SMARTER

SMAll RuminanTs breeding for Efficiency and Resilience

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New prototype and report for industry on GPS-generated phenotypes for behavioural adaptations to extensive grazing systems; artificial rearing adaptation phenotypes; lamb vigour scores linked to lamb survival; new foetal and neonatal survival phenotypes

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About the 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.

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1 Summary

The sustainability of small ruminant farming systems is largely dependent on behavioural adaptation of animals to rearing conditions. Therefore, the objectives of deliverable 2.4 were to (i) monitor key behavioural adaptation traits in extensive and intensive farming systems utilising new technologies and (ii) estimate phenotypic and genetic parameters for indicators of behavioural adaptation. Specifically, six studies were conducted to assess (i) GPS (global positioning system) generated phenotypes for behavioural adaptation to extensive grazing systems (AUTH), (ii) behavioural reactivity at weaning and maternal reactivity at lambing (INRAE), (iii) relationship between efficiency and behavioural adaptation (INRAE), (iv) foetal and neonatal survival phenotypes (IDELE & RDF), (v) lamb vigour and ewe mothering ability (TEAGASC & SRUC), and (vi) lamb temperament (INIA-UY).

A total of 300 Boutsko ewes from three semi-extensive farms in mountainous regions of Greece were monitored with GPS devices to study behavioural adaptation to extensive conditions. Daily grazing duration, distance, speed, altitude difference and elevation gain were recorded within the framework of SMARTER. Lamb sociability towards conspecifics, docility toward humans and maternal reactivity at lambing were studied using 1,133 lambs and 633 ewes of the Romane breed reared in one experimental farm of France under extensive conditions; records were collected partly within SMARTER. Moreover, a total of 80 Romane ewe lambs from two genetic lineages on feed efficiency (high vs low feed efficiency), which were reared under two different modes (artificial vs maternal rearing) and then exposed to challenging rangeland conditions were used to study the relationship between efficiency and behavioural adaptation; behaviour, health, welfare, growth, reproduction, and metabolism of lambs were studied (within SMARTER) until their first lambing. Foetal and neonatal survival phenotypes were studied using a total of 6,015 lambing data (new and background) from three meat sheep breeds (Blanche de Massif Central, Mouton Vendéen, and Rouge de l'Ouest) in France. Lamb vigour and ewe mothering ability were studied using background data from 22,414 and 18,125 meat sheep, respectively reared in flocks of Ireland. Finally, 4,317 and 1,702 Merino lambs were phenotyped and genotyped, respectively (within SMARTER) to study temperament.

Results from the above studies indicate that:

- Grazing behaviour of Boutsko sheep is characterised by high duration, distance, and speed. There is significant between-animal variation for most grazing behaviour traits to support management practices aiming to improve adaptation to extensive rearing conditions. Moreover, genomic selection practices could be implemented to improve grazing behaviour in the context of a multi-trait breeding programme.
- Sociability towards conspecifics, docility toward humans and maternal reactivity at lambing of Romane sheep are heritable traits. Early genetic selection for such behavioural traits could be used to improve adult behaviour in challenging environments. Moreover, results suggest that lambs from artificial rearing can adapt to extensive conditions, however care should be taken during the transition period.
- Lamb mortality in meat sheep breeds increases with litter size.
- Genetic selection for improved lamb vigour and ewe mothering ability in meat sheep is feasible and could help to improve overall lambing performance and animal welfare.
- Lamb temperament is a heritable multigenic trait that could be amenable to genomic selection.

2 Introduction

Small ruminant farming is characterised by great diversity in terms of production aims and farming systems (Arsenos et al., 2021). Semi-extensive farming systems have an important socio-economic and environmental role in marginal and mountainous regions. In such regions, sheep and goats especially, of indigenous breeds are reared providing employment opportunities and maintaining local heritage. On the other hand, in intensive farming systems, high producing breeds are reared with the goal of increasing farm profitability. However, the sustainability of both semi-extensive and intensive small ruminant farming systems is largely dependent on adaptation of animals to rearing conditions (Simm et al., 1996).

Semi-extensively reared small ruminants face many environmental challenges and welfare issues that are not easy to quantify. Nevertheless, the notion is that natural selection combined with subjective artificial selection have led to some animals being better adapted to extensive rearing conditions. In this regard, genetic variation may exist for key adaptation traits. Traits related to adaptation in such conditions include behavioural aspects such as grazing and maternal behaviour and physical attributes such as lamb vigour, foetal and neonatal survival (Simm et al., 1996; Dwyer et al., 2005; 2016). A better understanding of the phenotypic and genetic background of these traits could help towards the development of appropriate breeding programmes. However, recording of such traits is challenging. The use of new technologies such as global positioning systems (GPS) could help towards efficiently monitoring grazing and maternal behaviour and identifying new easy to measure indicators of resilience (Homburger et al., 2014; Feldt and Schlecht, 2016).

Likewise, in intensive farming systems, adaptation to management practices is considered essential for maximising productivity. One such practise is artificial rearing, which despite its benefits has not been greatly adopted by small ruminant farmers. One of the biggest issues is that success of artificial rearing largely depends on the ability of newborns to easily adapt to the artificial teat (Sevi et al., 2009). Management and genetic selection practices could be used to increase adaptation to artificial rearing if significant variation exists.

Taking into consideration the issues above, part of the H2020-SMARTER project was to study behavioural adaptation traits. Specifically, the objectives of this deliverable were to (i) monitor key behavioural adaptation traits in extensive and intensive farming systems utilising new technologies and (ii) estimate phenotypic and genetic parameters for indicators of behavioural adaptation.

In this regard, new and existing datasets from AUTH, INRAE, SRUC, TEAGASC, IDELE, RDF, and INIA-UY were used to evaluate behavioural adaptation of sheep under different rearing conditions. Specifically, in the present report the following studies are presented:

- GPS generated phenotypes for behavioural adaptations to extensive grazing systems – AUTH (new data)
- Behavioural reactivity (lambs) at weaning and maternal reactivity at lambing – INRAE (new and background data)
- Relationship between efficiency and behavioural adaptation – G*E interactions – INRAE (new data)
- Foetal and neonatal survival phenotypes – IDELE & RDF (new and background data)
- Lamb neonatal vigour and ewe mothering ability – TEAGASC & SRUC (background data)
- Candidate genes associated with lamb temperament – INIA-UY (new data)

3 GPS-generated phenotypes for behavioural adaptations to extensive grazing systems - AUTH

The objectives of this study were to (i) monitor the grazing behaviour of Boutsko sheep reared in semi-extensive conditions in mountainous regions using global positioning system (GPS) technology and (ii) estimate phenotypic and genetic parameters for key grazing behaviour traits.

3.1 Materials and methods

3.1.1 Animals, study area and design

A total of 300 dairy ewes, which were purebred representatives of the Boutsko breed (Figure 3.1) were randomly selected from three farms of Epirus in Greece (Farm A; n=200, Farm B; n=50, Farm C; n=50, Figure 3.2). Selected farms followed the transhumance farming system that is characterised by circular movement of animals between winter and summer rangelands. The study was performed from June to September 2021, corresponding to the period that the animals were semi-extensively reared in the mountains.

During the study period animals grazed natural pasturelands. Samples of grazing plants in the studied areas were collected for characterisation and chemical composition analysis. Moisture, energy, and protein content of samples were estimated using a DA 7250 NIR analyser (PerkinElmer, Waltham, Massachusetts, USA). Plant species and their chemical composition are presented in Table 3.1.

Figure 3.1. Boutsko dairy sheep breed used in the study.

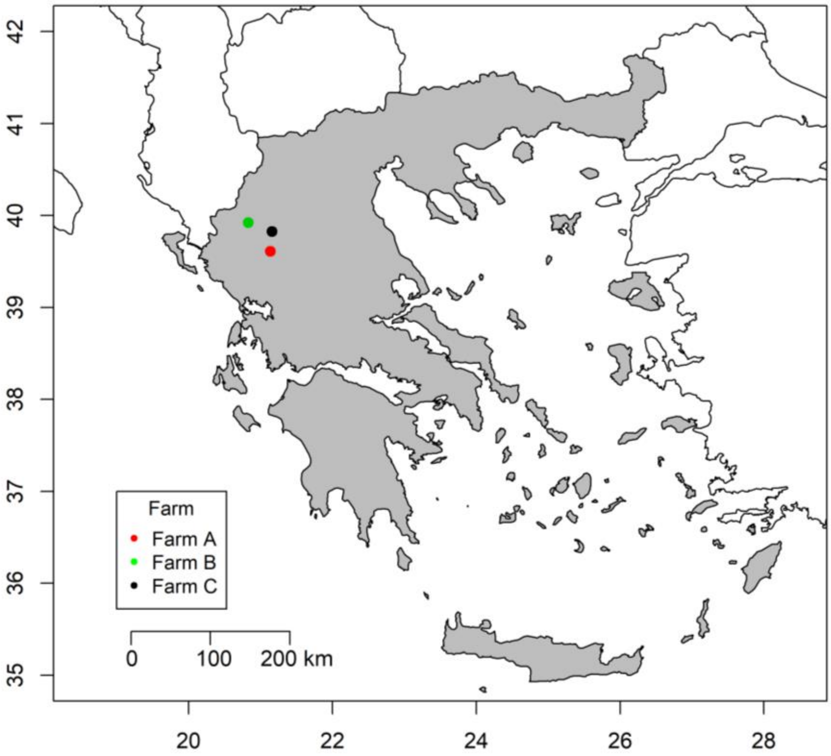


Figure 3.2. Map of Greece illustrating the regions in which the studied flocks were located.

Table 3.1. Plant species identified in the studied areas and their chemical composition.

Plant species	Moisture (%)	Energy (MJ/kg)	Protein (%)
Medicago lupulina	72.93	11.40	4.23
Bellardia viscosa	74.82	11.39	3.99
Acinos alpinus	67.06	10.53	4.09
Lotus corniculatus	74.68	11.90	3.54
Trifolium medium	73.26	11.37	3.40
Dorycnium herbaceum	71.68	11.13	2.76
Vicia angustofolia	70.24	12.64	5.55
Ononis spinosa	73.25	11.51	3.49
Pyrus spinosa Forssk.	55.58	12.85	7.12
Prunus domestica L. subsp. insititia	60.92	13.14	7.42
Lathyrus sp.	56.70	10.50	2.4
Festuca sp.	8.23	13.63	4.96

3.1.2 GPS monitoring of grazing behaviour

Rotational monitoring of animal grazing behaviour was performed using GPS devices (n=50, Tractive GPS, Tractive, Pasching, Austria), attached on designated collars (Figure 3.3). GPS devices weighted 28 grams and were accepted by the animals without any obvious irritation. Batteries with extended life were used that were able to provide sufficient energy for GPS tracking up to 10 days, depending on the available signal. GPS tracking of each animal was performed for 4-10 days at 2-60 minutes intervals; intervals were based on animal movement. In the case of 4 animals, GPS devices did not work and hence, no data were recorded.

GPS generated data of each animal for the total tracking period were exported in .gpx format using the location history function of MyTractive web app (n=296; https://my.tractive.com/#/). Then, the exported files were split by date using the software GPSBabel (version 1.8.0). For each animal, daily routes (Figure 3.4) and corresponding GPS data were visualized and extracted (n=1985) using Viking GPS data editor and analyser (version 2.0). Recorded grazing behaviour traits included daily grazing duration, distance, speed, minimum and maximum altitude, and total elevation gain. Based on minimum and maximum altitude, altitude difference was calculated. Moreover, energy expenditure for walking was calculated using the following formula of AFRC (Alderman and Cottrill, 1993):

$$EE = (0.0026 \times HD + 0.028 \times VD) \times BW$$

Where:

EE = energy expenditure for walking (MJ);

HD = horizontal distance (km, calculated as the difference between distance and elevation gain);

VD = vertical distance (km, corresponding to elevation gain);

BW = body weight (kg; 45 kg for the Boutsko breed);

Other useful metrics including number and average distance between tracking points, tracking duration and route followed by the animals were also extracted.

Figure 3.3. Boutsko sheep with GPS devices attached on designated collars.

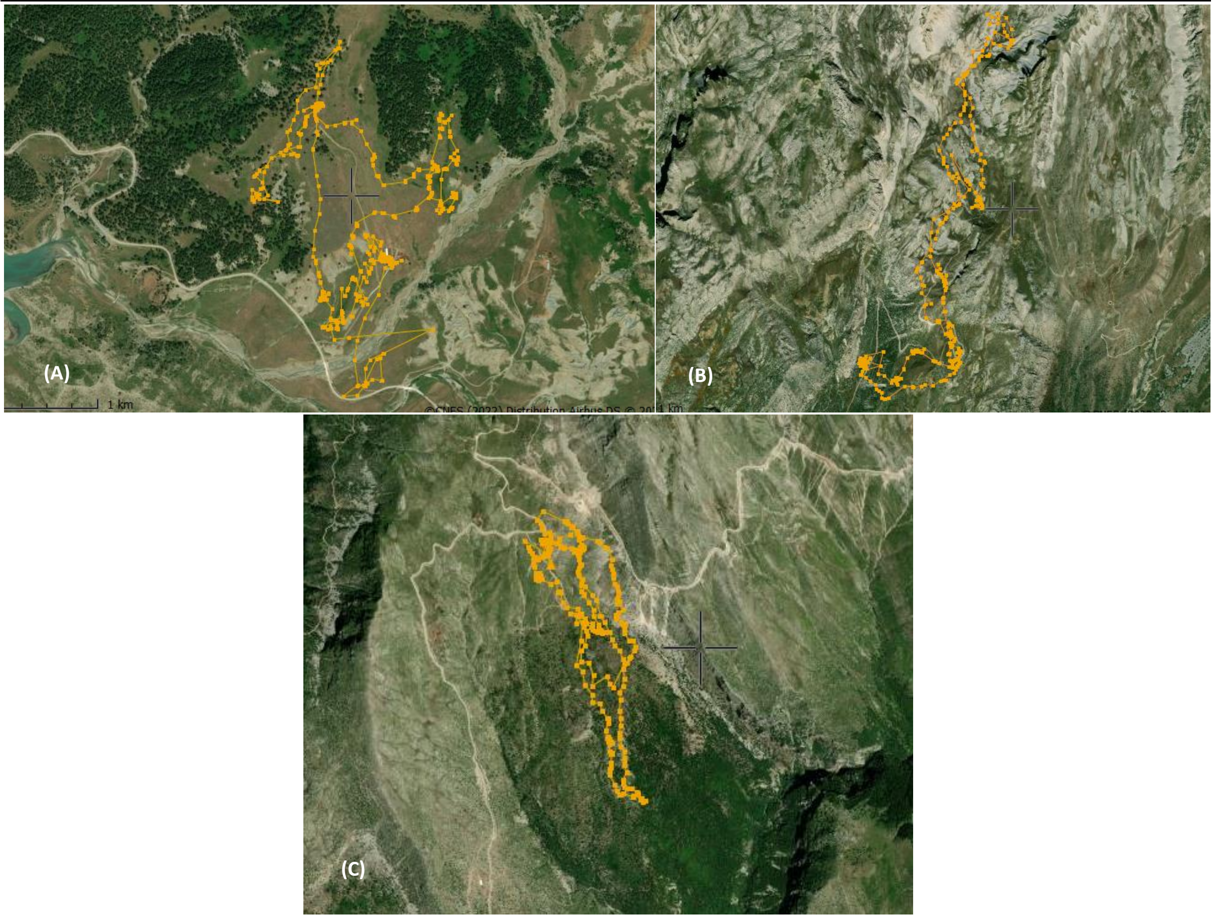


Figure 3.4. Indicative daily routes of animals in studied Farms A, B and C (visualized with Viking GPS data editor and analyser).

3.1.3 Collection of climatic data

For each of the tracking days, mean daily temperature and relative humidity were collected from the database of NASA Power Project. These traits were used to define mean daily temperature-humidity index (THI) according to the following formula described by Finocchiaro et al. (2005):

$$THI_m = T_m - [0.55 \times \left(1 - \frac{RH_m}{100}\right)] \times (T_m - 14.4)$$

where:

THI_m = mean daily temperature-humidity index;

T_m = mean daily temperature (^{o} C);

RH_m = mean daily relative humidity (%).

3.1.4 Blood sampling and genotyping

Blood samples of the studied animals (n=300) were collected from the jugular vein in EDTA vacutainers. Total genomic DNA was extracted according to the standard protocol of the QIAamp DNA Mini and

Blood Mini kit (QIAGEN, USA), following the manufacturer's recommendations with minor modifications. DNA samples were quantified using a Nanodrop spectrophotometer and stored at − 20 °C until use. Subsequently, DNA samples were oven-dried and 500 ng per sample were transferred to 96-well plates. All DNA samples were genotyped with the OvineSNP50 Genotyping BeadChip v2 that features 52,152 single nucleotide polymorphisms (SNPs) (Illumina, Inc., U.S.).

3.1.5 Data quality control

Quality control of GPS generated phenotypes was performed for extreme values and errors. Specifically, limits were set for minimum and maximum altitudes at 900 m and 2,150 m, respectively to reflect the real altitude of the studied mountainous regions. Tracking points beyond these limits were removed from the corresponding .gpx files and data were recalculated. Moreover, daily records for which GPS tracking of animals stopped before returning to their shed were excluded from the database. After quality control, a total of 1,923 GPS records for 296 animals remained.

Quality control of genotypes was performed by setting sample, marker call rate and minor allele frequency (MAF) thresholds at 0.95, 0.98 and 0.02 respectively, using PLINK v1.9 (Chang et al. 2015). In addition, all SNPs on the sex chromosomes were removed resulting in a final dataset of 292 individuals and 46,901 SNPs spread across the 26 ovine autosomes. SNP positions were assigned according to the Oar_v4.0 sheep genome assembly. A genomic relatedness matrix (GRM) was constructed and decomposed into eigenvalues and eigenvectors using GEMMA software (Zhou et al. 2012).

Based on all the above quality controls the dataset used for the estimation of genomic parameters included 288 animals with 1872 records.

