material and methods ... 7
    2.1. Animals and Management ... 7
    2.2. Phenotypes ... 7
    2.3. Genotypes ... 8
    2.4. Genome wide association analyses ... 9
    2.5. Data from published gwas ... 10
3. Results ... 10
4. Discussion ... 11
5. Conclusion ... 14
6. Declaration ... 14
    Ethics approval ... 14
    Competing interests ... 14
    Funding ... 14
    Acknowledgments ... 14
7. References ... 14
Deviation or Delays ... 17
Supplementary material: Tables 1-4 and Figures 1-3 ... 17

Preamble

The aim of the present study was i) to review several GWAS performed on resilience traits in the European SMARTER project and ii) to perform new GWAS on resilience traits not yet investigated in small ruminants. GWAS were performed for 11 resilience traits in seven breeds. The work done was drafted as a manuscript that will be submitted to Animal in the coming weeks.

Abstract

Livestock must adapt to new challenges raised by climate change, agro-ecological transitions and/or social concerns. One lever to improve adaptation of ruminants to more fluctuating environment relies on animals' biological capacities to cope with challenges through their resilience capabilities. Genetic variability for several resilience traits has already been reported in small ruminants including responses to disease and metabolic challenges. In order to investigate further genetic determinism of resilience traits, we performed genome wide association studies for such traits in small ruminants. The aim of the present study was i) to review several GWAS performed on resilience traits in the European SMARTER project, and ii) to perform new GWAS on resilience traits not yet investigated in small ruminants. GWAS were performed for 11 resilience traits in seven breeds. We reported QTL for parasitism using feacal egg count proxy on chromosomes 2, 7, 6, 11, 17 and 25 in Corriedale and Merino Uruguyan breeds. In the Texel breed, QTL for mastitis were found on chromosomes 14 and 17, and QTL for foot root were found on chromosomes 19, 23 and 26. Concerning metabolic plasticity investigated through body condition score or backfat thickness, QTL were found on chromosomes 1, 3, 13 in Romane and Merino sheep. Two QTL associated with plasma beta-hydroxybutyrate were located on chromosomes 1 and 19 in Saanen goats. Two QTL associated with colostrum immunoglobulin were mapped on chromosomes 4 and 17 in Alpine goats. We also found QTL associated with functional longevity on chromosomes 2, 4, 12 and 18 in Chios sheep. We didn't identify QTL overlapping between traits or breeds showing the importance of undertaking GWAS for resilience traits in each breed in order to identify associated genetic variants in the breed. Identifying genes underlying resilience traits in small ruminants could potentially be used to improve resilience traits through genomic selection.

Keywords: QTL, resilience, sheep, goat

1. Introduction

To ensure its long-term viability/sustainability, livestock must adapt all over the world to new challenges either posed by climate change, agro-ecological transitions (i.e. to reduce use of food inputs and increase their use of pastoral areas) and/or social concerns (i.e. welfare). Ruminants, and particularly extensively-reared sheep and goats, are especially impacted from these challenges. Firstly, they have a high risk of exposure to disease (e.g. endemic disease such as parasitism, footrot and mastitis). Resistance to anthelmintics is one of the most serious threats to the effective control of gastrointestinal nematodes in small ruminants throughout the world. Multiple resistance has reached levels which make sheep farming -unsustainable (Jackson and Coop, 2000). In several European countries, small ruminants are especially challenged to reduce their use of feed inputs and increase their use of pastoral areas, exposing them to more frequent natural variations in feed resources. To cope with feed supply fluctuations, sheep and goats rely on their energetic body reserves stored in adipose tissues in the form of lipids. Alternation of body reserve use and accretion periods provides animals with a metabolic plasticity that allows them to respond to energetic challenges (for a review see (Friggens et al., 2017; Lambe et al., 2003)).

One lever to improve adaptation of ruminants to more fluctuating environment relies on animals' biological capacities to cope with challenges (i.e. resilience traits including health, metabolic plasticity, overall lifetime resilience). The animal's resilience is the ability of an animal to respond (i.e. to cope with and recover) to short-term environmental perturbations by a dynamic pattern that may involve various mechanisms (physiologic, metabolic, behavioural…) (Colditz and Hine, 2016; Friggens et al., 2022). Since different environmental disturbances being faced by the animal (disease challenges, nutritional, climate related- challenges…), an overall resilience does not exist but several resilience traits specific to the challenge to which animals have to respond. Exploiting the genetic variability for resilience traits through genetic selection of more resilient animals is a relevant approach to improve animals' adaptation and livestock sustainability. The identification of animals with ability to withstand various challenges (environmental, disease…) whilst growing, reproducing and lactating to ensure economic outputs is one key issue for breeders. Currently, breeding programmes for sheep and goats in Europe are successful in increasing the production performance (milk, meat…). Some of these programmes include functional traits such as health traits and lamb survival.. Interestingly, the relatively recent development of genomic selection for small ruminants in several countries offers a new way to consider more efficient way to include hard-to-measure resilience traits in breeding programmes.

Investigation of genetic determinism has shown that resilience traits are heritable. Resilience traits associated with disease resistance, and especially on mastitis and gastrointestinal parasitism have been widely investigated in several breeds of small ruminants. A recent meta-analysis of genetic parameters for resilience traits in sheep and goats showed significant pooled heritabilities and are more variable compared to those estimated for production traits (somatic cell score: 0.21 and 0.13 in dairy goats and sheep; faecal egg count: 0.07, 0.14 and 0.29 in dairy goats, dairy sheep and meat sheep, respectively) (Mucha et al., 2022).

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 772787

Assessment of energetic body reserves relies on indirect measurements. In sheep, back subcutaneous fat is considered as a good indicator of global fatness level. Body condition score (BCS) and ultrasound backfat thickness (BFT) are practical and conventionally used proxies to estimate total body fat content (Russel et al., 1969; Mendizabal et al., 2003; Mendizabal et al., 2010). Genetic variability for adiposelevels has been previously described for several animal species including small ruminants. Heritability of body fat reserves estimated through BCS ranged between 0.06 and 0.37 depending on the breed and the physiological stage of the measurement (; Kenyon et al., 2014; Mace et al., 2018; Mucha et al., 2022). Similarly, that for backfat and muscle thickness measured by ultrasound iss reported to have moderate heritabilities (Simm and Dingwall, 1989 ; Conington et al., 1995; FitzMaurice et al., 2020 ;2021).

A key limitation for the incorporation of traits in breeding programmes is also the availability of low-cost and well-defined phenotypes in sufficient numbers. Thus, California mastitis trait (CMT) was used as the proxy trait to mastitis because of its high genetic correlation with milk somatic cell count (McLaren et al., 2018). This trait has a low heritability (0.07 to 0.11). Similarly, the FAMACHA test was developed as proxy for Haemonchus contortus gastrointestinal parasitism and was reported to be heritable (Marques et al., 2020**;** Werne et al.).

Pregnancy toxaemia is a common metabolic disease in pregnant ewes, as is hyperketonemia or subclinical ketosis, a similar metabolic disorder in early lactation dairy cows (Brozos et al., 2011; van der Drift et al., 2012). The disease is characterized by an increased blood concentration of ketone bodies, especially beta-hydroxybutyrate (BHB), and is caused by inadequate hepatic metabolism of non-esterified fatty acids excessively mobilized from adipose tissue during negative energy balance. BHB is used in dairy cattle as a proxy for such metabolic disorder and investigation of genetic determinism reported that blood and milk BHB concentrations were heritable traits (van der Drift et al., 2012; Lou et al., 2022). Among the components of animal longevity and health, survival in young age is a key period. In some situations, the mortality rate can be very high (Obrien and Sherman, 1993). The health of kids and lambs depends to a large extent on the ingestion of sufficient quality colostrum and it's uptake by young (Obrien and Sherman, 1993; Dwyer et al., 2016). Indeed, the immune system of small ruminants, which are born virtually agammaglobulinemic due to the synepitheliochorial placenta, is fully activated by the ingestion of colostrum (Borghesi et al., 2014). So, the immunoglobulin composition of colostrum is a major determinant of the health and survival of young animals. A few studies have estimated significant heritability for colostrum quality in sheep (Gilbert et al., 1988) and cattle (Conneely et al., 2013; Martin et al., 2021; Cordero-Solorzano et al., 2022) which assess genetic control of the host. In goats, however, little is known about the genetics of colostrum, and opportunities for selection. A complementary approach to the use of specific traits to improve resilience, includes the use of a global indicator. For example, functional longevity constitutes a fitness-related animal trait that is readily incorporated into genomic selection programmes to improve sheep resilience and adaptation. Herein, we studied the length of productive life (days between first lambing and culling date) adjusted for the amount of milk produced in lifetime, reflecting functional longevity in Chios dairy sheep. Previous studies investigated the genetic determinism of functional longevity have been conducted for such functional indicators in cattle (Zhang et al., 2021), goats (Palhiere et al., 2018) and sheep (Buisson et al., 2022)**.** In RP 1, the genetic background to these traits was also reported

This project has received funding from the European Union's Horizon 2020 research and innovation
program under grant agreement No 772787

for UK Dorset, Lleyn and Texel sheep. Despite this, these traits are still poorly investigated and genome-wide association analyses for these traits are scarce.

