Avec la contribution financière du compte d'affectation spéciale développement agricole et rural CASDAR
MINISTÈRE DE L'AGRICULTURE DE LA SOUVERAINETÉ ALIMENTAIRE ET DE LA FORÊT

SMARTER

SMAll RuminanTs breeding for Efficiency and Resilience

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

Guidelines for recording efficiency and resilience traits in sheep and goats

Jean-Michel Astruc[1], Cesare Mosconi[2], Juan-José Arranz[3], Bente Aspeholen[4], Ignacio de Barbieri[5], Donagh Berry[6], Beatriz Carracelas[5], Antonello Carta[7], Gabriel Ciappesoni[5], Marjorie Chassier[1], Joanne Conington[8], Arnaud Delpeuch[1], Philippe Hassoun[9], Dominique Hazard[9], Jette Jakobsen[10], Karolina Kaseja[8], Gilles Lagriffoul[1], Nicola Lambe[8], Coralie Machefert[9], Fiona McGovern[6], Isabelle Palhière[9], Carolina Pineda Quiroga[11], Suzanne Rowe[12], Ed Smith[8], Flavie Tortereau[9], Eva Ugarte[11], Sotiria Vouraki[13], Steffen Werne[14], Carole Moreno-Romieux[9] and Rachel Rupp[9]

[1,2,*] IDELE, ICAR – responsible for deliverable, [2] ICAR, [3] UNILEON,
[4] Norwegian University of Life Sciences, Norway, GrassToGas, [5] INIA-UY,
[6] TEAGASC, [7] Agris Sardegna (stakeholder platform, ICAR SGC-WG), [8] SRUC, [9] INRAE
[10] NSG, [11] NEIKER, [12] AgResearch NZ, stakeholder platform, GrassToGas
[13] AUTH, [14] FIBL-QUALITAS

* Deliverable leader – Contact: Jean-Michel.Astruc@idele.fr

DELIVERABLE D6.3

Workpackage N°6

Due date: M56

Actual date: 16/06/2023

Dissemination level: Public

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

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, agroecological 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.

Table of contents

1 Summary

The deliverable involves pulling together recommendations on recording efficiency and resilience traits in sheep and goat, with the aim to propose these recommendations to be published in ICAR guidelines. These recommendations are also extended to the record of the environment, especially the meteorological data and the diet. The materials used to write the recommendations are those produced by SMARTER in work packages 1 (efficiency) and 2 (resilience). The recommendations also use results obtained in other projects, such as ERA-GAS GrassToGas (on feed efficiency and greenhouse gas emissions in meat sheep), H2020 iSAGE and POCETFA ARDI (record of meteorological data). The ICAR network, with its working group on sheep, goat, and camelid, also contributed. The recommendations concern seven sections: two on efficiency traits (feed efficiency and greenhouse gases emissions), four on resilience traits (health and disease, survival of foetus and young, behaviour traits, lifetime resilience), one on the record of the environment. The different sections follow the same template: introduction, scope, definition, terminology and rationale, data recording and calculation of traits, use for genetic analysis and genetic evaluation. The harmonisation of the outline of each section, on the same pattern as the one proposed by ICAR will help to translate shortly the recommendations in ICAR guidelines. The phenotypes covered by the sections include the measures doable in experimental situations only and proxies that can be collected routinely on-farm. This deliverable was an opportunity to gather skills and inputs from different work packages to translate results from research into practical recommendations usable by breeding organisations for actual selection. The publication as ICAR guidelines will be handled in the next months by the ICAR working group on sheep, goat and camelid and will strengthen the impact of SMARTER towards a larger community, including academic and non-academic, as well as services organisations in selection.

2 Introduction

The aim of the work package 6 of SMARTER is to promote harmonisation and international cooperation on breeding processes in small ruminant, especially those concerning the selection of efficiency and resilience. The three case studies of across country genetic evaluation, implemented in SMARTER on existing traits as a proof of concept, have highlighted the importance of analysing traits that have been collected and/or calculated on a same way across country. Therefore, it appears fundamental that novel traits, such as those studied and suggested in SMARTER, which are not still widely routinely recorded on-farm for selection purposes, be recorded identically, or at least in the most similar way as possible.

For that purpose, recommendations must be proposed, for countries or breeding organisations that would like to start to record efficiency or resilience traits, or that would like to set up an across-country genetic evaluation on these traits. The more similar the traits, the higher the genetic correlation across country (at same level of connection across country).

The recommendations are basically written thanks to the work achieved in the work packages studying efficiency (WP1) and resilience (WP2). Therefore, this deliverable is the fruit of a close cooperation between several work packages, hence the large number of co-authors of this deliverable. As a similar deliverable (limited to feed efficiency and greenhouse gases emissions in meat sheep) was to be submitted in the ERA-GAS GrassToGas project contemporary to SMARTER, and as most of the research teams were similar across both projects, we decided that the section dedicated to feed efficiency and greenhouse gases emissions be used as deliverable in both projects, and we worked jointly to strengthen and consolidate the outputs. We also collected materials and results obtained in other projects (e.g. H2020 iSAGE, POCTEFA ARDI). Finally, we involved the members of the ICAR working group on sheep, goat and camelid to review the deliverable and bring their view, skills and inputs. By doing that, we have prepared the future transcription of the deliverable into ICAR guidelines, that is planned in the months beyond the project.

Tackling the record of the environment is a novelty in selection of small ruminant. Recording the diet is required to estimate the input component of feed efficiency. Recording the meteorological data (especially temperature and humidity) may help to study and select the thermotolerance of animals, with the aim to adapt the small ruminants to the climate change.

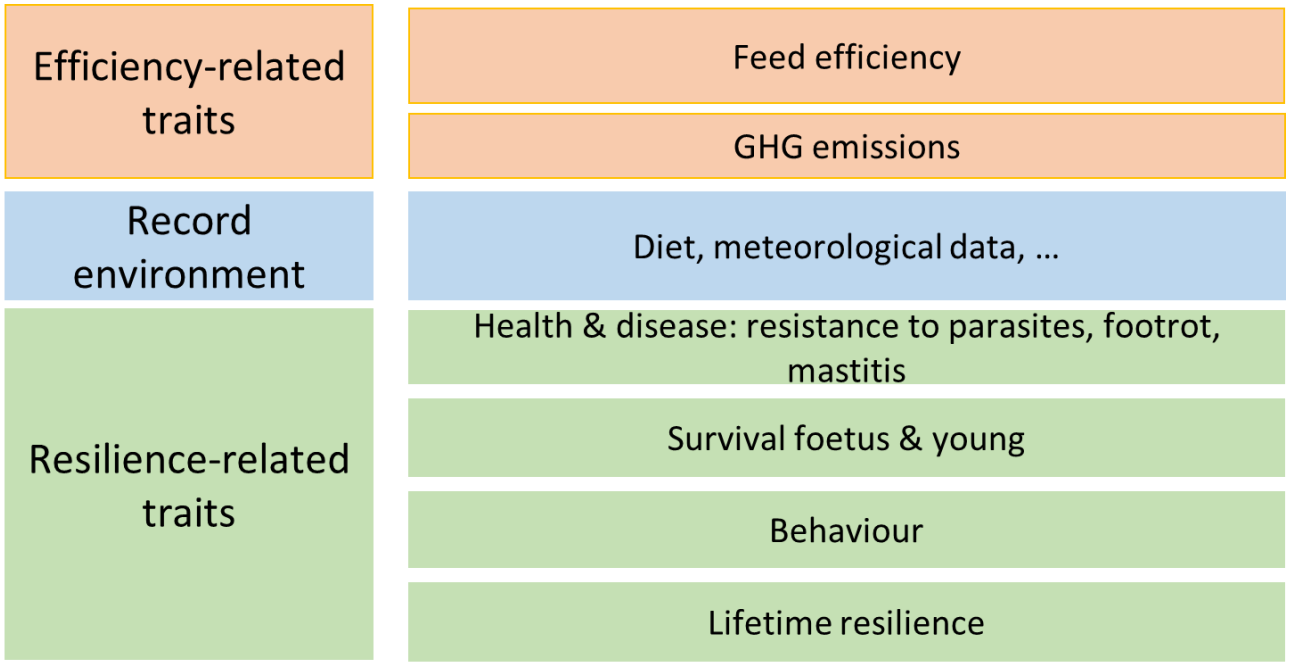
The recommendations, even though they target to suggest people measuring and calculating the traits the same way, are more informative than normative. The different ways to measure and calculate the traits are presented, without imposing one way, yet while suggesting some general features.

7 sub-sections of recommendations were written: feed efficiency, greenhouse gases emissions, record of the environment, health and disease, survival of foetus and young, behaviour, lifetime resilience. All sub-sections are written with the same template, which has been chosen to be similar as the current template of ICAR guidelines. This might ensure an easy transcription to ICAR guidelines. In theory, the same text should be taken as ICAR guidelines.

All the recommendations are based on the current state of the art. However, they are meant to evolve with new results and new research, and they are meant to be enhanced, consolidated, enriched. It is possible to add a new trait, a new proxy, a new sub-section. In brief, the recommendations must keep alive to stick to the evolving state of the art. This implies that the consortium that produced these recommendations, in some way, continue to contribute. ICAR, with its working group dedicated to sheep and goat, might be a relevant organisation to collect and integrate the different novelties and contributions. In this respect, we have suggested in the D6.4 of SMARTER that ICAR be part of a consortium (possibly with Interbull) for becoming an EU Reference Centre for harmonising & improving methods for performance testing and genetic evaluation in bovine species in small ruminants.

3 Scope of the recommendations

The SMARTER recommendations cover the following fields, shown in the figure 1.



The efficiency-related traits are those studied in the work package 1: feed efficiency and greenhouse gases (GHG) emissions. The recommendations benefited from inputs from the ERA-GAS GrassToGas project gathering similar research teams and working specifically on GHG emissions in meat sheep.

The resilience-related traits are those studied in the work package 2: health and disease (with a focus on resistance to parasites, to footrot, and to mastitis), survival foetus and young, behaviour traits (with a focus on behavioural reactivity towards conspecifics or humans, maternal reactivity, behaviour at grazing), lifetime resilience. The recommendations benefited from experience and results acquired in other projects.

The record of the environment covers the meteorological data and the diet. The record of the rations was studied in the on-farm protocols of WP1, especially in France. The record of the meteorological data benefited from works carried out in the H2020 iSAGE and POCTEFA ARDI projects, some of the SMARTER partners being committed in those projects.

The recommendations are conceived to be evolutive. Amendments can be brought in the next years, especially when the recommendations will turn into ICAR guidelines, either to strengthen results or include new insights, or to add new sub-sections or new traits. For example: (i) in the record of the environment, sensor data may be included; (ii) new proxies of feed efficiency or GHG emissions may be added in the future; (iii) new disease whose resistance has a genetic component may be added as well.

4 Sub-sections of recommendations

Each of the seven sub-sections constitutes a document *per se* with its own template and consistent by itself. Consequently, seven are provided with this deliverable, not as appendixes but as separate documents.

4.1 Feed efficiency recording in sheep and goat

See document:

"SMARTER D6.3 - Recommendations on recording feed efficiency_vfinal.docx"

4.2 Recording greenhouse gas emissions in sheep and goat

See document:

"SMARTER D6.3 - Recommendations on recording ghg_vfinal.docx"

4.3 Recording the environment in sheep and goat

See document:

"SMARTER D6.3 - Recommendations on recording the environment_vfinal.docx"

4.4 Health and disease: recording the resistance to parasites, to footrot and to mastitis in sheep and goat

See document:

"SMARTER D6.3 - Recommendations on recording health and disease traits_vfinal.docx"

4.5 Recording survival traits of foetus and young in sheep and goat

See document:

"SMARTER_D6.3_Recommendations on recording survival of foetus and young_vfinal.docx"

4.6 Recording behavioural traits in sheep and goat

See document:

"SMARTER_D6.3_Recommendations on recording behaviour traits_vfinal.docx"

4.7 Recording lifetime resilience in sheep and goat

See document:

"SMARTER D6.3 - Recommendations on recording lifetime resilience_vfinal.docx"

5 Deviations or delays

No delay nor deviation

6 Acknowledgements

All the people working in WP6 and beyond (other WP, other projects, ICAR working group on sheep, goat, camelids) have participated in this collaborative work.

The documents giving the recommendations of each sub-sections list in their own acknowledgements the persons involved in the writing of the guidelines.

7 References

The technical references (papers cited or used) are documented in each piece of recommendations.

8 Appendix

7 additional files:

SMARTER D6.3 - Recommendations on recording feed efficiency_vfinal.docx

SMARTER D6.3 - Recommendations on recording ghg_vfinal.docx

SMARTER D6.3 - Recommendations on recording the environment_vfinal.docx

SMARTER D6.3 - Recommendations on recording health and disease traits_vfinal.docx

SMARTER_D6.3_Recommendations on recording survival of foetus and young_vfinal.docx

SMARTER_D6.3_Recommendations on recording behaviour traits_vfinal.docx

SMARTER D6.3_Recommendations on recording lifetime resilience_vfinal.docx

Recommendations on recording feed efficiency in sheep and goat

SMARTER – Deliverable D6.3

Version 1 – 7 June 2023

Table des matières

Change Summary

Date of change	Nature of Change
March 2023	First draft
June 2023	Final version for SMARTER deliverable 6.3

1 Introduction

Feed is one of the most important costs in animal production systems. Considering the reported genetic variation of feed conversion efficiency of animals, improving it would be a relevant way to decrease costs without unfavourably affecting animal performance (Cammack *et al.* 2005; Paganoni *et al.* 2017). In the face of growing demand of animal products and of limiting resources, in particular feed (quantity and quality) and land for animal production, enhancing feed efficiency can help to address these challenges in sustainable small ruminant production systems.

Efficiency of feed resource use is a complex trait influenced by several factors such as food characteristics, behaviour, gut microbiota, genetic background and animal physiological state.

2 Scope

These guidelines are based on the experience and protocols set up by partners of the European projects SMARTER (H2020 – 772787) and GrassToGas (FACE ERA-GAS), both dealing partially or entirely with feed efficiency in small ruminants. The present guidelines are not set in stone: new protocols, and evolving scientific methods will help improve and enrich the recommendations on estimating feed efficiency.

This report first describes the different protocols currently in use in experimental (section 4.1) and commercial farms (section 4.2) to enable the recording of elementary traits used to estimate feed efficiency criteria presented in 4.4.

We then propose general recommendations that can be drawn from the different protocols (4.3).

In the section 4.5, we list a series of proxy traits that have been studied to predict feed efficiency traits, either in experimental or in commercial farms. For each of them, the protocol for recording the trait, the accuracy of the prediction and the level of ease of data recording for the proxy traits are given.

Section 5 deals with the genetic evaluation of the traits, with (i) suggestion of the genetic model and typical range of estimated genetic parameters.

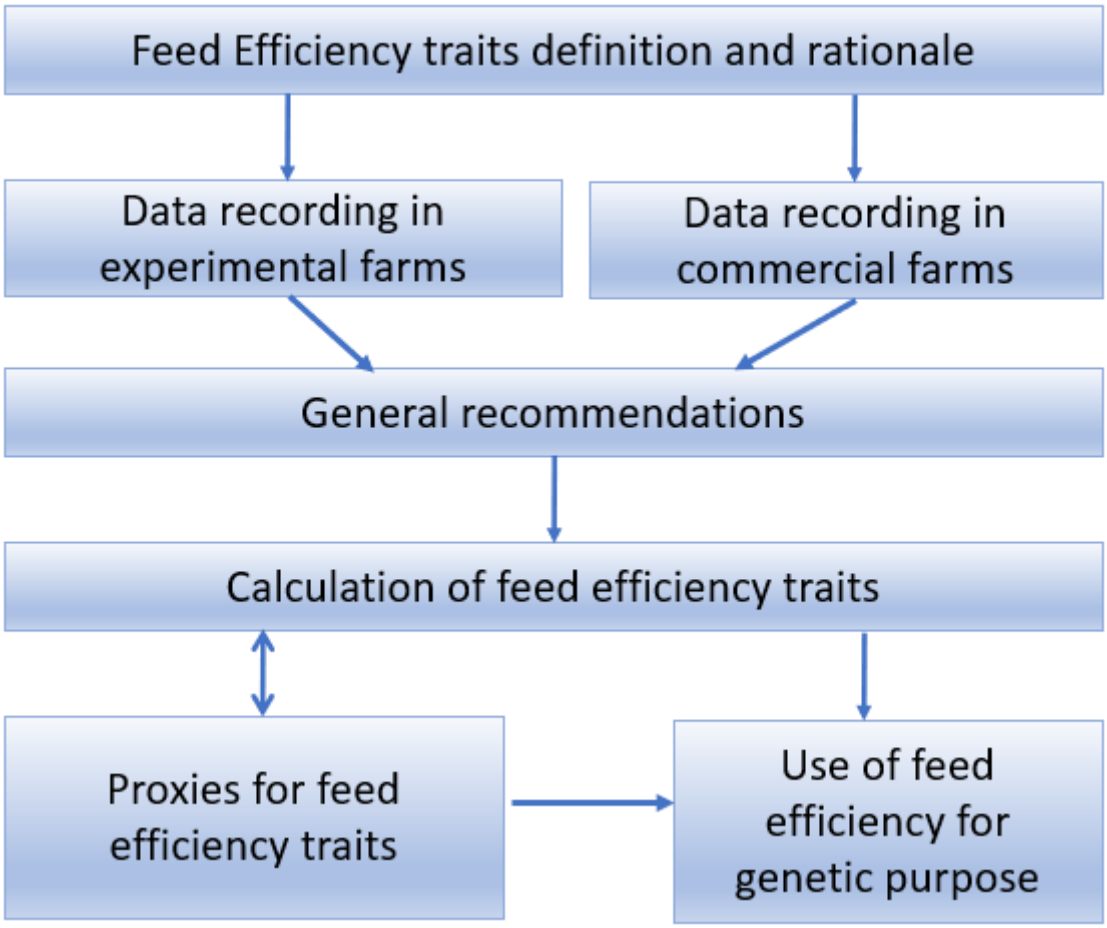


Figure 1. Scope of guidelines on Feed Efficiency recording in sheep and goat.

3 Feed efficiency definition, terminology, rationale

Feed efficiency can be seen as the ability of an animal to convert its feed intake into animal products for humans. This complex trait encompasses a number of underlying biological processes such as digestion, metabolism, thermoregulation, activity, etc… Indirect criteria of feed efficiency, such as relative growth rate and Kleiber ratio will not be considered in this document. Direct criteria have been proposed to characterize feed efficiency, such as feed conversion ratio and residual feed intake (RFI), based on feed intake and performance (growth, milk) records. Feed conversion ratio is the relationship between daily feed intake and body growth. RFI is the difference between observed and predicted feed intake based on requirements for growth, production (average daily gain, wool, or milk production) and maintenance (metabolic bodyweight) (Koch et al., 1963).
It has been shown that genetic selection for ratio traits can lead to unexpected responses (Gunsett, 1984). Therefore, in a perspective of integration of feed efficiency in ruminant breeding programs, RFI is the most commonly used trait.

Acronyms

ACF	Automated Concentrate Feeder
ADG	Average Daily Gain
ADFI	Average Daily Feed Intake
BCS	Body Condition Score
BFT	Backfat Thickness
BW	Body Weight
CW	Chest Width
DEI	Daily Energy Intake
DG	Dairy Goats
DIM	Days In Milk
DM	Dry Matter
DMI	Dry Matter Intake
DS	Dairy Sheep
DMY	Daily Milk Yield
EID	Electronic Identification
FC	Fat Content
FCR	Feed Conversion Ratio
LFCR	Lactation Feed Conversion Ratio
MD	Muscle Depth
MS	Meat Sheep
PAC	Portable Accumulation Chambers
PC	Protein Content
REI	Residual Energy Intake
RFI	Residual Feed Intake
SMY	Standardised Milk Yield
TMR	Total Mixed Ration

4 Recording of feed efficiency

The different components of feed efficiency are:

- the requirements of the output (energy, or protein), which are the production requirements for milk, wool and meat production as well as, the animal maintenance requirements, and body reserve variation.
- the feed intake

4.1 Protocols in experimental farm

4.1.1 Description protocol in INIA Uruguay and INRAE France – Meat sheep

This protocol is applied both at INIA-Uruguay and INRAE-France. When specificities exist for one of the two institutes, it is mentioned in the document.

Feed efficiency is influenced by a number of factors such as age, sex, breed, among others. Generally, the protocols are designed to limit the number of factors of variation or else they are accounted for statistically in the analyses.

At INRAE, only phenotypes from males of a given breed are used starting from 90 days of age on average. Matings are planned ahead in order to limit the variability of the age of the phenotyped lambs. Additional feed intake phenotyping can be performed until 12 months old, following the same protocol but different feed.

At INIA, several meat and wool breeds are phenotyped, starting from weaning until 13-months-old (late maturing ones are those phenotyped at the end). Each breed is phenotyped at the same age, every year. Male and female lambs can be phenotyped in the same or different tests depending on the breed and on the number of animals per breed.