3.1.6 Data analysis

Descriptive statistics of grazing behaviour phenotypes were performed using R statistical package "stats". Traits were logarithmically transformed to ensure normality of distribution. Mixed linear models were used to identify environmental factors with significant effects on the studied traits with R statistical package "lme4" (Bates et al., 2015). Specifically, the effects of farm, number of tracking points, distance between tracking points, tracking duration, time, GPS device, route, and THI were tested.

(Co)variance components of grazing behaviour phenotypes were estimated in a series of univariate and bivariate analysis using the ASReml software version 4.2 (Gilmour et al., 2021; Gilmour and Thompson, 2021). In the first instance, the following model was used:

$$Y_{imn} = \mu + F_i + b1 * TP + b2 * TD + b3 * TD + b4 * THI + b5 * T + A_m + e_{imn}$$

Where:

Y_{imn} = studied trait (n^{th} trait measurement on animal m);

μ = overall population mean;

F_i = fixed effect of farm (3 levels);

b1 = regression coefficient on number of GPS tracking points TP (number);

b2 = regression coefficient on GPS tracking duration TD (min);

b3 = regression coefficient on GPS distance between tracking points TD (m);

b4 = regression coefficient on temperature-humidity index THI;

b5 = regression coefficient on sampling time T;

A_m = random effect of animal m;

e_{imn} = random residual effect

Trait repeatability and correlations (animal and phenotypic) were calculated based on the corresponding variance and covariance values after convergence.

In a separate series of analyses, the random additive genetic effect (including the GRM) and random permanent environmental effect were fitted in the above model to disentangle variation between animals. Trait repeatability, heritability and between trait correlation estimates (genetic, permanent environmental and phenotypic) were calculated. Genomic breeding values (GEBVs) were also estimated and their reliability was calculated using the following formula proposed by Jamrozic et al. (2000):

$$Rel = 1 - \frac{PEV}{\sigma_A^2}$$

Where:

PEV = prediction error variance of GEBV;

σ^2_A = additive genetic variance of the trait.

Pairwise correlations between GEBVs for the studied traits were subsequently calculated. These correlations were adjusted for reliability according to the method of Calo (Calo et al., 1973) to derive an approximate estimate of the genetic correlation between traits as described below:

$$r_g = \frac{\sqrt{\sum_{i=1}^{n} \rho_{i,EBV} \times \sum_{i=1}^{n} \rho_{i,EBV}{}'}}{\sum_{i=1}^{n} \rho_{i,EBV} \times \rho_{i,EBV}{}'} \times r_{EBV,EBV}{}'$$

Where:

r_g = approximate genetic correlation between two traits;

$\rho_{i,EBV}$ = the reliability of the GEBV for one trait;

$\rho_{i,EBV'}$ = the reliability of the GEBV for the other trait;

$r_{EBV,EBV'}$ = Pearson correlation between the GEBVs for the two traits.

3.2 Results

3.2.1 Descriptive statistics

Descriptive statistics of the GPS generated grazing behaviour phenotypes and other recorded variables are presented in Tables 3.2 and 3.3, respectively. Mean daily grazing duration, distance, speed, altitude difference, elevation gain, and energy expenditure were 463.4 min, 10.7 km, 1.5 km/hour, 375.5 m, 2,740.7, and 1.3 MJ, respectively (Table 3.2). Daily recording of animal movement was performed with a mean tracking duration, number of tracking points and distance between them of 1222.6 min, 457.9, and 24.1 m, respectively (Table 3.3). Mean daily temperature, relative humidity and corresponding THI were 20.2 ° C, 55.5 %, and 18.6, respectively (Table 3.3).

Table 3.2. Descriptive statistics of GPS generated daily grazing behaviour phenotypes.

Phenotype	N	Mean	SD	Min	Max
Duration (min)	1,861	463.4	90.93	152	680
Distance (km)	1,903	10.7	2.01	4.51	20.55
Speed (km/hour)	1,841	1.5	0.42	0.47	4.11
Altitude difference (m)	1,903	375.5	174.84	119	1039
Elevation gain (m)	1,903	2740.7	968.95	625	6445
Energy expenditure (MJ)	1,903	1.3	0.23	0.53	2.40

Table 3.3. Descriptive statistics of other GPS generated metrics and climatic parameters.

Trait	N	Mean	SD	Min	Max
Tracking points (no)	1,903	457.9	107.46	185	968
Average distance between tracking points (m)	1,903	24.1	4.76	12.91	42.19
Tracking duration (min)	1,903	1222.6	202.11	623	1439
Lowest altitude (m)	1,903	1236.7	74.18	901	1426
Highest altitude (m)	1,903	1612.2	142.1	1424	2150
Mean temperature (° C)	1,923	20.2	4.21	13.75	27.48
Relative humidity (%)	1,923	55.5	10.58	37.69	76.56
THI	1,923	18.6	2.83	13.88	23.06

3.2.2 Significant effects on grazing behaviour phenotypes

Significant effects of farm, tracking points, tracking duration, and distance between tracking points on grazing behaviour phenotypes are presented in Figures S1, S2, S3, and S4, respectively (Appendix 1). Significant effects of THI on the studied phenotypes are presented in Table 3.4. One unit increase of THI was significantly ($P<0.001$) associated with a decrease of speed (0.8±0.20%) and altitude difference (2.0±0.40%), and an increase of duration (0.9±0.20%) and elevation gain (2.0±0.40%).

Table 3.4. Significant effects of temperature-humidity index (THI) on daily grazing behaviour phenotypes.

Phenotype	β-coefficient	SE	P-value
Duration (min, ln)	0.009	0.002	<0.001
Speed (km/hour, ln)	-0.008	0.002	<0.001
Altitude difference (m, ln)	-0.020	0.004	<0.001
Elevation gain (m, ln)	0.020	0.004	<0.001

Energy expenditure (MJ, ln)	0.011	0.003	<0.001

3.2.3 Phenotypic and genetic parameters

Estimates of repeatability and heritability for GPS grazing behaviour phenotypes are presented in Table 3.5. Statistically significant (P<0.05), high repeatability and heritability estimates were reported for daily grazing duration and speed. Significant low repeatability estimates were reported for elevation gain and energy expenditure; the respective heritability estimates were not significantly (P>0.05) different from zero.

Estimates of animal and phenotypic correlations between the studied traits are presented in Table 3.6. Significant (P<0.05) negative animal correlations were found between grazing duration and speed. Positive animal correlations were reported between elevation gain and energy expenditure and between duration and distance. Regarding phenotypic correlations, associations of grazing duration with altitude difference, distance with speed and speed with elevation gain and energy expenditure were not significantly different from zero (P>0.05). Significant (P<0.05) high negative correlations were reported between duration and speed. The rest of phenotypic correlations between traits were all significantly positive (P<0.05).

Estimates of genetic correlations and respective phenotypic correlations between the studied traits are presented in Table 3.7. Genetic correlations between traits were either non-significant (P>0.05) or not estimable. However, the further approximate estimation of genetic correlations based on GEBVs showed significant (P<0.05) negative associations between duration and speed (-0.99) and positive ones between duration and distance (0.28). Phenotypic correlations were similar to those reported with the non-genetic model. Finally, permanent environmental correlations were in all cases not estimable.

Table 3.5. Repeatability (r) and heritability (h^2) with respective standard errors in parenthesis of studied daily grazing behaviour phenotypes.

Trait	r_non-genetic model	r_genetic model	h^2
Duration (min, ln)	0.70 (0.02)*	0.85 (0.03)*	0.75 (0.12)*
Distance (km, ln)	0.02 (0.02)	0.05 (0.04)	0.05 (0.06)
Speed (km/hour, ln)	0.70 (0.02)*	0.86 (0.03)*	0.78 (0.11)*
Altitude difference (m, ln)	0.01 (0.01)	0.01 (0.01)	0.00 (0.00)
Elevation gain (m, ln)	0.14 (0.02)*	0.14 (0.06)*	0.00 (0.10)
Energy expenditure (MJ, ln)	0.14 (0.02)*	0.14 (0.02)*	0.00 (0.00)

*Indicates statistically significant estimates (P<0.05).

Table 3.6. Animal (above diagonal) and phenotypic (below diagonal) correlations (and standard errors in parentheses) between daily grazing behaviour phenotypes.

Trait	Duration (min, ln)	Distance (km, ln)	Speed (km/h, ln)	Altitude difference (m, ln)	Elevation gain (m, ln)	Energy expenditure (MJ, ln)
Duration (min, ln)		0.38 (0.17)*	-0.99 (0.00)*	-0.09 (0.31)	0.03 (0.09)	0.03 (0.09)
Distance (km, ln)	0.24 (0.02)*		-0.34 (0.18)	0.68 (0.85)	0.24 (0.22)	0.29 (0.21)
Speed (km/h, ln)	-0.96 (0.00)*	0.04 (0.03)		0.18 (0.33)	0.01 (0.09)	-0.00 (0.09)
Altitude difference (m, ln)	0.03 (0.02)	0.17 (0.02)*	0.01 (0.02)		NE	NE
Elevation gain (m, ln)	0.10 (0.03)*	0.30 (0.02)*	-0.01 (0.03)	0.40 (0.02)*		0.99 (0.00)*
Energy expenditure (MJ, ln)	0.11 (0.03)*	0.39 (0.02)*	-0.00 (0.03)	0.42 (0.02)*	0.99 (0.00)*	

*Indicates statistically significant estimates (P<0.05).

Table 3.7. Genetic (above diagonal) and phenotypic (below diagonal) correlations (and standard errors in parentheses) between daily grazing behaviour phenotypes.

Trait	**Duration (min, ln)**	**Distance (km, ln)**	**Speed (km/h, ln)**	**Altitude difference (m, ln)**	**Elevation gain (m, ln)**	**Energy expenditure (MJ, ln)**
Duration (min, ln)		0.70 (0.40)	NE	0.00 (0.00)	NE	0.70 (0.40)
Distance (km, ln)	0.26 (0.06)*		-0.67 (0.44)	NE	NE	NE
Speed (km/h, ln)	NE	-0.06 (0.07)		NE	NE	NE
Altitude difference (m, ln)	0.05 (0.06)	0.15 (0.03)*	0.01 (0.06)		NE	NE
Elevation gain (m, ln)	0.06 (0.09)	0.15 (0.03)*	NE	0.41 (0.02)*		NE
Energy expenditure (MJ, ln)	0.26 (0.06)*	0.54 (0.02)*	NE	NE	NE	

*Indicates statistically significant estimates (P<0.05).

3.3 Conclusions

In the present study, GPS devices were used to monitor the grazing behaviour of Boutsko sheep reared semi-extensively in mountainous regions of Greece. Results indicate that their grazing behaviour is characterised by high duration, distance, and elevation. THI was found to significantly affect all grazing behaviour traits apart from distance; farm and GPS tracking metrics were also identified as factors with significant effects on the studied traits. Moreover, results suggest that there is significant between-animal variation for grazing duration, speed, elevation gain and energy expenditure to support management practices aiming to improve animal adaptation to extensive rearing conditions. More importantly, grazing duration and speed were found to be highly heritable, which could further allow for genomic selection practices. Correlations between traits indicate that management and selection practices for higher grazing duration are expected to decrease speed and favourably increase distance. Limitations to disentangle additive genetic from permanent environmental variation for some of the studied traits could be related to the sample size. Therefore, future research should further investigate these traits using a higher sample size.

4 Behavioural reactivity (lambs) at weaning and maternal reactivity at lambing – INRAE

The objectives of this study were i) to estimate genetic parameters for behavioural traits facilitating adaptation to extensive conditions, ii) to investigate the efficiency of an early divergent genetic selection for social reactivity towards conspecifics or docility towards humans. Our hypothesis was that higher sociability, higher docility toward humans, higher maternal reactivity may improve adaptation to harsh environments.

4.1 Materials and methods

4.1.1 Animals and management

The experimental animals were Romane meat sheep, reared at the INRAE experimental farm of La Fage (Saint-Jean Saint-Paul, Causse du Larzac, France) exclusively outdoors under harsh environmental conditions. The flock comprised about 250 reproductive females reared on 280 ha of rangelands. The farming system and management characteristics have been described previously by Gonzalez et al.

(Gonzalez-Garcia et al., 2014). Ewes lambed for the first time at 2 years of age and all the lambs were born outdoor in the spring.

4.1.2 Behavioral tests and responses

Behavioural reactivity at weaning:

During each year of the SMARTER project, 250 to 300 female and male lambs were individually exposed just after weaning to two behavioural tests. A total of 1133 lambs were phenotyped. The arena test (AT) consisted of two successive phases evaluating 1) reactivity to social isolation (AT1), 2) the motivation of the lamb towards conspecifics in presence of a motionless human (AT2). The arena test pen consisted in an unfamiliar enclosure virtually divided into 7 zones as described in detail by Ligout *et al*. (Ligout et al., 2011). The corridor test (CT) consisted of two successive phases evaluating 1) reactivity to social isolation (CT1) and 2) reactivity to an approaching human (CT2). The test pen consisted in a closed, wide rectangular circuit and has been described in detail by Boissy *et al*. (Boissy et al., 2005). Several behaviours were measured: vocalizations (i.e frequency of high-pitched bleats), locomotion (i.e. number of virtual zones crossed), vigilance posture (i.e. time spent by the animal motionless), the proximity score (i.e. weighting of time spent in virtual zones, a high score indicated a high duration spent close to conspecifics and a human). The mean flight distance (DIST) separating the human and the lamb and the time during which the human saw the lamb (SEEN) were measured in CT2.

Maternal reactivity at lambing:

A total of 631 ewes were phenotyped for maternal reactivity at lambing. Maternal reactivity was assessed at the lambing site approximately 2h after lambing and 24h post lambing in an arena test. Measurement of maternal reactivity at the lambing site (LS) consisted of two successive phases: (1) when the shepherd approached the lambs; and (2) the capture and displacement of the lambs by the shepherd. The procedure was detailed in Hazard et al. (2021). A scoring system was developed for each of the two phases to evaluate maternal reactivity. In LS1, a maternal behavior score (LS1-MBS) was recorded on a 5-point scale as follows: 1 - ewe flees and doesn't return to the lambs within 60 s; 2 - ewe retreats (i.e., at least 2-3 m) but comes back to her lambs within 60 s; 3 - ewe retreats with at least one lamb and comes back; 4 - ewe retreats and returns repeatedly; 5 - ewe stays close to the lambing spot. In LS2, a second maternal behavior score (LS2-MBS) was recorded on a 4-point scale as follows: 1 - ewe flees; 2 - ewe stays close to the lambing spot, 3 - ewe follows but from a distance (i.e., 1 to 2 m), 4 - ewe follows, staying close to the shepherd (i.e., less than 1 m). Measurement of maternal reactivity in the arena test followed a similar procedure that the one applied at weaning. The test consisted of two successive phases evaluating the ewe's reactivity to social separation from her litter, and reactivity to a conflict between social attraction to her litter and avoidance of a motionless human. Vocalisations (ATL1/2-HBLEAT) and locomotor activity (ATL1/2-LOCOM) were assessed as the procedure applied at weaning.

4.1.3 Statistical handling

Behavioural reactivity at weaning for genetic divergent selection:

Two synthetic variables were constructed using the first component of each principal component analysis (PCA) (PRINCOMP procedure in the SAS® software). A PCA was performed on vocalizations across the different phases of behavioural tests (AT1, AT2 and CT1) and the resulting synthetic variable (HBLEAT) was used as indicator of the reactivity to social isolation. A PCA was performed on DIST and SEEN variables and the resulting synthetic variable (HUMAPPRO) was used as indicator of the reactivity to an approaching human. For divergent selection, individual estimated breeding values (EBVs) were calculated for HBLEAT and HUMAPPRO with univariate analyses under an animal mixed model using the REML methodology implemented in the ASREML software (Gilmour et al., 2009). In order to constitute the divergent lines, extreme animals were chosen as sires (in average 10 sires per generation and line) or dams (in average 70 dams per generation and line) at each generation according to their high or low EBV for HBLEAT or HUMAPPRO while intermediary for HUMAPPRO or HBLEAT, respectively. Selection intensities were in average 10 and 45% for sires and dams, respectively. Two generations of selected animals were produced using background and foreground data (in average 270 female and male lambs per generation and line). Linear models (proc GLM, SAS Institute Inc.) were applied to test whether the divergent selections on behavioural traits had a significant effect on the recorded traits considering the adequate fixed effects in the analyses. The linear model used was $y_{ijklm} = \mu + line_i + sex_j + lsr_k + age_l + year_m + e_{ijklm}$; where y_{ijklm} was the behavioural observations of lambs; μ was the overall mean of the population; line, sex, lsr, age and year were the fixed effects of the behavioural line *i*, the sex *j* of the lambs, the litter size born and reared *k*, age *l* of the dam, and year *m* of the experiment, respectively.

Genetic analysis of maternal reactivity

The (co)variance components for categorical behaviors were estimated by MCMC and Gibbs sampling methods using a threshold model in TM software (Legarra, Varona, & Lopez de Maturana, 2008). Random effects included a direct additive genetic effect of the animal (i.e., ewe) and a permanent environmental effect of the animal. The (co)variance components for quantitative behavioral traits were estimated by restricted maximum likelihood methodology applied to an animal model using ASREML 3.0 software (Gilmour et al., 2009). The following animal mixed model was fitted: $y = Xb + Za + Wc + e$ [I] where y is the vector of observations corresponding to the trait(s) in the analysis; b is the vector of appropriate fixed effects (age at first lambing, parity of the ewe, litter size and year of measurement); a is the vector of random genetic effects and c is the vector of permanent environmental effects; e is the vector of residual effects; X, Z and W are incidence matrices linking fixed effects, random animal genetic effects and random permanent environmental effects to the trait, respectively; a, c and e were assumed to be normally distributed with means equal to zero and (co)variances $A\sigma^2_a$, $I_c\sigma^2_c$, $I_e\sigma^2_e$ for a, c and e, respectively; A is the additive relationship matrix based on the pedigree; I are identity matrices of appropriate size, where σ^2_a is the additive genetic variance, σ^2_c is the variance due to the permanent environmental effect, and σ^2_e is the residual variance.