Genomic selection for small ruminants has begun in several countries including those involved in the present study, with already several years of practice depending on the breed and country. Such progress in genetic selection offers the possibility to include heritable DNA polymorphisms (i.e. SNP variants) for the improvement of various traits (Goddard et al., 2010) (Hayes and Goddard, 2010). Genome wide associations studies (GWAS) have been widely performed on production, reproduction traits, but less attention has been paid for resilience traits. Taking advantages of GWAS and considering the existing genetic variability for various resilience traits found in small ruminants, the aim of the present study was i) to review several GWAS performed on resilience traits in the European SMARTER project and ii) to perform QTL detection for new phenotypes for which GWAS have not been previously been reported in small ruminants.

2. Material and methods

2.1. Animals and Management

Functional longevity

The animals studied were Chios dairy sheep reared in semi-intensive and intensive farming conditions in Northern Greece for the period 2005-2019. Ewes usually lamb from September to May and their lactation period typically lasts 5-6 months (Basdagianni et al., 2019).

Beta-hydroxybutyrate

The data set came from a total of 338 primiparous Alpine and Saanen goats from 5 different farms, sampled between 2020 and 2022 as part of the SMARTER project.

Colostrum IgG

The data came from an experimental population of 201 Alpine goats, monitored in first lactation from 2017 to 2020 at the INRAE facility P3R (Osmoy, France, experimental approval: C18–174–01).

2.2. Phenotypes

Functional longevity

Individual records of purebred Chios dairy sheep were obtained from the database of the Chios Sheep Breeders' Cooperative Macedonia. Functional longevity was selected as a representative trait of lifetime resilience, referring to the time interval between the first lambing and the culling date. Data were adjusted for age at first lambing (12–48 months) and years of productive life after the first lambing date (at least 1 year). The final dataset consisted of 24,082 animals used to estimate the heritability of the trait. Animal pedigree was also obtained from the Cooperative including 101,493 animals, 2,324 sires and 26,008 dams. A subset of the animals was genotyped for downstream genome-wide association analysis.

Beta-hydroxybutyrate

Individual records of blood beta-hydroxybutyrate (BHB) concentration were measured from 2 to 4 times during the first lactation (3.2 times on average). BHB concentrations were determined on farms using portable FreeStyle® Precision Neo devices (Abbott), from a drop of blood taken from the jugular vein and applied directly to the dedicated strip. The experiment was carried out in agreement with French National Regulations for the humane care and use of animals for research purposes under the approval Avenant_SSA_2020_003V2 of committee SSA#115. For GWAS, repeated values were summarized into one single individual EBVs using the wombat software (Meyer, 2007), by accounting for year of sampling (2017 to 2020), farm (N=5), and for repeated values per animal.

Colostrum IgG

The immune quality of colostrum can be evaluated measuring IgG concentration and quantity in the hours following kidding. Accordingly, the colostrum of 201 primiparous goats was collected at the first milking, i.e. during the first 18 hours after kidding. The kids had been separated from their mothers as soon as they were born, to prevent them from consuming colostrum before sampling. The colostrum collected was weighed and a sample was sent to laboratory Agrolab's (Auch, France) for IgG dosage using the radial immunodiffusion method. IgG yield was then estimated by multiplying the IgG concentration by the quantity of colostrum. The average values for Igg concentration and yield in colostrum were 43.85 g/L (±18.42) and 47.40 g (±27.82), respectively. Values ranged from 10 to 112 g/L and from 7 to 175 g for IgG concentration and IgG yield, respectively. Because the distribution of both values was highly skewed, data was log-transformed to normalize the distributions for further statistical analyses. EBVs for both log-transformed IgG concentration and yield were then estimated using the wombat software (Meyer, 2007), to correct individual values for year of sampling (2017 to 2020) and litter size (1 to 3 kids). The experiment was carried out in agreement with French National Regulations for the humane care and use of animals for research purposes under the experimental approval APAFIS#8613–2017012013585646 V4.

2.3. Genotypes

Functional longevity

A total of 211 Chios female sheep intensively reared in three farms in Northern Greece was genotyped with the Illumina OvineSNP50 Genotyping BeadChip, featuring 54,241 single-nucleotide polymorphisms (SNPs). Quality control was performed by setting sample and marker call rate thresholds at 90% and minor allele frequency (MAF) at 98%, respectively, using Plink v1.9 software (Chang et al., 2015). SNP markers on sex and zero chromosomes and duplicate samples were removed. The final dataset consisted of 45,936 SNPs across the 26 ovine chromosomes.

Beta-hydroxybutyrate and colostrum IgG in goats

All 338 goats for BHB and 183 Alpine goats for IgG traits were genotyped using the Illumina Goat_IGGC_65K_v2 (59,727 SNPs). The DNA extraction from blood samples and genotyping were performed at LABOGENA. Data were filtering using PLINK software based on animal call rate (>98%), SNP call rate (>99 %), minor allele frequency (>2%), Hardy Weinberg equilibrium (p-value above 10-6), SNPs on autosomes. After filtering a total of 45,128, 45,372 and 47,260 SNPs in Alpine and Saanen breeds for BHB and Alpine goats for IgG traits, respectively, was retained for further analyses. Marker order and positions were based on the caprine Assembly ARS1.

2.4. Genome wide association analyses

Functional longevity

A genome-wide association study (GWAS) was performed to identify SNP markers associated with functional longevity in Chios dairy sheep, implementing a linear mixed model via GEMMA software (Zhou and Stephens, 2012). The model's equation is described below:

$$y = Wa + x\beta + Zu + \varepsilon$$

where y represents the vector of animal phenotypes, a is the vector of the fixed effects, x is the vector of SNP genotypes and β their associated fixed effects, u is the vector of random polygenic (additive genetic) effects normally distributed (0, VgG, with G being the genomic relatedness matrix and Vg the genomic variance of the trait), W and Z are the corresponding design matrices, and ε is the vector of residual effect. Fixed effects included in the model were the farm, first lambing year, lifetime milk production and total number of lactations.

A Bonferroni correction was applied for multiple testing to determine the adjusted genome-wide significance threshold, resulting in $P < 1.08E\text{-}06$ (0.05/46,290 SNPs), and corresponding to -log10 (P) of 5.97. Chromosome-wise significance thresholds were also obtained for chromosomes 2, 4, 12 and 18 estimated as –log10(0.05/n), where n is the number of SNP markers on each chromosome.

Gene annotation was conducted utilizing the Variant Effect Predictor software (McLaren et al., 2016) with distances of 1 Mb upstream and downstream of each SNP marker. The Animal QTL (Hu et al., 2022) database was used to identify relevant QTLs.

Beta-hydroxybutyrate and colostrum IgG

GWAS for Beta-hydroxybutyrate and colostrum IgG traits was carried out for each breed and trait separately, using GEMMA software (Zhou and Stephens, 2012). The linear mixed model used in this study was as follows:

$$y = W\beta + Zu + \varepsilon$$

where y denotes the vector of EBVs; β is the vector of fixed SNP effect; W is the incidence matrix of β corresponding SNP effect to individuals; u is the random genetic animal effect; Z is the incidence matrix accounting for relationship structure among individuals; ε is the random residual effect. Wald statistical tests were hired to test the null hypothesis $H0: \alpha=0$, and alternative hypothesis $H1: \alpha\neq0$.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 772787

Because of the test multiplicity, a Bonferroni correction of α=5% was applied for both genome-wide and chromosome-wide thresholds. Any SNP with P < 1.10 x 10 -6 were considered to be significantly associated at the genome-wide level. The chromosome-wide significance thresholds ranged from 1.67 x 10 -5 to 6.31 x 10 -5 depending on the chromosome size. The proportion of the genetic variance explained by the genotype of the significant SNPs was estimated by the formula: $2*p(1-p)*\beta^2$ where p is the frequency of the SNP and β the SNP effect.