In practice, to estimate residual feed intake, 20 lambs are gathered in each pen, according to their bodyweight. The connection among pens and tests is also considered (more than one sibling per pen, more than one sire per pen). Each pen is equipped with automatic concentrate or forage feeders (one or five per pen at INRAE and INIA respectively), delivering concentrate *ad libitum* (chopped Lucerne haylage at INIA, bins are filled three to four times a day). Each visit to the automatic feeder is recorded (hour, duration, quantity of fresh matter intake) and associated with the animal via electronic identification (EID). During the first two weeks, lambs are accustomed to their new environment and learn how to get feed from the automatic feeder. The number of visits of each animal to the feeder is monitored, so that animals that have difficulties to access to the feeder are trained by the technical staff. The time of occupation of the feeder is regularly checked during these two weeks: we set a maximum of 22 hours per day to ensure that each lamb is fed *ad libitum*. If the occupation time is above the threshold, one animal is removed from the experiment (the one which the largest duration of visit, or the one with the lowest average daily live weight gain (ADG). At INIA, lambs are weighed automatically on a daily basis. At INRAE, lambs are weighed before and after the two weeks and individuals with very low or negative ADG are removed from the experiment.

After these 2 weeks of adaptation, the feed intake recording period can start, for 6 weeks. At INRAE, lambs are weighed at the beginning, at the middle and at the end of the 6-week period. At INIA, lambs are automatically weighed when they drink water, via 2 weighing platforms installed in each pen. Backfat ultrasonic measurements are performed at the middle and at the end (INRAE) or at the end only (INIA) of the 6-week period, at the level of the last floating rib at 1 cm from the spine, to estimate muscle depth of the M. longissimus dorsi and backfat thickness. These measurements are accounted for in the analyses to ensure that the analysis of feed intake is independent of body carcass composition.

During the 6-week period, any information about health problems, technical defect, etc. is registered. At INIA, the dry matter content of the feed and its chemical composition are monitored twice per week.

At the end of the 6-week period, the raw datasets from INRAE contains on average one observation every 500 milliseconds, from which a table with each visit description (ID, day, hour, duration, quantity) is computed (after removal of multiple and/or unattributed visits or biological unlikely data). A similar dataset is obtained at INIA.

The second step sums up each visit per animal and per day in order to get daily feed intakes, which are subsequently converted to Kg dry matter intake (Kg DMI).

The final step is to average these daily feed intakes over the 42 days of measurements.

At the end of the experiment, all the required traits to calculate RFI are available: average daily dry matter feed intake, body weight (to calculate metabolic BW) daily or at the beginning and at the end of the control, from which we calculate the average daily gain, and the two ultrasound traits (backfat thickness and muscle depth or area).

4.1.2 Description protocol in AgResearch New Zealand – Meat sheep

This protocol is applied at AgResearch.

The AgResearch Invermay feed intake facility is a converted set of covered sheep yards that houses 20 custom built automated feeders. Each feeder can feed up to 10 sheep, and as such the maximum number of animals measured through the facility at one time is 200 animals. The facility has a dirt floor that is topped up every two weeks with fresh untreated sawdust. The animals are provided ad libitum access to clean water.

The automate feeders deliver pelleted feed into a tray that is mounted on weigh scales. All animals are tagged with EID tags. Each feeder has an EID reader mounted near the feed tray which registers which animal is present at any point in time. Each feeding event is electronically recorded, reporting: Feeder, Start and End Time of Feeding Event (from which the duration of the feeding event can be calculated) and the Amount of Feed Eaten. The animals are provided ad libitum access to the feeders and as such feed.

Any age of sheep can be measured through the facility, however, the most commonly used is young stock between 4-12 months of age. Any sex of stock can be measured through the facility, however, most commonly non-pregnant ewe lambs are used. Any type of pelleted feed can be used, however, the most commonly used is lucerne pellets.

The animals are in the facility for eight weights, which is made up of a 2-week adaptation period and then a 6-week recording period. During the 2-week adaptation period animals are initially weighed daily and feed intakes monitored, once animals have achieved eating 1000g per day they are no longer weighed daily but daily intakes continue to be monitored. There is always a small percentage of each group of animals that will not adapt, so more animals than are needed are often included in the training to account for this.

During the recording period animal intakes are monitored daily and the animals are weighed bi-weekly. Other measurements are often taken alongside the intake measurements which may include

ultrasound scanning for fatness at the beginning and end of the trial, or methane measurements through the Portable Accumulation Chambers (PAC).

The resulting data set is a collation of all individual feeding events for all animals across the 6-week recording period. From time to time there are issues with the feeders and or scales such that erroneous data may be included in the data set and a data cleaning step is required before the data can be used in any subsequent analysis. The data cleaning step for the live weight data involves fitting a regression model is fitted to the ~12 live weight data points obtained during the recording period and the 95% confidence boundaries determined, and any live weights that fall outside of the boundaries are excluded. The cleaned dataset is re-analysed fitting a regression model, the resulting model is used to determine the mid-recording live weight and the growth rate (which is the slope of the equation). The data cleaning step for intakes involves firstly excluding any individual feeding events that are greater than 1200g. Subsequently a regression model is fitted to the ~42 feed intake data points obtained during the recording period and the 95% confidence boundaries determined, and any feed intake weights that fall outside of the boundaries are excluded. The cleaned dataset is re-analysed fitting a regression model, the resulting model is used to determine the mid-recording daily feed intake.

The data set is also curated to determine other feeding behaviour traits for each animal including average number of feeding events per day, the average intake per individual feeding event, the average duration of an individual feeding event and the average feeding rate (average intake per feed per feeding event/average duration).

The resulting data set is most commonly analysed to estimate the trait of residual feed intake.

4.1.3 Description protocol in SRUC the UK – Meat sheep

This protocol is applied at SRUC.

Post-weaning, lambs are selected for feed intake recording to be representative of the population under investigation. Approximately 125 lambs are recorded in one batch. All lambs are housed in one pen with access to *ad-libitum* grass nuts fed through 16 BioControl feed bins. Sufficient water troughs are supplied to allow unlimited access to water.

Data are recorded for every meal eaten by every lamb, by using EID tags and BioControl software. Each visit to the automatic feeder is recorded (duration and quantity). A two-week adaptation period allows lambs to become accustomed to their new environment and diet, and to learn how to use the feeders. The number of visits of each animal to the feeder, and the daily amount of feed eaten, is monitored, so that animal that have difficulties accessing the feeder can be identified and trained by the technical staff. Live weights are taken weekly. If an animal shows signs of not training to use the feeders (low number of visits, or low daily intake) by the end of the 2-week training period, or if the animal fails to achieve acceptable growth rates, it is removed from the experiment.

The 2-week adaptation period is followed by a feed intake recording period of 6 weeks. Bodyweights are taken weekly. Fat and muscle levels are recorded at the start and the end of the recording period, by ultrasound (as previously described) and/or CT scanning (Lambe et al., 2003). Feed intake data is monitored daily (daily number of visits per lamb and per bin; daily feed intake per lamb) to identify any technical or animal-related issues. Bins are kept filled at all times and not allowed to run low on

grass nuts. Feed bins are emptied and calibrated once per fortnight. Reflectors (reflecting laser beam to identify when sheep are feeding) are cleaned regularly (at least weekly). Feed samples are sent for laboratory analysis from each batch (e.g. 1 tonne bag) of feed. During the 6-week period, a trial diary is maintained to record useful information about technical issues, animal handling, feed bin refill times etc.

At the end of the 6-week period, raw datasets are collated from across the feeding period into one spreadsheet with a description of each visit per lamb (RFID, day, hour, duration, quantity, eating speed). Data rows are removed from the dataset following a number of data cleaning rules. The visit record is removed if: feed eaten >1kg; eating speed >10 g/s; visit duration = 0; feed eaten = 0; duration <1min and feed eaten >300g; eating speed >2 and feed eaten >500g.

The second step is to sum each visit per animal and per day in order to have daily feed intakes. Average daily dry matter intake (DMI) is then calculated for each lamb using the dry matter content of the bag of grass nuts that was fed on that day.

The final step is to average these daily DMI values over the 6 weeks (=42 days) of the feeding period. For each lamb, a regression is plotted of DMI values against day and daily values are removed if they are >2 SD from the predicted regression line for that individual (usually only ~1-2 daily values removed per lamb). The remaining daily DMI values are then averaged per lamb to provide one value for average daily dry matter intake.

Using weekly lamb live weight records during the trial period, a regression is plotted of live weight against day and any outlying weight values for an individual lamb are removed (to achieve R2 >0.8). Mid-test metabolic live weight is then calculated for each lamb (MMWT = predicted LWt @ 21d ^0.75). Average daily live weight gain (ADG) is also calculated per lamb from the slope of the regression.

At the end of the experiment we have all the required traits to calculate RFI: average daily dry matter intake during the feeding period, weekly live weights throughout the trial, from which we calculate the average daily gain, ultrasound traits (backfat thickness and muscle depth) and/or CT predicted carcass fat and muscle weights.

4.1.4 Description protocol in Teagasc Ireland – Meat sheep

This protocol is applied at Teagasc, Ireland, in out of doors (grazing) of lambs (average age of 150days), hoggets (>365 days) and ewes (>730 days).

Dry matter intake is estimated in grazing animals using the n-alkane technique as developed by Mayes et al. (1986). All animals are dosed once daily, ideally in the morning, with paper boluses containing 132 mg of dotriacontane (C_{32} n-alkane) for 12 d with herbage and faeces samples collected during the measurement phase (days 7-12 of each period). The paper boluses are made by dissolving dotriacontane using a heptane solvent and then pipetting the solution onto the boluses before leaving them aside for the solvent to evaporate prior to oven drying. Faeces samples are stored at -20°C until required for further analysis. Daily faecal samples are then defrosted prior to being bulked per sheep per period. The faecal samples are dried at 60°C for 48 h or until dry before being milled through a 1 mm screen.

Samples of the offered herbage are collected daily and frozen immediately at -20°C before being bowl chopped and freeze-dried at -55°C for 72 h. The herbage samples are milled through a 1 mm screen

and bulked per treatment per period (6 days) for the analysis of n-alkane concentrations. The n-alkanes, pentacosane (C_{25}-alkane), hexacosane (C_{26}-alkane), heptacosane (C_{27}-alkane), octacosane (C_{28}-alkane), nonacosane (C_{29}-alkane), triacontane (C_{30}-alkane), hentriacontane (C_{31}-alkane), C_{32}-alkane, tritriacontane (C_{33}-alkane), (tetratriacontane (C_{34}-alkane), pentatriacontane (C_{35}-alkane) and hexatriacontane (C_{36}-alkane) concentrations in the faeces and herbage are analysed by gas chromatography (GC) using a modification of the method described by Mayes et al. (1986), which used direct saponification (Dillon, 1993). Peak areas are converted to amounts (mg/kg DM) of n-alkane by reference to the internal standard (C_{34}-alkane). As per Dove and Mayes (2002), the relationship between the n-alkane pairs C_{31} and C_{32} and C_{33} and C_{32}. The herbage DMI/sheep per period is calculated using the following modified equation (Mayes et al., 1986):

$$\text{Intake}\left(\text{kg}\frac{\text{DM}}{\text{sheep}}\text{per day}\right) = \frac{\text{F}i\text{D}j}{\text{F}j\text{H}i - \text{F}i\text{H}j} \qquad (1)$$

where F_i and H_i represent the concentrations (mg/kg DM) of odd-chain faecal and herbage n-alkanes respectively, and F_j and H_j are the respective concentrations (mg/kg DM) of even-chain length faecal and herbage n-alkanes.

As we don't have automated feed intake equipment for sheep, dry matter intake is recorded on animals indoors using a feed and weigh method. Animals are housed individually for the duration of the study. Each morning their feed refusals are weighed and a fresh quantity of feed is weighed into animals. Daily feed allocations are +10% of the previous day's intake to ensure *ad libitium* feeding is provided.

Animal live-weight is recorded weekly and average daily gain of the animals is calculated where required using regression analysis. This enables calculation of RFI.

4.1.5 Description protocol in NMBU Norway – Meat sheep

This protocol is applied at the Norwegian University of Life Sciences (NMBU)

The research farm at NMBU has a capacity to measure batches up to 48 animals kept in individual pens. Feed intake is continuously recorded using the BioControl system, where each feeding event is recorded (quantity and duration). The pens have slatted floors, with no bedding. Individual water intake can be recorded, in addition to individual manure collection. The set-up is suitable for both adults and lambs. The University farm stocks sheep of two breeds, Norwegian White Sheep (modern, heavy long-tailed composite breed, 90 kg live weight) and Old Norwegian Spæl (Nordic short-tailed land race, 60 kg live weight).
The following section describes the protocol carried out in the "Grass to Gas"-project in order to study feed intake in adult ewes.

40 (20 of each breed) adult, dry ewes in early pregnancy are recorded in one batch. Study animals are balanced for ewe age and number of lambs in the previous litter (2 lambs). The ewes are offered grass silages consisting mainly of Timothy and Perennial ryegrass, with some white clover and various herbs. The ewes are observed in individual pens for six weeks, split into two periods: one grass silage quality in each period, half of the animals starting on each quality. Each period is made up of one-week of adaptation and two weeks of feed intake recording. The ewes are offered the grass silage *ad libitum*, aiming for approximately 10% refusals. The feed is given twice a day (7 am and 17 pm), with leftovers

removed, and weighed, before feeding. The grass silage is processed through a mixer wagon to reduce the grass silage particles to 3-5 cm to reduce the potential for feed selection. No concentrate is offered. Live weight is recorded twice during the adaptation week (mid-day), and daily for the two following weeks.

Samples of feed are taken before the start of the experiment and analysed at a commercial lab (Eurofins); thereafter representative samples are collected twice a week from each new roundbale (Mondays and Thursdays) for the duration of the experiment. The samples are analysed for chemical composition (dry matter contents, protein, fibre) and fermentation products in the silage. The energy content is estimated by determining the rumen digestible organic matter.

Individual daily DMI is calculated from daily feed intake and corresponding DM analysis. Individual daily energy intake (Net energy lactation, MJ/day) is calculated from DMI and energy density of the silage.

Other measurements taken in addition to feed intake include methane measurements through Portable Accumulation Chambers (PAC), rumen fluid and tissue sampling for DNA extraction and genotyping.

4.1.6 Description protocol in INRAE France – Dairy sheep

This protocol is applied at INRAE, on mature dairy ewes. Within each batch, ewes are of the same parity.

The measurement period of DMI starts after the weaning of the lambs, i.e. 35 days after lambing (DIM35), during a period of 8 weeks, until 90 days in milk (DIM90), during which ewes are machine-milked twice daily.

The ewes are housed in sheepfolds, in a pen of 48 on straw-bedding and have permanent access to fresh water. Ewes have access to an individual feeding post, controlled by an EID tags. The training of the ewes to their individual feeding post starts before the measurement period, 2 months before lambing.

During the measurement period, ewes are individually fed with a standard *ad libitum* (15% refusal) total mixed ration (TMR). The TMR is prepared and offered twice daily, one-third in the morning and two-thirds in the afternoon, at about 8 am and 4 pm, respectively. The distribution is adjusted to an allowance rate of 115% of the previous day's voluntary intake. In addition, 100 g (fresh weight) of the same commercial protein concentrate is offered at each milking in the milking parlour. Twice a day, before the distribution of the mixed ration, the refusals of the previous meal are weighed and removed. Individual intake is measured every day all over the experiment. Dry matter of TMR offered (three samples per distribution) and individual DM of refusals are determined four times a week for measuring individual DMI. Average DMI is thus individually calculated weekly, and further used to evaluate individual feed efficiency per ewe.

The individual daily energy intake (DEI, Unité Fourragère Lait per day (UFL/d)) is calculated multiplying individual DMI by energy density of the feed in the diet. One unit of UFL equals to 1.7MCal.

For calculating RFI, DEI must be compared to the ewes' energy requirements for milk production, maintenance, growth, and variation in body reserves.

<u>Evaluation of milk production requirements</u>

Daily milk yield (DMY, L/d) and milk fat (FC, g/L) and protein (PC, g/L) contents are assessed once a week. Standardised milk yield (SMY, L/d) proposed by Bocquier et al. (1993), is calculated as follow:

$$SMY = DMY \times (FC \times 0.0071 + PC \times 0.0043 + 0.2224) \quad (2)$$

Net energy of one litre SMY (SMYe, UFL/d) is calculated as per Hassoun and Bocquier, 2007:

$$SMYe\ (UFL/d) = SMY \times 0.71 \quad (3)$$

Evaluation of variation in body reserves

Around each milk sampling date every week, ewes are individually weighed, and body condition score (BCS) measured by the same trained observer based on a 6 points scale (0 emaciated to 5 very fat) established by Russel et al, (1969). Variation of BCS (BCSΔ), difference between two successive scoring, is converted into net energy (BCSΔe, UFL/d) according to the following formula (Hassoun et al., 2018) corrected for the previous INRA feeding system:

$$BCS\Delta e = (-0.43 \times BW \times BCS\Delta / Day\Delta) \times 0.956 \quad (4)$$

where BW is the body weight, BCS_{Δ} is the BCS variation between two scorings, and Day_{Δ} is the number of days between the two successive scorings.

Maintenance

Maintenance energy requirement (UFL/d) is calculated as per Hassoun and Bocquier, 2007:

$$\text{Maintenance energy requirement} = 0.033 \times \text{metabolic BW } (BW^{0.75}) \quad (5)$$

This value is increased by 10% when ewes are grazing (de Boissieu et al., 2019).

Growth

Because primiparous lamb at one year old, they still have growth requirements. Growth energy requirement (UFL/d) based on average daily gain (ADG, g/d) is calculated as per Hassoun and Bocquier, 2007

$$\text{Growth energy requirements} = (ADG / 100) \times 0.26 \quad (6)$$

4.1.7 Description protocol in University of León Spain – Dairy sheep

This protocol is applied at the University of León in Spain, on dairy ewes.

At the University of León, feed efficiency is assessed in first-lactation dairy ewes for which oestrus synchronisation is deployed to ensure that the lambing occur over a short period. A total of forty ewes are phenotyped at the same time. The recording of feed intake lasts 28 days during which ewes are tethered in individual stalls.

Ewes are milked twice a day, at approximately 08:30 and 18:30. Total milk produced by each animal during morning and evening milking is collected and weighed to calculate individual milk yield and standardized milk yield as per Bocquier et al., 1993 (equation (2)).

Ewes are fed a total mixed ration (TMR) from a commercial supplier and the offer is adjusted daily to ensure *ad libitum* intakes (10-15% orts). The TMR is formulated from alfalfa hay (particle size > 4cm)

and concentrates (50:50 forage:concentrate ratio), including sugar beet molasses, to hinder selection of dietary components. Clean drinking water is always available.

Feed intake is measured daily by weighing the amount of dry matter (DM) offered and refused 24h after by each animal. Each day before the distribution of the feed, the refusals of the previous day are removed and weighed. The DM of the TMR offered and the individual DM of the refusals are determined weekly for measuring individual DM intake (DMI).

Ewes are weighed on two consecutive days at the beginning and at the end of the experiment.

4.2 Protocols in commercial farms

4.2.1 Description protocol in France – Dairy sheep and goat

This section is an example of measurements and algorithms used that were carried out during the SMARTER project in French commercial farms, in dairy sheep and dairy goats, in order to study feed efficiency.

Note that recording the diet is described in the section of the guidelines dedicated to the recommendations on 'recording the environment'.

Recording feed intake is often not feasible at an individual level in commercial farms, except for concentrate in the milking parlour equipped with ACF (automatic concentrate feeder).

The diet should be analysed for feed quality parameters (including pasture) at regular intervals and particularly when different batches of feed are consumed. An average of three consecutive days of recording feed intake may be considered (optional because time-consuming).

Total Dry matter intake (DMI) must be calculated, assuming a percentage (10% for example) of refusal for forages offered ad libitum and in cases where refusals are not weighed. The total amount of each forage and concentrate must be weighed and sampled for dry matter (DM) content determination. No refusals are considered for all concentrates.

To calculate individual DMI, the total DM amount measured at the flock/herd/mob must be divided by the number of animals.

For grazing animals, it is possible to propose an estimation of the ingestion at pasture according to the time spent grazing. In dairy sheep the following equivalences are used: 2h = 0.4 kg DM, 4h = 0.8 kg DM, 6h = 1 kg DM.

The individual daily energy intake (DEI) must be calculated by multiplying individual DMI by energy density of the feed in the diet at each test day.

To evaluate ewes' energy requirements for milk production, daily milk yield (DMY, L/d), and milk fat (FC, g/L) and protein (PC, g/L) are assessed at each test-day. As an example, in the French dairy sheep, the standardised milk yield (SMY, L/d) is calculated using equation (2).

SMY must be converted into net energy, in the unit used in the country.

In the French system, we apply equation (3) which returns net energy of one litre SMY in UFL/d. UFL is the net energy unit and corresponds to the net energy requirements for lactation equivalent of one kg of standard air-dried barley.

Body reserve dynamics is assessed by measuring body condition scores (BCS) at different key physiological stages: one month before lambing/kidding, at the end of suckling (case of dairy sheep with a 30-days suckling period after lambing), at the first test-day, one month before and one month after mating. Because lambing/kidding occur during a more or less long period, the visits at the flock/herd level must be fixed so that they aim at the best the targeted physiological stages.