4.2 Results

For gregarious species such as sheep, vocalization and locomotion behaviours in response to social separation are interpreted as an active way to re-establish social links (Boissy et al., 2005; Beausoleil et al., 2012). Estimation of genetic parameters confirmed that sociability towards conspecifics in lambs was highly heritable (h^2=0.5) and docility towards an approaching human was moderately heritable (h^2=0.2, Table 4.1). Maternal reactivity of ewes was moderately to highly heritable (h^2=0.10 to 0.45) depending on the behavioural traits considered. Sociability at weaning was highly genetically correlated with maternal reactivity at lambing considering vocalisation behaviours.

Table 4.1 Genetic parameters for behavioural reactivity traits at weaning and maternal reactivity at lambing

Animals	Traits	Heritability	Genetic correlations	Divergence between lines
Lambs (at weaning)	Sociability	HBLEAT: 0.5 (±0.06)	0.3 (±0.11) with Docility	S-/S+: 1.8 σ_g
	Docility	HUMAPPRO: 0.2 (±0.04)		H-/H+ : 1.0 σ_g
Ewes (at lambing)	Maternal reactivity	LS1/2-MBS : 0.20 (±0.06) ATL1/2-HBLEAT: 0.45 (±0.05) ATL1/2-LOCOM: 0.10 (±0.03)	0.7 to 0.8 (±0.11) with Sociability considering HBLEAT	

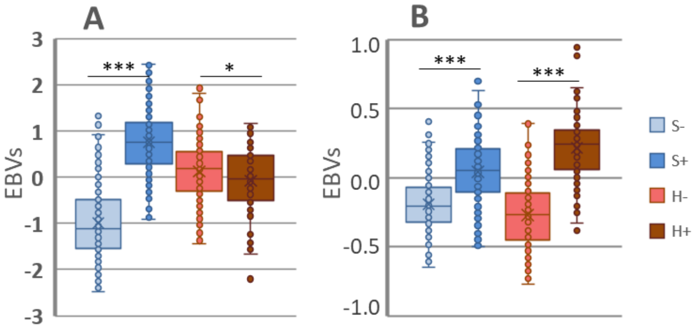
σ_g, genetic standard deviation

The divergence between low (S-) and high (S+) lines selected on sociability towards conspecifics reached 1.8 genetic standard deviation after two generations of selection. The divergence between low (H-) and high (H+) lines selected on docility toward humans reached 1.0 genetic standard deviation after two generations of selection. Responses to selection obtained were consistent with those expected considering the heritabilities previously estimated for both traits and the intensity of selection applied.

As expected, the divergent lines for docility toward humans were intermediary for social attractiveness compared to S- and S+ lines even if a slight difference was observed between H+ and H- lines for the social criterion (Figure 4.1). A high difference was observed between S- and S+ lines for docility toward humans while no difference was expected between these lines for this criterion. Such difference could be linked to the low positive genetic correlation previously reported between criteria for sociability toward conspecifics and tolerance toward humans (r_g=0.3 ± 0.11). This difference may also be due to the selection criterion that involved measurement of reactivity to social separation in presence of a human (i.e. vocalizations in AT2).

Figure 4.1 Estimated breeding values for sociability toward conspecifics (A) and docility toward humans (B) in the four divergent lines after two generations of selection. S- and S+ lines, low and high sociability toward conspecifics, respectively; H- and H+ lines, low and high docility toward humans, respectively. Pairwise comparison between Least Squares Means for line effect: *, and *** for P-value < 0.05 and 0.001, respectively.

Effects of genetic divergent selections on the different behaviours expressed in response to social separation with or without human presence in arena and corridor tests were also investigated (Table 4.2). Selection for sociability toward conspecifics resulted in a two-fold higher expression of vocalizations in response to social isolation without or with human presence. This selection for social attractiveness also resulted in a higher locomotor activity in response to visual isolation from



conspecifics (i.e. AT1 phase) as well as a higher proximity score with conspecifics in presence of a human (i.e. AT2 phase). Thus, selection for social attractiveness based on vocalizations not only affected vocalization behaviour but also locomotor activity in a context specific way. As expected, the flight distance of lambs from an approaching human decreased with the selection for docility toward humans (Table 4.2). This selection also resulted in a slight decrease in vocalization and locomotion behaviours in response to social isolation as well as an increase in the proximity score toward conspecifics in presence of a human. We hypothesized that genetic selection for lambs more tolerant toward humans also reduced the overall reactivity to social separation and exposure to humans, may be by reducing overall stress responses.

Table 4.2 Behaviours at weaning in the four divergent lines after two generations of selection.

Behaviours	Test	S-[1]	S+	Line[2]	H-	H+	Line
Vocalizations	AT1	8.0	16.2	***	13.6	11.9	***
Vocalisations	AT2	3.0	7.6	***	5.7	5.0	NS
Locomotion	AT1	23.3	27.2	***	26.5	24.0	*
Locomotion	AT2	6.6	7.2	NS	7.2	4.9	**
Vigilance	AT1	18.6	18.2	NS	17.7	19.3	NS
Proximity	AT2	23.9	27.9	*	25.0	29.1	*
Flight distance	CT2	5.5	5.3	*	5.6	5.1	***

[1] S- and S+ lines, low and high reactivity to social separation, respectively; H- and H+ lines, low and high tolerance toward a human, respectively. AT1, arena test 1 (visual social isolation); AT2, arena test 2 (social isolation in presence of a human); CT2, corridor test 2 (reactivity to an approaching human); Vocalizations: frequency; Locomotion: number; Vigilance: duration in sec; Proximity: duration in sec; Flight distance: meter.

[2] Line effect: *, ** and *** for P-value < 0.05, 0.01 and 0.001, respectively

4.3 Conclusions

Including behavioural traits in genetic selection could be an advantageous strategy for improving adaptation and welfare of farm animals to extensive farming systems in an agroecological perspective. Using standardized behavioural tests, we phenotyped a large number of sheep for behavioural reactivity at weaning and maternal reactivity at lambing. Using these phenotypes and pedigree information, we estimated genetic parameters for several behavioural traits. Thus, in the present study we demonstrated that sociability towards conspecifics, docility toward humans and maternal reactivity at lambing are heritable traits in sheep. By developing genetic divergent lines for behaviours, we also demonstrated in sheep the efficiency of an early selection for sociability toward conspecifics or docility toward humans. These new results offer a new opportunity to improve resiliency in sheep by including behavioural traits in breeding programs. Further studies will be developed to investigate impacts of such selections for behaviours on different traits including production.

5 Relationship between efficiency and behavioural adaptation – G*E interactions – INRAE

To promote/improve robustness and/or efficiency, one approach is genetic selection. Another approach is the environment that the individual experiences, especially during the early stages of its life (Langenhof and Komdeur, 2018). The present project combines these two approaches in the context of sheep farming in disadvantaged rangeland areas. These disadvantaged areas are characterised by significant spatiotemporal variations in feed resources and climatic conditions, associated with large open spaces that present social constraints, diminished human relations and challenges related to space utilization. The aim of the present study is to explore and understand the mechanisms underlying the development of adaptive capacities, particularly behavioural ones, in animals living in these environments. These adaptive capacities help to enhance the well-being of animals while grazing in these areas.

The originality of the study lies in: 1- an analysis of adaptation on multiple dimensions combining behaviour (activity on rangeland, behavioural reactivity and robustness, response to humans, maternal behaviour), performances, metabolism and health; 2- the interaction between genetics on feed efficiency and early experience using existing and contrasted rearing modes; and 3- a long-term follow-up of these animals from birth until the weaning of their first lambs.

The current report presents the data analysed to date, concerning the period from birth to the first lambing of the animals.

5.1 Materials and methods

5.1.1 Animals, study area and design

The study involved 80 ewe lambs from Romane breed and four treatments. We considered two genetic lineages that derived from a divergent selection on feed efficiency, based on residual feed intake, and applied on their fathers (Tortereau et al., 2020). We crossed these two lineages (high feed efficiency: **HFE**; low feed efficiency: **LFE**) with two early experiences based on contrasted rearing modes from birth to three months of age. The first mode was an indoor artificial rearing (**AR**) that involved an indoor environment, no mother or adult models, artificial milk feeding, then low diversified feed resources, and high human proximity. The second mode was an outdoor maternal rearing (**MR**) that involved a rangeland environment, the presence of the mother and other adults, maternal milk feeding, then high diversified feed resources, and reduced human contact. At three months of age, all ewe lambs were grouped into sets of 20 based on their rearing experience, with two groups of AR and two groups of AM lambs, for replication. Each group was composed of a nearly equal number of lambs from both genetic lineages. From this date (D100, Figure 5.1), all lambs lived under the same full-time outdoor rangeland living conditions.

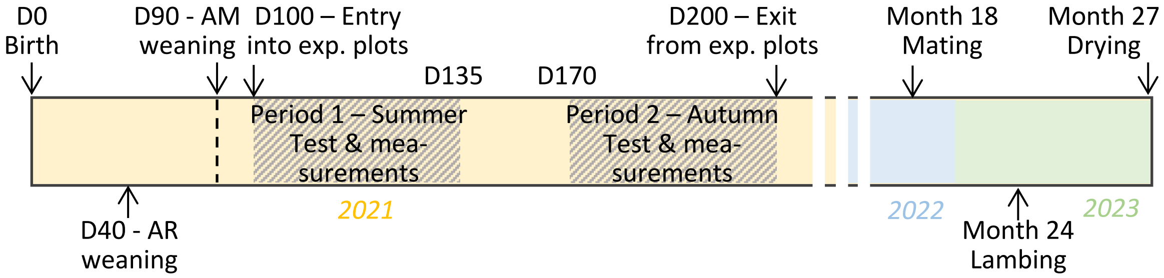


Figure 5.1. Time schedule of the study with key dates

The study took place at the INRAE experimental farm of La Fage, located in the south of the French Massif Central, at an altitude of about 800 m. According to Köppen's classification, the climate of the site is Mediterranean with a temperate summer. The soil is very shallow and the vegetation consists of highly diverse herbaceous species (70 species recorded) supplemented with boxwood shrubs and some junipers (Figure 5.2). The site was divided into four plots of approximately 1.1 hectares each (Figure 5.3), and each of the four groups was introduced into one of the plots on D100. They stayed there for 2 periods between D100 and D200 (Period 1 “Summer” and Period 2 “Autumn, Figure 5.1), with a temporary exit between the two to preserve the vegetation.

Following D200, the 80 ewe lambs were gathered into a single group and still reared on the rangeland, just like the main ewe flock of the farm.

Figure 5.2. Experimental Romane ewe lambs in the rangeland

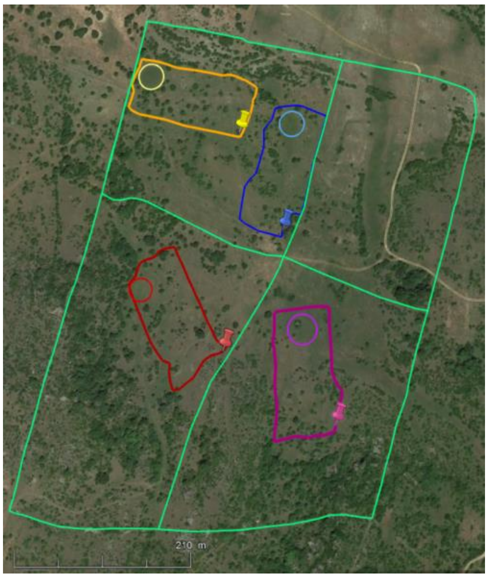


Figure 5.3. Satellite view of the four experimental plots (Google earth)

Period 1 and Period 2 were privileged moments of testing, observation, and measurements for investigating the adaptation of ewe lambs to the rangeland conditions, according to their genetic background and early experience. These tests and measurements are detailed below.

5.1.2 Tests, behavioural observations and measurements

5.1.2.1 Behavioural tests

- Risk-taking test

The risk-taking test was developed to evaluate the animal's ability to take a risk when confronted with an unknown obstacle to achieve a motivational drive. It was conducted twice - once during Period 1 (approximately 3 weeks after the animals entered the plots) and again during Period 2 (about two months later). Following a group passage through the whole device, each individual is led alone into an arena through a side corridor. The animal is left in this arena for one minute (phase 1: isolation) then a sliding door is opened on a central corridor leading to a large waiting area with other sheep and access to food and water. The isolated individual is given a maximum of three minutes to join his conspecifics and the latency to do this is recorded (phase 2: motivation). Next, the animal is brought back into the arena through the lateral corridor and given 10 seconds to acclimatize. Then, the sliding door on the central corridor is opened, but this time, a 1.2 m-long obstacle in the form of a yellow and black crosshatched board, along with transparent suspended plastic bands, is added. Once again, the individual is given 3 minutes to join its conspecifics (phase 3: risk-taking) and the latency to pass through the obstacle is recorded. Throughout phases 1 and 3, all fear and exploration behaviours are noted, along with the occupied zones (Figure 5.4A).

- Human preference test

The human preference test evaluates the lambs' response to humans and their possible preference for a particular human which had been associated with a positive reward, i.e. a food distribution in the past. Firstly, a learning phase was carried out in the rangeland, during the first 10 days after entry onto the plots (D100 to D110). Two disguised persons (Figure 5.4B) fed the lambs with concentrate, one different for each group of AR or AM (after that all the animals were fed by a green full-body suit). The human preference test was carried out three weeks after this learning period (Period 1) and repeated two months after (Period 2). The dispositive consists of an arena virtually divided into six areas, where the two disguised humans are positioned on each side of the back of the arena. Their respective positions, on each side of the device, are balanced within each treatment. Each animal is individually led to the entrance of the arena. When the door is closed, the animal is observed for 3 minutes and the time spent in each area and in contact with each person is recorded.

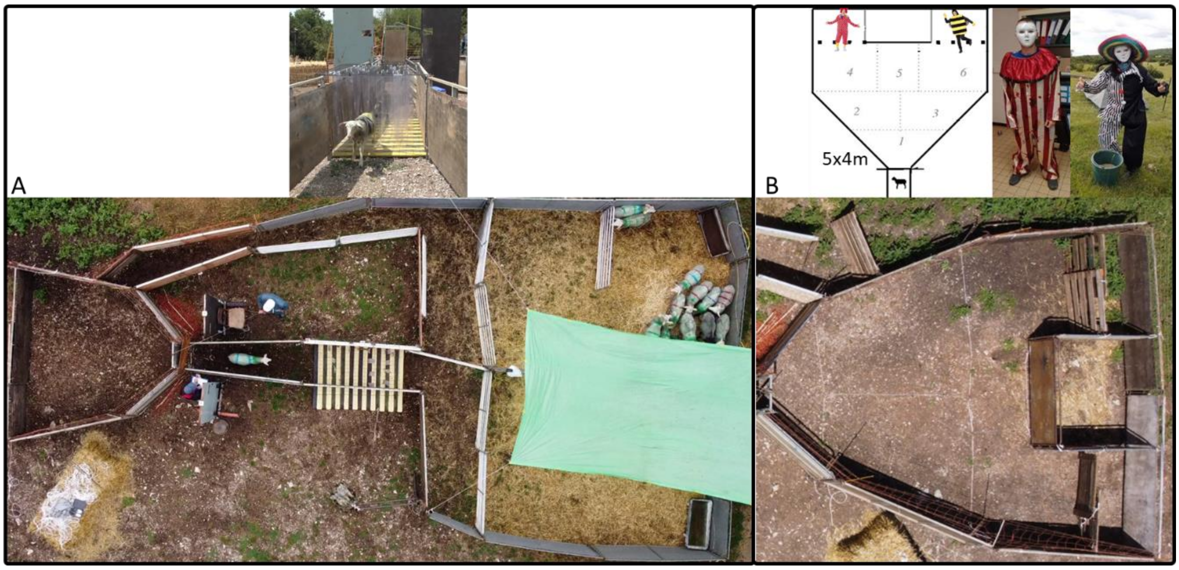


Figure 5.4. Views of the device for the Risk-taking test (A) and Human preference test (B).

- Collective reactivity test

This test is a first attempt to investigate the collective reactivity to a sudden and novel moving stimulus while feeding. A tent was installed before the lambs entered the plots, in front of the feeder in the pasture, for familiarity. The test was conducted four times, at 1, 4, 11 and 14 weeks after the first entry onto the plots, so twice in Period 1 and twice in Period 2.

After a stockperson feeds the animals in the feeder with concentrate then went away, a sudden stimulus appears. In our test, it took the form of a person disguised as a ghost (Figure 5.5) emerging from the tent and making two full rotations around the feeder at a consistent regular pace (for

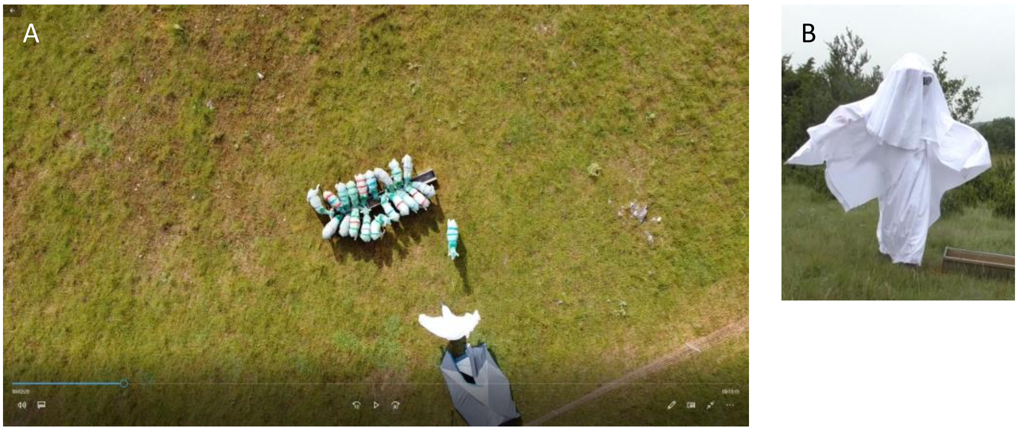


Figure 5.5. Overhead view of the conditions for the collective reactivity test, showing the tent (grey), the feeder and the stimulus emerging from the tent (A); View of the stimulus (B).

approximately 20 seconds) before returning to hide in the tent. Both a drone at an altitude of 50m and a camera in front of the tent recorded the events for 20 and 60 minutes, respectively. The presence of the animals within concentric zones surrounding the feeder, each spaced by one feeder length, was recorded every 3 seconds in order to calculate a flight index (Table 5.1). Additionally, the flight latency of each individual was also recorded.