2.5. Data from published gwas

In addition to the new gwas described above, we used gwas results from Smarter partner studies already published or under review. These studies focused on metabolic plasticity as phenotyped by ultrasonic backfat thickness in Texel sheep (Kaseja et al., 2023 in press), or body condition score in Merino (Ramos et al., 2023) and Romane sheep (Macé et al., 2018; Macé et al., 2022). These studies also focused on health, including footrot and mastitis (CMT) in UK Texel sheep (Kaseja et al., 2023 in press), and parasite resistance (FEC) in FEC Uruguayan Merino (Vera et al., 2023 PhD) and Corriedale (Carracelas et al., 2022). Finally, we used the results of a Greek study on reproduction (age at first lambing) and maternal lamb survival in Chios sheep (Tsartsianidou et al., 2023).

All phenotypes and breeds studied are presented in Table1.

3. Results

Beta-hydroxybutyrate

The GWAS for beta-hydroxybutyrate in goat was conducted within breed. No significant SNP was found in the Alpine breed. Two QTL were significant at the chromosome-wise level in the Saanen breed (Table 4). One QTL mapped on CHI 1 (snp44252-scaffold-1187060) around 7.3 Mb and the second QTL on CHI 19 (snp28387-scaffold303-1179769) around 31.2 Mb (Figure 1).

Colostrum IgG

The GWAS for colostrum immune quality in goats was applied to both IgG concentration and IgG yield. No significant SNP was found for IgG yield. Two SNPs were significant at the chromosome-wise level: One mapped on CHI4 around 20.9 Mb and the second QTL on CHI 17 around 4.2 Mb (Tables 5; Figure 2A).

Functional longevity

Genome-wide association results for functional longevity (FL) in Chios dairy sheep are presented in Table 4. Four QTLs were detected, reaching the chromosome-wise (CW) significance threshold (Figure 3). In particular, three QTLs were located on chromosomes 2, 12 and 18, respectively, each with one chromosome-wise SNP marker. Also, a QTL region consisting of two chromosome-wide SNP markers was detected on chromosome 4, spanning a region of 28,930 bp. The closest annotated genes to the most significant SNP markers are also shown in Table 4. No genome-wide significant SNPs were detected for FL.

Results from already published studies are presented in Tables 2 to 4.

4. Discussion

There is an urgent need for improvement of resilience traits in small ruminants' populations to cope with current and future challenges. Small ruminants demonstrate genetic variability within and between breeds for several resilience traits, either considering specific or global criteria, suggesting that genetic selection for these traits will be feasible. In addition, appropriate use of genome-wide high-throughput genotyping and associated models used in genomic selection will further enhance rapid improvements of resilience traits. In the present study, we report and discussed several QTL associated with resilience traits which have been found during the European SMARTER project. Comparison of genomic regions identified between breeds for a similar trait or between traits for a given breed is of great interest to identify potential pleiotropic QTL. Resilience traits in the present study are considered as traits contributing to the ability of the animal to adapt transitory, and respond to various challenges in order to maintain health, welfare and production in challenging environments. We focused more particularly on resilience traits for disease and nutritional challenges.

Metabolic plasticity

Sheep and goats can use natural resources (i.e. forages) in extensive environments that do not compete with food production for humans. Small ruminants rely on their biological capacity to use and constitute their body energy reserves stored in the form of lipids to cope with fluctuations in feed resources (Friggens et al., 2017). Investigation of body reserves involved to consider body reserves (BR) levels and BR changes over time. Macé et al (Mace et al., 2022) reported several QTLs associated with BR levels and BR changes, based on body condition score criteria measured at key physiological stages throughout the productive cycle in Romane ewes. They didn't report pleiotropic QTL since distinct QTLs were found for BR levels and BR changes. Their findings were of particular interest for the genomic region mapped on chromosome 1 (OAR1) (Table 3) due to the overlapping of several QTLs associated with BR levels at several physiological stages of the BR mobilization period, the high level of significance level, and the candidate gene present in the QTL region. Among the significant SNPs identified, Macé et al (Mace et al., 2022) reported a candidate mutation in the gene coding the receptor of the leptin hormone (LEPR). This mutation in LEPR gene was associated with higher fatness in sheep (Haldar et al., 2014; Mace et al., 2022), consistently with effects of various mutations in leptin and LEPR genes reported to cause obesity in human and animal models (Ghalandari et al., 2015; Berger and Kloting, 2021).

Concerning QTLs associated with BR changes, only one QTL reached the genome-wise significance level on chromosome 16 and was associated with accretion during the post-weaning period (Mace et al., 2022). The authors didn't observe overlapping for QTL associated with BR changes over time either considering BR accretion or BR mobilization periods.

In Merino sheep (i.e. productive ewes), Ramos et al (Ramos et al., 2023) reported a QTL on chromosome 1 associated with BCS at mating (Table 3) but in a different region that the genomic

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 772787

region found in Romane sheep. These authors reported an additional QTL on chromosome 13 associated with BCS at mating and explaining a higher percentage of genetic variance. Among genes present in this region, TMC2, SIRPA and CPXM1 have been reported as candidate genes for tail fat deposition in sheep (Yuan et al., 2017). In Texel sheep (i.e. 21 weeks of age), Kaseja et al (Kaseja et al., 2023) reported a QTL on chromosome 3 associated with backfat thickness measured by ultrasound. This region is close to a QTL associated with body weight previously reported in Awassi-Merino sheep using microsatellite markers (Raadsma et al., 2009). This genomic region was not identified in Romane and Merino sheep using BCS as proxy of fatness in productive ewes..

Health

In the present study, resilience traits for health address gastrointestinal parasitism, lameness, mastitis, metabolic disorder and immune response. In Corriedale sheep (i.e. growing lambs), Carracelas et al (Carracelas et al., 2022) reported various QTL associated with gastrointestinal nematodes (GIN) resistance using fecal egg count (FEC) as proxy (Table 3). Among these QTL, QTL reported on chromosome 7 was of greatest interest with a candidate gene (INO80) present in the region which has not been previously reported in association with GIN resistance. Additional QTLs associated with GIN resistance were found in Merino sheep (i. e. growing lambs or yearling lambs) on chromosomes 2, 6 and 11 using the same proxy (i.e. FEC) without identifying any signal on chromosome 7. Interestingly, Salle et al (Salle et al., 2012) reported 5 QTL associated with GIN resistance in Romane X Martinik Black Belly backcross lambs on chromosomes 5, 7, 12, 13 and 21. The QTL mapped on chromosome 7 in backcross lambs and Corriedale didn't overlap while the same proxy was used for measuring GIN resistance and animals of similar ages but of different breed. Pacheco et al (2023) reported 15 significant SNPs for parasites, faecal soiling and associated immunological traits (Animal, submitted), although the majority of these were significant at the chromosome-wise level only. Regardless, following gene ontology analyses, they were associated with 52 known genes implicated in immune function and disease.

Elevation of beta-hydroxybutyrate concentrations in the blood is indicative of hyperketonemia or subclinical ketosis due to an excessive mobilization of body energy reserves during negative energy balance as in the early lactation in goats. The QTL mapped on chromosome 19 in Saanen goats for BHB overlapped with another QTL of great interest previously found in Saanen. Indeed, the significant SNP is located within a notable pleiotropic region associated with milk production traits, udder conformation and somatic cell score (SCS) in French, UK, New-Zealand dairy goat populations (Martin et al., 2018; Mucha et al., 2018; Scholtens et al., 2020; Talouarn et al., 2020). No potential functional candidate genes involved in metabolic diseases or lipid metabolism were identified among positional candidate genes. In cattle, a QTL on chromosome BTA20 associated with milk BHB has been found in two breeds (Simmental, (Falchi et al., 2021); Holstein, (Nayeri et al., 2019)). These authors described a candidate gene ANKH (Inorganic Pyrophosphate Transport Regulator) already described to be associated with lactose percentage, alpha-lactalbumin and protein composition in milk.