The BCS are evaluated by palpation of the lumbar region according to the 6-point scale proposed by Russel et al. (1969) ranging from 0 (emaciated) to 5 (very fat), with 0.25 intervals. BCS must be scored by evaluators previously trained to ensure the harmonisation of the scoring and therefore make the comparison possible between evaluators (if there are more than one).

Variation of BCS (BCSΔ) between two successive scorings must be converted into net energy (BCSΔe, UFL/d), in the unit used in the country. In the French system, the conversion is done with equation (4). Missing data (for example because an animal is out of the targeted period during the flock/herd visit) may be imputed by statistical method (the method used in dairy sheep in SMARTER was the copyMean method implemented by Genolini and Falissard (2011) in the kml package of R software).

Body weight – Either the animal is weighed, or the animal is not weighed (general case in dairy farms). In the absence of weighing, body weight (BW) must be estimated:

- a parity-dependent reference BW can be assigned to all animals (for example one BW for primiparous and one for multiparous according to the expertise of technicians).
- the chest width (CW) can be used as a proxy of the body weight and be measured once during the lactation (for example in the middle of the lactation). The prediction of BW from CW was obtained with equation 7:

$$BW = 1.3048 * CW - 63.678 \quad (7)$$

Maintenance energy requirement is estimated according to the method adopted by each country. In the French system, we apply equation (5) and the result is increased by 10% when ewes are grazing (de Boissieu et al., 2019).

When maintenance energy requirement is applied to primiparous with lambing/kidding at one year old, growth requirements must be considered and are calculated using equation (6). In absence of measure of growth, we assume as an approximation that growth requirements are the same for all the females.

4.3 Synthesis and recommendations (what can be generalised across the protocols)

General recommendations

- Batches of at least 40-50 animals phenotyped at the same time, particularly if the criterion is obtained through a regression such as RFI.
- An adaptation period of 2 weeks is needed to train the animals getting feed from the feeders and to get accustomed to their new physical and social environments.

- The protocols of phenotyping must last 6-8 weeks.
- Meat sheep are generally measured during the growing period (3-10 months). But mature sheep might as well be measured.
- Dairy animals are measured in lactation, whatever the parity. The measure on dairy animals is recommended in the first part of the lactation to avoid additional variation due to previous parities. Measurements should be undertaken out of the grazing period, to avoid the difficulty of estimation of intake at grazing unless intakes at grass can be measured accurately.

Recommendations on measuring the input

- The number of individuals per feeder varies according to the different feeding systems. Regardless of the feeding system, all animals should be fed *ad libitum* for this metric to be estimated.
- The calibration and the cleaning of the equipment depend on the feeding systems. Calibration must be performed at least at the beginning and every 2-3 weeks.
- All feed should have nutritional parameters estimated to at least include dry matter, energy, and protein. Diet analyses are used to assist in the estimation of converting feed intake to dry matter intake.

Recommendations on measuring the output

- In growing animals (lambs, kids), the protocols of phenotyping must include at least 2 body weight records (beginning and end). If the facility exists to record body weight more frequently (some equipment includes a weighing platform at the feeder) then that is preferable to use to estimate ADG.
- For dairy, milk recording must be performed to measure milk yield and milk composition (fat and protein content).
- When measures are done over a large period (dairy animal on-farm), recording of body condition and/or composition where possible (BCS / ultrasound / CT) is recommended to estimate changes in body reserves and composition during the test period. For shorter monitoring periods (growing animals over 8 weeks), weighing the animals should be enough, as there is not much variation in BCS.

Recommendations on data cleaning / editing

- For weight and growth, as well as for intake measurement, data considered biologically unlikely (e.g. according to breed, sex and age) would be excluded. Outlier body weight data can be tested for and eliminated (e.g. outliers <-3 SD and >+3 SD). In automatic feeders, several data are obtained per day with their corresponding time and length, so they can be edited by amount of feed intake per time (e.g. delete intake >1 kg in 3 minutes). Additional criteria could be applied to daily feed intake (e.g. greater intake than certain percentage of body weight).

Protocols in experimental unit vs on-farm protocols

- Recording feed efficiency in commercial / breeding farms is difficult, especially regarding collecting feed intake. When possible, an alternative would be to bring the commercial animals (for genetic purpose) in experimental facilities and to apply the experimental protocol to evaluate these animals. The experimental facilities would thus be considered as a central station for candidate males from commercial populations, in the context of breeding programmes.

4.4 Definition of traits, calculation of variables

Feed efficiency relates feed intake to production and maintenance requirements. Different indicators have been proposed and used to express feed efficiency. They are normally implemented at an individual animal level, where possible.

The main indicators are ratios and residuals from multiple regression of feed intake over energy sinks such as metabolic weight, growth and milk production.

Ratio indicators:

Feed conversion ratio (FCR) is the main ratio indicator. The numerator consists of dry matter intake. The denominator depends on production level. For meat sheep, the usual denominator is average daily gain (ADG) whereas in dairy sheep and dairy goats, it is usually the standardized milk yield (SMY).

To summarize:

- In meat sheep: FCR = DMI/ADG
- In dairy sheep and goat FCR = DMI/SMY

Another ratio, named lactation FCR (LFCR) can be computed from the different recorded traits:

$$LFCR = \frac{SMYe}{DEI - (maintenance\ and\ growth\ requirements) + BCS\Delta e}$$

The ratios have the advantage of being calculated for one individual: they do not use data from other phenotyped animals.

Residual feed intake:

RFI measures the feed efficiency of an animal based on what we expect it to eat (based on body size, stage of lactation or growth and /or other physiological measurements) compared to what it is actually eating. Measuring animals individually that are part of a cohort of animals being monitored at the same time enables the comparisons of animals to be undertaken simultaneously so that the differences cannot be attributed to differences in the environment (eg such as differing levels of daylight and temperature etc). By definition, RFI is centred on zero, (where expected vs actual feed intakes are equal). An individual animal that is eating more than expected has a positive RFI, and an animal eating less than expected has a negative RFI.

In growing animals (meat sheep lambs in particular), maintenance requirements are estimated from the metabolic body weight (body weight to the power of 0.75), and production requirements (e.g. growth requirements) and (where applicable) body composition such as backfat thickness (BFT) and muscle depth (MD)or CT-measured fat and muscle weights.

In mature animals (including dairy, but not only), maintenance requirements are estimated also by use of $BWT^{0.75}$as well as accounting for production (e.g. number of lambs reared, or stage of lactation) as well as potentially BCS variation (ΔBCS) or also through the interaction body weight (BW) by body

weight variation (ΔBW). In dairy sheep and goat, production requirements are estimated by accounting for milk yield, fat and protein contents (or a combination of yield and contents into standardised milk yield).

Depending on the availability of the different traits, the following regressions can be proposed:

In meat sheep:

- $DMI = \mu + a \times BW^{0.75} + b \times ADG + c \times BFT + d \times MD + RFI$ (from Koch et al., 1963)

  BFT and MD are obtained with ultrasound. They can be replaced by CT-scan body composition values.

In dairy sheep and goats:

- $DMI = \mu + a \times SMY + b \times BW^{0.75} + c \times BW \times \Delta BW + RFI$,
- Or, $DMI = \mu + a \times DMY + b \times FC + c \times PC + d \times \Delta BCS + e \times BW + RFI$

When dairy ewes or goats are not weighed, body weight can be replaced as a predictor by chest width, and the following equation can be proposed:

- $DMI = \mu + a \times DMY + b \times FC + c \times PC + d \times$ chest width $+ RFI$

In dairy sheep and goats, these equations are often applied on the energy expressed traits.

Based on the ratios and residuals definitions, efficient animals have low ratio values and negative RFI values.

4.5 Proxies measurable routinely or not in experimental or commercial farms

The following Table 1 lists information from the SMARTER and GrassToGas projects. It presents the performance of different proxy traits for feed efficiency, measured in the SMARTER and GrassToGas projects in small ruminant populations to predict the phenotypes considered as the gold standard of feed efficiency. Residual Feed Intake (RFI) and Feed Conversion Ratio (FCR) are the two key traits of interest. The results have been performed using cross-validation and/or machine-learning algorithms such as random forest or sparse Partial Least squares regressions. When possible, the feed efficiency (FE) prediction have been estimated by means of a cross-validation process and Machine Learning (ML) prediction methods. The parameter measuring the efficiency of prediction for each proxy is either the coefficient of determination (r^2) or the Pearson correlation coefficient (r) between FE traits using the RFI-FCR from the complete data set (i.e., not divided between training and validation groups) and the FE parameter estimated using the proxy. The heritability and/or the genetic correlation parameter between FE and the proxy is provided in the table if the performance has not been estimated using Machine Learning methods (shadow cells).

Table 1: Candidate phenotypes for indirect measurements of feed efficiency.

Partner(s)[1]	Group of traits	Trait[2]	Species[3] (DS, MS, DG)	Experimental (E) or Commercial (C)	Proxy of which (reference FE) trait[4]	Measurement protocol	Prediction accuracy [5]	# animals	Level of ease of collection on-farm[6]
INRAE	Zootechnical	Body weights	MS	E	RFI	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.26 ±0.05	277	1
	Zootechnical	Body weights	MS	E	FCR	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.45 ±0.09	277	1
	Zootechnical	Body weights	MS	E	Feed intake	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.84 ±0.05	277	1

Partner(s)[1]	Group of traits	Trait[2]	Species[3] (DS, MS, DG)	Experimental (E) or Commercial (C)	Proxy of which (reference FE) trait[4]	Measurement protocol	Prediction accuracy [5]	# animals	Level of ease of collection on-farm[6]
	Zootechnical	Body weights	MS	E	RFI	4.1.1 (8 months old lambs, 2/3 forage +1/3concentrate ad libitum)	R^2=0.32 ±0.10	166	1
	Zootechnical	Body weights	MS	E	Feed intake	4.1.1 (8 months old lambs, 2/3 forage +1/3concentrate ad libitum)	R^2=0.59 ±0.09	166	1
	Ruminal microbiota	16S or 18S sequencing	MS	E	RFI	4.1.1 (growing lambs, 100%concentrate ad libitum)	16S :R^2=0.02 ±0.02 ; 18S : R^2=0.02 ±0.02	277	3
	Ruminal microbiota	16S or 18S sequencing	MS	E	FCR	4.1.1 (growing lambs, 100%concentrate ad libitum)	16S :R^2=0.17 ±0.08 ; 18S : R^2=0.05 ±0.04	277	3
	Ruminal microbiota	16S or 18S sequencing	MS	E	Feed intake	4.1.1 (growing lambs, 100%concentrate ad libitum)	16S : R^2=0.08 ±0.06; 18S : R^2=0.04 ±0.04	277	3
	Ruminal microbiota	16S or 18S sequencing	MS	E	RFI	4.1.1 (8 months old lambs, 2/3 forage +1/3concentrate ad libitum)	16S : R^2=0.09 ±0.07; 18S : R^2=0.06 ±0.06	166	3
	Ruminal microbiota	16S or 18S sequencing	MS	E	Feed intake	4.1.1 (8 months old lambs, 2/3 forage +1/3concentrate ad libitum)	16S : R^2=0.08 ±0.06; 18S : R^2=0.12 ± 0.09	166	3
	NMR	plasma	MS	E	RFI	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.07	277	2

Partner(s)[1]	Group of traits	Trait[2]	Species[3] (DS, MS, DG)	Experimental (E) or Commercial (C)	Proxy of which (reference FE) trait[4]	Measurement protocol	Prediction accuracy [5]	# animals	Level of ease of collection on-farm[6]
	NMR	plasma	MS	E	FCR	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.19	277	2
	NMR	plasma	MS	E	Feed intake	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.20 ±0.09	277	2
	Metabolites	β-HB, NEFA, total proteins, and hormones	DG	C	Feed intake	cf. D1.1	ongoing	534	2
	NIRS	faecal NIRS	MS	E	RFI	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.02	91	1
	NIRS	faecal NIRS	MS	E	FCR	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.04	91	1
	NIRS	faecal NIRS	MS	E	Feed intake	4.1.1 (growing lambs, 100%concentrate ad libitum)	R^2=0.12	262	1
	NIRS	faecal NIRS	MS	E	FCR	4.1.1 (8 months old lambs, 2/3 forage +1/3concentrate ad libitum)	R^2=0.15	47	1
	NIRS	faecal NIRS	MS	E	Feed intake	4.1.1 (8 months old lambs, 2/3 forage +1/3concentrate ad libitum)	R^2=0.19	164	1
UNILEON	Epigenetic marks in milk somatic cells	DNA--DML (Differencial Metilated Loci)	DS	E	RFI, FCR	4.1.6 (Lactating animals)	R^2 = 0.344 (RFI) R^2 = 0.332 (FCR)	28	3
	Milk composition	Milk fatty acid profile	DS	E	RFI, FCR	4.1.6 (Lactating animals)	R^2= 0.720 (RFI) R^2 = 0.745 (FCR)	39	2

Partner(s)[1]	Group of traits	Trait[2]	Species[3] (DS, MS, DG)	Experimental (E) or Commercial (C)	Proxy of which (reference FE) trait[4]	Measurement protocol	Prediction accuracy [5]	# animals	Level of ease of collection on-farm[6]
	Milk composition	Untargeted metabolome	DS	E	RFI, FCR	4.1.6 (Lactating animals)	ongoing	39	2
	Milk somatic cells whole transcriptome (DEG)	RNA-seq	DS	E	RFI, FCR	4.1.6 (Lactating animals)	ongoing	24	3
INIA-UY	Zootechnical	Feed intake	MS	E	RFI	4.1.1	R^2 = 0.75	930Merinos (WCGALP)	1
	Zootechnical	Backfat depth	MS	E	RFI	4.1.1	(Ph) = R^2 = 0.57 (Ph) = R^2 = 0.74 (Ph) = R^2 = 0.61	811 Merinos 281 Corriedale 214 Dohne	1
	Zootechnical	GHG Methane and Carbon dioxide	MS	E	RFI	4.1.1	O2 = R^2 = 0.15 CH4 = R^2 = 0.02 CO2 = R^2 = 0.11	811 Merinos 281 Corriedale 214 Dohne	2
AgResearch	Zootechnical and ruminal microbiota	Body weight, ADG, body composition, GHG emissions, ruminal data	MS	C	RFI	4.1.2 (9-12 month ewe lambs)	R^2 =0.69 to 0.78		3
SRUC	Zootechnical	Body weight, ADG, body composition	MS	E	ADFI	4.1.3	R^2=0.27 to 0.35		1
	Feeding behavior	Average daily number of meals	MS	E	ADFI	4.1.3	R^2=0.69		2
	Feeding behavior	Average daily number of meals	MS	E	RFI	4.1.3	R^2=0.59		2
	Feeding behavior	Average daily number of meals	MS	E	FCR	4.1.3	R^2=0.21		2

Partner(s)[1]	Group of traits	Trait[2]	Species[3] (DS, MS, DG)	Experimental (E) or Commercial (C)	Proxy of which (reference FE) trait[4]	Measurement protocol	Prediction accuracy [5]	# animals	Level of ease of collection on-farm[6]
TEAGASC	Zootechnical	Body weight, ADG, body composition	MS	C	ADFI	4.1.4 (nulliparous lambs and hoggets)	R^2=0.88		1
	Zootechnical	Body weight, ADG, body composition	MS	C	RFI	4.1.4 (nulliparous lambs and hoggets)	R^2=0.06		1
	Zootechnical	Body weight, ADG, body composition	MS	C	FCR	4.1.4 (nulliparous lambs and hoggets)	R^2=0.80		1
	Zootechnical	Body weight, ADG, body composition, GHG emissions	MS	C	ADFI	4.1.4 (nulliparous lambs and hoggets)	R^2=0.88		2
	Zootechnical	Body weight, ADG, body composition, GHG emissions	MS	C	RFI	4.1.4 (nulliparous lambs and hoggets)	R^2=0.10		2
	Zootechnical	Body weight, ADG, body composition, GHG emissions	MS	C	FCR	4.1.4 (nulliparous lambs and hoggets)	R^2=0.78		2
	Zootechnical	Body weight, ADG, body composition, BCS	MS	C	ADFI	4.1.4 (ewes)	R^2= 0.69		1
	Zootechnical	Body weight, ADG, body composition, BCS	MS	C	RFI	4.1.1 (ewes)	R^2=0.28		1

Partner(s)[1]	Group of traits	Trait[2]	Species[3] (DS, MS, DG)	Experimental (E) or Commercial (C)	Proxy of which (reference FE) trait[4]	Measurement protocol	Prediction accuracy [5]	# animals	Level of ease of collection on-farm[6]
NMBU	zootechnical	Body weight	MS	Experimental	ADFI	4.1.5	R^2=0.82	40	1
	zootechnical	Feeding behavior	MS	E	ADFI	4.1.5	R^2=0.74 to 0.82	40	2
	Zootechnical	GHG	MS	E	ADFI	4.1.5	R^2=0.80 to 0.87	40	2

[1]The organisations/institutes involved: INRAE (France), UNILEON=University of Leon (Spain), SRUC (the UK), INIA_UY (Uruguay), AgResearch (New Zealand), TEAGASC (Ireland), NMBU=Norwegian University of Life Sciences (Norway)

[2]The type of trait considered as a proxy

[3]Species are defined as Dairy Sheep (DS), Meat Sheep (MS) and Dairy Goat (DG).

[4]Acronyms for feed efficiency traits: RFI: Residual Feed Intake, FCR: Feed Conversion Ratio, REI: Residual Energy Intake, ADFI: Average Daily Feed Intake, FI: Feed Intake.

[5]Prediction performances: The FE prediction performances from the proxies have been estimated, when possible, by means of a cross-validation process and Machine Learning (ML) prediction methods. The parameter measuring the efficiency for each proxy is either the coefficient of determination (r^2) or the Pearson correlation coefficient (r) between FE predictions using the RFI-FCR from the complete data set (i.e., not divided between training and validation groups) and the FE parameter estimated using the proxy.

[6]Scale of difficulty in measuring the proxy on-farm: 1=routinely collected; 2=possible but hard/costly to collect in routine; and 3=very difficult to collect in routine".

5 Use for genetic evaluation

5.1 Models for genetic analysis / evaluation

To improve feed efficiency, selection programmes use different models for the genetic analyses. Table 2 shows the models used by different analyses of feed efficiency criteria.

Table 2: List of fixed and random effects used to analyse feed efficiency criteria.

Trait name[1]	Species (DS, MS, DG)[2]	Definition	Recording[3]	Fixed effects	Random effects	Notes
RFI	MS	In a population	4.1.1	Series of control; pen; early life management (litter size, nb of reared lambs)	animal	Tortereau et al., 2020
RFI	MS	Within a series of control	4.1.1	pen; early life management (litter size, nb of reared lambs)	animal	As it is performed in the frame of the French breeding programs
ADFI	MS	In a population	4.1.1	Serie of control; pen; early life management (litter size, nb of reared lambs), body weight as a covariate	animal	Tortereau et al., 2020
FCR	MS	In a population	4.1.1	Serie of control; pen; early life management (litter size, nb of reared lambs), body weight as a covariate	animal	Tortereau et al., 2020
REI	DG	In commercial populations	4.2.1	Herd, year of lactation, Herd test day, Physiological Stage	Permanent effect of the goat + animal	Chassier et al., 2022
LFCR	DS	In commercial populations	4.2.1	parity (1/2/3/4+), litter size (single/multiple), lambing period (start/end) according to parity, birth mode (AI/AI return/natural mating), lactation month crossed to test-day x year, flock x birth mode and flock x year	Permanent effect of the ewe + animal	Machefert et al., 2022

[1]Acronyms for feed efficiency traits: RFI: Residual Feed Intake, FCR: Feed Conversion Ratio, REI: Residual Energy Intake, ADFI: Average Daily Feed Intake, LFCR: Lactation Feed Conversion Ratio.

[2]Species are defined as Dairy Sheep (DS), Meat Sheep (MS) and Dairy Goat (DG).

[3]Recording gives the paragraph describing the protocol from which data were considered

5.2 Genetic parameters

Genetic parameters obtained from the previously described protocols and using models presented in Table 2 are given in the following Table 3.

Table 3: Genetic parameters for feed efficiency traits estimated in small ruminants

Traits[1]	Species (DS, MS, DG)[2]	Heritability	Genetic standard deviation	Coefficient of variation (%)	Notes
RFI	MS	0.45 ± 0.08	78.5 g/d	Not estimable	Tortereau et al., 2020
RFI	MS	0.42 ± 0.09	0.86 MJ/d	Not estimable	Johnson et al., 2022
ADFI	MS	0.28 ± 0.08	131.8 g/d	12.67	Tortereau et al., 2020
ADFI	MS	0.35 ± 0.10	2.70 MJ/d	17.85	Johnson et al., 2022
FCR	MS	0.30 ± 0.08	0.44	13.94	Tortereau et al., 2020
REI	DG	0.19 ± 0.08	0.06 UFL/d	Not estimable	Chassier et al., 2022
LFCR	DS	0.12 ± 0.02	0.07	7	Machefert et al., 2022

[1]Acronyms for feed efficiency traits: RFI: Residual Feed Intake, FCR: Feed Conversion Ratio, REI: Residual Energy Intake, ADFI: Average Daily Feed Intake, LFCR: Lactation Feed Conversion Ratio.

[2]Species are defined as Dairy Sheep (DS), Meat Sheep (MS) and Dairy Goat (DG).