Table 5.1. Boundaries for the calculation of the flight index

Observed distance between lamb and feeder	< 1 feeder length	>1 and <2 feeders	>2 and <3 feeders	>3 and <4 feeders	>4 feeders
Flight Index	0	0.25	0.5	0.75	1

5.1.2.2 Behavioural observations

When animals are exposed to a new and complex outdoor environment, their adaptation relies on their ability to explore the surroundings and locate sources of food and shelter. The following observations were conducted to assess some of these abilities.

- Behavioural activities at the entry onto the experimental plots

At the start of Periods 1 and 2, upon the animal's entry onto the experimental plots, their activity was recorded over three 2.5-hour-long time slots: on the first morning, first afternoon, and the following morning. Twelve lambs out of 20 per group were observed by a total of eight observers (two per plot), with each observer observing 6 individuals. We utilised the scan sampling method to record the posture (standing; lying), activity (exploring; feeding; walking; resting; ruminating) and position relative to shelter (at least the head under shelter; out of shelter) of the animals every 3 minutes.

- Shelter use

During Period 1, approximately one month after entering the plots, the same 12 individuals per group were observed for a more detailed recording of their shelter use during two sunny and hot days. We used the scan sampling method to record over four 2-hour-long time-slots (09:00 to 11:00 then 16:00 to 18:00 each day), every 5 minutes, the main activity (grazing; resting + ruminating; other), the position of the head and body of the lambs relative to shelter (under boxwood or other low-lying shrubs; near boxwood, shrubs or water tank; in open area) and shade (under shade; in the sun).

5.1.2.3 Animal performances, welfare and health

- Welfare and health measurements

Around 24 hours after birth, the rectal temperature and vigour of lambs were evaluated using a 4-point scale ranging from lying very weak to lively and strong with a filled stomach. Only lambs with a score of 4 were selected for the study. Throughout the study, the health status of the lambs was regularly monitored (7 dates[1]). All lambs were monitored for the presence of nasal and ocular discharge, lameness and perianal dirtiness. Additionally, faecal samples were collected in 8 animals per group, to determine individual parasitic load (gastrointestinal nematodes) using the McMaster technique.

- Growth and reproductive performances

The lambs were weighed regularly at appropriate intervals from birth until the end of the experiment (12 dates[2]). Average daily gains (ADG) were calculated for relevant time periods. Their body condition score was evaluated at 285 days of age, during their first winter at pasture, and again at one year of age, as well as at the beginning of the reproduction period at 555 days of age. The individual reproductive performances, including the prolificacy (number of lambs born per pregnant ewe) and the maternal behaviour were also documented. A total of 32 ewes were evaluated for their maternal behaviour, 24 hours after lambing. To assess their selectivity towards lambs, each ewe was tested individually in a small enclosure. The study recorded their responses towards their own lamb as well as towards a lamb born from a different ewe, including the proximity and willingness to suckle.

- Metabolism

Individual blood samples were collected from the jugular vein in EDTA vacutainers on the 80 lambs at 4 occasions[3]. The plasma samples were stored at -20°C until they were analysed for NEFA (Non-Esterified Fatty Acids, using the acyl-CoA synthase method), BHB (Beta hydroxy Butyrate, using the d-β-hydroxybutyrate-dehydrogenase method), and urea (using the glutamate dehydrogenase method). Analyses were performed with an automatic analyzer (Arena 20XT, Thermo Fisher Scientific, Cergy Pontoise, France).

5.1.3 Data analysis

The data were analysed using analysis of variance (ANOVA) for testing the main effects of the genetic lineage (GL – HFE and LFE), the early experience (EE – AR and MR) and the GL*EE interaction. Depending on data, a factor related to date was included with the associated interactions with the main factors. Mixed models were used at first intention, with the group considered as the random factor. The subjects were the individual lambs (excepted for the collective reactivity test).
The behavioural observation data (scans of activity and shelter use), performance and health data (body weight, daily gain, metabolism, parasitism) were analysed using the SAS® Enterprise Guide (7.1 version) software.

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[1] Average age in number of days from birth: D090, D120, D135, D170, D200, D365.

[2] D0, D015, D040, D060, D0902, D120, D135, D170, D200, D285, D365, D555.

[3] D120, D135, D170, D200.

The data from the behavioural individual tests (risk-taking and human preference tests) were analysed using the R software with the packages car, lmerTest, and emmeans.

Regarding the collective reactivity test, the analysis of the flight index relied on the calculation of the area under the curve (AUC) that represents the evolution of the index over the successive scans. Such curves were calculated for each lamb using the multiple trapezoid method. The AUCs, as well as the latencies to flight, were analysed using the non-parametric Mann-Whitney test to test for the effects of the treatments (GL, EE) in each test occurrence.

5.2 Results

5.2.1 Behavioural adaptation traits

5.2.1.1 Risk-taking ability (Period 1 presented)

In Period 1, during the isolation phase, we observed a significant effect of GL*EE on the bleating behaviour of the lambs. Specifically, all the AR lambs bleated more than the MR ones and the LFE/MR lambs bleated more than the HFE/MR lambs (Figure 5.6A).

During the motivation phase, we observed no difference between treatments in the motivation to pass through the corridor (GL*EE, EE, GL effects, P>0.05), at the exception of one individual that did not cross the corridor and was removed from the analyses.

During the risk taking phase, none effect of GL nor of GL*EE was observed. However, the capacity to pass through the obstacle was greater in AR lambs (35 out of 40 lambs succeeded) than in MR ones (12/39) (Fisher test, P< 0.0001). The AR lambs also displayed a higher frequency of exploration of the obstacle (Figure 5.6B), while the MR lambs exhibited a higher frequency of observation of the humans (MR: 1.25±0.61; AR: 0.54±0.84 (nb/min), P<0.01).

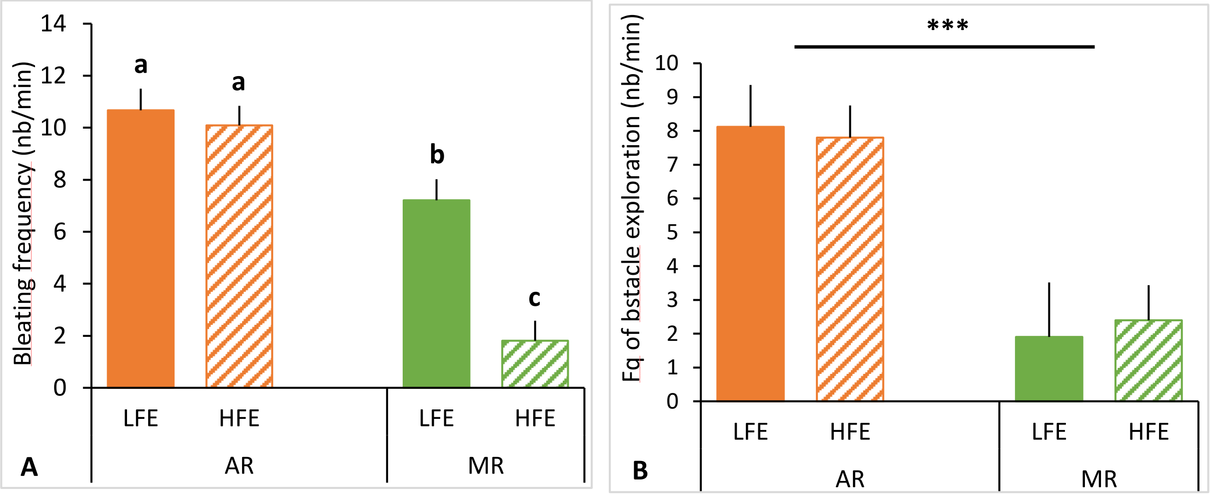


Figure 5.6. Frequency of bleating (A, isolation phase) and of obstacle exploration (B, risk-taking phase) during the Risk-taking test (Period 1) according to genetic lineage (low feed efficiency in plain bars, high feed efficiency in hatched bars) and early experience (artificially reared lambs in orange, maternally reared ones in green).

5.2.1.2 Relationship with Humans

In Period 1, the LFE lambs tended to spend less time close to the humans (P=0.07) than the HFE ones (Figure 5.7A) but this was no longer observed in Period 2.

These LFE lambs also vocalised more during the test than the HFE ones (P<0.01, Figure 5.7B) at both periods. This effect is greater for LFE-MR lambs compared to HFE-MR ones in Period 1 (GL*EE, P<0.01).

Whatever the Period, the AR lambs spent more time close to the experimenters (P<0.01) and vocalised more than the MR lambs (P<0.01), Figure 5.7AB). Lambs did not show any preference between the two persons present in the test pen.

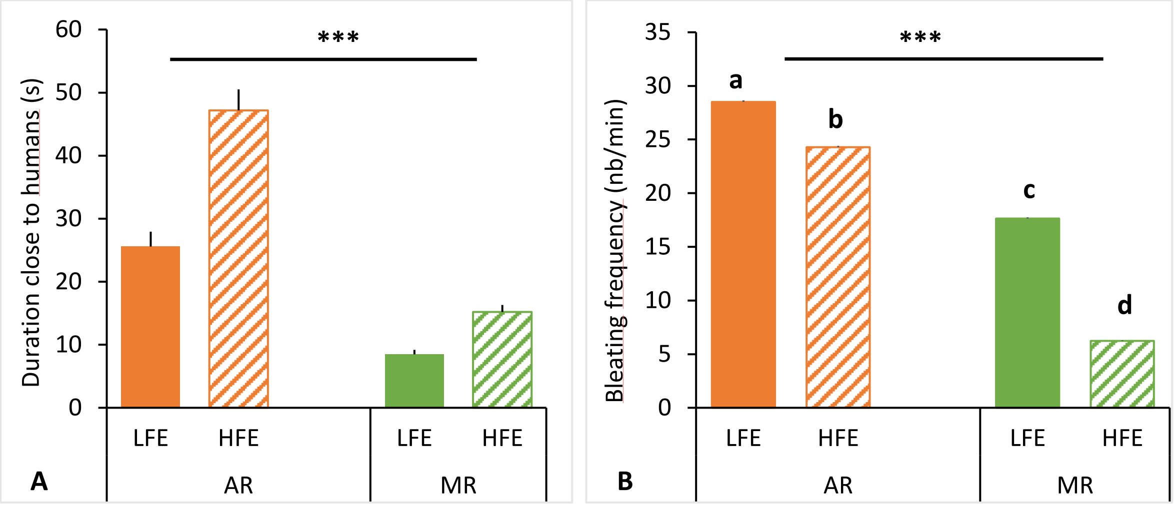


Figure 5.7. Duration of time spent close to humans (A) and bleating frequency (B) during the Human preference test in Period 1, according to early experience (artificially reared lambs in orange, maternally reared ones in green) and genetic lineage (low feed efficiency in plain bars, high feed efficiency in hatched bars).

5.2.1.3 Collective reactivity (Period 1 presented)

No effect of the genetic lineage was observed on the latency to flight after the emergence of the ghost stimulus, in any of the tests in Period 1 (Mann-Whitney, test 1: U=730, P>0.1; test 2: U=632, P>0.1) (Figure 5.8). Both tests showed a significant impact of the early experience, as the AR lambs took approximately twice as long to initiate flight compared to the MR lambs (Mann-Whitney, test 1: U=495, P<0.01; test 2: U=396, P<0.001).

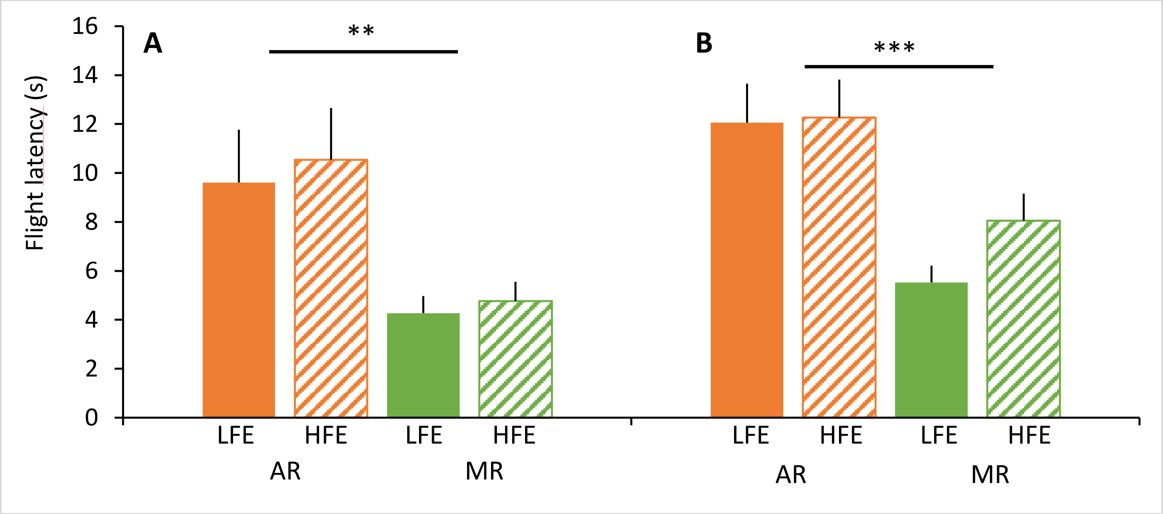


Figure 5.8. Latency to flight during the Collective reactivity tests (A: test 1, B: test 2) of Period 1, according to genetic lineage (low feed efficiency, LFE, in plain bars; high feed efficiency, HFE, in hatched bars) and early experience (artificially reared lambs in orange and maternally reared ones in green).

With regard to the approximation of the flight distance (flight index), there was no effect of the genetic lineage in any of the tests (Mann-Whitney, test 1: U=659.5, P>0.1; test 2: U=664, P>0.1) (Figure 5.9). In return, the early experience affected the index, as MR lambs fled further away than the AR ones in both tests (Mann-Whitney, test 1: U=15, P<0.001; test 2: U=20, P<0.001), even if the reactivity seems to decrease over time (Figure 5.9).

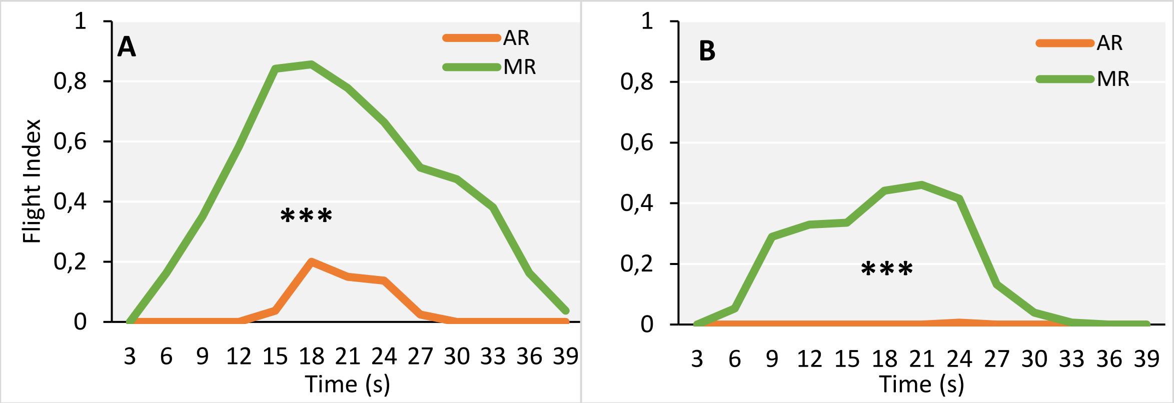


Figure 5.9. Evolution of the flight index (approximation of the flight distance) following the emergence of the aversive stimulus (ghost) at time 0, for the artificially (AR) and maternally (MR) reared lambs, in test 1 (A) and test 2 (B) of Period 1.

5.2.1.4 *Ability to explore environment, to find feed and shelter*

Data relative to the behavioural activities at the entry onto the experimental plots are presented for time-slots 1 and 3 (morning 1 and morning 2). There was no effect of genetic lineage, either as a main factor or in interaction with early experience, whatever the period.

At the very first entry (period 1, time-slot 1), AR lambs exhibited more exploration and less feeding than MR lambs (EE effect: P<0.0001, Figure 5.10). The AR lambs' exploratory behaviour decreased significantly as early as the following morning (EE*time-slot effect, P<0.0001), and was replaced by increasing resting and rumination, which also increased in MR lambs (time-slot effect: P<0.0001). During Period 2, we observed little difference overall between AR and MR lambs, with a pattern similar to that of MR lambs in time-slot 3 in Period 1.

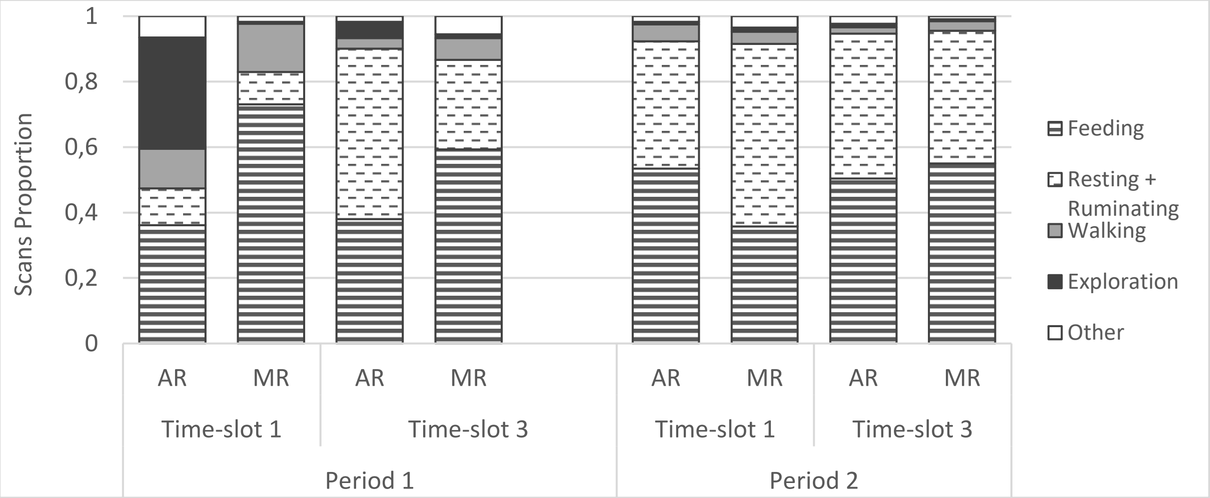


Figure 5.10. Proportions of main lambs' activities on the two successive mornings (time-slots 1 and 3) after entry onto the experimental plots in periods 1 (summer) and 2 (autumn), according to early experience (AR: artificial rearing; MR: maternal rearing).