In goats, the present QTL associated with BHB in Saanen goat is breed-specific consistently with the previously identified QTL on chromosome 19 in this breed (which was not found in Saanen). Despite similar breeding goals that have been applied in Saanen and Alpine breeds in France over the past 60 years, genetic heterogeneity in the determinism of traits may still reflect original breed differences.

Colostrum IgG

The immune quality of colostrum in goats was analysed thought individual IgG dosage in colostrum 18 hours after kidding. Two SNPs were significant, highlighting the existence of genetic control of this trait in this species. To our knowledge this is the first GWAS result for colostrum related traits in small ruminants. Two studies have been published in dairy cattle. In Jersey cattle, Kiser et al. (2019) identified 7 loci associated with colostrum quantity and 1 locus associated with colostrum IgG concentration estimated by BRIX refractometer methodology. In Chinese Holstein cows, Lin et al. (2022) identified 47 loci associated with colostrum IgG (IgG1, IgG2, IgGM and IgGM) measured by Elisa tests. None of the loci identified in the two studies, however, was located within the orthologous regions (± 10Mb) to our 2 caprine SNPs (BTA17 - 69 Mb; BTA4 - 100 Mb). No straightforward candidate gene could be identified in the present goat QTL regions and it is likely that the underlying control is polygenic. Further data analyses are required to more fully understand underlying mechanisms and genes.

Functional longevity

Functional longevity can be used in livestock as an overall indicator of resilience/robustness at the scale of the productive life and should be a promising way to improve livestock sustainability. Investigation of genetic determinism of functional longevity began relatively recently in small ruminants in France. In the present study, we reported new QTL for functional longevity in small ruminants.

Four QTL on four distinct chromosomes have been found to be associated with functional longevity in Chios dairy sheep. The candidate genes highlighted for longevity in Chios breed (i.e. SCN3A, NEUROD6) have not been previously reported for this trait but some genomic regions overlapped with other traits. The significant SNP marker detected on chromosome 12 is located within QTLs previously reported in Animal QTL database for health traits, especially associated with parasite resistance (Beh et al., 2002) and levels of immunoglobulin A (IgA) (Atlija et al., 2016). In addition, QTLs related to muscle depth (Matika et al., 2011) and meat fatty acid content (Karamichou et al., 2006) are located within the genomic region of interest located on chromosome 18. Interestingly, both QTLS detected here on chromosomes 4 and 12 are located 0.5-0.9 Mb away from QTL regions previously associated with seasonal reproduction (Mateescu and Thonney, 2010; Martinez-Royo et al., 2017).

The most significant SNPs found in the present study were located within previously identified QTL region for health, reproduction and production. This situation has also been observed in previous GWAS for several longevity traits in Chinese Holsteins where SNPs were located within the previously identified QTL for calving traits, health traits, fertility traits and immunity (Zhang et al., 2021). Similar phenomenon has also been reported in previous GWAS for longevity in other cattle populations (North America: (Nayeri et al., 2017); United States: (Cole et al., 2011); Nordic: (Zhang et al., 2016)). Present findings are consistent with the fact that longevity, defined in the present study as the time interval between first lambing and culling, measures resilience to various challenges including health problems or reproduction disorders. Overlapping of genomic regions between longevity and other specific resilience traits is also consistent with favourable genetic correlations between longevity and specific resilience traits as previously reported between functional longevity and mammary health in goats and sheep (Palhiere et al., 2018; Buisson et al., 2022). These results highlight the importance of performing GWAS for specific and global resilience traits in each breed to identify potential pleiotropic QTL that could be investigated further via fine-mapping.

Reproduction

The age at first lambing and maternal lamb survival are important reproductive traits affecting sheep longevity and resilience. Very few genome association studies have been conducted for these reproductive traits in small ruminants and GWAS revealed two new QTL for maternal lamb survival and age at first lambing on chromosomes 1 and 2, respectively. Functional annotation revealed a set of genes in these two genomic regions among which *ZPLD1* and *WDR75* were the closest candidate genes (Tsartsianidou et al., 2023).

5. Conclusion

The work described here helps to identify the most relevant QTL associated with resilience traits in small ruminants. We reported various QTL associated with resilience traits specific to health or metabolic plasticity in sheep and goat, and also QTL for lifetime resilience which combines probably several resilience traits. Some of the QTL regions contain interesting candidate genes. Ultimately, the identification of genes underlying the resilience traits investigated in our study will provide opportunities for deeper understanding the genetic components involved and will contribute to the general understanding of resilience and adaptation in animals. QTL or SNP markers reported here could potentially be used to improve resilience traits in small ruminants through genomic selection.

6. Declaration

Ethics approval

The experiments described here fully comply with applicable legislation on research involving animal subjects in accordance with the European Union Council directive (2010/63/UE). The investigators who carried out the experiments were certified by their relevant governmental authority in each country. All experimental procedures were performed according to the guidelines for the care and use of experimental animals and approved by each governmental authority and local ethics committee.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by the European Union’s Horizon 2020 Research and Innovation Action through the SMARTER project under the grant agreement No. 772787.

Acknowledgments

We would like to thank Chios Sheep Breeder’s Cooperative Macedonia for animal records provided. The authors are also indebted to Emilie Cobo, Florian Mosnier and Morgane Bastide for their assistance in samples collection for BHB measurement in goats.

7. References

Atlija, M., J. M. Prada, B. Gutierrez-Gil, F. A. Rojo-Vazquez, M. J. Stear, J. J. Arranz, and M. Martinez-Valladares. 2016. Implementation of an extended ZINB model in the study of low levels of natural gastrointestinal nematode infections in adult sheep. Bmc Veterinary Research 12.

Basdagianni, Z., E. Sinapis, and G. Banos. 2019. Evaluation of reference lactation length in Chios dairy sheep. Animal 13: 1-7.

Beh, K. J., D. J. Hulme, M. J. Callaghan, Z. Leish, I. Lenane, R. G. Windon, and J. F. Maddox. 2002. A genome scan for quantitative trait loci affecting resistance to Trichostrongylus colubriformis in sheep. Animal Genetics 33: 97-106.

Berger, C., and N. Kloting. 2021. Leptin Receptor Compound Heterozygosity in Humans and Animal Models. International Journal of Molecular Sciences 22.

Borghesi, J., L. Mario, M. Rodrigues, P. Favaron, and M. Miglino. 2014. Immunoglobulin Transport during Gestation in Domestic Animals and Humans—A Review. Open Journal of Animal Sciences 04: 323-336.

Brozos, C., V. S. Mavrogianni, and G. C. Fthenakis. 2011. Treatment and Control of Peri-Parturient Metabolic Diseases: Pregnancy Toxemia, Hypocalcemia, Hypomagnesemia. Veterinary Clinics of North America-Food Animal Practice 27: 105-+.

Buisson, D., J. M. Astruc, L. Doutre, and I. Palhière. 2022. Toward a genetic evaluation for functional longevity in French dairy sheep breeds. In: 12th World Congress on Genetics Applied to Livestock Production, Rotterdam

Conington, J., Bishop, S.C.,Waterhouse, A., Simm,G. (1995). A genetic analysis of early growth and ultrasonic measurements in hill sheep. Animal Science 61:85-93

Carracelas, B., E. A. Navajas, B. Vera, and G. Ciappesoni. 2022. Genome-Wide Association Study of Parasite Resistance to Gastrointestinal Nematodes in Corriedale Sheep. Genes 13.

Chang, C. C., C. C. Chow, L. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4.

Colditz, I. G., and B. C. Hine. 2016. Resilience in farm animals: biology, management, breeding and implications for animal welfare. Animal Production Science 56: 1961-1983.

Cole, J. B., G. R. Wiggans, L. Ma, T. S. Sonstegard, T. J. Lawlor, B. A. Crooker, C. P. Van Tassell, J. Yang, S. W. Wang, L. K. Matukumalli, and Y. Da. 2011. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary US Holstein cows. Bmc Genomics 12.

Conneely, M., D. P. Berry, R. Sayers, J. P. Murphy, I. Lorenz, M. L. Doherty, and E. Kennedy. 2013. Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. Animal 7: 1824-1832.