6 Acknowledgements

We gratefully acknowledge the contributions to small ruminant Feed Efficiency guidelines by the following people:

- Flavie Tortereau, INRAE, France
- Juan Jose Arranz, University of Leon, Spain
- Bente Aspeholen, NMBU, Norway
- Jean-Michel Astruc, IDELE, France
- Ignacio de Barbieri, INIA, Uruguay
- Marjorie Chassier, IDELE, France
- Gabriel Ciappesoni, INIA, Uruguay
- Joanne Conington, SRUC, UK
- Jette Jakobsen, Norwegian Sheep and Goat Association, Norway
- Patricia Johnson, AgResearch, New Zealand
- Arjan Jonker, AgResearch, New Zealand
- Nicola Lambe, SRUC, UK
- Hélène Larroque, INRAE, France

- Coralie Machefert, INRAE, France
- Christel Marie-Etancelin, INRAE, France
- Fiona Mc Govern, Teagasc, Ireland
- Elly Navajas, INIA, Uruguay
- Suzanne Rowe, AgResearch, New Zealand

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

This work received funding from the European Unions' Horizon2020 Research & Innovation Programme under grant agreement No 696356—GrassToGas.

7 References

Bocquier E., Barillet F., Guillouet P. and Jacquin M. (1993) Prévision de l'énergie du lait de brebis à partir de différents résultats d'analyses: proposition de lait standard pour les brebis laitières Ann Zootech 42(1):57-66.

De Boissieu C., Fanca B. et Hassoun P., 2019. L'alimentation des brebis laitières- Références et conseils pratiques. [Synthèse]. Institut de l'Elevage, Paris, France. 119p.

Chassier M., Mosnier F., Rupp R., Bluet B., Bailly-Salins A., Palhière I. Genetic parameters of feed efficiency in dairy goats under commercial conditions, Proc 12th World Congr Genet App Livest Prod, 2022

Genolini C. and Falissard B. (2011) Elsevier 104(1):112-121. https://doi.org/10.1016/j.cmpb.2011.05.008

Gonzalez-Garcia et al., 2015 (Domestic Animal Endocrinology, http://dx.doi.org/10.1016/j.domaniend.2014.07.001),

Gonzalez-Garcia et al., 2020 (animals, doi:10.3390/ani10091593)

Hassoun P. and Bocquier F. (2007). Alimentation des ovins. In: Inra Editions (eds). Alimentation des bovins, ovins et caprins. Editions Quae, Paris, France, pp. 123-138.

Hassoun P., Berthelot V. and Bocquier F. (2018). Dairy, growing and fattening sheep. In (Nozière P., Delaby L. and Sauvant D. Eds.) INRA Feeding System for Ruminants. Wageningen Academic Publishers, Wageningen, the Netherlands, 319-337.

Koch RM, Swiger LA, Chambers D and Gregory KE 1963. Efficiency of feed use in Beef Cattle1. Journal of Animal Science 22, 486–494.

Lambe et al., 2003 (Animal Science, https://doi.org/10.1017/S1357729800053443)

Machefert C., Robert-Granié C., Lagriffoul G., Astruc J.M, Parisot S., Allain C., Portes D., Hassoun P., Larroque H. Validation and genetic analisis of a feed efficiency criterion in French Lacaune ewes, Proc 12th World Congr Genet App Livest Prod, 2022

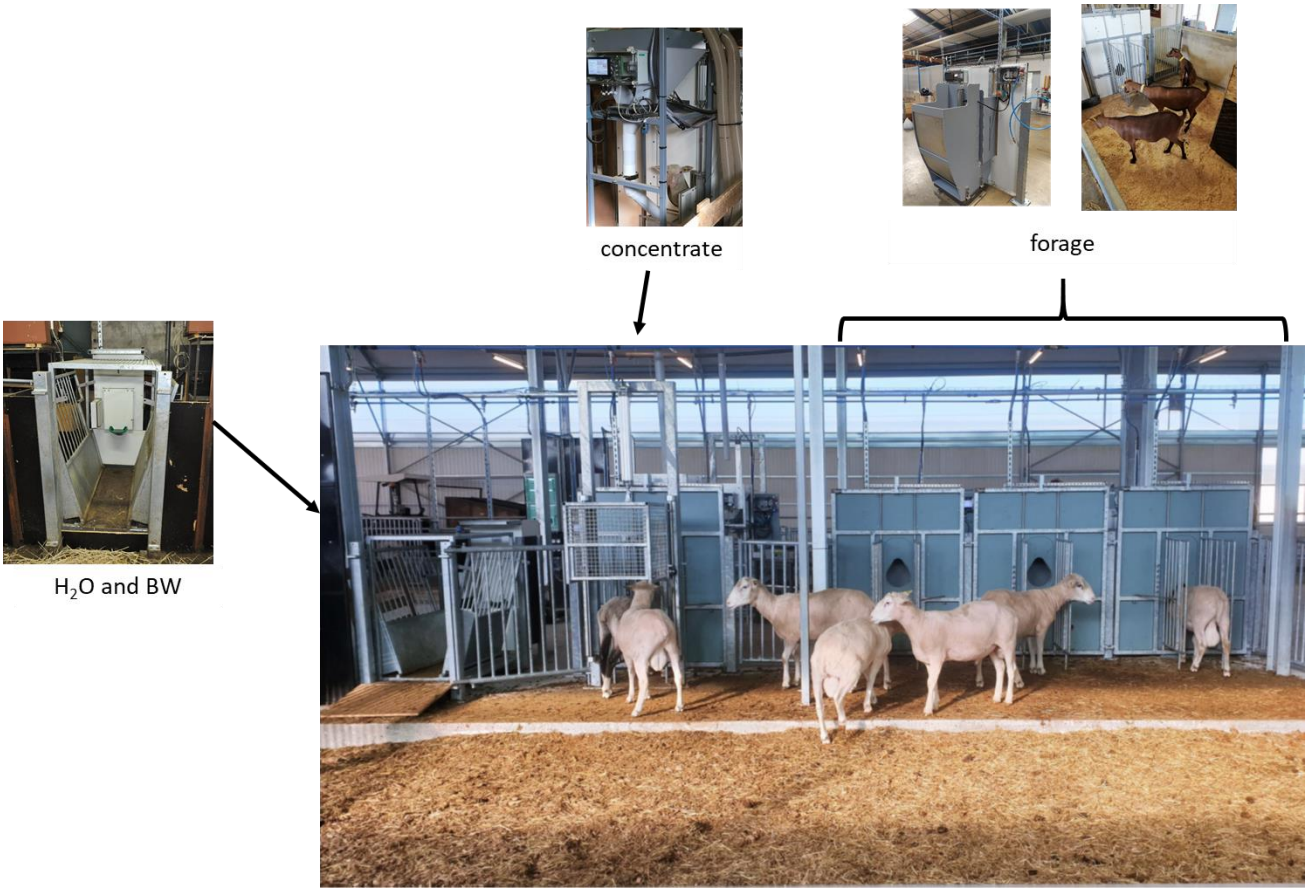
Russel, A.J.F., Doney, J.M., Gunn, R.G., 1969. Subjective assessment of body fat in live sheep. J. Agric. Sci. 72, 451-454. https://doi.org/10.1017/S0021859600024874.

Tortereau et al., 2020 (animal, doi:10.1017/S1751731119002544)

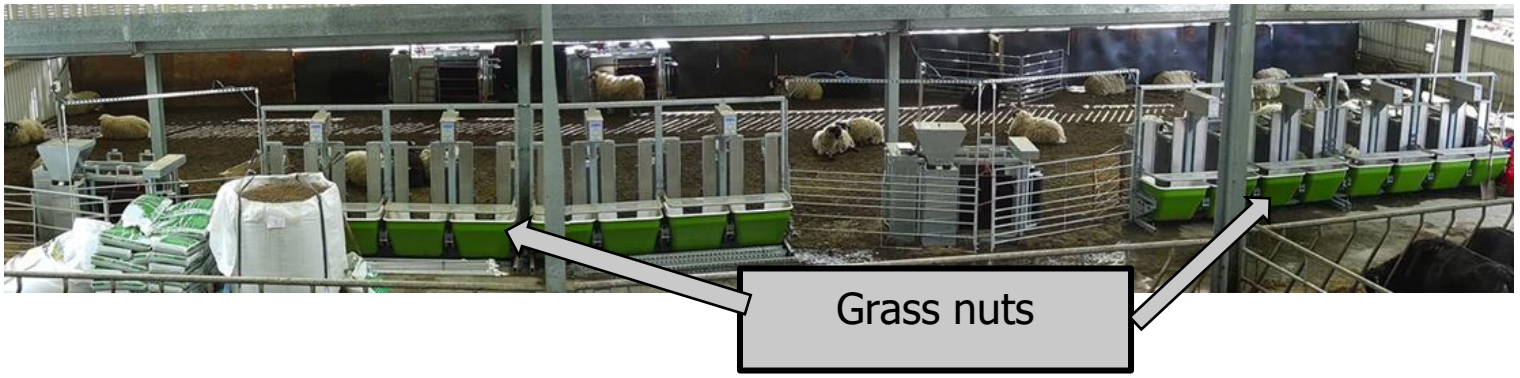
8 Appendixes

8.1 Appendix 1 – Pen at INRAE facilities

A pen in INRAE facilities to where protocol described in 4.1.1 is led:



8.2 Appendix 2 – Feed intake recording at SRUC (protocol 4.1.3)



Recommendations on recording greenhouse gases emissions in sheep and goat

SMARTER – Deliverable D6.3

Version 1 – 16 June 2023

Table des matières

Change Summary

Date of change	Nature of Change
May 2023	First draft
June 2023	Final version for SMARTER deliverable 6.3

1 Introduction

The environmental impacts of livestock production are a major concern, particularly in the context of climate change. Ruminants are often pointed out because of the ruminal process which results in methane emissions. Main greenhouse gases (GHG) produced by ruminants are methane (CH_4) and carbon dioxide (CO_2). GHG emissions impacts the environment, but they can also be considered, to a certain extent, as a loss of energy for the animal, due to the loss of carbon. Different devices are being used in sheep to record GHG emissions: portable accumulation chambers (PAC) and Sheep Greenfeed (manufactured by the C-lock company).

2 Scope

These guidelines are based on the experience and protocols set up by partners of the European projects SMARTER (H2020 – 772787) and GrassToGas (FACE ERA-GAS), both dealing partially with GHG emissions in small ruminants. The present guidelines are not set in stone: new protocols, and evolving scientific methods will help improve and enrich the recommendations on phenotyping GHG.

This report first describes the different protocols currently in use in experimental (section 4.1) and commercial (section 4.2) farms to enable the recording of elementary traits used to phenotype GHG emissions presented in 4.4.

We then intend to propose general recommendations that can be drawn from the different protocols (section 4.3). However, this section is empty at this stage.

In the section 4.5, we list a series of proxy traits that have been studied to predict GHG emissions traits, either in experimental or in commercial farms. For each of them, the protocol for recording the trait, the accuracy of the prediction and the level of ease of data recording for the proxy traits are given.

Section 5 deals with the genetic evaluation of the traits, with suggestion of the genetic model and typical range of estimated genetic parameters.

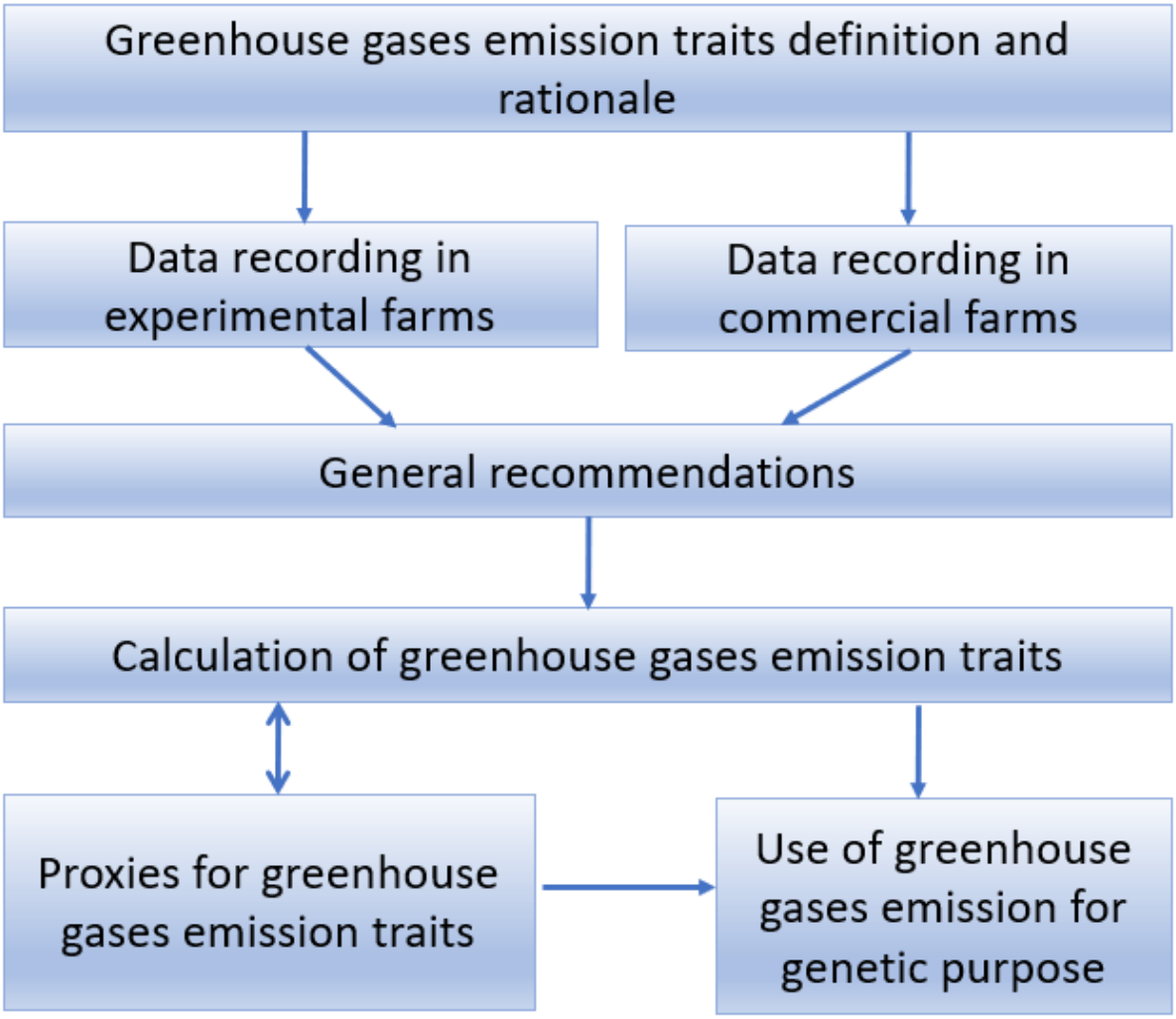


Figure 1. Scope of guidelines on GHG emissions recording in sheep and goat.

3 GHG emissions definition, terminology, rationale

Ruminant methane emissions contribute to GHG concentrations in the atmosphere and represents a loss of energy from the eaten feed by the animal.

International commitments to reduce methane emissions to mitigate the impacts of climate change have been made by over 50 countries through the Paris agreement to limit global temperature rises to less than 1.5 degrees C. In this respect, effective methane mitigation solutions are urgently needed to reduce CH_4 emissions for sustainable small ruminant production systems worldwide (Conington et al, 2022).

In addition, emissions of CH_4 and CO_2 can be seen as an indicator of the yield of conversion of feed intake into animal products. It is therefore relevant to increase this yield and decrease GHG emissions to improve the sustainability of small ruminant production systems.

The level of emissions of O_2 is often provided by gas analysers and can be seen as a proxy of animal metabolism to consider in the characterization of maintenance requirements.

Genetic improvement is well known to be permanent, sustainable, cumulative and highly cost-effective with an excellent penetrance rate. It has been shown that genetic selection can be used to achieve cumulative reductions in CH_4 per animal (Rowe et al. 2021).

As there is no consensus about how best to implement direct and indirect measurements of CH_4 into breeding programmes internationally, these recommendations describe the different protocols and measurements implemented in the SMARTER project, as well as the proxies studied in both the SMARTER and the GrassToGas projects. Further research may lead to a consensus in the next future.

Acronyms

GHG	Greenhouse gas(es)
CO_2	Carbon dioxide
CH_4	Methane
O_2	Oxygen
PAC	Portable Accumulation Chambers
DMI	Dry Matter Intake
BW	Body Weight
RME	Residual Methane Emissions

4 Recording of GHG emissions

4.1 Protocols in experimental farm

4.1.1 Description protocol in INRAE France – Meat sheep

At INRAE, CH_4 and CO_2 emissions are recorded with two Sheep GreenFeed devices manufactured by the C-Lock company (US) (Rozier et al., 2021).

One device is installed per pen of 30-40 individuals. A drop (small amount of concentrate) is delivered by the device in order to attract animals. To be valid, a visit of animal must last at least 2 minutes. To optimize the number of efficient visits and considering that we don't want too much concentrate to be delivered by GreenFeed devices, we set the following parameters: a given day is divided in 4 periods of 6 hours each. During each period, each animal can get a maximum of 6 drops of concentrate, these drops being delivered with an interval of 30 seconds. Animals can visit the device even if they can't get any drop, and if this visit lasts more than 2 minutes, GHG are recorded.

Similarly to the phenotyping of feed intake, animals are accustomed to the pen and the device during a minimum of 2 weeks, and the control lasts 6 weeks after the adaptation period. During this period of control, animals are fed as usual. Feed intakes can be recorded over the same period, but it must be

reminded that the concentrate delivered by the Sheep GreenFeed devices to a given animal can be eaten by another one. This can bias daily feed intake calculation.

During the control period, animals are weighed on a weekly basis, mainly for the computation of different GHG traits.

With Sheep GreenFeed, raw data are not available: GHG emissions, expressed in gram per day are downloaded from the C-Lock website, with one value per visit.

For Sheep GreenFeed, animals may visit the device whenever they want, and several times a day. However, the production of methane is not constant over 24 hours. Therefore, GHG emissions have to be corrected for the moment of the visit. This moment can be either the hour of the day (24 levels) or the period (from 4 to 6 periods can be defined, depending on how often animals are fed during the day). Individual emission rates are obtained with a repeatability model:

$CH_4 = \mu + Pen + Day + Animal + Hour + Ɛ$

The animal emission rates are the animal LSMeans estimates from this model.

Individual CO_2 estimates are obtained by applying the same repeatability model on CO_2.

4.1.2 Description protocol in INIA Uruguay – Meat sheep

The main device used in small ruminants are the PAC. They were first developed in Australia and described by Goopy et al. (2011; 2016), Robinson et al. (2014), and Paganoni et al. (2017).

In brief, the animal must be placed in a sealed chamber (860-880 litres; for large animals/breeds, larger chamber should be considered) for a known period of time, between 40 to 60 minutes, after at least three weeks of constant feeding in terms of quantity and type of feed. More than one measure (2 to 3) per animal is recommended, with a period no shorter than 7 days between estimates. The traits to evaluate would be the concentration of Oxygen, Carbon dioxide and Methane. On the measurement day with the animal placed into the chamber, CH_4, CO_2, and O_2 are recorded using a portable multi-gas detector (in parallel with a background estimation) every ten or twenty minutes. Air temperature and pressure will be also registered for the calculation of methane emission at standardized conditions. Multi-gas detector calibration, bump tests and chambers leak tests should be performed routinely. Sealing of the chamber is mandatory to guarantee isolation, which is highly recommended. Transparent chambers can be used to reduce stress, accounting for animal welfare. Records of body weight will be necessary to estimate actual gas volume in the chamber and to estimate methane intensity. Also, dry matter intake on the measurement day and previous days will be required to assess methane yield. When possible, animals can be off feed from one hour before the estimate, if extra handling is necessary and records of eaten feed and hour of last meal are available, they can stay on feed until the estimate (Robinson et al., 2020).

Data from each batch of methane measurements can be then transformed considering the body weight of the animal, the time between measures and start of measurement, the gas concentration inside and outside of the chamber, the temperature and atmospheric pressure, following the procedure described by Jonker et al. (2018).

4.2 Protocols in commercial farms

4.2.1 Description protocol in Norway – Meat sheep

Greenhouse gas emission (CH_4 and CO_2) and consumption (O_2) are measured in portable accumulation chambers (PAC) set in a truck. The truck is easier than a trailer to drive on icy roads, there is a possibility for heating, for cleaning, for carrying wastewater. The truck allows to make measurement in commercial farm. 10 chambers are used, so that gases are measured in lots of 10 animals at a time. A hand-held Eagle2 instrument is used to capture accumulated 50 min gas emissions / consumption following a measurement protocol developed in New Zealand (Jonker et al., 2020). The sheep are lambs (under 1 year) with a live weight averaging 50-60 kg and adult ewes with a live weight averaging 80-85 kg. They are placed in a sealed chamber (box) and all gases emitted accumulate in the chamber, from which gas production can be calculated. The measurements last 50 minutes. Sheep are either fed fresh grass or grass silage and are required to be off feed for at least one and less than four hours prior to entering the chamber and are in addition weighed prior to measurement. Fifty-minute CH_4 concentration is converted to CH_4 g/hr.