Regarding the ability to use shelter on sunny and warm summer days, we did not observe any differences related to the genetic lineages nor to the interaction with early experience in the proportion of scans observed under boxwoods (0.50 (HFE) vs. 0.49 (LFE), sem 0.03, P>0.1) or in open areas (0.22 (HFE) vs. 0.20 (LFE), sem 0.01, P>0.1). The AR lambs were observed less often under boxwoods than the MR ones (0.25 (AR) vs. 0.74 (MR), sem 0.03, P<0.0001) and more often in open areas (0.37 (AR) vs. 0.04 (MR), sem 0.01, P<0.0001), which may be partly due to their lower proportion of time spent resting or ruminating over the observation periods than MR lambs (EE effect, P<0.0001).

Besides behavioural observations, analysing the lambs' performance and metabolism, including indicators of body reserve mobilization, is another way of assessing their ability to locate food and shelter.

Regarding the growth of lambs from birth to D555, the interaction between genetic lineage and early experience was never significant, whether for live weight or average daily gain (ADG, Figure 5.11). For the effects of genetic lineage, some trends were observed for ADG (0.05<P<0.1), but these trends did not result in any significant differences in live weight between HFE and LFE lambs at any date. The contrasted rearing modes applied during the first three months tended to lower ADG and live weight (D90) in artificially-reared lambs (EE effect, P<0.10). The main difference between AR and MR lambs occurred during the first few weeks that followed the entry onto the plots, with a lower ADG between D90 and D120 (AR: 47 g/d vs MR: 128 g/d, sem 13) and a lower live weight at D120 (EE effect, P<0.0001). As soon as D120, the average daily gains were similar, but the live weight difference persisted until the time of breeding (D555: 3.4 kg less for AR compared to MR lambs, P<0.001).

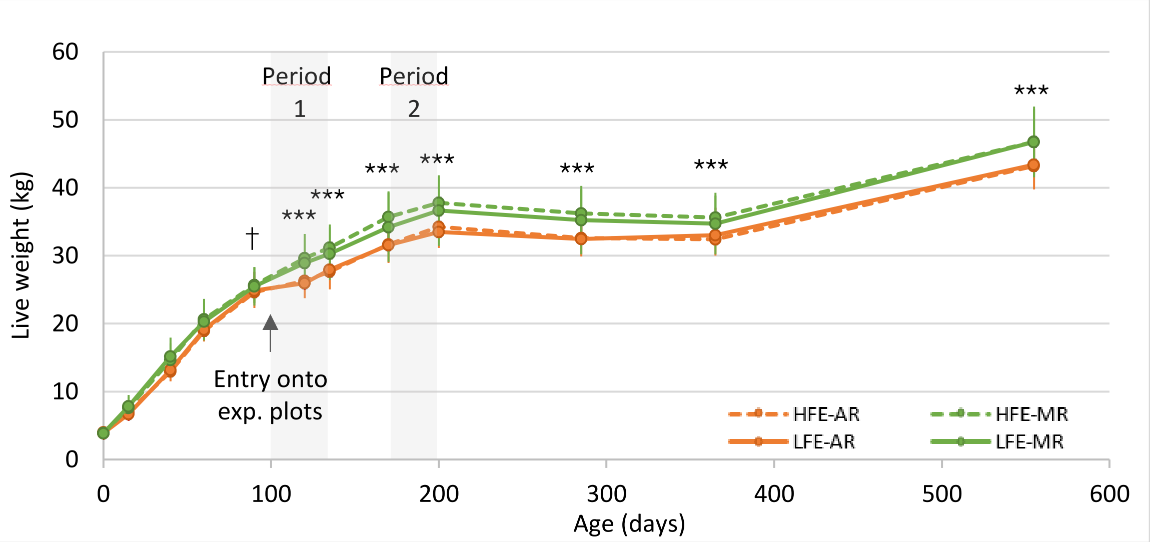


Figure 5.11. Live weight of lambs (mean ± sd) over the study from birth (D0) to mating (D555) depending on the genetic lineage (low feed efficiency, LFE, in solid line; high feed efficiency, HFE, in dashed line) and early experience (artificially reared lambs in orange; maternally reared ones in green).

Regarding lambs' metabolism, we did not observe any significant interaction between genetic lineage and early experience, whatever the metabolite analysed. After spending three weeks on the experimental plots (D120), the LFE lambs showed higher levels of plasmatic non-esterified fatty acids than the HFE lambs, and this difference persisted until the end of Period 1 (D135, Table 5.2). This suggests that the low-efficient lambs mobilized their body reserves to a greater extent than the high-efficient lambs. A similar pattern, although more significant quantitatively, was observed for AR and MR lambs over the same period of time. The artificially-reared lambs mobilized their body reserves more than the maternally-reared ones (Table 5.2). The plasmatic level of urea was greater in MR lambs than in AR lambs at D120 and D135, which may indicate the selection of a diet richer in nitrogen by MR lambs.

From the end of Period 2 (D200), no differences were observed between the lambs, regardless of their lineage or experience.

Table 5.2: Plasmatic levels of certain metabolites, at key dates, in lambs with different genetic lineage (GL) and early experience (EE) and significance of these effects and of their interactions

		Mean at key dates			**Statistical significance**					
Item	**Lineage and experience**	**D120 mid-summer**	**D135 end summer**	**D200 end autumn**	**GL**	**EE**	**Date**	**GL*EE**	**GL*Date**	**EE*Date**
NEFA (mmol/L)	LFE	0.295^a	0.193^a	0.104	0.008	0.0001	0.0001	0.7	0.2	0.0001
	HFE	0.219^b	0.144^b	0.091						
	AR	0.338^x	0.203^x	0.095						
	MR	0.176^y	0.134^y	0.10						
BHB (mmol/L)	LFE	0.57	0.58	0.52	0.8	0.7	0.0001	0.6	0.4	0.05
	HFE	0.58	0.60	0.50						
	AR	0.59	0.58	0.51						
	MR	0.56	0.60	0.51						
Urea (g/L)	LFE	0.205	0.240	0.361	0.06	0.02	0.0001	0.09	0.8	0.1
	HFE	0.226	0.247	0.380						
	AR	0.194^x	0.234^x	0.369						
	MR	0.237^y	0.253^y	0.371						

NEFA = non-esterified fatty acids; BHB = Beta hydroxybutyrate – Genetic lineage: LFE = low efficient; HFE = high efficient; Experience: AR = artificially-reared; MR = maternally-reared.

$^{a,\,b}$ or $^{x,\,y}$: means with different letters differ between treatments at a given date ($p<0.05$)

On a longer term, the analysis of the reproductive performances revealed that the HFE ewes that were artificially reared at an early age, gave birth to a higher number of single lambs (11 out of 19) compared to the LFE ewes reared under the same conditions (3 out of 13), or to those reared under the dam, regardless of their genetic lineage (3 out of 15 or 2 out of 13, respectively; Fisher exact Probability test, P<0.05). Their maternal behaviour in the selectivity test did not differ based on their early rearing conditions or genetic lineage (P>0.1).

5.2.2 Health and welfare traits

The regular monitoring of some health indicators (rear end dirtiness, ocular and nasal discharge, lameness) varied by date but did not appear to vary according to genetic lineage or early experience.

The gastrointestinal parasitic load showed no effect of genetic lineage or of interaction with early experience (Figure 5.12). Despite a significant EE*date interaction (P<0.05), there was no clear trend in the effect of early experience, probably because of the initial difference in parasite load between AR and MR lambs at D90, due to their different living conditions before weaning. We can also see that the infestation levels are moderate and characterised by a high individual variability in all treatments.

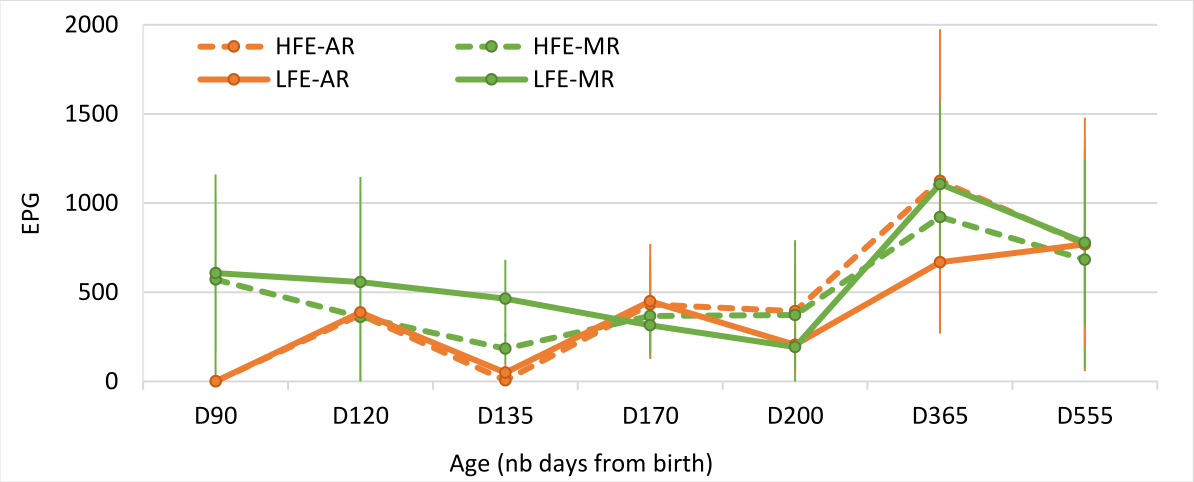


Figure 5.12. Parasitic load from gastrointestinal nematodes (EPG, eggs per gram, mean ± sd) from D90 to D555 depending on the genetic lineage (low feed efficiency, LFE, in solid line; high feed efficiency, HFE, in dashed line) and early experience (artificially reared lambs in orange; maternally reared ones in green).

5.3 Conclusions

The experimental setup successfully tested the robustness/resilience of sheep raised under contrasted conditions from birth and then all exposed to challenging rangeland conditions after weaning. The artificially reared animals were significantly disadvantaged in terms of growth and body reserve mobilisation compared to those reared with their dam on the rangeland, but this was only temporary.

The study has led to the development of new tools for phenotyping individuals, particularly with regards to their behavioural robustness. These tests have demonstrated their capacity to differentiate between individuals subjected to different treatments. This enables exploration of individual variability and a better understanding of how it is influenced by the origin and experiences of the individuals.

In this context, the analysis of the genotype*environment interaction reveals a lineage effect on body reserve mobilisation, with lambs from the low feed efficiency lineage exhibiting a higher mobilisation. Additionally, these animals demonstrated lower prolificacy when reared artificially, and exhibited more stress (bleating) when isolated, particularly when reared with their dam on the rangeland during their young age.

The effects of the genetic lineage are still limited compared to those of the early experience, but the divergent selection is still recent (Touitou et al. 2022). Nonetheless, the results obtained appear to be consistent with the selection objectives of the lineages.

6 Foetal and neonatal survival phenotypes – IDELE & RDF

6.1 Materials and methods

For three meat sheep breeds in France, 6,015 lambing data (Table 6.1) were available with complete lambs' mortality records. Perinatal mortality (stillbirth and mortality in the 20 first days) was recorded on commercial meat sheep farms involved in the Smarter project for 4 years (2019-2022).

Table 6.1. Lambing data for three meat sheep breeds in France.

Meat sheep breed	Count lambing	Count lambs born	Count flocks	Years of data collection
Blanche du Massif Central - 24	3112	5088	6	From 1 to 3 : 2019-2021
Mouton Vendéen -41	1730	2817	5	From 2 to 3 : 2019-2021
Rouge de l'Ouest-43	1173	1952	5	From 2 to 4 : 2019-2022
Total	**6015**	**9857**	**16**	**From 1 to 4 : 2019-2022**

6.2 Results

A total of 6,015 lambing data from meat ewes were registered with mortality (stillbirth + until 20 days) records. Stillbirth was lower than 1% regardless of the studied breed. Mortality rate ranged between 4 to 5.35% depending on the breed (Table 6.2).

Table 6.2. Stillbirth and mortality rates in the three studied meat sheep breeds in France.

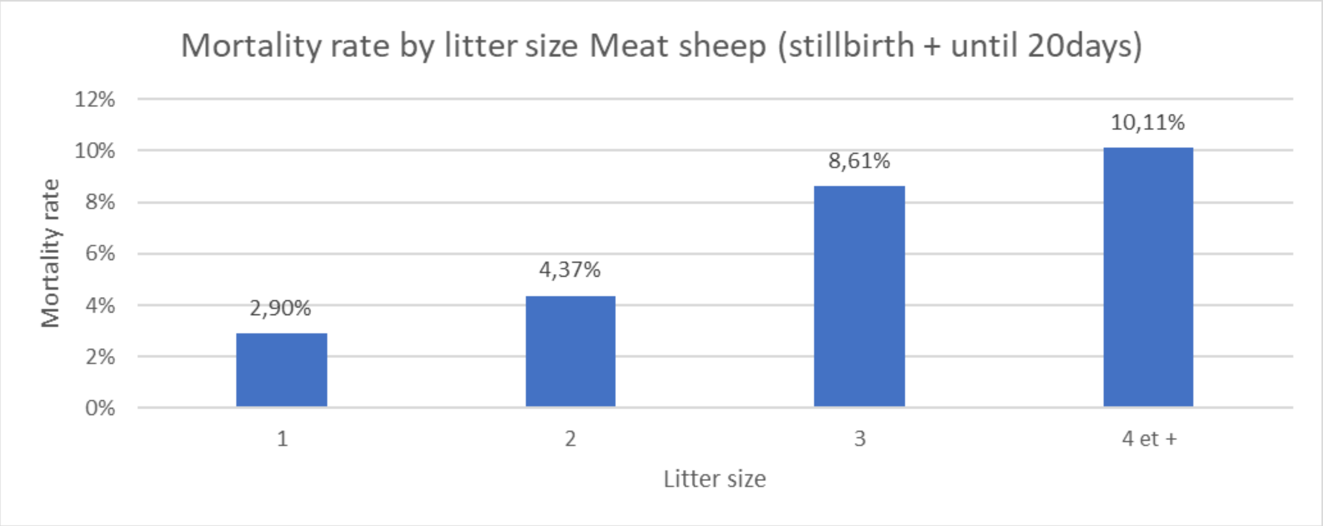
Meat sheep breed	Count lambing	Count lambs born	Count lamb stillborn	Mortality rate (Stillbirth)	Count lamb mortality (stillbirth + until 20d)	Mortality rate (stillbirth + until 20d)
Blanche du Massif Central -24	3112	5088	5	0.98%	272	5.35%
Mouton Vendéen -41	1730	2817	3	0.11%	113	4.01%
Rouge de l'Ouest-43	1173	1952	0		81	4.15%
Total	**6015**	**9857**	8	0.81%	462	4.69%

As expected, mortality rate increased with litter size in French meat sheep to reach up to 10% when litter size was higher than 4 lambs. A two-fold increase in the mortality rate was observed between twins and triple litter (Table 6.3 and Graph 6.1). Increase in mortality rate by litter size varied depending on the studied breed. The Mouton Vendéen breed showed a lower increase in mortality rate by litter size compared to the two other breeds (Table 6.4). Moreover, mortality rate varied widely depending on the studied farm (Table 6.5).

Table 6.3. Lamb mortality rate by litter size in French meat sheep.

Litter size	Count young born	Count young dead	Mortality rate (%)
1	2376	69	2.90
2	5900	258	4.37
3	1393	120	8.61
4	178	18	10.11
5	10	1	10.00

Total	**9857**	**462**	**4.69**



Graph 6.1. Lamb mortality rate by litter size in French meat sheep.

Table 6.4. Lamb mortality rate by litter size in French meat sheep breeds

Litter size	Lamb Mortality rate BMC - 024	Lamb mortality rate MVD - 041	Lamb mortality rate RO - 043
1	2.82%	3.02%	2.99%
2	4.79%	3.94%	3.83%
3	12.37%	5.46%	6.42%
4	22.45%	4.65%	5.81%
5	14.29%	0.00%	
Total	**5.35%**	**4.01%**	**4.15%**

BMC = Blanche du Massif Central; MVD = Mouton Vendéen; RO = Rouge de l'Ouest

Table 6.5. Lamb mortality rate by studied farm in French meat sheep.

Breed	Recoding farms	Mortality rate	Average of births recorded per year	Years of data collection
Blanche du Massif Central	A	7.73%	194	2020
Blanche du Massif Central	B	1.11%	853	2020-2021
Blanche du Massif Central	C	5.73%	629	2019-2020-2021
Blanche du Massif Central	D	12.03%	574	2019-2020-2021
Blanche du Massif Central	E	2.21%	249	2019-2020-2021
Blanche du Massif Central	F	5.94%	143	2019-2020
Mouton Vendéen	G	3.38%	168	2019-2020-2021
Mouton Vendéen	H	7.53%	153	2020-2021
Mouton Vendéen	I	1.89%	207	2019-2020-2021
Mouton Vendéen	J	3.81%	186	2020
Mouton Vendéen	K	5.71%	383	2019-2020-2021
Rouge de l'Ouest	L	0.60%	477	2020-2021
Rouge de l'Ouest	M	1.75%	13	2019-2020-2021-2022
Rouge de l'Ouest	N	3.66%	183	2020-2021
Rouge de l'Ouest	O	8.00%	446	2020-2021
Rouge de l'Ouest	P	11.17%	316	2020-2021

6.3 Conclusions

Smarter project has given the opportunity to massify recording of lamb mortality in France and better describe the neonatal survival in meat sheep farms. Some limitations were identified due to the difficulty of collecting exhaustive mortality data in the field.