Cordero-Solorzano, J., D. J. de Koning, M. Traven, T. de Haan, M. Jouffroy, A. Larsson, A. Myrthe, J. A. J. Arts, H. K. Parmentier, H. Bovenhuis, and J. J. Wensman. 2022. Genetic parameters of colostrum and calf serum antibodies in Swedish dairy cattle. Genetics Selection Evolution 54.

Dwyer CM, Conington J, Corbiere F, Holmøy IH, Muri K, Nowak R, Rooke J, Vipond J, Gautier JM., 2016. Invited review: Improving neonatal survival in small ruminants: science into practice. Animal 10(3):449-59.

Falchi, L., G. Gaspa, A. Cesarani, F. Correddu, L. Degano, D. Vicario, D. Lourenco, and N. P. P. Macciotta. 2021. Investigation of beta-hydroxybutyrate in early lactation of Simmental cows: Genetic parameters and genomic predictions. Journal of Animal Breeding and Genetics 138: 708-718.

Fitzmaurice, S., Conington J., Fetherstone, N., Pabiou, T., McDermott, K. Wall, E, G. Banos, G and McHugh, N. 2020 Genetic analyses of live weight and carcass composition traits in purebred Irish Texel, Suffolk and Charollais lambs. Animal 14: 5: 899-909.

Fitzmaurice, S, Conington, J, McHugh, N, Banos G. 2021 Towards future genetic evaluations for live weight and carcass composition traits in UK sheep. Small Ruminant Research 196: 106327 March 2021.

Friggens, N. C., I. Adriaens, R. Boré, G. Cozzi, J. Jurquet, C. Kamphuis, F. Leiber, I. Lora, T. Sakowski, J. Statham, and Y. De Haas. 2022. Resilience: reference measures based on longer-term consequences are needed to unlock the potential of precision livestock farming technologies for quantifying this trait. Peer Community Journal 2.

Friggens, N. C., F. Blanc, D. P. Berry, and L. Puillet. 2017. Review: Deciphering animal robustness. A synthesis to facilitate its use in livestock breeding and management. Animal 11: 2237-2251.

Ghalandari, H., F. Hosseini-Esfahani, and P. Mirmiran. 2015. The Association of Polymorphisms in Leptin/Leptin Receptor Genes and Ghrelin/Ghrelin Receptor Genes With Overweight/Obesity and the Related Metabolic Disturbances: A Review. International journal of endocrinology and metabolism 13: e19073.

Gilbert, R. P., C. T. Gaskins, J. K. Hillers, C. F. Parker, and T. C. McGuire. 1988. GENETIC AND ENVIRONMENTAL-FACTORS AFFECTING IMMUNOGLOBULIN-G1 CONCENTRATIONS IN EWE COLOSTRUM AND LAMB SERUM. Journal of Animal Science 66: 855-863.

Goddard, M. E., B. J. Hayes, and T. H. E. Meuwissen. 2010. Genomic selection in livestock populations. Genetics Research 92: 413-421.

Haldar, A., M. C. French, R. Brauning, S. J. Edwards, A. R. O'Connell, P. A. Farquhar, G. H. Davis, P. D. Johnstone, and J. L. Juengel. 2014. Single-Nucleotide Polymorphisms in the LEPR Gene Are Associated with Divergent Phenotypes for Age at Onset of Puberty in Davisdale Ewes. Biology of Reproduction 90.

Hayes, B., and M. Goddard. 2010. Genome-wide association and genomic selection in animal breeding. Genome 53: 876-883.

Hu, Z. L., C. A. Park, and J. M. Reecy. 2022. Bringing the Animal QTLdb and CorrDB into the future: meeting new challenges and providing updated services. Nucleic Acids Research 50: D956-D961.

Jackson, F., and R. L. Coop. 2000. The development of anthelmintic resistance in sheep nematodes. Parasitology 120: S95-S107.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 772787

Karamichou, E., R. I. Richardson, G. R. Nute, K. P. Gibson, and S. C. Bishop. 2006. Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. Journal of Animal Science 84: 3228-3238.

Kaseja, K., S. Mucha, J. Yates, E. Smith, G. Banos, and J. Conington. 2023. Genome-wide association study of health and production traits in meat sheep. Animal: in press.

Kenyon, P. R., S. K. Maloney, and D. Blache. 2014. Review of sheep body condition score in relation to production characteristics. New Zealand Journal of Agricultural Research 57: 38-64.

Lin S, Ke C, Liu L, Gao Y, Xu L, Han B, Zhao Y, Zhang S, Sun D., 2022. Genome-wide association studies for immunoglobulin concentrations in colostrum and serum in Chinese Holstein. BMC Genomics 10;23(1):41.

Lou, W., H. Zhang, H. Luo, Z. Chen, R. Shi, X. Guo, Y. Zou, L. Liu, L. F. Brito, G. Guo, and Y. Wang. 2022. Genetic analyses of blood beta-hydroxybutyrate predicted from milk infrared spectra and its association with longevity and female reproductive traits in Holstein cattle. Journal of Dairy Science 105: 3269-3281.

Lambe N.R., Young M.J., Brotherstone, S, Kvame, T, Conington J.E>, Kolstad, K., Simm, G. 2003. Body composition changes in Scottish blackface ewes during one annual production cycle. Animal Science 76; 211-219.Mace, T., E. Gonzalez-Garcia, D. Foulquie, F. Carriere, J. Pradel, C. Durand, S. Douls, C. Allain, S. Parisot, and D. Hazard. 2022. Genome-wide analyses reveal a strong association between LEPR gene variants and body fat reserves in ewes. Bmc Genomics 23.

Mace, T., E. González García, J. Pradel, S. Parisot, F. Carriere, S. Douls, D. Foulquie, and D. Hazard. 2018. Genetic analysis of robustness in meat sheep through body weight and body condition score changes over time. Journal of Animal Science 96: 4501-4511.

Marques, C. B., V. Goldberg, and G. Ciappesoni. 2020. Genetic parameters for production traits, resistance and resilience to Nematode parasites under different worm burden challenges in Corriedale sheep. Veterinary Parasitology 287.

Martin, P., I. Palhiere, C. Maroteau, V. Clement, I. David, G. T. Klopp, and R. Rupp. 2018. Genome-wide association mapping for type and mammary health traits in French dairy goats identifies a pleiotropic region on chromosome 19 in the Saanen breed. Journal of Dairy Science 101: 5214-5226.

Martin, P., A. Vinet, C. Denis, C. Grohs, L. Chanteloup, D. Dozias, D. Maupetit, J. Sapa, G. Renand, and F. Blanc. 2021. Determination of immunoglobulin concentrations and genetic parameters for colostrum and calf serum in Charolais animals. Journal of Dairy Science 104: 3240-3249.

Martinez-Royo, A., J. L. Alabart, P. Sarto, M. Serrano, B. Lahoz, J. Folch, and J. H. Calvo. 2017. Genome-wide association studies for reproductive seasonality traits in Rasa Aragonesa sheep breed. Theriogenology 99: 21-29.

Mateescu, R. G., and M. L. Thonney. 2010. Genetic mapping of quantitative trait loci for aseasonal reproduction in sheep. Animal Genetics 41: 454-459.

Matika, O., S. Sechi, R. Pong-Wong, R. D. Houston, A. Clop, J. A. Woolliams, and S. C. Bishop. 2011. Characterization of OAR1 and OAR18 QTL associated with muscle depth in British commercial terminal sire sheep. Animal Genetics 42: 172-180.

McLaren, A., K. Kaseja, J. Yates, S. Mucha, N. R. Lambe, and J. Conington. 2018. New mastitis phenotypes suitable for genomic selection in meat sheep and their genetic relationships with udder conformation and lamb live weights. Animal 12: 2470-2479.

McLaren, W., L. Gil, S. E. Hunt, H. S. Riat, G. R. S. Ritchie, A. Thormann, P. Flicek, and F. Cunningham. 2016. The Ensembl Variant Effect Predictor. Genome Biology 17.

Mendizabal, J. A., R. Delfa, A. Arana, P. Eguinoa, C. Gonzalez, T. Treacher, and A. Purroy. 2003. Estimating fat reserves in Rasa Aragonesa ewes: A comparison of different methods. Canadian Journal of Animal Science 83: 695-701.