The measurements are:

- CH_4, ppm in ~50 min
- O_2, % in ~50 min
- CO_2, % in ~50 min
- Time of measurements
- Air pressure outside chamber, mBar
- Temperature, C
- Body Weight, kg
- Feeding
- Hours since last feeding

The computation of CH_4, CO_2 and O_2 are realised as follows:

CH_4 / CO_2 emission and O_2 consumption are measured in gram/hour:

<u>Conversion from weight to litres:</u>

Litres CH_4/hour = ppm CH_4/hour*(1146-Weight*1.01)/1000000
Litres CO_2/hour = ppm CO_2/hour*(1146-Weight*1.01)/100
Litres O_2/hour = ppm O_2/hour*(1146-Weight*1.01)/100

1.01 is the density of a sheep
1146 is the air volume (in litre) in the PAC

<u>Converting mBar to kPa:</u>

gram_CH_4/hour = Litres CH_4/hour * (0.1 * Air Pressure, mBar) * 16.043/(8.3145*(Temp in °C+273.15)
gram_CO_2/hour = Litres CO_2/hour * (0.1 * Air Pressure, mBar) * 44.01/(8.3145*(Temp in °C+273.15)
gram_O_2/hour = Litres O_2/hour * (0.1 * Air_Pressure, mBar) * 31.998/(8.3145*(Temp in °C+273.15)

16.043 is the molar mass (g) of methane
44.01 is the molar mass (g) of carbon dioxide

31.998 is the molar mass (g) of oxygen
8.3145 is the gas constant
273.15 is to convert the temperature from °C to Kelvin

4.3 Synthesis and recommendations (what can be generalised across the protocols)

Not tackled at this stage.

4.4 Definition of traits, calculation of variables

GHG emissions can be expressed as raw outputs, in grams per day, or in relation to feed intake or body weight. Different indicators have been proposed and used to express GHG emissions.

Ratio indicators:

CH_4 or CO_2 yields express gas emissions in relation to dry matter intake

$$CH_4\ yield = \frac{CH_4}{DMI}$$

CH_4 or CO_2 intensities express gas emissions in relation to live weight

$$CH_4\ intensity = \frac{CH_4}{liveweight}$$

Residual CH_4:

Similarly to residual feed intake, residual methane emissions (RME) has been proposed as an indicator of methane emissions.

In meat sheep, residual methane can be obtained as the residual from the following equation:

- Daily methane = $\mu + a \times BW^{0.75} + b \times DMI + CG + RME$ (from Smith et al., 2021)

BW and DMI are body weight and dry matter intake, respectively, and have to be recorded over the same period as gas emissions. CG is the contemporary group.

4.5 Proxies measurable routinely or not in experimental or commercial farms

The following Table 1 lists information from the SMARTER and GrassToGas projects. It presents the performance of different proxy traits for GHG emissions, measured in the SMARTER and GrassToGas projects in small ruminant populations to predict the phenotypes. The parameter measuring the efficiency of prediction for each proxy is either the coefficient of determination (r^2) or the Pearson correlation coefficient (r) between GHG emissions traits using the the complete data set (i.e., not divided between training and validation groups) and the GHG emission parameter estimated using the proxy.

Table 1: Candidate phenotypes for indirect measurements of feed efficiency.

Partner(s) [1]	Trait[2]	Species[3] (DS, MS, DG)	Experimental Commercial	Proxy of which GHG emission trait	Protocol of measure	Prediction performances[4]	# animals	Level of ease of collection on-farm[5]
NMBU	body weights	MS	experimental	CH_4 (g/day)	adult dry ewes	R^2=0.79 ; RMSE=4.85	40	1
	body weights	MS	experimental	CH_4 yield (g/g DMI)	adult dry ewes	R^2=0.33 ; RMSE=0.003	40	1
	body weights	MS	experimental	CH_4 intensity (g/kg BW)	adult dry ewes	R^2=0.30 ; RMSE=0.06	40	1
	feed intake	MS	experimental	CH_4 (g/day)	adult dry ewes	R^2=0.85 ; RMSE=4.20	40	3
	feed intake	MS	experimental	CH_4 yield (g/g DMI)	adult dry ewes	R^2=0.36 ; RMSE=0.003	40	3
	feed intake	MS	experimental	CH_4 intensity (g/kg BW)	adult dry ewes	R^2=0.51 ; RMSE=0.05	40	3
	daily nb of meals	MS	Experimental	CH_4 (g/day)	adult dry ewes	R^2=0.69 ; RMSE=6.03	40	2
	daily nb of meals	MS	Experimental	CH_4 yield (g/g DMI)	adult dry ewes	R^2=0.33 ; RMSE=0.003	40	2
	daily nb of meals	MS	Experimental	CH_4 intensity (g/kg BW)	adult dry ewes	R^2=0.31 ; RMSE=0.06	40	2
Teagasc	body weights	MS	Experimental	CH_4 (g/day)	immature and mature sheep	R^2=0.82 ; RMSE=3.57	4867	1
	body weights	MS	Experimental	CH_4 yield (g/g DMI)	immature and mature sheep	R^2=0.87 ; RMSE=0.226	517	1
	body weights	MS	Experimental	CH_4 intensity (g/kg BW)	immature and mature sheep	R^2=0.75 ; RMSE=0.06	4867	1

Partner(s) [1]	Trait[2]	Species[3] (DS, MS, DG)	Experimental Commercial	Proxy of which GHG emission trait	Protocol of measure	Prediction performances[4]	# animals	Level of ease of collection on-farm[5]
	body composition (ultrasound)	MS	Experimental	CH_4 (g/day)	immature and mature sheep	R^2=0.87 ; RMSE=3.05	800	1
	body composition (ultrasound)	MS	Experimental	CH_4 yield (g/g DMI)	immature and mature sheep	R^2=0.88 ; RMSE=0.221	800	1
	body composition (ultrasound)	MS	Experimental	CH_4 intensity (g/kg BW)	immature and mature sheep	R^2=0.68 ; RMSE=0.07	800	1
INIA	feed intake	MS		CH_4 (g/day)	male and female lambs	AIC from 9199 to 9305 (based model AIC = 9560)	1400	3
	body weights	MS		CH_4 (g/day)	male and female lambs	AIC from 9312 to 9489 (based model AIC = 9560)	1400	1
AgResearch								

[1]The organisations/institutes involved: INIA_UY (Uruguay), AgResearch (New Zealand), TEAGASC (Ireland), NMBU=Norwegian University of Life Sciences (Norway)

[2]The type of trait considered as a proxy

[3]Species are defined as Dairy Sheep (DS), Meat Sheep (MS) and Dairy Goat (DG).

[4]Prediction performances: The parameter measuring the efficiency for each proxy to predict GHG is either the coefficient of determination (r^2) or the Pearson correlation coefficient (r) between GHG predictions using the gold standard measure from the complete data set (i.e., not divided between training and validation groups) and the GHG parameter estimated using the proxy.

[5]Scale of difficulty in measuring the proxy on-farm: 1=routinely collected; 2=possible but hard/costly to collect in routine; and 3=very difficult to collect in routine".

5 Use for genetic evaluation

5.1 Models for genetic analysis / evaluation

To decrease GHG emissions, selection programmes can use different models for the genetic analyses. Table 2 shows the models used by different analyses of GHG emissions criteria.

Table 2: List of fixed and random effects used to analyse GHG emissions criteria.

Trait name[1]	Species (DS, MS, DG)[2]	Fixed effects	Random effects	Notes
Gram CH_4 / CO_2 /O_2 per hour	MS	Flock, age, lot (pen / trial), sex, birth type, dam age, live weight (fixed regression), age at measurement (fixed regression)	Animal	

[2]Species are defined as Dairy Sheep (DS), Meat Sheep (MS) and Dairy Goat (DG).

5.2 Genetic parameters

Genetic parameters obtained from the previously described protocols and using models presented in Table 2 are given in the following Table 3.

Table 3: Genetic parameters for GHG emissions traits estimated in small ruminants.

Traits	Species (DS, MS, DG)[1]	Heritability	Genetic standard deviation	Coefficient of variation (%)	Notes
CH_4 (gram per hour)	MS (adult ewes)	0.18			SMARTER, Jette Jakobsen, NSG (Jakobsen et al, 2022)
CO_2 (gram per hour)	MS (adult ewes)	0.31			SMARTER, Jette Jakobsen, NSG (Jakobsen et al, 2022)
CH_4 (gram per day)	MS (wool)	0.23			Marques et al 2022
CO_2 (gram per day)	MS (wool)	0.27			Marques et al 2022
O_2 (gram per day)	MS (wool)	0.26			Marques et al 2022

[1]Species are defined as Dairy Sheep (DS), Meat Sheep (MS) and Dairy Goat (DG).

6 Acknowledgements

We gratefully acknowledge the contributions to small ruminant Feed Efficiency guidelines by the following people:

- Flavie Tortereau, INRAE, France
- Christel Marie-Etancelin, INRAE, France
- Jean-Michel Astruc, IDELE, France
- Ignacio de Barbieri, INIA, Uruguay
- Gabriel Ciappesoni, INIA, Uruguay
- Jette Jakobsen, Norwegian Sheep and Goat Association, Norway
- Patricia Johnson, AgResearch, New Zealand
- Arjan Jonker, AgResearch, New Zealand
- Nicola Lambe, SRUC, UK
- Fiona Mc Govern, Teagasc, Ireland
- Bente Aspeholen, NMBU, Norway
- Elly Navajas, INIA, Uruguay
- Suzanne Rowe, AgResearch, New Zealand

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

This work received funding from the European Unions' Horizon2020 Research & Innovation Programme under grant agreement No 696356—GrassToGas.

7 References

Conington J., Lambe N., Tortereau F., McGovern F., Navajas E., De Barbieri I., Ciappesoni G., Jakobsen J., Smith E., Yates J., Le Graverand Q., McDermott K., Steinheim G., Aspeholen Aby B., Dønnem I., McHugh N., Farrell L., Marie-Etancelin C., Johnson P., Rowe S. (2022). Strategies to mitigate greenhouse gas emissions from pasture-based sheep systems – an EU project consortium view. Proc. of WCGALP 12, Rotterdam, the Netherlands, 3-8 July 2022.

Goopy J, Woodgate R, Donaldson A, Robinson DL, Hegarty RS. 2011. Validation of a short-term methane measurement using portable static chambers to estimate daily methane production in sheep. Anim. Feed Sci. Technol. 166–167, 219–226.

Goopy J, Robinson DL, Woodgate RT, Donaldson AJ, Oddy VH, Vercoe PE, Hegarty RS. 2016. Estimates of repeatability and heritability of methane production in sheep using portable accumulation chambers. Anim. Prod. Sci. 56, 116-122.

Jakobsen J.H., Blichfelct T., Linneflaatten L.-B., Gloersen M.O., Wallin L.E., McEwan J.C. (2022). Methane emission has low genetic correlations to lamb growth traits in Norwegian White sheep. Proc. of WCGALP 12, Rotterdam, the Netherlands, 3-8 July 2022.

Jonker A, Hickey SM, Rowe SJ, Janssen PH, Shackell G, Elmes S, Bain W, Wing J, Geer G, Bryson B, MacLean S, Dodds K, Pinares-Patiño C, Young E, Knowler K, Pickering N, MacLean S. 2018. Genetic parameters of methane emissions determined using portable accumulation chambers in lambs and ewes grazing pasture and genetic correlations with emissions determined in respiration chambers. Journal of Animal Science 96(8), 3031–3042.

Jonker, A., Hickey, S.M., McEwan, J.C., and Waghorn G. (2020). Guidelines for estimating methane emissions from individual ruminants using: GreenFeed, 'sniffers', hand-held laser detector and portable accumulation chambers. Ministry for Primary Industries, Wellington, New Zealand. https://www.mpi.govt.nz/resources-and-forms/publications/

Marques C.B, De Barbieri I., Velazco J., Navajas E.A. and Ciappesoni G (2022). Genetic parameters for feed efficiency, gas emissions, oxygen consumption and wool traits in Australian Merino. Proc. of WCGALP 12, Rotterdam, the Netherlands, 3-8 July 2022.

Paganoni B, Rose G, Macleay C, Jones C, Brown DJ, Kearney G, Ferguson M, Thompson AN. 2017. More feed efficient sheep produce less methane and carbon dioxide when eating high-quality pellets. J. Anim. Sci. 95, 3839–3850.

Robinson DL, Goopy JP, Hegarty RS, Oddy VH, Thompson AN, Toovey AF, Macleay A, Briegal JR, Woodgate RT, Donaldson AJ, Vercoe PE. 2014. Genetic and environmental variation in methane emissions of sheep at pasture. J. Anim. Sci. 92, 4349–4363.

Robinson DL, Dominik S, Donaldson AJ, Oddy VH. 2020. Repeatabilities, heritabilities and correlations of methane and feed intake of sheep in respiration and portable chambers. Animal Production Science 60, 880-892.

Rozier A., François D., Maupetit D., Legoff Y., and Tortereau F. 2021. First estimations of methane emissions using Sheep GreenFeed in the Romane breed. 72nd EAAP, 2021, Davos, Switzerland.

Rowe S.J., Hickey S.M., Johnson P.L., Bilton T.P., Jonker A., Bain W., Veenvliet B., Pile G., Bryson B., Knowler K., Amyes N.C., Newman S.A. and McEwan J.C. (2021). The contribution animal breeding can make to industry carbon neutrality goals. Proc. of AAABG. Adelaide, Australia, 2-4th Nov. 2021. http://www.aaabg.org/aaabghome/proceedings23.php.

Smith et al., 2021. Effect of divergence in residual methane emissions on feed intake and efficiency, growth and carcass performance, and indices of rumen fermentation and methane emissions in finishing beef cattle. JAS Volume 99, Issue 11, November 2021, skab275 https://doi.org/10.1093/jas/skab275

Recommendations on recording the environment

SMARTER – Deliverable D6.3

Version 1.0 – 17 May 2023

Table des matières

Change Summary

Date of change	Nature of Change
October 2022	First draft
May 2023	Final version for SMARTER deliverable 6.3

1 Introduction

In the genetic evaluation process, the genetic model includes environmental effects (generally fixed effects, in some cases random effects) to correct the phenotypes from these effects, not related to the genetic value of the animal. These environmental effects that affects the expression of the genotypes depend on the traits and the method of phenotyping, the environment itself (flock/herd, year, parity, season of lambing, number of born or reared lambs/kids, scorer, gender of the lamb/kid, management of mob groups, etc). The quality of the record of the environment is important to correct relevantly the performance of the animal.

Some other environmental effects that are usually included in a general flock/year or management mob group effect could be identified, such as the feeding effect or the climate effect. By including these effects in the genetic model, we could get less biased and more precise EBVs, especially when these effects are individualised or are period-specific (feeding might depend on such and such groups of animals, climate might influence the performance of such and such test-day). Moreover, the more precise knowledge of environmental effect might be valorised for flock/herd management and extension services towards farmers.

Moreover, feeding can be considered as an environmental effect, but as well be constitutive of a performance. This is typically the case for feed efficiency where the quantity and the quality of the diets allows to calculate the phenotype.

Likewise, with the climatic change, breeding for animals more resistant or more resilient to higher temperatures (especially thermal stress) becomes a selection objective per se (example of heat tolerance). In this context, the conditions of temperatures (or temperature/humidity combination) not only might be an environmental factor, but be part of the phenotype.

Other environmental effects can be described and should enrich this document in the future.

2 Scope

This document focuses on those data that are worth recording the precise the environment or to calculate novel traits of interest.

Following SMARTER work, the document will describe the record of the diet (section 3) and the record of meteorological data (section 4).

Further factors might be described later, letting this document open to new section in the future.

Recording the diet in small ruminant

Recording meteorological data

Other environmental records

3 Recording the diet

3.1 Definition, terminology, rationale

Recording the diet consists in collecting data on the quantity and quality of a ration that an animal, a group of animals of a flock/herd consumes at a given period.

The characterisation of the ration, in terms of energy and protein depends upon the countries. For example, the French INRAE Feeding System for Ruminants (Nozière et al., 2018) is different from the British one (AFRC, 1993). This is the reason for which we will describe in this section general recommendations, that can be applied, translated to the domestic feeding system used.

Breeding for more efficient animals is more and more important for economic reason (the feeding resources are costly, might be rare in years with climatic excess such as heat or drought) and for environmental reasons (feed/food competition, emission of green-house gases). Feed efficiency is a trait of high interest in this context. Even though it is deceptive to calculate gold standard efficiency trait in private farm, the knowledge of diets in those farms should help to correctly manage the proxies that are promoted in SMARTER. Diet could also be used as a corrective factor in evaluation models in the future. In addition, it might be a support to better understand the herd/flock effect and its variation across year, and therefore give more acute and relevant advice to the farmers.

It is difficult and time-consuming to collect the data for establishing the diet in the flock/herds. The diet is collective in most of the situations (the same amount of forage is given to all animal because the forage is not given individually). When the concentrate is given through Automated Concentrate Feeder (ACF) in the milking parlour, the individualisation is not at the animal scale but at a limited number of groups scale. That's why we suggest recommendations that must be adapted to each situation.

The aim is to tend to the better possible estimation of the forage ingestion, given that the direct measurement is impossible in commercial farms. Proxies are studied to get indirect measurement of the intake, but they are not validated so far (Near Infra Red Spectra technique). As soon as validated results are available, these recommendations will be updated.

3.2 Data recording

3.2.1 When to record the diet

The diet may be recorded at relevant period of the physiological status of the animals in the flock/herd. It is possible to take advantage of the visit of a technician to record the ration (for example when performance recording such as at each (or some of the) test-day when milk recording, or at weighing visit in meat sheep performance recording.

Below are examples of relevant physiological status:

- At mating (or before the mating and after the mating)
- End of gestation (in the month preceding the lambing/kidding)
- After lambing/kidding
- At weaning or just after weaning (peak of production in dairy animals)
- Dairy animals: at each test-day or at some of the test-day

In case of ACF (Automatic Concentrate Feeder), it is possible to record the distribution of concentrate more frequently.

It may be useful to establish the requirements of animals (on average) at each point of diet record. The requirements must concern the energy (in the unit usually used in the country) and the protein (in the unit usually used in the country).

3.2.2 How to record the diet

Individual diet

- This can be obtained through ACF for concentrate, mainly in the milking parlour.
- Intake of forage cannot be collected individually but can be predicted through the intake capacity system, such as the one proposed by INRAE (Nozière et al., 2018).

Collective diet (at the flock/herd scale or at the mob scale)

- Forage (hay, or haylage): some bales of each preservation technic can be weighed once a year with a dry matter (DM) measurement for haylage (it can substantially vary). For hay, DM can be estimated at 85%. Afterward, we can just record how many bales of a given quality (several cutting stages are preserved and not given at random) are distributed per flock per time unit. For silages, it is more complicated, but based on the same procedure, we can weigh one distribution (assuming that it will be constant over time) and simultaneously measured DM. In both situations, if refusals

cannot be measured, they must be sufficient for assuming an ad libitum distribution. When the feeding system used in the country can predict the DM intake through the intake capacity of the animal and the quality of the feed, individual diet can be estimated.

- Grazing: for dairy sheep grazing within a short duration per day or the full day, intake can be estimated through ad hoc system. As an example, the new French INRATion feeding software (INRATion V5®) proposes such estimation based on grazing duration, biomass availability and quality.

3.2.3 Defining the constitution of the diet

3.2.3.1 Precise the type of distribution of the ration:

- collective ration
- individual ration (concentrate when ACF)
- pasture

3.2.3.2 Categories of feedstuff

- Hay
- Partially or fully fermented fodder and fodder preserved by silaging or wrapping:
  - Silage
  - Wrapped bales
- Pasture
- Straw
- Green feeding
- Dehydrated alfalfa
- Pulp (dehydrated beet pulp, citrus pulp, etc)
- Cake (soybean, rapeseed or sunflower seed)
- Cereals grain (wheat, barley, maize, etc)
- Complete commercial concentrate
- Other by-products of agro-food industry (cereal brans, brewer's grains, hulls etc.)

3.2.3.3 Species

For each category, specify the species (rye grass, alfalfa, clover, maize, wheat, barley, etc), physiological stage or age of regrowth, and harvest conditions (cutting length of the forage and added preservative or not for silages, conditions of hay making drying in the field or mechanically dried).

3.2.4 Characterizing the diet

3.2.4.1 Quantity

Quantity distributed, refused, consumed. Check that these amounts are regularly distributed, refused and consumed because it can markedly influence the animal performance specifically for dairy animals at test day.

The quantity of each feedstuff may be expressed in kg dry matter for forage, in kg gross matter for concentrate. However, final diet for requirement calculation must be expressed as DM.

3.2.4.2 Requirements

Requirements for the main categories of animals: it depends on the physiological status (maintenance, production, growing, pregnancy)

Average requirement coverage ratio (energy and nitrogen). For example, the requirement coverage ratio in French dairy sheep is roughly 115% for energy and about 125% for nitrogen of the requirements of the average ewe. That allows covering the requirements of about 85-90% of the flock. Difference between energy and nitrogen is assumed to be covered through the body reserve mobilisation.

3.2.4.3 Quality characterization

The feedstuffs and the ration must be characterized at least in terms of

- Energy
- Protein (or nitrogen)

In case of commercial concentrate, data written on the label are used.