7 Lamb vigour scores and ewe mothering ability – TEAGASC & SRUC

7.1 Materials and methods

Data used in the present study originated from the Sheep Ireland (https://www.sheep.ie) national database. The traits considered in the present study were ewe mothering ability and lamb vigour both of which were extracted from the database. Also available was information pertaining to the flock the ewe gave birth in, the date of birth, lamb gender(s), and the breed composition of both the ewe and lamb.

7.1.1 *Recorded traits*

Both lamb vigour and ewe mothering ability were measured on-farm by either trained technicians (present at lambing) or producers in the immediate post-natal period. Ewe mothering ability was subjectively scored on a five-point scale for each lambing event (regardless of litter size), where: 1 = ewe has no interest in her lamb(s); 2 = ewe stands well away and is slow to lick her lamb(s); 3 = ewe licks her lamb(s) and follows her lamb(s) to the post-lambing individual pen; 4 = ewe licks her lamb(s), is protective, and follows closely to the individual pen; and 5 = ewe is very protective, licks her lamb(s) immediately, follows her lamb(s) very closely to the individual pen and bleats for her lamb(s). Lamb vigour was scored on a five-point scale on each lamb individually, where: 1 = lamb is still not standing after 60 minutes; 2 = lamb is standing within 60 minutes; 3 = lamb is standing within 30 minutes; 4 = lamb is standing within ten minutes; and 5 = lamb is standing within five minutes.

As well as ewe mothering ability and lamb vigour, a number of other birth-related phenotypes were also recorded. Lambing difficulty was scored as a single record per lambing event (irrespective of the number of lambs born) on a four-point scale where 1 = no lambing assistance or the lambing event was unobserved; 2 = slight assistance; 3 = severe assistance which may include lamb malpresentation; and 4 = significant intervention by the producer and/or the veterinary surgeon up to and including a caesarean section. Lamb mortality was recorded as whether the lamb was dead (lamb mortality = 1) or alive (lamb mortality = 0) at 24 hours of age. Lamb birth weight was recorded within 24 hours of age and only lambs with a recorded birth weight of between 2 and 9 kg were retained; weight at birth was rounded to the nearest half kilogram. Litter size was defined as the number of lambs born (dead and/or alive) for a given lambing event; only litter sizes between one (singleton) and four (quadruplet) were retained.

7.1.2 *Data*

Between the years 2018 and 2022 inclusive, a total of 141,333 lambing events from 46,729 ewes lambing in 883 flockbook-recorded flocks (70,822 lambing events from 24,513 ewes) or 78 commercial crossbred flocks (42,521 lambing events from 15,873 ewes) were available. The remaining 75 flocks included both flockbook-recorded and commercial crossbred animals (i.e., combined flocks; 9,390 lambing events from 3,418 flockbook-recorded ewes and 9,069 lambing events from 3,351 crossbred ewes).

Both ewe live weight and body condition score (BCS) data were available on ewes at various time points throughout the production year. Ewe BCS was subjectively measured by both trained technicians and producers on a five-point scale where 1 = emaciated and 5 = obese. Ewe live-weight was defined as the weight of a ewe that had at least one recorded lambing event; only recorded ewe live-weights between 45 and 130 kg were retained. For the present study ewe BCS and live weight measured at two time-points were retailed including the pre-lambing (between three weeks before to one week prior to lambing) and the post lambing period (between two and eight weeks post lambing). Ewe BCS and live weight change was defined as the difference (positive or negative) in both BCS and live weight between the pre- and post-lambing period.

For all traits, records were discarded if the date of birth, flock of birth were unkown. For lamb specific traits (i.e. lamb vigour, lambing difficulty, lamb mortality and lamb birth weight) records with unknown dam or sire were discarded; for ewe specific traits (i.e. mothering ability and litter size), ewes were only retained where the sire of the ewe was known. All data from 9,460 lambing events that were classified as embryo transfer events were discarded. All records from parity 11 or greater ewes were also removed and ewe parity was subsequently categorised as 1, 2, 3, 4, 5, 6 or ≥7. Age of the ewe at first lambing was categorised as: 1) lambing between 8 and 18 months of age (i.e., lambing as ewe lambs), or 2) lambing between ≥18 and 28 months of age (i.e., lambing as hoggets). Ewes recorded to have lambed for the first time less than eight months of age were discarded.

The breed proportion of each ewe and lamb was categorised as Belclare, Charollais, EasyCare, Lleyn, Rouge de l’Ouest, Suffolk, Texel, and Vendeen (i.e., the 8 most recorded breeds in the Sheep Ireland database); all other breeds were collectively classified as “other”. Coefficients of general heterosis and general recombination loss were calculated for each ewe and lamb using the formulae $1 - \sum_{\mathrm{i}=1}^{\mathrm{n}} \mathrm{sire}_{\mathrm{i}} \cdot \mathrm{dam}_{\mathrm{i}}$ and $1 - \sum_{j=1}^{n} \frac{sire_j^2 + dam_j^2}{2}$ respectively where $\mathrm{sire}_{\mathrm{i}}$ and $\mathrm{dam}_{\mathrm{i}}$ are the proportion of a specific breed (i) in the sire and dam, respectively (VanRaden and Sanders, 2003). Heterosis and recombination loss, for both the ewe and lamb, were subsequently grouped into distinct classes based on the frequency distribution of the respective coefficients; for heterosis 3 distinct classes were formed: less than 10%, 10% to 99%, and 100%. For recombination loss 2 classes were formed: less than 10% and 10% to 50%.

Contemporary groups were defined for each trait separately as flock-year-week of lambing. Only contemporary groups with variability in the phenotypic score and at least five records were retained. Following edits, 26,590 ewe mothering ability records from 18,125 ewes as well as 58,161 lamb vigour records from 22,414 ewes remained.

7.1.3 Statistical analyses

Variance components for both lamb (i.e. lamb vigour, lambing difficulty, lamb mortality and birth weight) and ewe (i.e. ewe mothering ability and litter size) traits were estimated using animal linear mixed models in ASReml (Gilmour et al., 2021). In all models, a direct additive genetic effect was included as a random effect enabled through the pedigree linkages via the numerator relationship matrix. When traits of the lamb were under investigation, other random effects considered were: a maternal genetic effect (also captured through the pedigree relationship matrix), a within-litter maternal permanent environmental effect and an across-parity maternal permanent environmental effect. For ewe traits an across-parity ewe permanent environmental effect was also considered. The log-likelihood ratio test between nested models was used to determine the improved fit to the data by including each additional random term. When a significant maternal genetic variance was detected, a direct-maternal covariance was also estimated and its improvement to the fit of the data determined using the log-likelihood ratio test compared to where no covariance was assumed. The pedigree of each animal was traced back to the founder population and consisted of 454,702 animals. A series of bivariate models were used to estimate the phenotypic and genetic correlations between all lamb and ewe lambing traits.

7.2 Results

The frequency distribution of lambing events throughout the year is in Figure 7.1. The highest frequency of lambing events occurred in March (35.35%) followed by January and February with lambing event frequencies of 29.97% and 16.33%, respectively. Lambing events that occurred between May and November cumulatively accounted for just 1.26% of the overall lambing events throughout the year. The frequency distribution of lambing difficulty and number of lambs born is in Figure 7.2.

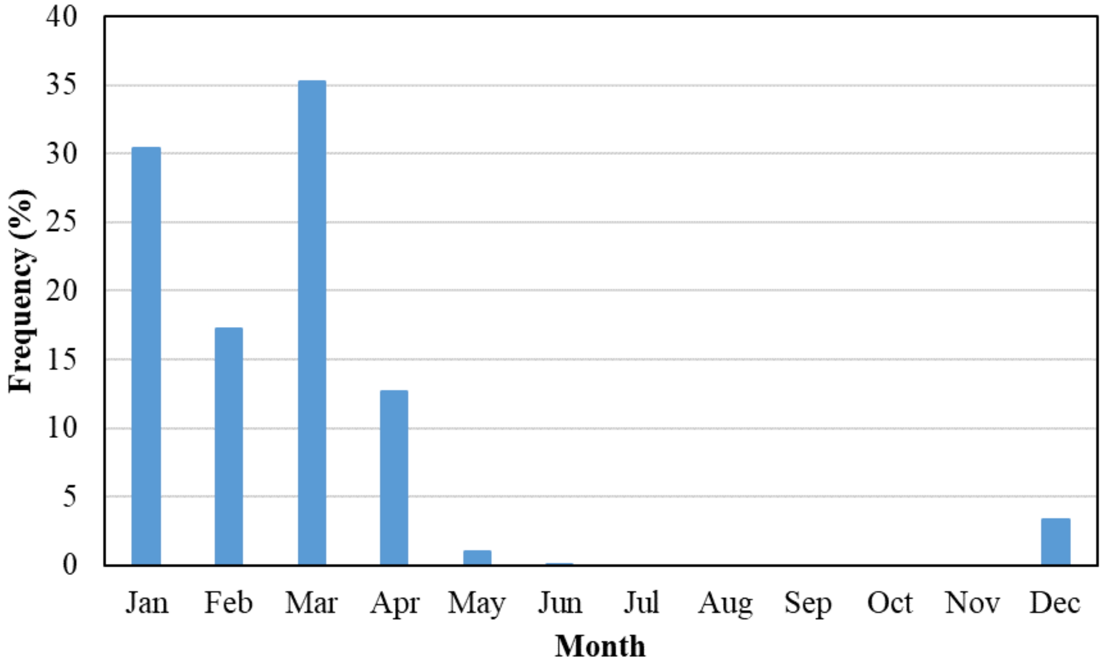


Figure 7.1 Frequency distribution of lambing events across the calendar year.

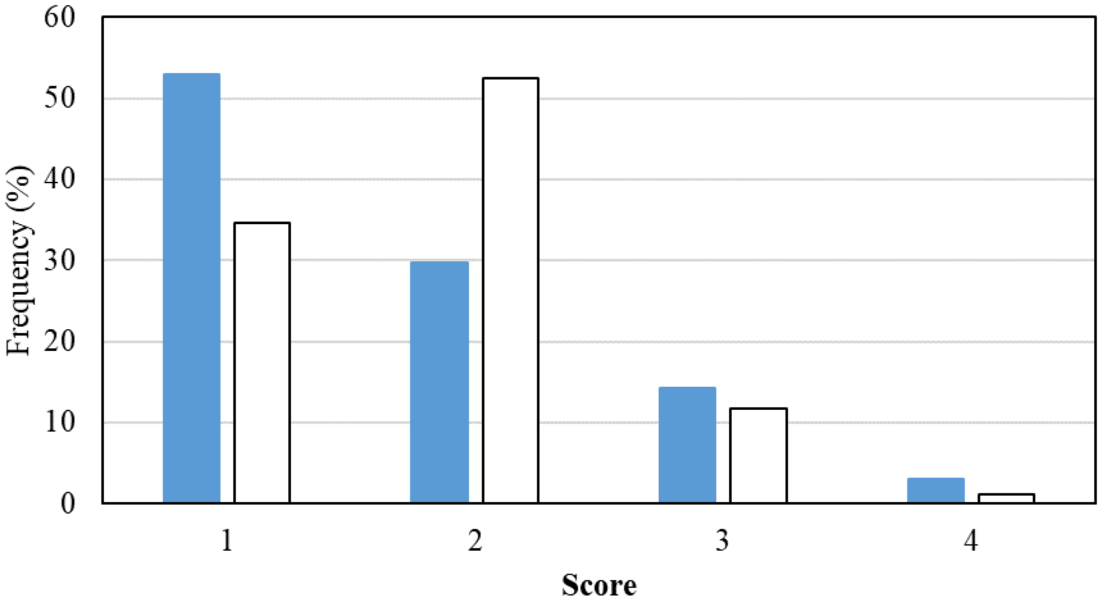


Figure 7.2. Frequency distribution of lambing difficulty (black bars) and number of lambs born (white bars).

7.2.2 Lamb vigour

The prevalence of good (i.e., lamb standing within 10 minutes) and very good (i.e., lamb standing within 5 minutes) lamb vigour was 40.06% and 42.88%, respectively (Table 7.1). The prevalence of lambs that took at least 30 minutes to stand after birth (i.e., score 1 and 2) was 4.09%. The direct heritability of lamb vigour was 0.12 (0.02) while the maternal heritability was 0.03 (0.01).

7.2.3 Ewe mothering ability

The majority of ewes scored for ewe mothering ability fell into the good and very good categories with 35.95% and 50.20% of ewes having a score of 4 and 5, respectively (Table 7.1). The prevalence of poor ewe mothering ability (i.e., ≤ score 2) was 2.99%. The average mothering ability across all ewes was 4.33 (SD= 0.81). The genetic SD and direct heritability of ewe mothering ability was 0.19 and 0.07 (0.02), respectively. The ewe repeatability estimate for ewe mothering ability was 0.10 (0.01).

7.2.4 Other lambing traits

The average lamb birth weight and lambing difficulty score was 4.78 kg and 1.67, respectively. The mean incidence of lamb mortality was 11.84%. The direct heritability for lamb birth weight, lambing difficulty score and lamb perinatal mortality was 0.09 (0.01), 0.06 (0.02) and 0.02 (0.01). A significant maternal heritability was estimated across all three lambing traits and ranged from 0.01 (0.005) to 0.10 (0.01; lamb birth weight).

7.2.5 Genetic Correlations among lambing traits

The genetic correlation between lamb vigour and other lambing traits ranged from -0.48 (lamb mortality) to 0.27 (lamb birth weight). The genetic correlation between ewe mothering ability and number of lambs born was 0.70 (0.04,) while the genetic correlation between ewe mothering ability and direct lamb mortality was -0.25 (0.02). A strong genetic correlation was recorded between lamb birth weight and lambing difficulty score (0.76). The genetic correlation between lamb birth weight and lamb mortality was positive but close to zero (0.09). The genetic correlation between lambing difficulty score and lamb mortality was 0.52 (0.05).

Table 7.1. Brief trait description, number of records, and prevalence of each score for ewe mothering ability and lamb vigour.

	Score	Trait description	Records	Prevalence
Mothering ability	1	No interest in lamb(s)	366	1.04
	2	Hesitant and slow to lick lamb(s)	686	1.95
	3	Licks and follows lamb(s)	3,826	10.86
	4	Licks lamb(s), is protective, and follows lamb(s) closely	12,660	35.95
	5	Licks immediately, very protective, follows lamb(s) closely, and bleats	17,682	50.20
Lamb vigour	1	Standing after 60 minutes	1,456	2.05
	2	Standing within 60 minutes	1,444	2.04
	3	Standing within 30 minutes	9,200	12.97
	4	Standing within 10 minutes	28,423	40.06
	5	Standing within 5 minutes	30,429	42.88

7.3 Conclusions

Results conducted as part of the SMARTER project show that low to moderate heritability estimates, coupled with ample genetic variation, and the availability of routinely collected data on two novel lambing traits (i.e. lamb vigour and mothering ability) clearly indicate that breeding should be strongly considered as a tool to improve overall lambing performance and reduce labour requirements during the lambing period. The genetic correlation estimated between the two novel traits and routinely available lambing traits show that improving ewe mothering ability and lamb vigour will result in benefits for improved standards of ewe and lamb welfare, with reduced intervention and could lead to reduced risk of infections as well as improved ewe-lamb bonding.

8 Candidate genes associated with temperament trait in Merino -INIA-UY

8.1 Materials and methods

A total of 4,317 lambs were tested for temperament between one- and three-months post-weaning, including four progeny: 2010, 2011, 2018 and 2019. Temperament was measured using the Isolation Box Test (IBT) (Blache and Ferguson, 2005). Each lamb was gently pushed inside the box, held there for 30 seconds, and agitation was objectively measured by an agitation meter. The agitation meter registered the vibrations of the box induced by the lamb's movements and high-pitched vocalizations. The higher the agitation score, the more nervous the sheep. The IBT was calibrated with an electronic unit to high and low agitation score. There was no previous selection of animals and they had no previous experience with IBT.

Genotype data: A total of 1,697 lambs were genotyped for 43,705 SNP and the molecular information was obtained using the Geneseek Genotypong Profile panel (GeneSeek ® Genotyping Profile, GGP, Illumina, San Diego, California). Quality Control (QC) was conducted using PREGSF90 by animals and SNP. Animals were removed from the analysis when the call rate was < 95 % and when presenting Mendelian conflicts. The SNP were removed when the call rate was < 90 %; SNP with Minor Allele Frequency < 95 % (MAF) and monomorphic SNP. Sex chromosomes were not included in the analysis. After QC, a total of 38,268 effective SNP were retained for subsequent genomic analyses.

Pedigree data of 10,799 Merino sheep was used and the Off-Diagonal correlation between genomic information and pedigree data was 0.86. The genealogy level considered was level 3.

8.1.1 Statistical analyses

The model used for analysis was according to Zambra et al. (2015). The mixed model to perform a ssGWAS included fixed effects (genetic and environmental) and random effects. The fixed effects were contemporary group (year, flock, sex and rearing group), dam age (< 2 years, 2 to 3 years or > 3 years) and type of birth (single or twin). The age of the lamb at the time of measurement with the IBT was considered as a co-variable. Through BLUP suite programs, it was possible to include the genomic information into this model to obtain the genomic estimated breeding value (GEBV) for temperament and the SNP effects. The variance components necessary for the model were estimated by airemlf90 program version 1.148 of BLUPF90.

A Single Step Genome Wide Association Studies (ssGWAS) were performed by the BLUPF90 family programs (Misztal et al., 2020). To identify markers associated with temperament, we worked with the p-value of the SNP and the % var explained by windows or adjacent segments of 0.5 mbp. The statistical threshold of –log value of 5.0 and % var ≥ 0.5 % were used.