Mendizabal, J. A., R. Delfa, A. Arana, and A. Purroy. 2010. A comparison of different pre- and post-slaughter measurements for estimating fat reserves in Spanish Blanca Celtiberica goats. Canadian Journal of Animal Science 90: 437-444.

Meyer, K. 2007. WOMBAT: a tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). Journal of Zhejiang University. Science. B 8: 815-821.

Mucha, S., R. Mrode, M. Coffey, M. Kizilaslan, S. Desire, and J. Conington. 2018. Genome-wide association study of conformation and milk yield in mixed-breed dairy goats. Journal of Dairy Science 101: 2213-2225.

Mucha, S., F. Tortereau, A. Doeschl-Wilson, R. Rupp, and J. Conington. 2022. Animal Board Invited Review: Meta-analysis of genetic parameters for resilience and efficiency traits in goats and sheep. Animal 16.

Nayeri, S., M. Sargolzaei, M. K. Abo-Ismail, S. Miller, F. Schenkel, S. S. Moore, and P. Stothard. 2017. Genome-wide association study for lactation persistency, female fertility, longevity, and lifetime profit index traits in Holstein dairy cattle. Journal of Dairy Science 100: 1246-1258.

Nayeri, S., F. Schenkel, A. Fleming, V. Kroezen, M. Sargolzaei, C. Baes, A. Cánovas, J. Squires, and F. Miglior. 2019. Genome-wide association analysis for β-hydroxybutyrate concentration in Milk in Holstein dairy cattle. BMC Genetics 20: 58.

Obrien, J. P., and D. M. Sherman. 1993. SERUM IMMUNOGLOBULIN CONCENTRATIONS OF NEWBORN GOAT KIDS AND SUBSEQUENT KID SURVIVAL THROUGH WEANING. Small Ruminant Research 11: 71-77.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 772787

Palhiere, I., C. Oget, and R. Rupp. 2018. Functional longevity is heritable and controlled by a major gene in French dairy goats 11. World Congress on Genetics Applied to Livestock Production (WCGALP). Massey Universtiy, Auckland, New Zealand.
Raadsma, H. W., P. C. Thomson, K. R. Zenger, C. Cavanagh, M. K. Lam, E. Jonas, M. Jones, G. Attard, D. Palmer, and F. W. Nicholas. 2009. Mapping quantitative trait loci (QTL) in sheep. I. A new male framework linkage map and QTL for growth rate and body weight. Genetics Selection Evolution 41: 34.
Ramos, Z., D. J. Garrick, H. T. Blair, B. Vera, G. Ciappesoni, and P. R. Kenyon. 2023. Genomic Regions Associated with Wool, Growth and Reproduction Traits in Uruguayan Merino Sheep. Genes 14.
Russel, A. J. F., J. M. Doney, and R. G. Gunn. 1969. SUBJECTIVE ASSESSMENT OF BODY FAT IN LIVE SHEEP. Journal of Agricultural Science 72: 451-&.
Simm, G. and Dingwall, W.S. 1989. Selection Indices for Lean Meat Production in Sheep. Livestock Production Science, 21 (1989) 223-233.
Salle, G., P. Jacquiet, L. Gruner, J. Cortet, C. Sauve, F. Prevot, C. Grisez, J. P. Bergeaud, L. Schibler, A. Tircazes, D. Francois, C. Pery, F. Bouvier, J. C. Thouly, J. C. Brunel, A. Legarra, J. M. Elsen, J. Bouix, R. Rupp, and C. R. Moreno. 2012. A genome scan for QTL affecting resistance to Haemonchus contortus in sheep. Journal of Animal Science 90: 4690-4705.
Scholtens, M., A. Jiang, A. Smith, M. Littlejohn, K. Lehnert, R. Snell, N. Lopez-Villalobos, D. Garrick, and H. Blair. 2020. Genome-wide association studies of lactation yields of milk, fat, protein and somatic cell score in New Zealand dairy goats. Journal of Animal Science and Biotechnology 11.
Talouarn, E., P. Bardou, I. Palhiere, C. Oget, V. Clement, and C. VarGoats. 2020. Genome wide association analysis on semen volume and milk yield using different strategies of imputation to whole genome sequence in French dairy goats. Bmc Genetics 21.
Tsartsianidou, V., A. Pavlidis, E. Tosiou, G. Arsenos, G. Banos, and A. Triantafyllidis. 2023. Novel genomic markers and genes related to reproduction in prolific Chios dairy sheep: a genome-wide association study. Animal 17.
van der Drift, S. G. A., K. J. E. van Hulzen, T. G. Teweldemedhn, R. Jorritsma, M. Nielen, and H. C. M. Heuven. 2012. Genetic and nongenetic variation in plasma and milk beta-hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. Journal of Dairy Science 95: 6781-6787.
Werne, S., K. Schwarz, S. Thuer, and B. Bapst. Breeding options for nematode resistance in Lacaune dairy sheep. Animal: submitted.
Yokoo, M. J., L. G. Albuquerque, R. B. Lobo, L. A. F. Bezerra, F. R. C. Araujo, J. A. V. Silva, and R. D. Sainz. 2008. Genetic and environmental factors affecting ultrasound measures of longissimus muscle area and backfat thickness in Nelore cattle. Livestock Science 117: 147-154.
Yuan, Z., E. Liu, Z. Liu, J. W. Kijas, C. Zhu, S. Hu, X. Ma, L. Zhang, L. Du, H. Wang, and C. Wei. 2017. Selection signature analysis reveals genes associated with tail type in Chinese indigenous sheep. Animal Genetics 48: 55-66.
Zhang, H. L., A. X. Liu, Y. C. Wang, H. P. Luo, X. Y. Yan, X. Y. Guo, X. Li, L. Liu, and G. S. Su. 2021. Genetic Parameters and Genome-Wide Association Studies of Eight Longevity Traits Representing Either Full or Partial Lifespan in Chinese Holsteins. Frontiers in Genetics 12.
Zhang, Q. Q., B. Guldbrandtsen, J. R. Thomasen, M. S. Lund, and G. Sahana. 2016. Genome-wide association study for longevity with whole-genome sequencing in 3 cattle breeds. Journal of Dairy Science 99: 7289-7298.
Zhou, X., and M. Stephens. 2012. Genome-wide efficient mixed-model analysis for association studies. Nature Genetics 44: 821-U136.

Deviation or Delays

Due to Covid-19, the achievement GWAS analyses from WP2 were delayed. The total duration of the project, including the 8month extension, has been used to try and achieve all expected analyses. We also took advantage of the final meeting to collate the different partner’s advice on the manuscript. Thus, the paper was drafted very lately and has not been submitted yet, but the draft has been prepared to submit to the journal ‘Animal’ in the coming weeks.

Supplementary material: Tables 1-4 and Figures 1-3

Table 1 Breed and resilience traits used for GWAS

Species	Breed	Country	Production	Biological functions	Resilience traits
Goat	Alpine	France	milk	Metabolic plasticity	Blood BHB concentrations
				Health and Lifetime resilience	Colostrum IgG concentration Colostrum IgG yield
	Saanen	France	milk	Health and metabolic plasticity	Blood BHB concentrations
Sheep	Texel	Scotland	meat	Health	Footroot Mastitis: CMT
				Metabolic plasticity	body reserves: Ultrasound backfat thickness
		Uruguay	meat	Metabolic plasticity	body reserves: Ultrasound backfat thickness
	Merino	Uruguay	meat	Health	Parasitism: FEC FAMACHA
				Metabolic plasticity	Body reserves: BCS, Ultrasound backfat thickness
	Corriedale	Uruguay	meat	Health	Parasitism: FEC
	Chios	Greece	milk	Lifetime resilience	Functional longevity: time interval between first lambing and culling
				Reproduction	Maternal lamb survival Age at first lambing
	Romane	France	meat	Metabolic plasticity	Body reserves: BCS

BHB, beta-hydroxybutyrate; IgG, immunoglobulin G; CMT, California Mastitis Test ; FEC, Fecal Egg Count; BCS, body condition score.