Energy and protein can be expressed in the current unit used in the country.

For example, in France, energy is expressed in UFL which is equal to 1.7 Mcal Net energy (Nozière et al., 2018).

It may also be expressed in the international unit, which can be Mcal or MJ.

3.3 Use for genetic analysis / genetic evaluation

Diet as part of a phenotype

Calculation of feed efficiency phenotypes: see recommendations on feed efficiency.

Diet as part of a factor in the evaluation model

In most of situations it is impossible in small ruminants to establish individual consumption, for practical reason. The collective effect of the diet is explained in the flock/year effect. The intermediate situation should be when ACF allows to identify several groups within the flock/herd, at a specific test-day or visit. It is possible in this case to put in the model a mob effect grouping animals being given the same amount of concentrate. This should result in a more precise calculation of the breeding value of the animal. Nevertheless, this approach has so far not be used to our knowledge.

4 Meteorological data

4.1 Definition, terminology, rationale

Meteorological conditions may affect the environment effect on the traits of interest. Even though they may be absorbed in a flock effect at the scale of the year or at the scale of a given test-day, it is relevant to be able to quantify the effect of such and such meteorological parameter (and especially the heat stress) ot the zootechnical traits. The global warming and the higher temperature in which the animals are bred emphasises this interest. It is possible to better assess the comfort zone of the populations, that means the meteorological conditions in which the zootechnical traits are not affected. It is also possible to identify animals better adapted to an increase in temperatures or able to be resilient to a wide range of temperatures, that means to maintain their productive ability. In this case, meteorological data, combined with a production trait (growth, milk production, milk composition) or fertility trait, are used as a resilience characterisation by assessing the ability of the animals to recover their production following meteorological challenges.

Meteorological data are mostly temperature, humidity, precipitations, wind speed and radiations.

An issue in small ruminants is to select for adapted animals to new environmental challenges, without artificializing their environment of breeding. Mainly because the economic and societal constraints are such as breeding animals outdoors on pasture is desired and breeding indoors in artificialized environment may be costly in terms of energy.

4.2 Data recording

4.2.1 Meteorological data from weather station

The aim is to affect outdoors meteorological data to a farm. This can be obtained by assigning to the farm the meteorological data of the closest or more relevant weather stations, using the geographical coordinates of both the farm and the weather station.

The following data may be used:

- Temperature (minimum, maximum, average)
- Relative humidity (amount of moisture in air compared to the maximum amount of moisture it can have at a specific temperature). Expressed in %.
- Specific humidity (ratio of water vapor mass to the total mass of air and water vapor.
- Wind speed
- Precipitations and precipitation type
- Solar radiation
- Atmospheric radiation
- Evapotranspiration

Different index accounting for weather factors have been proposed. One of the most popular is the Temperature Humidity Index (THI) which may be calculated to get a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. Different formulas of THI are proposed in the literature. Below is an example of formula proposed by Finocchiaro (et al., 2005):

$$THI = T - [0.55 \times (1 - RH)/100] \times (T - 14.4)$$

Where T is the mean daily in °C and RH is the mean relative humidity expressed in percent.

Quite often, the parameter used in the analysis model is the temperature of the THI (mainly because temperature and relative humidity are the most available parameters).

Let us also mention the Heat Load Index, referred to as the 'HLI', which is an index that brings together all the weather factors into one number to allow easy interpretation of the cooling capacity of the environment.

The assignation of meteorological data to a farm depends on the countries and on the availability of weather data.

In some countries, the territory may be cut out in a grid, each cell of the grid being considered to have the same meteorological parameters because they are close to the same weather station of reference. As an example, this is the case in France with a grid named SAFRAN cutting the territory into 9892 cells of 64 square kilometres each [8 km by 8 km] (Annex 1). This grid was used, thanks to specific permission from Meteo France, to affect each farm of a given project (by using its GPS coordinate) to a single cell of the grid and thus get relevant meteorological parameters.

The meteorological spatialised data are collected from weather station, on which specific interpolation are applied to present these data on the SAFRAN grid.

The meteorological data key period to consider must be thought according to the production system associated to the breed, type of traits measured and analysed. For example, for milk production (milk recording), we may consider the 3 days preceding the test-day. For semen production, we may consider the meteorological data either at the day of the semen collection, or during the spermatogenesis, which is around 50 days before the semen collection. For the insemination itself (which is in case of fresh semen the same day as semen production), we may consider climate data either the very day of the insemination operation or during a week preceding it.

4.2.2 Environmental data from sensor in the farm

Temperature and humidity may also be collected on site, thanks to sensors situated on-farm, for example in the sheep pen or the stable.

The number of sensors may depend upon the situation and configuration of each building, the goal being to be representative of the pen. In the practical situations of the SMARTER project, 2 to 3 sensors were set in the pen where animals are indoors at a height of 2 meters above the ground, so that they are protected from the animals. If the pen is already equipped by sensors, it is possible to retrieve the data from the existing sensors. The sensors must cover

all the relevant groups of animals (primiparous, multiparous, etc), even if they are in different buildings. Measures might be collected several times a day, for example once an hour, to get a precise evaluation of the daily temperature and hygrometry. To relevantly collect the atmosphere of the building, the sensors must be set in a place free from too much air flow or too much sunshine. It is important to regularly check the batteries to avoid loss of data.

4.3 Use for genetic analysis / genetic evaluation

Effect of meteorological parameters (eg. temperature or THI) may be estimated on zootechnical traits, using different types of linear models.

The parameter may be considered as a categorical variable (each degree of the parameter being defined as a different class). Or it may be considered in a linear regression on degrees of the parameter.

Reaction norms model, using Legendre polynomial for example, may be used to assess populational losses of the zootechnical trait due to high or low temperature and/or humidity.

Two types of analysis can be made:

-a populational analysis (populational response to the effect of temperature or THI). It gives the comfort rage of each population and how much the loss is with lower or higher temperature or THI.

-an analysis of the genetic components using a random regression model. It permits to estimates genetic parameters of traits according to the temperature or THI and to calculate EBVs of animals at different temperatures or THI levels. Such EBVs allow to identify less vulnerable animals along a range of climate values, so as to identify and select the most robust animals.

5 Other environmental record

To be completed (or not) when necessary

6 Acknowledgements

We gratefully acknowledge the contributions to these lifetime resilience guidelines by the following people:

- Jean-Michel Astruc, IDELE, France
- Antonello Carta, Agris, Italy
- Philippe Hassoun, INRAE, France,
- Gilles Lagriffoul, IDELE, France
- Carolina Pineda Quiroga, NEIKER, Spain

- Eva Ugarte, NEIKER, Spain

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

7 References

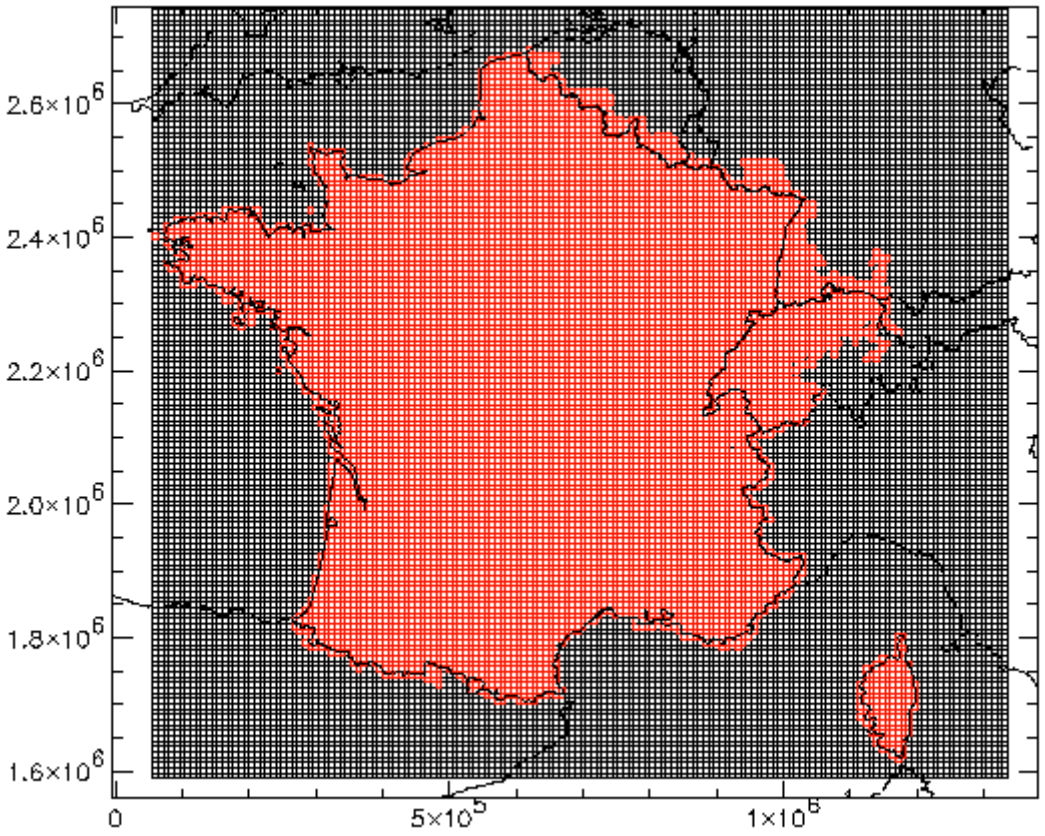
Finocchiaro R, van Kaam JB, Portolano B, Misztal I. Effect of heat stress on production of Mediterranean dairy sheep. J Dairy Sci. 2005 May;88(5):1855-64. doi: 10.3168/jds.S0022-0302(05)72860-5. PMID: 15829679.

Nozière, P., Sauvant, D., Delaby, L. 2018. INRA Feeding System for Ruminants. Wageningen Academic Publishers, 640 p., 2018, 978-90-8686-292-4. ⟨10.3920/978-90-8686-292-4⟩. ⟨hal-02791719⟩

AFRC (Agricultural and Food Research Council). 1993. Energy and protein requirements of ruminants. CAB International, Wallingford.

8 Annexes

8.1 Annex 1: SAFRAN grid from Meteo France in the case of France



Recommendations on recording health and disease traits

SMARTER – Deliverable D6.3

Version 1.0 – 17 May 2023

Contents

Change Summary

Date of change	Nature of Change
December 2022	First draft
May 2023	Final version for SMARTER deliverable 6.3

1 Introduction

Health and resistance to disease are keys factors for increasing resilience in farm animals in general and in small ruminants in particular. Among the challenges that sheep and goats must face, the infectious challenges are among the most important. They lead to losses of production and difficulties of reproduction. They also generate an increase in the consumption of chemical input. Beyond actual extra cost that may hamper the sustainability of the farms, but also of the breeding programs, there is a risk for the environment and the occurrence of resistance to drugs.

In most cases, an integrated approach is the more beneficial and efficient, mixing the different leverages. Among them, the control of the challenges by the host through its genetic resistance has shown its efficiency for some disease (resistance to scrapie, resistance to mastitis in dairy species) or is promising (resistance to parasites, resistance to footrot).

These guidelines on health and disease phenotypes are dedicated to any kind of health and disease resistance indicators. However, to start, we focus on the traits studied in SMARTER, which are the resistance to parasites and the resistance to footrot and mastitis in meat sheep and dairy sheep and goats.

2 Scope

This section on recording health and disease in sheep and goats is intended to evolve and to be completed in the following years.

Starting from the task achieved in SMARTER, the section starts with the three following sub-sections:

- Resistance to parasites
- Resistance to mastitis
- Resistance to footrot

3 Resistance to parasites

Definition, terminology, rationale

The resistance to parasites described here corresponds to the resistance to gastro-intestinal nematodes (GIN). They are one of the main constraints for grazing sheep. They cause substantial economic losses due to lower production levels, the costs of anthelmintic treatments and the mortality

of severely affected sheep. GIN control strategies mainly rely on treatment with anthelmintics. In many regions of the world, studies have reported the development of GIN resistance to most anthelmintic molecules due to their extensive use. Additionally, the possible presence of drug residues in animal products and the negative impact of these molecules on the micro and macro fauna of the soil are of concern. Therefore, sustainable GIN control may be a priority with schemes that do not only rely on anthelmintics but include complementary strategies such as nutritional supplementation with tannins and/or proteins, pasture management and genetic selection of resistant animals. This latter strategy relies on the existence of genetic variation of host resistance to GIN both between and within breeds. The faecal egg count (FEC), which is the number of parasite eggs per gram of faeces, is the most commonly used indicator to assess this resistance to GIN.

In many countries, the selection for parasite resistance is based on FEC measures in natural infestation conditions under natural grazing conditions. As FEC measurements in sheep and goats are extremely costly and laborious, and because response to artificial challenges is highly correlated to response to natural infestation, it is therefore possible to implement a protocol of experimental infestation, as it is the case in France.

3.1 Data recording

3.1.1 Indicators of parasite resistance or resilience

3.1.1.1 Faecal Egg Count

Faecal Egg Count (FEC) is the main indicator that measures the egg excretion intensity. It measures the number of parasite eggs per gram of faeces. FEC is determined for each sample using a modified MacMaster technique (Whitlock, 1948 or Raynaud, 1970) with a sensitivity of 100 or 15 eggs per gram, respectively.

The measure may be done in natural infestation or in experimental infestation.

FEC can be applied to one species (for example Haemonchus contortus) or several species (including Hc, Teladorsagia circumcincta, Trichostrongylus colubriformis, etc).

The distribution of the FEC has an asymmetric distribution (some high value, many low or medium value). A transformation must be applied to process a genetic analysis. The most frequent transformations are a root (fourth, third or square root) or a log transformation.

This trait is related to the resistance of the animal (ability to limit the installation, the development and the prolificacy of the nematode inside the digestive tract (especially the abomasum).

3.1.1.2 Packed Cell Volume

Packed Cell Volume (PCV) - Blood samples were collected in EDTA coated tubes and PCV values were determined individually by centrifugation in microhematocrit tubes with a relative centrifugal force of 9500 for 10 min.

PCV can be exploited as a single value of more relevantly as a gain/loss of PCV between two points.

Variation of PCV is a relevant indicator of the resilience of the sheep / goat.

3.1.1.3 FAMACHA score

FAMACHA® score – As the anaemia provoked by some hematophagous parasites is at some stage visible on the mucosa (especially ocular mucosa), a scale of grading, based on the colour of the ocular mucosa, ranging from 1 (dark red mucosa) to 5 (white mucosa) has been built. This score was developed in South Africa to facilitate the clinical identification of anaemic sheep infected with H. contortus (Van Wyk and Bath, 2002).

As drawbacks, the FAMACHA® score does not allow to detect the non-hematophagous parasites and it appears quite belatedly: a FAMACHA® score over 3 concerns animals with a PCV below 20%. The method is not specific, anaemia being possibly caused by other reason than Haemonchus contortus. It is however interesting to detect the anaemia.

FAMACHA® score is related to the resilience of the sheep / goat.

3.1.1.4 DAG score

DAG score is an indicator for assessing dagginess, which measures faecal soiling in sheep.

DAG score uses a 5-point or 6-point scoring scale ranging from 0 (no dags) to 5 or 6 (very daggy). Dag score scale shows the degree or extent of faecal contamination of the fleece.

The key is to be consistent when scoring a mob of sheep and for these sheep to have been run under similar conditions. Faecal contamination should not be confused with urine stain in ewe lambs and hoggets.

3.1.1.5 Immunological traits

Immunological and physiological profiles may be linked to phenotypes of resistance to parasites (strongyles).

These new immunological and physiological profiles are blood lymphocytes cytokine production and serum levels of nematode parasite-specific Immunoglobulin A (IgA) that are produced upon whole blood stimulation. In SMARTER experiment in SRUC, blood was stimulated with pokeweed mitogen (a lectin that non-specifically activates lymphocytes irrespectively of their antigen specificity), and Teladorsagia circumcincta (T-ci) larval antigen to activate parasite-specific T lymphocytes.

Adaptive immune response may be determined by quantifying:

- the cytokines interferon-gamma (IFN-γ), which relate to T-helper type 1 (Th1)
- The interleukin IL-4, which relates to T-helper type 2 (Th2)
- The interleukin IL-10, which relate to regulatory T cell (Treg) responses

Each immune trait displays a significant genetic variation (heritabilities ranging from 0.14 to 0.77). Heritability of IgA is moderate (0.41). Correlations with FEC are rather weak, from 0 to 0.27 but not significantly different from 0.

3.1.1.6 Blood Pepsinogen dosing

Blood pepsinogen is an indicator of the integrity of the gastric mucosa.

The determination of serum pepsinogen is therefore a proxy in the diagnosis of abomasal strongylosis of ruminants (pepsinogen in blood is caused by an increase in the permeability of the abomasum

mucosa due to presence of nematodes). There is a correlation between the concentration of pepsinogen in the blood and the number of worms harboured by the host.

3.1.2 Natural infestation

3.1.2.1 General considerations

Measurements (FEC or other proxies) are mainly undertaken in natural infestation under natural grazing conditions. In natural condition of infestation, frequency and amounts of yearly samplings have to be assessed according to the climate and epidemiological conditions and breeds. Local knowledge is essential for adjusting protocols to each country, as the level of infestation is strongly influenced by seasonality and the grazing system.

Several countries (e.g. Australia, New Zealand, and Uruguay), have incorporated the genetic evaluation of faecal egg count at various ages into their national evaluation systems.

In any case, in order to have data useful for the genetic evaluation, a representative sample of sheep in the flock involved in the selection scheme has to be periodically monitored to decide whether to sample the whole flock, i.e. when the number of infected animals and the level of infestation are considered sufficient to appreciate individual variability, individual FEC can be measured on the whole flock.

Further data related to environmental factors affecting the level of infestation should be recorded to be included in the genetic model for estimating the breeding values:

- Farm management mainly grazing system
- Age
- Parity
- Lambing date
- Sampling date
- Frequency, date, and molecule of anthelmintic administration

Additionally, stool cultures can be performed from the faecal samples taken (one per management group).

3.1.2.2 Description of the protocol and the measures (Uruguayan protocol)

At weaning, lambs undergo anthelmintic treatment, and their treatment efficacy is checked 8-14 days later through the analysis of FEC samples from 20 randomly selected lambs to confirm the absence of egg excretion. Subsequently, FEC is monitored every 15 days by collecting samples (based on epidemiological conditions) from 10-15% of lambs in each management group. The first individual FEC sampling is conducted when the FEC arithmetic mean exceeds 500 with no more than 20% samples exhibiting zero FEC. At this point, the lambs undergo anthelmintic treatment again, and their treatment efficacy is evaluated after 8-14 days. If the FEC mean remains above 500, a second individual sampling is conducted. Throughout the protocol, faecal egg counts (FEC1 and FEC2) are measured at the end of the first and second natural infestations. Generally, with some variations based on the breed, these samplings correspond to lambs at 9 and 11 months of age, respectively.

Currently, to simplify the protocol, only one sampling is conducted, and the control begins on a fixed date (early autumn) when the most significant parasite, H. contortus, prevails.

Along with the FEC records (FEC1 and FEC2), other records, such as body weight, FAMACHA®, and body condition score, can also be taken.

3.1.3 Experimental infestation (French protocol)

As mentioned above FEC measurements on sheep in commercial flocks are extremely costly and laborious. It has been shown that sheep that are selected on the basis of their response to artificial challenges respond similarly when exposed to natural infestation, and a high positive genetic correlation was estimated between FEC recorded under artificial or natural infestation. Moreover, it has been shown that selection of rams for parasite resistance after artificial challenges allows to improve the resistance of their female offspring for parasite infestation in natural conditions.

Thus, an alternative approach may be to select rams gathered for AI progeny-testing or performance-testing by artificially infecting them with standardized doses of larvae.

In most cases, resistance to GIN is assessed in natural infestation conditions at grazing. However, the intensity of natural infestation in grazing animals depends on climatic conditions and may vary from season to season leading to over- or under-estimation of the genetic parameters of resistance. In France, sheep breeds are selected collectively on selection stations and the strategy is to take advantage of this organization to implement the GIN control selection by phenotyping rams after experimental infestation. There are two main advantages. Firstly, a large diffusion of the genetic progress is expected via these rams, which are the future elite males. Secondly, the experimental infestation performed in control stations allow to evaluate these rams in homogeneous conditions (standardization of doses of infestation, farming conditions, climatic conditions, etc) during the ram evaluation period. Previous studies (Gruner et al, 2004) estimated high genetic correlations between resistances to experimental and natural infestation, between infestation by different parasite species (Haemonchus contortus and Trichostrongylus colubriformis) and between resistance in adult sheep and lambs. Moreover, recent works have shown that the genetic correlation between the resistance of rams in experimental conditions and the resistance of pregnant or milking ewes in natural conditions of GIN infestation are high.

3.1.3.1 Description of the protocol and the measures

An original protocol for phenotyping resistance to gastro-intestinal parasitism has been conceived and developed in France, targeted to rams (or bucks) gathered in a breeding centre or station, or an AI centre (Jacquiet et al, 2015; Aguerre et al, 2018). Males bred indoors, supposed to be naïve, are artificially infected twice with L3 larvae of a given strain of Haemonchus contortus susceptible to anthelminthic. Males are subjected to a first infestation (after a coprological examination be performed to confirm that no eggs were excreted before the artificial infestation) with a given dose of L3 larvae (D0). At D30, the males are phenotyped (FEC30 and possibly PCV30) and treated with an anthelminthic. After a 15-day recovery period, the rams are challenged again with a given dose of L3 larvae of Haemonchus contortus. At that time (D45), the efficacy of anthelmintic treatment is ensured in each male. Thirty days after (D75) the second challenge, the males are phenotyped (FEC30 and possibly PCV30) and treated again. The protocol lasts 2 and a half months.