We used genetic enrichment analysis as a tool to identify the biological and molecular terms associated with a phenotype of interest. For this analysis, the 5 % of SNP with the greatest effect were selected. From the two approaches (% var and SNP effect) a list of genes was obtained. To assign the genes to functional categories, the DAVID (Database for Annotation, Visualization and Integrated Discovery, latest available version 6.8) was used.

8.2 Results

8.2.1 Estimation of genetic parameters

The genetic variance was 111.78 ± 22.8 (SEM) and the residual variance was 472.6 ± 20.80 (SEM). The heritability of the temperament trait was 0.19 ± 0.038 (SEM).

8.2.2 Single Step Genome Wide Association Studies (ssGWAS)

The SNP with highest statistical association to the –log p value was found on chromosomes 2, 3, 6, 8 and 18 (Figure 8.1a). Another approach of analysing the genetic variance explained by windows of 0.5 Mbp of adjacent SNP, identified genomic regions of interest associated with chromosomes 6, 10 and 21 (var≥0.5) (Figure 8.1.b).

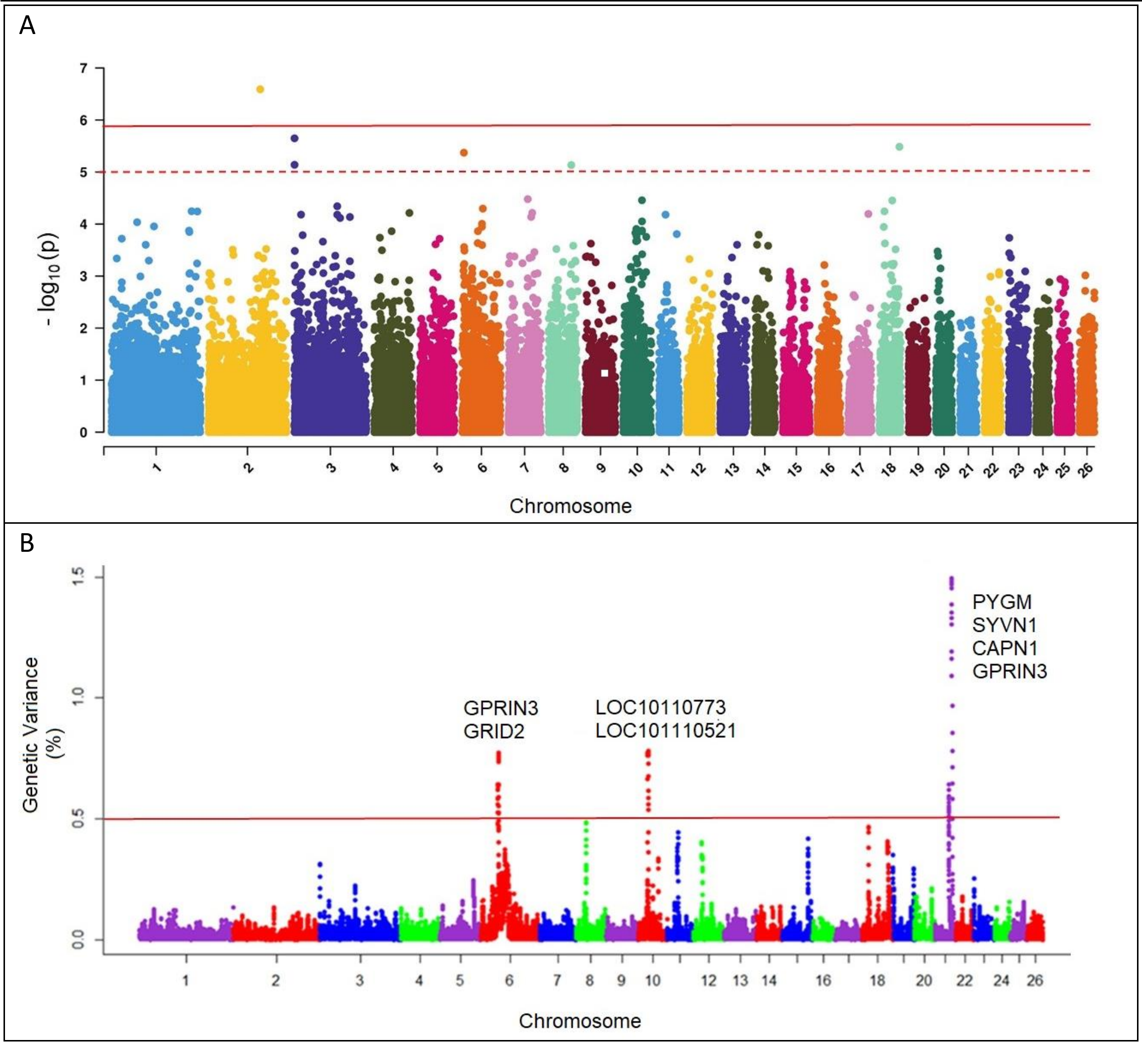


Figure 8.1 A): Manhattan plot of 38,268 effective SNP for 1,697 Australian Merino genotyped animals. Chromosome number and statistical significance threshold of –log10(p) according to Bonferroni adjustment (solid horizontal line) and strategic adjustment (dashed horizontal line). B) Manhattan plot for temperament according to the % variance explained by adjacent segments of 0.5 mbp single nucleotide polymorphism (SNP) windows. The solid horizontal line is the threshold, % var ≥ 0.5%. The nine candidate genes involved in the temperament trait on chromosomes 6, 10 and 21 were inserted with their respective codes within each chromosome.

The information on the most relevant SNP found in these regions about % var, shows that there are nine candidate genes for the temperament trait (Table 8.1), which are: **PYGM** (21:42,295,599-42,307,126), **SYVN1** (21:42,650,559-42,655,527), **CAPN1** (21:42,712,976-42,740,799), **LOC101110773** (10:29,275,771-29,457,586), **LOC101110521** (10:28,986,741-29,188,660), **GRID2** (6:30,768,380-31,534,647), **FADS1** (21:39,652,537-39,665,108), SYT7 (21:39,390,965-39,426,334) and the **GPRIN3** gene (6:35,511,293-35,513,635). The genes and annotation of the regions on chromosomes 6, 10 and 21 is show in Table 8.1.

Table 8.1. SNP detected in the GWAS with the greatest genetic variance, rs code, chromosome, position in the reference genome version oar_v3.1 in base pairs, type of variant and candidate genes.

rs code	pos (bp)	CHR	% Var	Variant type	Candidate gene
rs402505013	42295749	21	1,4691	Splice acceptor var	PYGM
rs419347404	42601748	21	1,4519	Intron variant	-
rs161627521	42654067	21	1,3288	Intron variant	SYVN1
rs413708295	42714381	21	1,1617	Intron variant	CAPN1
rs421553713	42714613	21	1,0906	Intron variant	CAPN1
rs161627624	42715850	21	0,8537	Synonymous variant	CAPN1
rs408317317	29353089	10	0,7803	Intron variant	LOC101110773
rs428995675	29072930	10	0,7738	Intron variant	LOC101110521
rs407693533	35491698	6	0,7722	Intron variant	-
rs422603241	31453177	6	0,7688	Intron variant	GRID2
rs422288687	29304176	10	0,7678	Intron variant	LOC101110773
rs427220269	29054709	10	0,7670	Intron variant	LOC101110521
rs421383362	29188403	10	0,7636	Intron variant	LOC101110521
rs409829992	29162222	10	0,7624	Intron variant	LOC101110521
rs399382510	29202499	10	0,7619	Intergenic variant	-
rs411759303	35511497	6	0,7430	Missense variant	GPRIN3
rs400430030	29421760	10	0,6745	Intron variant	LOC101110773
rs419116702	29030595	10	0,6646	Intron variant	LOC101110521
rs398879843	35191867	6	0,6423	Intergenic variant	-
rs161618576	39638962	21	0,6420	Downstream gene variant	-
rs424142667	35511899	6	0,6415	Missense variant	GPRIN3
rs410592527	35184703	6	0,6228	Intergenic variant	-
rs408222545	39642723	21	0,6204	Upstream gene variant	-
rs419203432	29415140	10	0,6173	Intron variant	LOC101110773
rs161618641	39646260	21	0,5915	Upstream gene variant	-
rs416558978	35254368	6	0,5873	Intergenic variant	-
rs417392501	39651749	21	0,5832	Downstream gene variant	-
rs404318469	42719476	21	0,5826	Intron variant	CAPN1
rs403363266	39653383	21	0,5657	3 prime UTR variant	FADS1
rs419203432	29415140	10	0,5595	Intron variant	LOC101110773
rs403382566	35512106	6	0,5508	Missense variant	GPRIN3
rs421709693	39432569	21	0,5434	Intergenic variant	SYT7
rs427110197	39654860	21	0,5384	Intron variant	FADS1
rs398157763	29455959	10	0,5363	3 prime UTR variant	LOC101110773
rs399480023	31217615	6	0,5249	Intron variant	GRID2
rs399060511	35275766	6	0,5247	Intergenic variant	-
rs406335698	35276244	6	0,5219	Intergenic variant	-

8.2.3 Enrichment analysis

The association study evaluated 38,268 SNP contained in the GGP_50k_ovi panel. For the enrichment analysis 2,185 SNP were considered (5 % SNP greatest effect). This set of SNP defined a set of 908 genes in the sheep reference genome. The enriched p-value of each biological term indicated the importance of the term with respect to the set of genes analyzed (Table 8.2, Figure 8.2). Several significant metabolic pathways (p-value ≤ 0.05) were identified and grouped into four categories: signaling, metabolism, steroidogenesis, and others (Table 8.2).

Table 8.2: Pathways detected in DAVID from the list of capture genes with the 5 % of SNP with the greatest effect and their classification into 4 classes associated with the p-value of enrichment.

Pathways	**P-value**
Signaling	
Phosphatidylinositol signaling system	0.0042
Adherens junction	0.0094
Phoaphopase D signaling pathway	0.0240
Pathways in cancer	0.0310
Axon guidance	0.0370
Calcium signaling pathway	0.0480
Metabolism	
Glycosaminoglycan biosynthesis - heparin sulfate/heparin	0.0070
ECM - receptor interaction	0.0270
Metabolic pathway	0.0340
Steroidogenesis	
Ovarian steroidogenesis	0.0170
Others	
Arrhythmogenic right venticular cardiomyopathy	0.0370

The gene ontology of functional enrichment is shown in Figure 8.2. There are several terms linked to the regulation of ATP, calcium, cell activity, locomotion and behaviour, lipid and carbohydrate metabolism, modulation of synaptic transmission, GTPase activity among others.

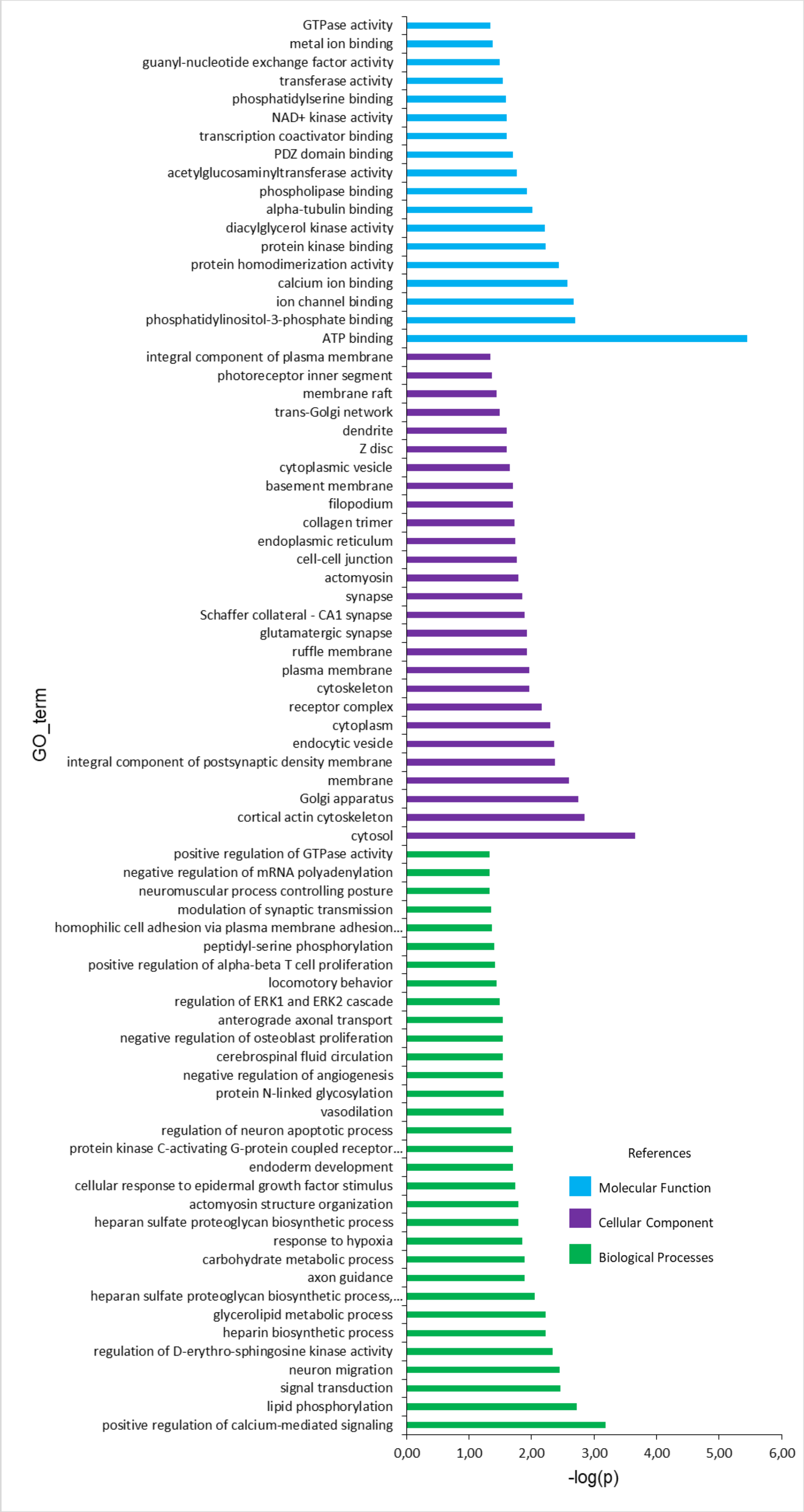

GTPase activity
metal ion binding
guanyl-nucleotide exchange factor activity
transferase activity
phosphatidylserine binding
NAD+ kinase activity
transcription coactivator binding
PDZ domain binding
acetylglucosaminyltransferase activity
phospholipase binding
alpha-tubulin binding
diacylglycerol kinase activity
protein kinase binding
protein homodimerization activity
calcium ion binding
ion channel binding
phosphatidylinositol-3-phosphate binding
ATP binding
integral component of plasma membrane
photoreceptor inner segment
membrane raft
trans-Golgi network
dendrite
Z disc
cytoplasmic vesicle
basement membrane
filopodium
collagen trimer
endoplasmic reticulum
cell-cell junction
actomyosin
synapse
Schaffer collateral - CA1 synapse
glutamatergic synapse
ruffle membrane
plasma membrane
cytoskeleton
receptor complex
cytoplasm
endocytic vesicle
integral component of postsynaptic density membrane
membrane
Golgi apparatus
cortical actin cytoskeleton
cytosol
positive regulation of GTPase activity
negative regulation of mRNA polyadenylation
neuromuscular process controlling posture
modulation of synaptic transmission
homophilic cell adhesion via plasma membrane adhesion…
peptidyl-serine phosphorylation
positive regulation of alpha-beta T cell proliferation
locomotory behavior
regulation of ERK1 and ERK2 cascade
anterograde axonal transport
negative regulation of osteoblast proliferation
cerebrospinal fluid circulation
negative regulation of angiogenesis
protein N-linked glycosylation
vasodilation
regulation of neuron apoptotic process
protein kinase C-activating G-protein coupled receptor…
endoderm development
cellular response to epidermal growth factor stimulus
actomyosin structure organization
heparan sulfate proteoglycan biosynthetic process
response to hypoxia
carbohydrate metabolic process
axon guidance
heparan sulfate proteoglycan biosynthetic process,…
glycerolipid metabolic process
heparin biosynthetic process
regulation of D-erythro-sphingosine kinase activity
neuron migration
signal transduction
lipid phosphorylation
positive regulation of calcium-mediated signaling
GO_term
0,00
1,00
2,00
3,00
4,00
5,00
6,00
-log(p)
References
Molecular Function
Cellular Component
Biological Processes

Figure 8.2: Gene Ontology categories represented by Molecular Functions (light blue color), Cellular Component (purple color) and Biological Processes (green color), for the set of positional candidate genes with the 5 % of SNP with the greatest effect in function del –log of the enriched p-value.

8.3 Conclusions

The present study allowed to know SNP associated with the temperament trait in sheep. These SNP were not previously reported, which opens an exploration window for future work, not only linked to temperament but also to different metabolic pathways that could have an impact on aspects of production and welfare.

The genetic variance of the SNP revealed that there are regions of the genome associated with greater variability and that temperament is not regulated by the effect of a major gene, but rather is a multigenic characteristic. Nine genes were detected in the genomic regions on the chromosome 6, 10 and 21 (genes: GRID2, GPRIN3, LOC101110773, LOC101110521, PYGM, SYVN1, CAPN1, FADS1, SYT7), linked to the energetic activity of the organism, synaptic transmission, meat tenderness and activity of calcium. The identification of these genes, metabolic pathways and their respective functions should contribute to a better understanding of the genetic mechanisms that regulate the temperament trait in sheep.

The functional enrichment analysis allowed to identify gene functions and complement the results of the association study. The 5 % of the SNP with the greatest effect determined a set of 900 genes linked to various metabolic pathways, where the gene ontology analysis shows that there are several processes linked to the regulation of ATP, calcium, cell activity, locomotion and behaviour, lipid and carbohydrate metabolism, modulation of synaptic transmission, among others. Several of these metabolic pathways are of interest for the temperament trait and lead to the need for further exploration.