Table 2 Description of the resilience traits used for GWAS

Trait[1]	Breed	N animal (genotyped)	N obs.	Age	Physiological stage	mean	SD	Heritability (± se)	Reference
BHB	Alpine	210	634	Primiparous	Lactating	0.56	0.21	ND	This study
BHB	Saanen	197	436	Primiparous	Lactating	0.39	0.13	ND	This study
IgG	Alpine	201	201	Primiparous	Lactating	43.85	18.42	ND	This study
IgG yield	Alpine	201	201	Primiparous	Lactating	47.40	27.82	ND	This study
FL	Chios	211	979	adult	Lactating	1791.40	733.60	0.11 (0.02)	This study
AFL	Chios	528	2 084	adult	Lactating	509.69	106.26	0.07 (0.01)	Tsartsianidou et al., 2023
MLS	Chios	528	2 084	adult	Lactating	0.85	0.20	0.21 (0.01)	Tsartsianidou et al., 2023
FR*	Texel	4 506	9 123	adult	Lactating	0.35	0.63	0.12	Kaseja et al., 2023 (in press)
CMT*	Texel	4 343	4 787	adult	Lactating	0.70	0.74	0.07	Kaseja et al., 2023 (in press)
FEC1*	Merino	1 610	26 638	lamb	growth	6.63	1.14	0.20 (0.01)	Vera et al., 2023 (*PhD*)
FEC2*	Merino	1 513	18 971	yearling	growth	6.64	1.13	0.19 (0.02)	Vera et al., 2023 (*PhD*)
FAMACHA	Merino	2 525		lamb	growth	2.06	0.86	0.19 (0.04)	This study
FEC*	Corriedale	375-702	19 547	lamb	growth	6.49	1.24	0.18 (0.02)	Carracelas et al., 2022
BFT	Texel	5 590	5 590	adult	-	2.82	1.36	0.31	Kaseja et al., 2023 (in press)
BFT	Texel	637	8549	lamb	growth	2.16	1.02	0.24 (0.03)	This study
BFT	Merino	4 065		yearling	growth	2.24	1.04	0.29 (0.04)	This study
BCS	Merino	1 205		lamb	growth	2.81	0.39	0.40 (0.09)	This study
BCS	Merino	6 442	6442	adult	mating	3.2	0.65	0.16 (0.02)	Ramos et al., 2023
BCS	Romane	1034	2167	adult	P/L/S	2.9/2.6/2.5	0.22	0.26 to 0.37 (0.05)	Macé et al., 2018

[1] BHB, beta-hydroxybutyrate (mmol/L); IgG, Immunoglobulin G concentration in colostrum (g/L); IgG yield, Immunoglobulin G quantity in colostrum (g); FL, Functional longevity (days); AFL, age at first lambing (days); MLS, maternal lamb survival (ratio); FR, Foot Root; CMT, California Mastitis Test; FEC, Fecal Egg Count; BFT, Ultrasound Backfat thickness (mm); BCS, body condition score. P, Pregnancy; L, Lambing; S, Suckling; * Log transformed; N obs., total number of observations; ND, Not Determined

N obs., number of observations; SD, Standard Deviation; Ref., Reference for previously published results;

Table 3. Summary of QTLs associated with resilience traits previously published.

Trait[1]	Breed	Chr	Position[2] (bp)	Significance level	SNP or window effect[3]	Closest gene/QTL[4]	Article
BCS-S	Romane	1	40,821,987	GW	2.8	*LEPR*	Macé et al., 2022
BCS-P	Romane	1	40,857,869	GW	3.5	*LEPR*	Macé et al., 2022
BCS-L	Romane	1	40,890,859	GW	3.9	*LEPR*	Macé et al., 2022
BCS	Merino	1	173,862,929–190,851,636	0.25% PVE	0.88[5]	*ATP6V1A, CD200, ATG3, CFAP44, CCDC191, NECTIN3, NEPRO, PLCXD2, SLC9C1*	Ramos et al., 2023
BCS	Merino	13	51,269,879–54,158,422	0.25% PVE	13.89[5]	*TMC2, SIRPA, CPXM1, KCNQ2, RBBP8NL, DNAAF9*	Ramos et al., 2023
BFT	Texel	3	192,372,203	CW	0.49	'Body weight (56 weeks)' (Raadsma et al., 2009)	Kaseja et al., 2023 (in press)
FR	Texel	19	22,759,405	CW	0.05	-	Kaseja et al., 2023 (in press)
FR	Texel	23	2,728,076	CW	0.06	-	Kaseja et al., 2023 (in press)
FR	Texel	26		CW	0.06	Parasitism (Marshall et al., 2009)	Kaseja et al., 2023 (in press)
CMT	Texel	14	14,511,245	CW	0.04	Meat traits and parasitism (Davies et al., 2006; Silva et al., 2012)	Kaseja et al., 2023 (in press)
CMT	Texel	17	32,936,496	CW	0.05	Parasitism (Silva et al., 2012), aseasonal reproduction (Mateescu and Thonney, 2010)	Kaseja et al., 2023 (in press)
FEC	Corriedale	7	33,565,208	CW	-	*INO80*	Carracelas et al., 2022
FEC1 & 2	Merino	2		-	-	*PCSK5*	Vera et al., 2023 (*PhD*)
FEC1 & 2	Merino	6		-	-	*HERC3*	Vera et al., 2023 (*PhD*)
FEC1 & 2	Merino	11		-	-	*SLC25A35*	Vera et al., 2023 (*PhD*)
FEC1 & 2	Merino	17		-	-	*TLL1*	*Vera et al., 2023 (PhD)*
FEC1 & 2	Merino	25		-	-	*CTNNA3*	*Vera et al., 2023 (PhD)*
MLS	Chios	1	183,753,694	CW	*2.9*	*ZPLD1*	*Tsartsianidou et al., 2023*
AFL	Chios	2	129,887,607	GW	*0.3*	*WDR75*	*Tsartsianidou et al., 2023*

[1] BCS, body condition score; BFT, Ultrasound Backfat thickness ; FR, Foot Root; CMT, California Mastitis Test; FEC, Fecal Egg Count; AFL, age at first lambing; MLS, maternal lamb survival. P, Pregnancy; L, Lambing; S, Suckling; [2] Position of the highest significant SNP or interval window; [3]Percentage of variance explained by SNP. [4]Annotated protein coding genes closest to the top SNP of the QTL region, or closest QTL previously identified. Proportion of additive genetic variance explained by window. Chr, chromosome. GW, Genome-wide significance level; CW, chromosome-wide significance level. 0.25% PVE, higher than the suggestive threshold of 0.25% of the expected proportion of genetic variance explained

Table 4. Summary of new QTLs detected in GWAS at the chromosome-wise significance level.

Trait[1]	Breed	Chr	Nb SNPs[2]	Top SNP[3]	Position (bp)	-log10(P-value)	Chromosome-wise significance threshold	SNP effect[4]	Closest gene[5]
FL	Chios	2	1	OAR2_152193248.1	155,993,633	5.07	4.99	3.8	*SCN3A*
FL	Chios	4	2	OAR4_68678863.1	71,739,222	5.08	4.68	3.8	*NEUROD6*
FL	Chios	12	1	s61001.1	76,260,347	5.18	4.48	3.4	*KCNH1*
FL	Chios	18	1	OAR18_55758204.1	52,364,795	5.68	4.41	3.8	*ENSOARG00020006279*
BHB	Saanen	1	1	snp44252-scaffold601-1187060	7,290,5625	4.82	4.78	0[6]	ADAMTS5
BHB	Saanen	19	1	snp28387-scaffold303-1179769	31,220,004	4.54	4.37	0[6]	ARHGAP44
log(IgG)	Alpine	4	1	snp46580-scaffold646-639533	20,958,056	5.01	4.66	0.022[6]	NUP205
log(IgG)	Alpine	17	1	snp25646-scaffold264-2016061	4,179,626	5.49	4.43	0.020[6]	LOC108637869

[1] FL, Functional longevity; BHB, beta-hydroxybutyrate; log(IgG), immunoglobulin concentration in colostrum

[2] Number of significant SNPs in the 1-Mb window. [3] The reported top SNP is the SNP that has the highest –log10(P-value) among the significant SNPs that are in a 1-Mb window. [4] Percentage of variance explained by SNP. [5] Annotated protein coding genes closest to the top SNP of the QTL region. Chr, chromosome. [6] Percentage of genetic variance explained by SNP.