During the protocol, the measures carried out are as follows:

-faecal egg counts (FEC30 and FEC75) at the end of the first and second infestation (from faecal sample).

-packed cell volumes PCV0, PCV30, PCV45 and PCV75 at the start and the end of both infestation (from blood sample).

3.1.3.2 Calculation of variables

The FEC30 and FEC75 are used per se.

Variations of PCV are calculated:

- PCV_loss_inf1 = PCV0-PCV30 (or ratio PCV30/PCV0)
- PCV_loss_inf2 = PCV45-PCV75 (or ratio PCV75/PCV45)
- PCV_recovery = PCV45-PCV0

PCV_loss_inf1 and PCV_loss_inf2 represent the loss of PCV after each infestation.

PCV_recovery represents the males' capacity to recover after the first infestation.

PCV variations might be interpreted as an indicator of resilience of the animal, i.e. its ability to maintain its blood parameters despite the parasitical challenge.

3.2 Use for genetic analysis / genetic evaluation

3.2.1 Model for genetic analysis

The genetic analysis of experimentally infected animals that are raised indoors may include:

- Fixed effects: contemporary group (mob x doses of larvae), age of animals (eg. 1 year, 2 years, 3years, 4 years and older)
- Random additive effect of the animals
- Random residual effect

The genetic analysis of naturally infected animals that are raised outdoors may include:

- Fixed effects: they obviously will depend of the type of animals (females in lactation *vs* lambs/kids). They should include flock/herd, year x season (e.g. spring, summer, autumn, winter), anthelmintic treatments (e.g. eprinomectin, ivermectin, moxidectin …) in interaction with the number of days between the date of treatment and the sampling date (e.g. less than 70 days, between 70 and 100 days, more than 100 days). For females in lactation: age and/or parity, litter size before lactation (single or multiple new-born lambs). For lambs or kids: age of the dam, type of birth or rearing, and age at the time of the records, expressed in day.
- Random additive effect of the animals
- Random permanent environment effect if repeated measures (e.g. for FEC 1 & 2)
- Random residual effect

3.2.2 Genetic parameters

(From Mucha et al, 2021 - Meta-analysis of genetic parameters for resilience and efficiency traits in goats and sheep)

Table 1. Pooled estimates of heritability of resistance to gastrointestinal parasites from meta-analysis in dairy goats

Trait[1]	Pooled h^2 (± SE)	Min[2] h^2	Max[3] h^2	N obs	N studies
FEC	0.07±0.01	0.04	0.15	8	2

[1] Trait: FEC – faecal egg count
[2] minimum h^2 from individual studies included in meta-analysis
[3] maximum h^2 from individual studies included in meta-analysis

Table 2. Pooled estimates of heritability of resistance to gastrointestinal parasites from meta-analysis in dairy sheep

Trait[1]	Pooled h^2 (± SE)	Min[2] h^2	Max[3] h^2	N obs	N studies
FEC	0.14±0.04	0.09	0.35	6	3

[1] Trait: FEC – faecal egg count
[2] minimum h^2 from individual studies included in meta-analysis
[3] maximum h^2 from individual studies included in meta

Table 3. Pooled estimates of heritability of resistance to gastrointestinal parasites from meta-analysis in meat sheep

Trait[1]	Pooled h^2 (± SE)	Min[2] h^2	Max[3] h^2	N obs	N studies
DAG	0.30±0.06	0.06	0.63	37	15
FCons	0.14±0.02	0.03	0.27	13	5
NBW[4]	0.10±0.02	0.00	0.54	11	3
Par-Ab	0.18±0.07	0.05	0.29	6	3
Par-Ig	0.36±0.06	0.13	0.67	24	8
FEC	0.29±0.03	0.00	0.82	116	32
HC	0.32±0.14	0.08	0.56	5	2

[1] Trait: DAG – dagginess, FCons – faecal consistency, NBW – number of worms, Par-Ab – parasitism anitbodies, Par-Ig – parasitism immunoglobulin, FEC –faecal egg count, HC - Haematocrit
[2] minimum h^2 from individual studies included in meta-analysis
[3] maximum h^2 from individual studies included in meta-analysis

[4] pooled heritability obtained from a simple random effects model as the three level meta-analysis model did not converge

(From Aguerre et al, 2018 - Resistance to gastrointestinal nematodes in dairy sheep: Genetic variability and relevance of artificial infestation of nucleus rams to select for resistant ewes on farms)

Table 4. Estimates of heritability of resistance to gastrointestinal parasites from meta-analysis in dairy sheep in experimental infestations

Trait[1]	h^2
Root FEC30	0.14
Root FEC75	0.35
PCV_loss_inf1	0.24
PCV_loss_inf2	0.18
PCV-recovery	0.16

4 Resistance to mastitis

4.1 Definition, terminology, rationale

In small ruminants, mastitis mainly consists in subclinical infections caused by coagulase-negative staphylococci, which is much more frequent than clinical mastitis (Bergonier et al., 2003). Under these conditions, somatic cell count (SCC) is an accurate, indirect measure to predict mammary gland infection. Therefore, SCC could be used as an indirect selection criterion for mastitis resistance as is widely done in dairy cattle. Moreover, selection for mastitis resistance in dairy sheep and goats could mainly focus on selection against subclinical mastitis using SCC, considering the low incidence of clinical cases in these species (<5%), compared to dairy cattle for which clinical cases occur frequently (Bergonier et al., 2003).

Clinical mastitis is not recorded in dairy small ruminants, mainly because of its low incidence and because SCC is a relevant and simple indicator of intra-mammary infections. Work completed in France has developed two lines of ewes (experimental farm INRAE-La Fage) and goat (experimental farm INRAE-Bourges), a high line generated from sires with unfavourable EBVs for somatic cells and a low line generated from sires with favourable EBVs for somatic cells. For both sheep (Rupp et al., 2009) and Goats (Rupp et al., 2019), the low line has the lowest SCC, the lowest incidence of clinical mastitis and the lowest incidence of chronic mastitis (abscesses or unbalanced udder) and subclinical mastitis (assessed by milk bacteriology).

Even though SCC is the established indicator for use in animal breeding, the use of the California Milk test (CMT) is a very good indicator of SCC for monitoring udder health in flock/herd management in dairy and meat-producing small ruminants.

4.2 Data recording

4.2.1 Somatic Cell Count (SCC)

Large scale somatic cell counting relies on the application of routine methods, such as fluoro-opto-electronic counting. The somatic cell counter must be properly calibrated against a reference and laboratories must frequently verify the calibration settings are still correct.

The design for recording somatic cell count (SCC) will depend upon the objective. For flock/herd management related to high bulk SCC, the whole flock/herd should be sampled and analysed to identify the animals with the highest SCC. For genetic purpose, simplified designs might be implemented.

In dairy species, somatic cell counting is achieved within the milk recording design and the sampling design, as for milk components such as fat and protein content. As in small ruminants, most of the designs are simplified ones compared to the A4 method (all daily milkings recorded, once a month) (see ICAR guidelines – section 16 – dairy sheep and goats), SCC are quite often available at one out of the two daily milkings. In this case, use of SCC must be handled accordingly.

As for milk composition, with the aim of simplifying and decreasing further the cost of recording, it is possible/recommended to measure SCC on only a part of the flock/herd (first parity or first two parities).

It is also possible to go further in the simplification of the design, for example by sampling only a part of the lactation within a part-lactation sampling as proposed in the section 16 of the ICAR guidelines. The genetic parameters of test-day and lactation mean for Somatic Cell Score (SCS - log-transformed SCC) show that the records of the middle of the lactation appear as the most representative of the whole lactation. Two to four individual samples per female and per lactation, collected monthly in the middle part of the lactation are highly correlated (around 0.98) with SCS determined from samples collected over the complete lactation (A4 method) but are hardly less heritable compared with the A4 homologous traits (negligible loss of precision for SCS) (Astruc et Barillet, 2004). The balance between cost and genetic efficiency, depending on the genetic correlations close to 1, is clearly in favour of the part-lactation sampling compared to A4 testing.

4.2.2 California Mastitis Test (CMT)

The California mastitis test is an animal-side diagnostic test that provides an estimate of the level of infection within a mammary gland. A sample of milk (~3ml) from each udder half is combined with an equal volume of reagent in a CMT paddle and mixed for 15 to 20s. The reaction is scored based on the level of thickening of the mixture from zero (no thickening) consistent with no, or low, levels of infection, to four (gel formation with elevated surface) indicating high levels of infection.

A previous study (McLaren et al, 2018) has demonstrated the strong correlation between CMT score and SCC from samples collected from pedigree meat sheep in the UK.

4.3 Calculation of traits

Test-day SCC must be transformed to Somatic Cell Score (SCS) by the logarithmic transformation of Ali and Shook (1980) to achieve normality of distribution.

Example: SCS = log2+(SCC/100,000)+ 3

The table 5 gives correspondence between SCC and SCS

Table 5. Correspondence between somatic cell score and somatic cell count

Somatic Cell Count (SCC)	Somatic Cell Score (SCS)
12,500	0
25,000	1
50,000	2
100,000	3
200,000	4
400,000	5
800,000	6
1,600,000	7

SCS can be adjusted for days-in-milk (DIM). In this case, the adjustment procedure must be defined from a study based on healthy ewes/goats with enough number of test-days over the lactation.

Then a lactation SCS (LSCS) may be calculated (case of lactation model in genetic evaluation).

LSCS can be computed as the weighted arithmetic mean of test-day SCS (adjusted or not for DIM). Weights are either 1 (equivalent to no weight) or r2, where r is the correlation between one measure and the mean of all other records.

4.4 Use for genetic analysis / genetic evaluation

4.4.1 Genetic model

The genetic model might include the following fixed effects:

- Flock x year (x parity)
- Month of lambing/kidding
- Age at lambing/kidding
- Number of lambs/kids born

4.4.2 Genetic parameters

(From Mucha et al, 2021 - Meta-analysis of genetic parameters for resilience and efficiency traits in goats and sheep)

Table 6. Pooled estimates of heritability of somatic cell score from meta-analysis in dairy sheep

Trait[1]	Pooled h^2 (± SE)	Min[2] h^2	Max[3] h^2	N obs	N studies
SCS	0.13±0.02	0.03	0.27	29	22

Table 7. Pooled estimates of heritability of somatic cell score from meta-analysis in dairy goats

Trait[1]	Pooled h^2 (± SE)	Min[2] h^2	Max[3] h^2	N obs	N studies
SCS	0.21±0.01	0.19	0.24	5	3

Table 8. Pooled estimates of genetic correlations between resilience (SCS, FEC) and efficiency (MY, FC, PC) traits from meta-analysis in dairy goats

Traits[1]	Pooled r_g (± SE)	Min[2] r_g	Max[3] r_g	N obs	N studies
SCS & MY	0.35±0.31[ns]	0.00	0.59	3	2
SCS & FC[4]	-0.19±0.01	-0.20	-0.18	3	2
SCS & PC	-0.06±0.05[ns]	-0.13	0.00	3	2
FEC & MY	0.17±0.35[ns]	-0.21	0.63	4	2

Table 9. Pooled estimates of genetic correlations between resilience (SCS) and efficiency (MY, FY, PY, FC, PC) traits from meta-analysis in dairy sheep

Traits[1]	Pooled r_g (± SE)	Min[2] r_g	Max[3] r_g*	N obs	N studies
SCS & MY	-0.05±0.10[ns]	-0.30	0.23	16	11
SCS & FC	0.04±0.05[ns]	-0.16	0.16	8	8
SCS & PC	0.12±0.03	0.02	0.24	12	9
SCS & FY	0.11±0.15[ns]	-0.04	0.31	4	4
SCS & PY	0.17±0.10[ns]	0.06	0.31	4	4

[1] Traits: SCS – somatic cell score, FEC – faecal egg count, MY – milk yield, FY – fat yield, PY – protein yield, FC – fat content, PC – protein content

[2] minimum r_g from individual studies included in meta-analysis

[3] maximum r_g from individual studies included in meta-analysis

[4] pooled correlations obtained from a simple random effects model as the three level meta-analysis model did not converge

[ns] – pooled estimate did not differ significantly from zero

Table 10. Estimates of heritability of somatic cell score, clinical mastitis and CMT in meat and dairy sheep (source Oget et al., 2019)

Species (breed)	Trait	Heritability (SE)	reference
Dairy sheep (Chios)	California mastitis test (CMT)	h^2=0.12 (0.06)	Banos et al., 2017
Meat sheep (Belclare, Charollais, Suffolk, Texel and Vendeen breeds)	Clinical mastitis (examination and palpation of the udder)	h^2=0.04 (0.03)	O'Brien et al., 2017
Meat sheep (Texel)	SCS	h^2=0.11 (0.04)	McLaren et al., 2018
Meat sheep (Texel)	CMT	h^2=0.08-0.09 (0.04)	McLaren et al., 2018
Meat sheep Texel	CMT	h^2=0.07	Kaseja et al., 2023 - submitted paper (SMARTER, D2.3)

5 Resistance to footrot

5.1 Definition, terminology, rationale

Footrot is caused by Dichelobacter nodosus and is a major cause of lameness in sheep. The disease is highly contagious and endemic in many countries that causes pain and welfare issues in affected animals. In addition to the direct impacts on time and veterinary / medicine costs, the disease has further, indirect, impacts through reducing fertility and milk supply.

The presence of footrot is assessed by inspection of the hooves of lame animals.

5.2 Data recording

5.2.1 Scoring methods

Each hoof is assessed individually and scored based on the five-point scale (used in UK): clean, unaffected hoof (score 0), mild inter-digital inflammation (score 1), inter-digital necrosis (score 2), under-running of the sole of the hoof (score 3) and fully under-run to the abaxial wall of the hoof (score 4) (Conington et al., 2008).

The sum of scores is calculated by adding all four scores (for each hoof), hence the animal can obtain the phenotype in a range from zero to 16.

In France, where footrot is usually not recorded, a simplified scoring system has been developed using a scale (0 normal and severity of lesions scored from 1 to 3) adapted from the Victorian Farmers Federation and Coopers Animal Health.

Additionally, the health of feet is assessed in France and the UK for other important hoof lesions including white line degeneration, contagious ovine digital dermatitis, horn growth, presence of abscess, granuloma, interdigital hyperplasia, and panaritium).

5.3 Calculation of traits

Sum of scores are log-transformed in order to normalise the data using the formula ln(Sum of scores + 1). The addition of one prevents to logarithm the value of sum of scores equal to zero. Each animal can obtain transformed score ranging between zero and 2.83.

5.4 Use for genetic analysis / genetic evaluation

5.4.1 Genetic model

The genetic model might include the following fixed effects:

- age of the dam
- scorer (if more than one)
- vaccine status (if some animals treated with the vaccination against ovine foot-rot)
- Flock or Flock x Year interaction

5.4.2 Genetic parameters

The estimated heritability for UK meat sheep varies between 0.12 (Kaseja et al., 2021, unpublished results) to 0.23 Nieuwhof et al. (2008).

Table 11. Estimates of heritability of resistance to footrot in meat sheep

Species (breed)	Trait	Heritability (SE)	reference
Meat sheep (Texel)	Resistance to foorot	0.12(0.02)	Kaseja et al., 2023 in press.
Meat sheep (Scottish Blackface)	Clinical mastitis (examination and palpation of the udder)	0.19 to 0.23	Kaseja et al., 2023 in press.
Meat sheep (Scottish Blackface lambs)	SCS	0.12	Nieuwhof et al., 2008
Meat sheep (Texel)	CMT	0.18	Mucha et al., 2016
Meat sheep			Irish parameters?

6 Acknowledgements

We gratefully acknowledge the contributions to small ruminant health and disease guideline by the following people:
• Joanne Conington, SRUC, the UK
• Jean-Michel Astruc, IDELE, France
• Rachel Rupp, INRAE, France
• Beat Bapst, Qualitas AG, Switzerland

- Donagh Berry, TEAGASC, Ireland
- Beatriz Carracelas, INIA, Uruguay
- Antonello Carta, Agris Sardegna, Italy
- Gabriel Ciappesoni, INIA, Uruguay
- Arnaud Delpeuch, IDELE, France
- Frédéric Douhart, INRAE, France
- Karolina Kaseja, SRUC, the UK
- Ed Smith, SRUC, the UK
- Flavie Tortereau, INRAE, France
- Stefen Werne, FiBL, Switzerland

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

This work also used deliverable from the Eurosheep project (Horizon 2020 under agreement N° 863056).

7 References

Aguerre, S.; P. Jacquiet, H. Brodier, J.P. Bournazel, C. Grisez, F. Prévot, L. Michot, F. Fidelle, J.M. Astruc, C.R. Moreno, Resistance to gastrointestinal nematodes in dairy sheep: Genetic variability and relevance of artificial infection of nucleus rams to select for resistant ewes on farms, Veterinary Parasitology, Volume 256, 2018, Pages 16-23, ISSN 0304-4017, https://doi.org/10.1016/j.vetpar.2018.04.004.

Astruc J.M. et Barillet F., 2004. Current challenge for milk recording in dairy sheep and goats: the simplification of milk sampling design for chemical composition and somatic cell counts of milk. Proceedings of the 34th ICAR session, Sousse, Tunisia, 31 May-3 June 2004.

Banos, G., Bramis, G., Bush, S.J., Clark, E.L., McCulloch, M.E.B., Smith, J., Schulze, G., Arsenos, G., Hume, D.A., Psifidi, A., 2017. The genomic architecture of mastitis resistance in dairy sheep. BMC Genom. 18, 624.

Bergonier, D., Crémoux, R. de, Rupp, R., Lagriffoul, G., Berthelot, X., 2003. Mastitis of dairy small ruminants. Vet. Res. 34, 689–716.

Conington, J., Hosie, B., Nieuwhof, G.J., Bishop, S.C., Bünger, L. (2008). Breeding for resistance to footrot-the use of hoof lesion scoring to quantify footrot in sheep. Veterinary Research Communication, 32(8):583-9. https://doi.org/10.1007/s11259-008-9062-x

Gruner, L., Bouix, J., Brunel, J.C., 2004. High genetic correlation between resistance to
Haemonchus contortus and to Trichostrongylus colubriformis in INRA 401 sheep. Vet. Parasitol. 119, 51–58.

Jacquiet et al., 2015. 25th International Conference of the WAAVP, Liverpool, UK,16-20 August

McLaren A, Kaseja K, Yates J, Mucha S, Lambe NR, Conington J. New mastitis phenotypes suitable for genomic selection in meat sheep and their genetic relationships with udder conformation and lamb live weights. Animal. 2018 Dec;12(12):2470-2479. doi: 10.1017/S1751731118000393. Epub 2018 Mar 26. PMID: 29576020.

Mucha S, Bunger L, Conington J. Genome-wide association study of footrot in Texel sheep. Genetics Selection Evolution, 2015, 47 (1), pp.35. <10.1186/s12711-015-01193>. <hal-01309642>

Mucha S, Tortereau F, Doeschl-Wilson A, Rupp R, Conington J. Animal Board Invited Review: Meta-analysis of genetic parameters for resilience and efficiency traits in goats and sheep. Animal. 2022 Mar;16(3):100456. doi: 10.1016/j.animal.2022.100456. Epub 2022 Feb 18. PMID: 35190322.

Nieuwhof, G.J., Conington, J., Bünger, L., Haresign, W. and Bishop, S.C. (2008) Genetic and phenotypic aspects of resistance to footrot in sheep of different breeds and ages. Animal 2 issue 9: 1289-1296. https://doi.org/10.1017/S1751731108002577

O'Brien, A.C., McHugh, N., Wall, E., Pabiou, T., McDermott, K., Randles, S., Fair, S., Berry, D.P., 2017. Genetic parameters for lameness, mastitis and dagginess in a multi-breed sheep population. Animal 11, 911–919.

Oget C., G. Tosser-Klopp, R. Rupp (2019). Genetic and genomic studies in ovine mastitis. Small Ruminant Research 176, 55-64. https://doi.org/10.1016/j.smallrumres.2019.05.011.

Rupp, R., Bergonier, D., Dion, S., Hygonenq, M.C., Aurel, M.R., Robert-Granié, C., Foucras, G., 2009. Response to somatic cell count-based selection for mastitis resistance in a divergent selection experiment in sheep. J. Dairy Sci. 92, 1203–1219.