9 Conclusion

The present deliverable focused on (i) monitoring key behavioural adaptation traits in extensive and intensive farming systems utilising new technologies and (ii) estimating phenotypic and genetic parameters for indicators of behavioural adaptation. Specifically, six studies were conducted to assess (i) GPS generated phenotypes for behavioural adaptation to extensive grazing conditions in Boutsko sheep, (ii) behavioural reactivity at weaning and maternal reactivity at lambing in Romane sheep, (iii) relationship between efficiency and behavioural adaptation in Romane sheep, (iv) foetal and neonatal survival phenotypes in French meat sheep breeds, (v) lamb neonatal vigour and ewe mothering ability in meat sheep reared in Ireland, and (vi) lamb temperament in Merino sheep. In Boutsko sheep, results suggest that there is significant between-animal variation for most grazing behaviour traits to support management practices aiming to improve adaptation to extensive rearing conditions. Genomic selection practices could be further implemented for grazing duration and speed. In Romane sheep, behavioural reactivity at weaning and maternal reactivity at lambing could be improved through genetic selection practices. Importantly, early selection for sociability toward conspecifics or docility toward humans could help to improve adult behaviour and increase adaptation in challenging environments. Moreover, results indicate that Romane lambs from artificial rearing could adapt to extensive conditions after weaning; however, during the transition period, their growth rate and body reserve mobilisation might be lower and higher, respectively compared to lambs reared with their mothers in extensive conditions. In French meat sheep breeds, results suggest that lamb mortality increases proportionally with litter size. In meat sheep reared in Ireland, lamb mortality could be reduced through genetic selection for lamb vigour and ewe mothering ability. Finally, in Merino sheep, lamb temperament is heritable with results indicating that it is a multigenic characteristic that could be amenable to genomic selection.

10 Deviations or delays

Deviations are described below:

AUTH: Monitoring of animals was scheduled to start in May 2020. However, due to COVID-19 restrictions it started in June 2021 (monitoring animals in the mountain is possible from May until mid of September). Hence, monitoring of 800 animals (foreseen in the DoA) was not feasible. The sample size of 300 animals allowed for the estimation of phenotypic parameters as well as genomic parameters for most of the studied traits. Moreover, body condition score, footrot and litter size were not considered in the analyses since relevant data were not available in the studied farms and recording was difficult to perform in extensive conditions.

11 Acknowledgements

The contribution of farmers that were involved in the presented studies is gratefully acknowledged.

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13 Appendix

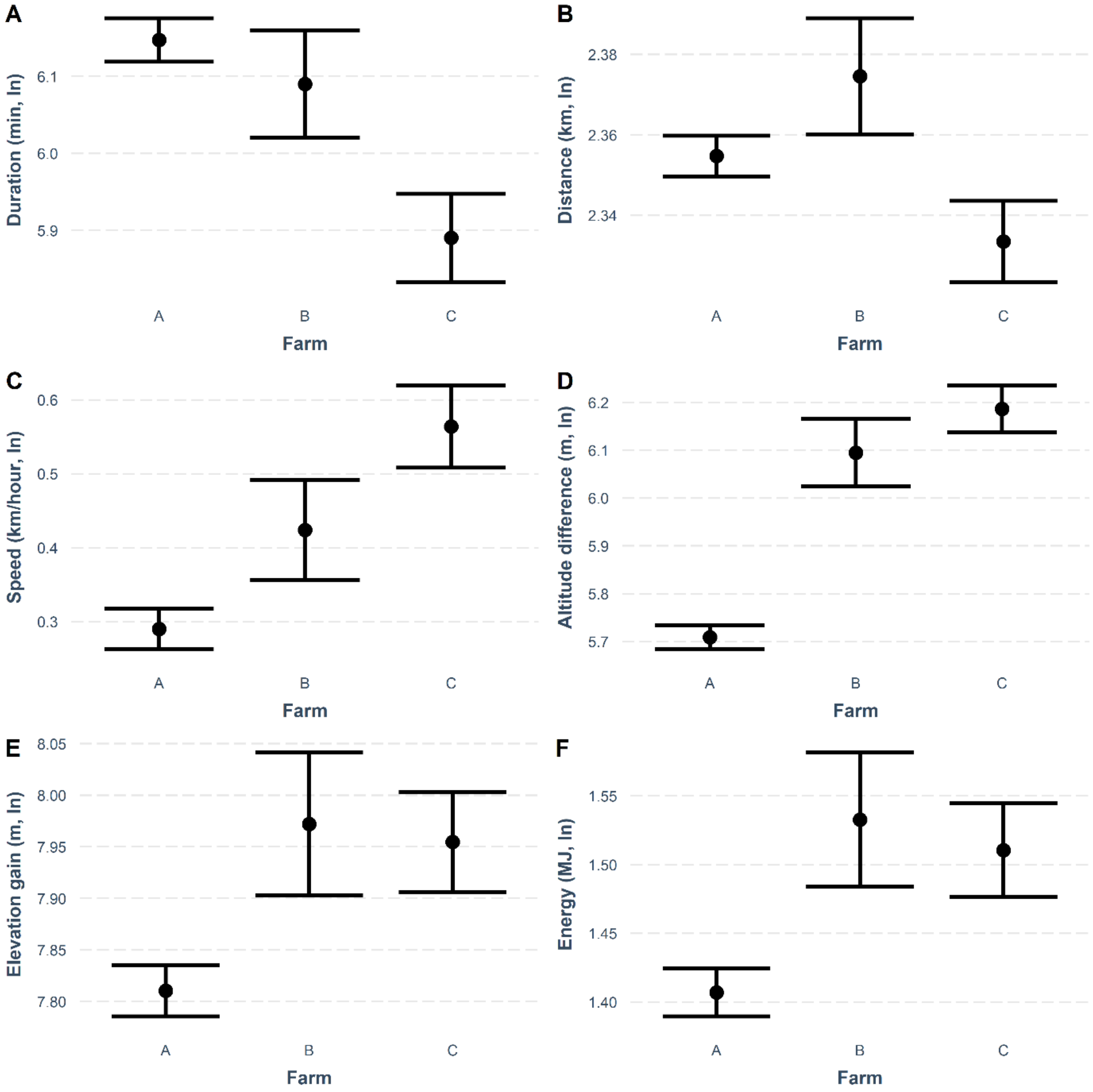

Figure S1. Plots of significant effects ($P<0.05$) of farm on daily grazing behaviour phenotypes of Boutsko sheep.

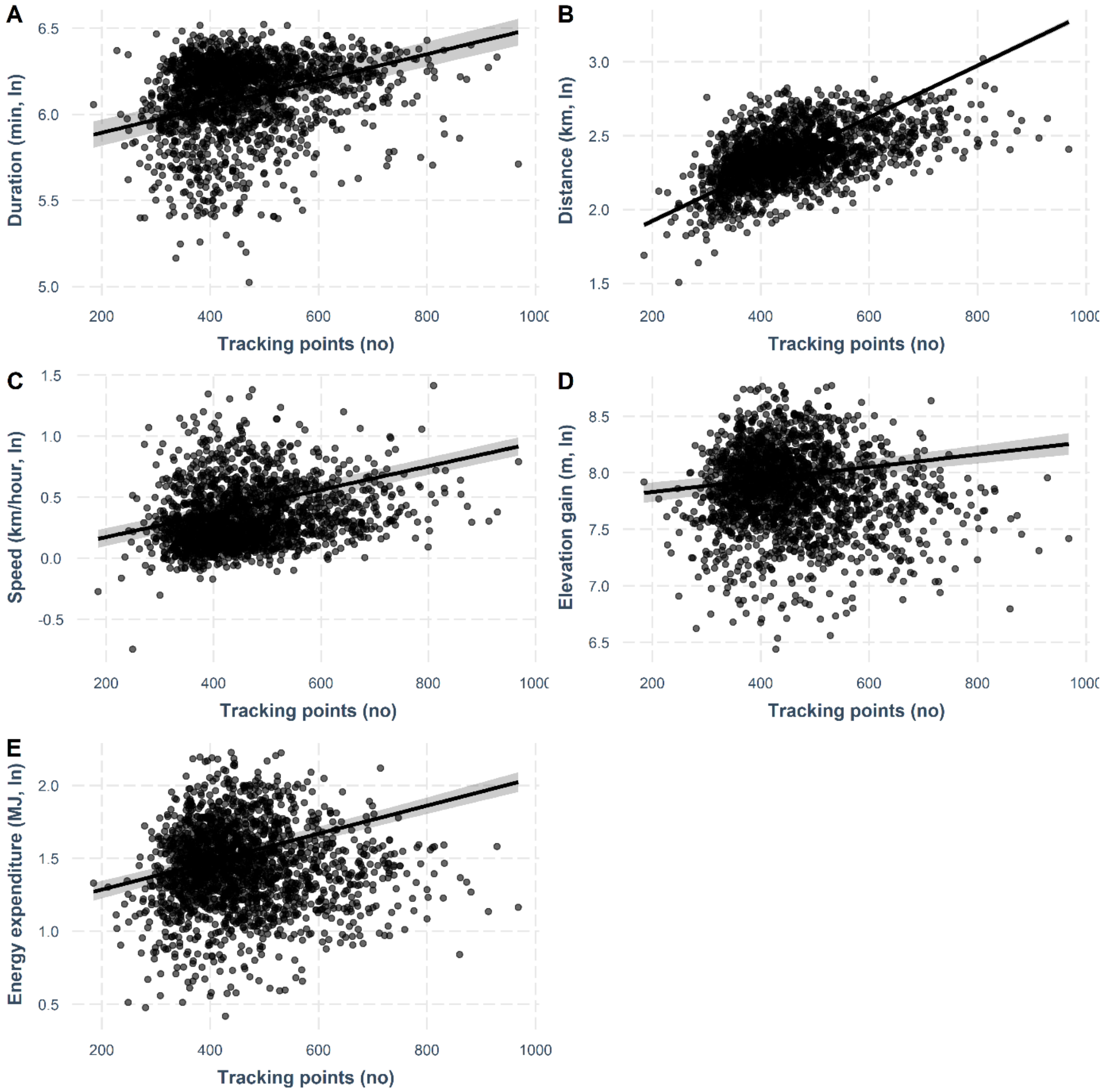

Figure S2. Plots of significant effects (P<0.05) of GPS tracking points on daily grazing behaviour phenotypes of Boutsko sheep.

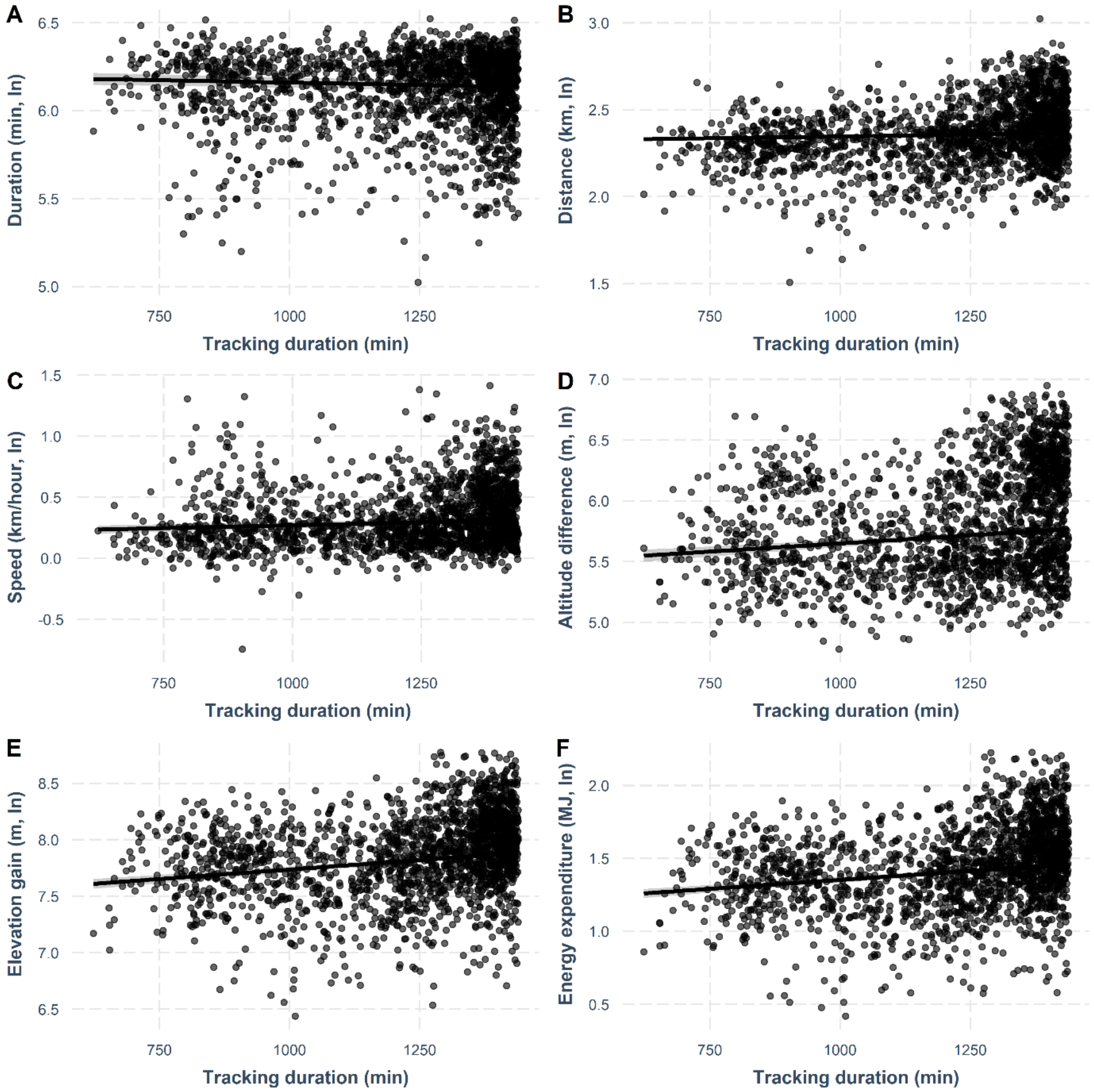

Figure S3. Plots of significant effects (P<0.05) of GPS tracking duration on daily grazing behaviour phenotypes of Boutsko sheep.

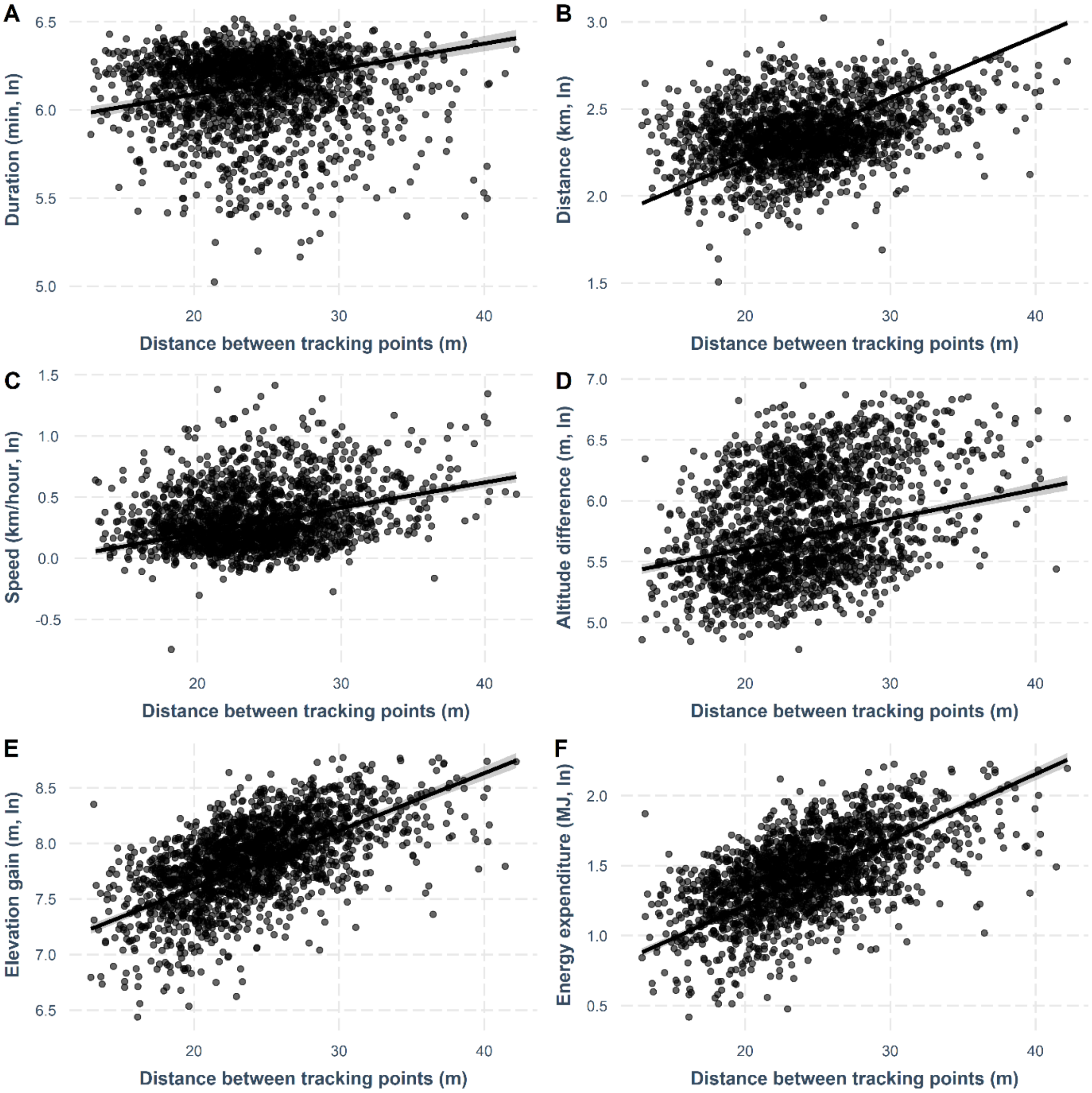

Figure S4. Plots of significant effects ($P<0.05$) of distance between GPS tracking points on daily grazing behaviour phenotypes of Boutsko sheep.