Figure 1: Manhattan plot of genome wide association study for blood BHB concentration in Saanen goats. -log10 P-values for each SNP are shown on the vertical axis. The red horizontal line represents 5% genome-wide threshold and the blue horizontal line represents 5% chromosome-wide threshold.

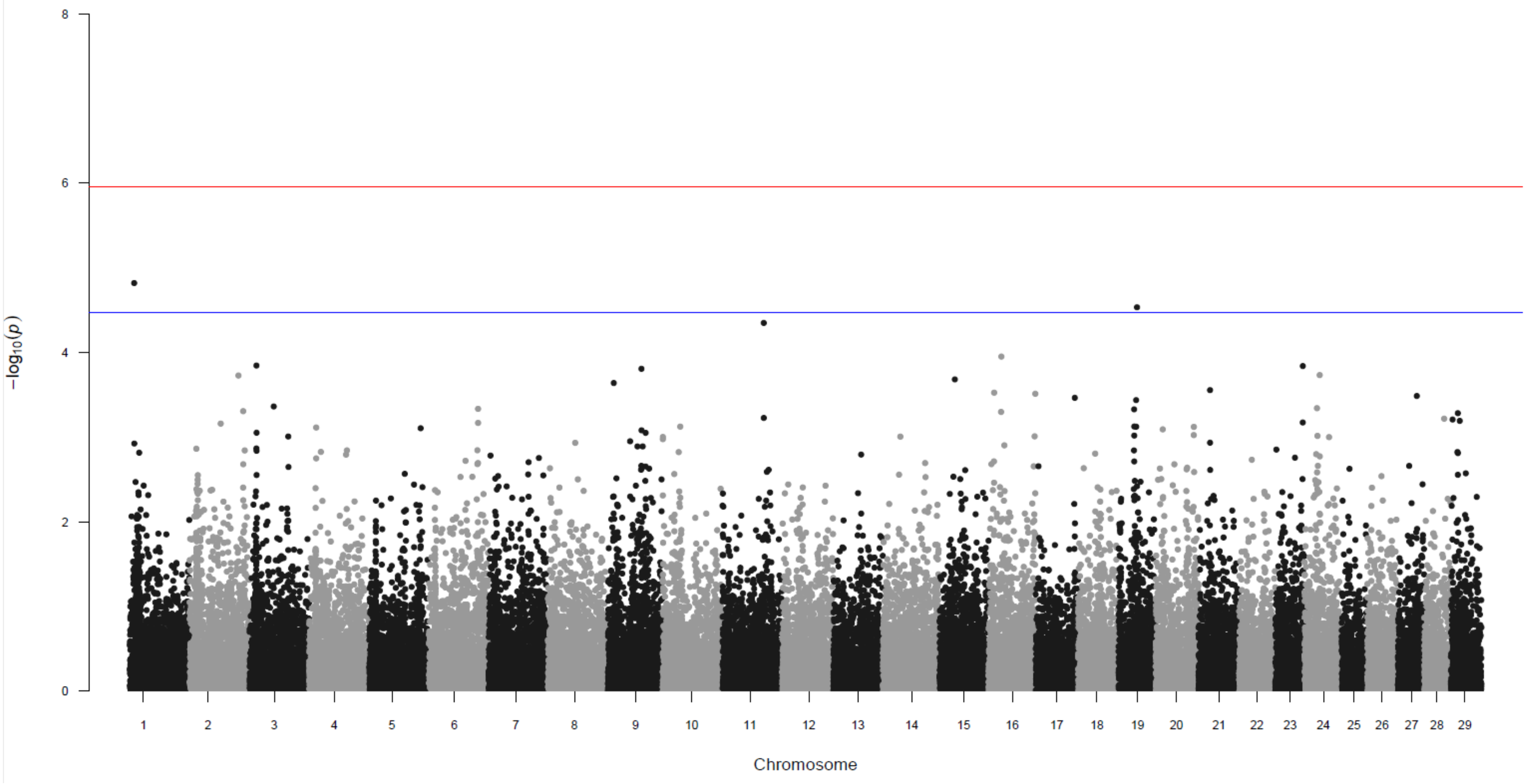
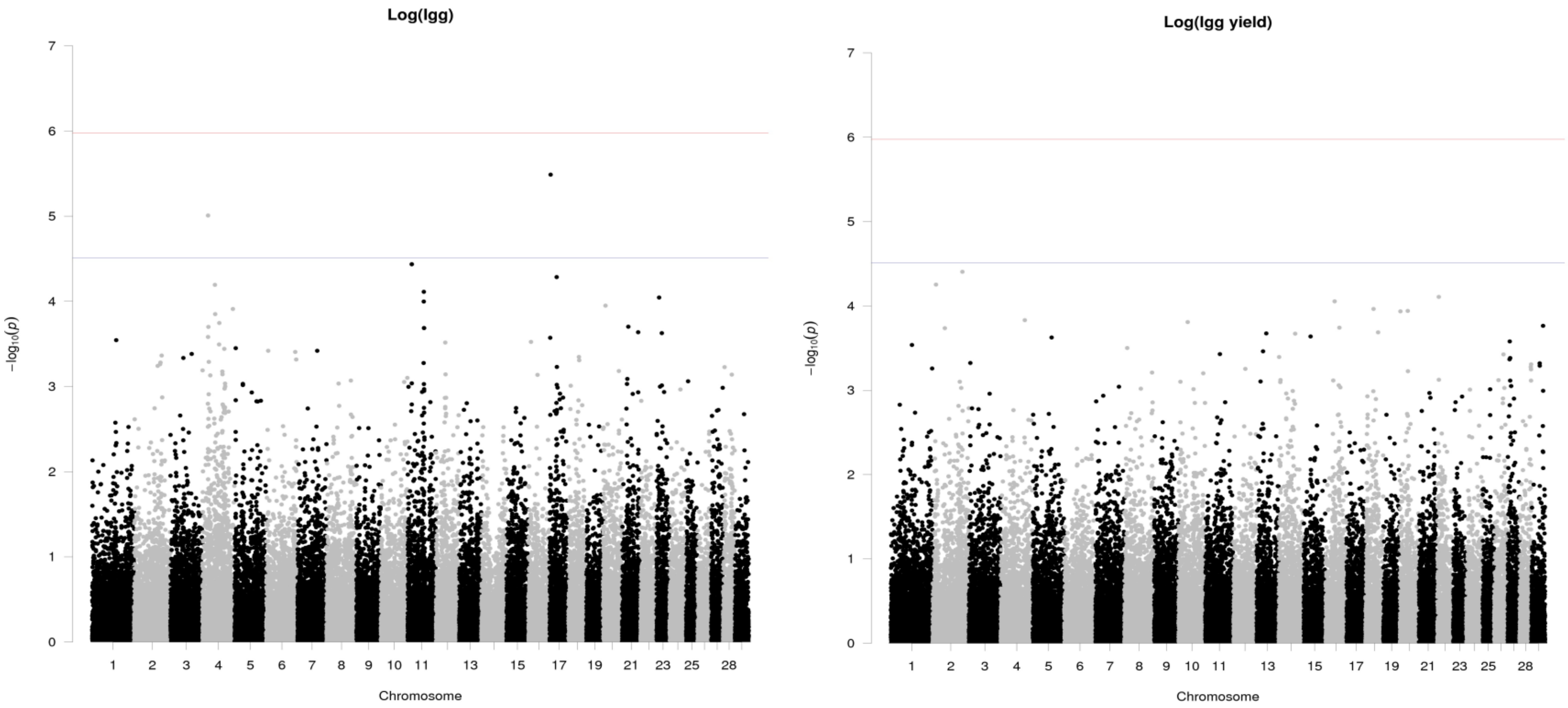


Figure 2: Manhattan plots of genome-wide association study for colostrum IgG in Alpine goats: (**A**) immunoglobulin G concentration (log(IgG)) and (**B**) immunoglobulin yield (log(IgG Yield)). -log10 P-values for each SNP are shown on the vertical axis. The red horizontal line represents 5% genome-wide threshold and the blue horizontal line represents 5% chromosome-wide threshold.



(A)

(B)

Figure 3: Manhattan plots of genome-wide association study for functional longevity in Chios dairy sheep. Red and blue lines indicate the genome-wide (Bonferroni correction) and suggestive (one false positive per genome scan) significance thresholds, respectively