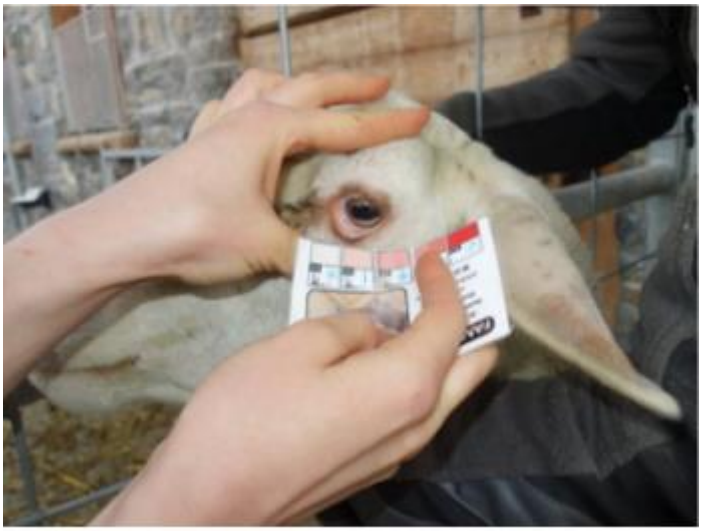
Rupp R, Huau C, Caillat H, Fassier T, Bouvier F, Pampouille E, Clément V, Palhière I, Larroque H, Tosser-Klopp G, Jacquiet P, Rainard P. Divergent selection on milk somatic cell count in goats improves udder health and milk quality with no effect on nematode resistance. J Dairy Sci. 2019 Jun;102(6):5242-5253. doi: 10.3168/jds.2018-15664. Epub 2019 Mar 21. PMID: 30904305.

Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529.

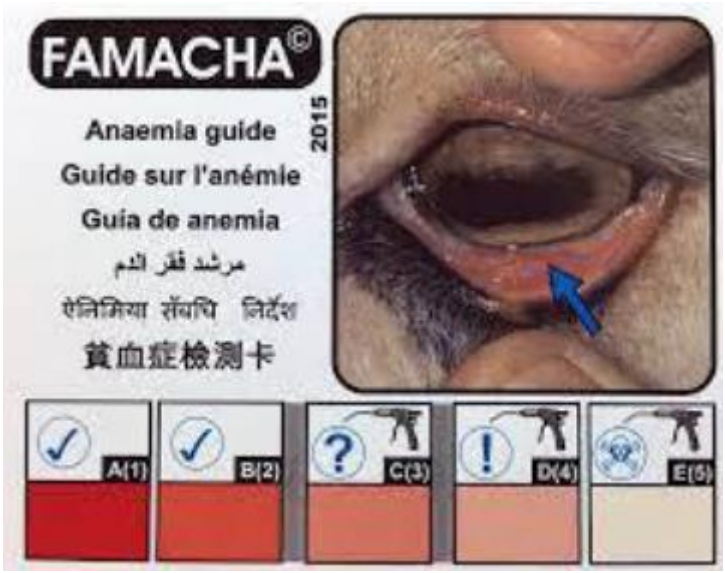
Whitlock, H.V., 1948. Some modifications of the McMaster helminth egg counting technique and apparatus. J. Coun. Sci. Ind. Res. 21, 177.

8 Annexes

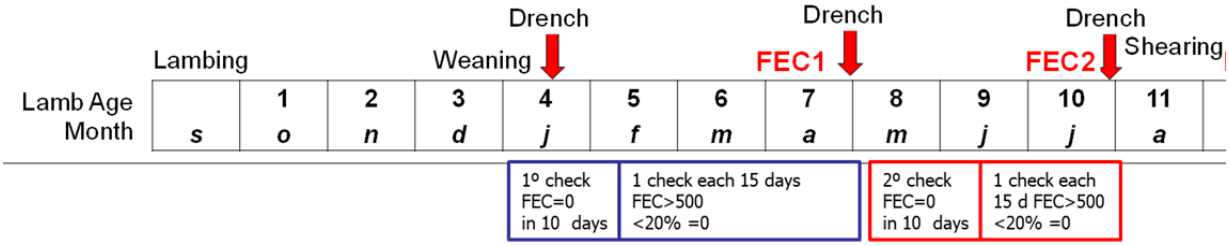
8.1 Picture of FAMACHA score (source FiBL – Qualitas)



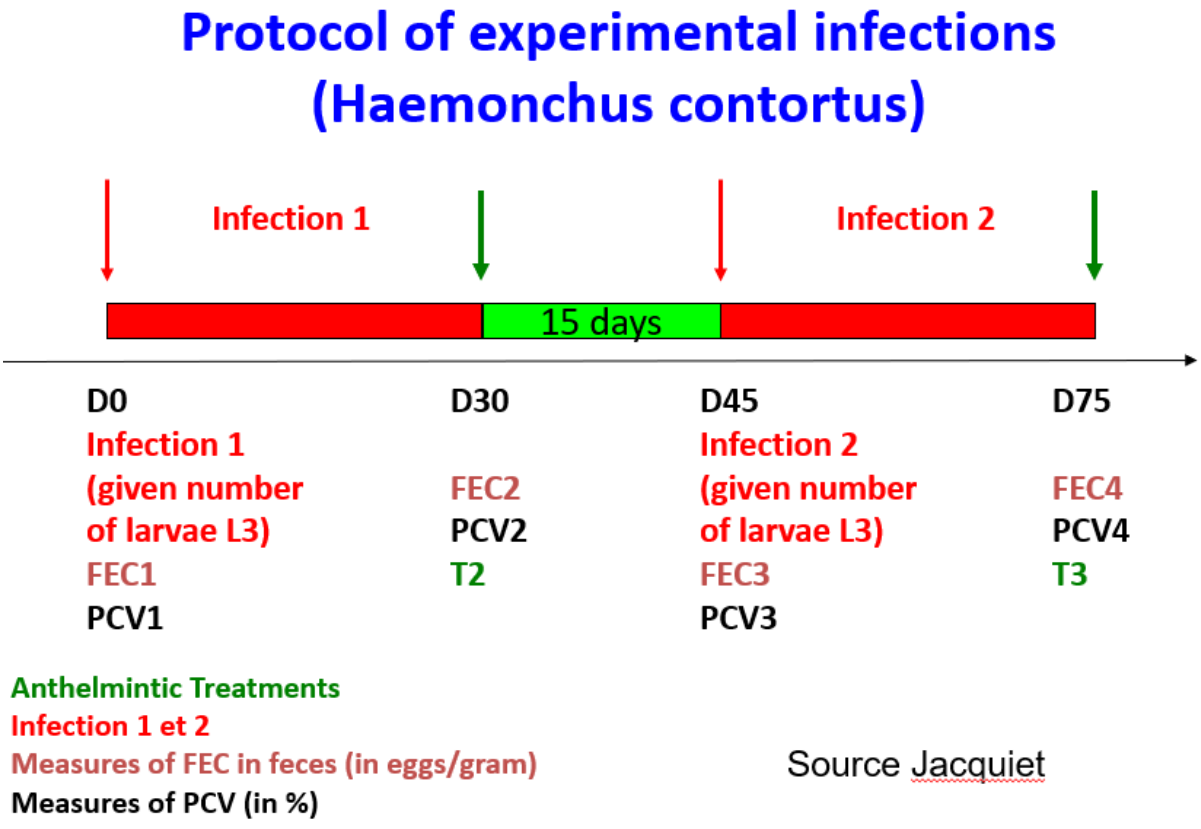
FAMACHA Score



8.2 Uruguayan protocol of natural infestation for recording the resistance to gastrointestinal parasites



8.3 French protocol of experimental infestation for recording the resistance to gastrointestinal parasites



Recommendations on recording survival of foetus and young in small ruminants

SMARTER – Deliverable D6.3

Version 1 – 17 May 2023

Table des matières

Change Summary

Date of change	Nature of Change
January 2023	First draft
May 2023	Final version for SMARTER deliverable 6.3

1 Introduction

Foetal and young survival are parameters linked to neonatal vigour scores, maternal and young behaviours, stress responses, immunity transfer and traits related to dam fertility and longevity. Minimising mortality, either in utero (e.g., embryo/foetus) or pre-weaning, are crucial to profitable small ruminant production systems. Despite this, pre-weaning survival in many species is far from ideal (Binns et al., 2022; Yapi et al., 1990, Chaarani et al., 1991, Green and Morgan, 1993, Nash et al., 1996). This can be particularly worse in small ruminant production systems which are typically more extensive and therefore prevailing weather conditions can be an additional stressor as well as predators. Moreover, the poly-ovulatory nature of species such as sheep and goats also predisposes such species to greater foetal and pre-weaned young losses (Scales et al., 1986).

Litter size can be determined using trans-abdominal ultrasonography of the uterine horns at ideally 40-70 days post-fertilisation. Good accuracy in determining foetal number has been reported from trans-abdominal ultrasonography (Taverne et al., 1985). The number of young eventually born can then be used to assess foetal loss since the time of scanning. At birth, young survival is usually based on dead or not in the first 24 h post-birth; stillborn individuals or those dead within 24 hours are usually defined as failed to survive. Young survival can also be considered as different age group categories until weaning – for example from 1 day to 7 days of age. Young animals (i.e., < 7 days) are greatest at risk of mortality (Binns et al., 2002) and tend to die of exposure to hypothermia, starvation, septicaemia, or repercussions from trauma suffered at birth.

2 Scope

To define approaches for the definition of foetal and lamb survival as well as the data editing and downstream analyses (including statistical models)

3 Definition, terminology, rationale

A plethora of different definitions exist depending on whether defined at the level of the individual (i.e., binary trait) or that of the litter. A non-exhaustive list is given below.

Foetal survival (at a litter level):

- Whether or not some foetal mortality has occurred defined as a binary trait (i.e., the number of individuals born is less than the number scanned in utero)
- Number of individual foetuses scanned alive (along with gestational age)
- Number of foetuses scanned minus the number that were born (dead or alive) – this is a measure of foetal mortality as opposed to survival and assumes stillborn young are considered in the definition of a young survival trait. It is a count trait
- The number of young born divided by the number of foetuses scanned (this is mortality rate figure but per little with a penalty on losses for smaller litter sizes)

Foetal survival (at an individual level):

this can be defined as a binary trait of 0 (died between scanning and birth) or 1 (survived between scanning and birth). A dummy ID for the dead foetus would need to be constructed but the parentage would still potentially be known (especially if generated from AI).

Young survival (at a litter level):

- Number of lambs born alive (NLBA)
- Number of lambs dead within 24 hours of birth
- Number of lambs dead within 24 hours of birth divided by the total number of lambs born

Young survival (at an individual level):

this can be defined as a binary trait of 0 (dead within 24 hours of birth) or 1 (alive after 24 hours of birth). The dead animal would need to receive an ID and can, of course, be genotyped to verify parentage (but also used for downstream genomic analyses discussed later).

4 Recording survival of foetuses and young in small ruminants

In all instances, accurate data is crucial. Data should be collected on the animal/dam itself (dead or alive) but also potential confounding effects that could be considered for inclusion in the statistical model as fixed effects. Examples include contemporary group (e.g., flock-date of scanning, flock-year-season of birth (for each NLB separately), ewe parity, litter size). Ideally also all individuals should be genotyped. Because the heritability of foetal or young animal mortality in small ruminants is relatively low (<0.1; Safari et al., 2005; Brien et al., 2014), a

large number of records are required to achieve accurate genetic/genomic evaluations. Care should also be taken when interpreting the scoring (and also the downstream genetic evaluations); some jurisdictions may record mortality rather than survival or may record mortality but present genetic evaluations as survival (i.e., positive value is favourable).

4.1 Pregnancy scanning records

Ideally scanning should be undertaken 40 to 70 days post-fertilisation. This may be possible to (easily) achieve where extensive AI has been used but, otherwise, should ideally be 30 days after the last female has been marked as been served by natural mating. Skilled operators should be able to determine the number of foetuses from 30 to 100 days of gestation; usually only one operator will scan a flock on a given day so will be confounded with flock-date of scanning contemporary group. If AI is solely used or if single sire mated then the parentage of the foetus should be known; if mob mated or single sire mated at AI, then superfecundation could cause a discrepancy in recorded sire.

4.2 Young survival

Young survival can be defined at birth, ideally as a binary trait as to whether the animal was born stillborn or died within 24 hours (survival=0) or was still alive 24 hours after birth (survival = 1). If information is also available on the reason for death (i.e., autopsy results) then, where sufficient data exists for any one ailment, it could be analysed separately as separate traits. This could be particularly important for generating separate genetic evaluations for the main ailments thereby not only possibly increasing the heritability through more accurate data, but also provide genetic evaluations specific to individual ailments which could enable more selection pressure on these traits in situations where they are more impactful. Ideally a genotype of the dead animal should be generated. Any obvious external defects should be noted.

4.3 Ancillary information

Having ancillary information coinciding with an event is useful for several reasons:

- For helping data editing (e.g., comparing actual birth date to expected birth date based on recorded service information)
- For adjustment in the statistical model (e.g., dam parity)
- Understanding the risk factors associated with survival
- Enabling more precise estimates of correlations with other performance traits by having information on multiple features from the same animal
- Adjusting for possible selection in multi-trait genetic evaluation models

Possible ancillary information can be divided into those associated with 1) the past of prevailing environmental conditions, 2) the dam (or sire), or 3) the individual. Examples include:

1. Environment:
    - Weather related factors (rainfall, temperature, wind including direction)
    - Flock
    - Date of scanning or date of birth
2. Dam
    - Parity
    - Age
    - Breed
    - Genotype
    - Litter size
    - Mating type (i.e., AI versus natural)
    - BCS (change) and live-weight (change)
    - Mothering ability
    - Colostrum quality and yield
3. Individual
    - Days since service (for foetal survival trait)
    - Birthing difficulty
    - Birth weight
    - Gender
    - Genotype
    - Sire
    - Autopsy results if possible

5 Use for genetic analysis / genetic evaluation

5.1 Data editing & statistical modelling

In order to estimate contemporary group effects well, the larger the contemporary group, the better the group estimates. Therefore, imposing a minimum contemporary group size prior to data analysis should be considered as should good genetic connectedness with other contemporary groups. Genetic connectedness can be an issue with small ruminant populations in particular, especially where natural mating prevails.

5.1.1 Data editing

Foetal survival - Each flock-scanning date can be firstly investigated at a macro level to gauge ultrasound quality control. Simple cross-references between the number of females with scanning data versus those presented as well as the ID numbers of both is useful to ensure all data were properly recorded. High foetal mortality rates could simply be indicative of high foetal loss (e.g., abortions due to causes like chlamydial and toxoplasma) as well as poor operator competence – assessing the rate for individual operators across flocks (and time) could be useful to assess operator proficiency. A high proportion of litters where the number of young born (dead or alive) exceeds that recorded at scanning suggests a poor accuracy of recording and consideration should be given to discarding the data from that date but also investigating the operator in more detail across other flocks; irrespective, the scanning results from that litter at least should be discarded. The proportion of scanned litters with >3 detected foetuses should also be calculated; depending on the expected prolificacy of the animals (e.g., breed), then the appropriate editing of either the individual data points or the date in its entirety should be assessed.

Young mortality - A high incidence of young mortality per contemporary group could simply be a consequence of some underlying issue (e.g., predation, disease) or indeed a high fecundity rate; a low incidence of young could be indicative of a good stock person. Therefore, it can be difficult to distinguish between high and low quality data. Using guaranteed high quality and reliable data, it is possible to estimate the expected distribution of the incidence of young animal mortality for different population strata such as flock size, ewe age, breed, litter size. Using these distributions, the probability that the mean mortality for a contemporary group fits this distribution can be estimated and a decision made as to whether or not to include the data in the downstream analyses.

5.1.2 Statistical modelling

In all instances, both a direct genetic and a maternal genetic effect should be considered as random effects in the statistical model. Traditionally, relationships were accounted for though the recorded ancestry, but this can often now be supplemented with genome-wide genotype information to generate a H matrix (i.e., combines genomic and ancestry information). A covariance between the direct and maternal genetic effect could also be considered should the data structure allow it. A litter permanent environmental effect should also be considered as a random effect where the trait is that of the individual (and not the ewe); also, consideration should be given to a ewe permanent environmental effect across parities as a random effect in the model. Whether the estimation of these additional covariance components improve the fit to the data can be deduced by a likelihood ratio test but ideally a metric such as the AIC or BIC to account for the increased complexity of the model.

The choice of fixed effects included in the model will depend on the population being studied and should be decided upon in conjunction with the relevant subject matter expert. Factors that could be considered include:

- Contemporary group (e.g., flock-date of scanning for foetal survival and flock-year-season of birth or flock-year-season-birth rank of birth)
- Lamb gender (may not be possible for foetal survival trait)
- Dam parity
- Mating type (i.e., AI versus natural)
- Dam age nested within parity
- Day of gestation (for foetal survival) if available or defined as a categorical variable
- Litter size (at scanning or birth)
- Heterosis and recombination loss of the dam and foetus/young
- Inbreeding coefficient of the dam and foetus/young
- Age of the sire
- Breed composition of the dam and foetus/young

Adjusting for factors such as dystocia or birth weight may not be appropriate in the statistical model for young survival as they are likely to be genetically correlated with survival and thus may remove some of the true genetic variance – nonetheless, the eventual decision will be based on the genetic evaluation system employed and how the economic value on the traits within the overall breeding objectives are constructed.

5.2 Genomic association analyses

Where genotypes are available, then a genome-wide association study (or candidate gene study) can be undertaken. Although it is not possible to have the genotype of the aborted foetus, it could still be possible to undertake a genomic analysis especially by focusing on the genotype/haplotype of the living animals versus the expectation based on the genotype/haplotype of the parents (Maxime Ben Braiek et al., 2021).

6 Acknowledgements

We gratefully acknowledge the contributions to these recording of behaviour guidelines by the following people:

- Donagh Berry, TEAGASC, Ireland
- Joanne Conington, SRUC, the UK
- Maxime Ben Braiek, INRAE, France
- Arnaud Delpeuch, IDELE, France

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

7 References

Ben Braiek, M., Fabre, S., Hozé, C. et al. Identification of homozygous haplotypes carrying putative recessive lethal mutations that compromise fertility traits in French Lacaune dairy sheep. Genet Sel Evol 53, 41 (2021). https://doi.org/10.1186/s12711-021-00634-1

Binns, S.H., I.J.Cox, S. Rizvi, L.E.Green. 2002. Risk factors for lamb mortality on UK sheep farms. Preventive Veterinary Medicine. 52, 287-303

Brien, FD, Cloete, SWP, Fogarty, NM, Greeff, JC, Hebart, ML, Hiendleder, S, Hocking Edwards, JE, Kelly, JM, Kind, KL, Kleeman, DO, Plush, KL and Miller, DR 2014. A review of genetic and epigenetic factors affecting lamb survival. Animal Production Science 54, 667–693.

Chaarani, B., R.A. Robinson, D.W. Johnson. 1991. Lamb mortality in Meknes Province (Morocco). Prev. Vet. Med., 10 (1991), pp. 283-298

Green, L.E., K.L. Morgan. 1993. Mortality in early born, housed lambs in south-west England. Prev. Vet. Med., 17 (1993), pp. 251-261

Nash, M.L., L.L. Hungerford, T.G. Nash, G.M. Zinn. 1996. Risk factors for perinatal and postnatal mortality in lambs. Vet. Rec., 139 (1996), pp. 64-67

Safari, E, Atkins, KD, Fogarty, NM and Gilmour, AR 2005. Analysis of lamb survival in Australian Merino. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 16, 28–31.

Scales, G. H.,R. N. Burton & R. A. Moss. 1986. Lamb mortality, birthweight, and nutrition in late pregnancy. New Zealand Journal of Agricultural Research. 29: 1

M. A. M. Taverne , M. C. Lavoir , R. van Oord & G. C. van der Weyden (1985) Accuracy of pregnancy diagnosis and prediction of foetal numbers in sheep with linear-array real-time ultrasound scanning, Veterinary Quarterly, 7:4, 256-263, DOI: 10.1080/01652176.1985.9693997

C.V. Yapi, W.J. Boylan, R.A. Robinson. 1990. Factors associated with causes of preweaning lamb mortality. Prev. Vet. Med., 10 (1990), pp. 145-152

Recommendations on recording behavioural traits

SMARTER – Deliverable D6.3

Version 1 – 17 May 2023

Table des matières

Change Summary

Date of change	Nature of Change
March 2023	First draft
May 2023	Final version for SMARTER deliverable 6.3

1 Introduction

Genetic selection including behavioural traits could be an advantageous strategy for improving robustness and welfare of farm animals in various farming conditions by minimizing unsuitable responses to changes in their social and physical environment, limiting an excessive fear of humans and improving sociability (Mignon-Grasteau et al., 2005). Farm animals are social and gregarious, and relational behaviours are essential for ensuring social cohesion, social facilitation, offspring survival and docility toward humans. Breed differences and genetic variation within breed have been reported in lambs for early social behaviours and found to be heritable, and associated with some QTL, suggesting such behaviours could be selected early (Boissy et al., 2005; Beausoleil et al., 2012; Hazard et al., 2014; Cloete et al., 2020). In addition, such early social reactivity of lambs towards conspecifics or humans was identified as a robust trait (Hazard et al., 2016). We recently reported that selection for early social reactivity of lambs towards conspecifics or humans is feasible (Hazard et al 2022).

The behaviour of both ewes and lambs, and their interaction at lambing, have been widely described. Such behaviour is important for the survival of the offspring, especially in extensive farming conditions as reviewed by Dwyer et al. (2014). Moreover, it has been shown that primiparous ewes are more prone to abandon their lambs due to their lack of maternal experience (Dwyer, 2008) and that lamb survival at birth is lowly heritable (Brien et al., 2014). Taken together these factors could hinder the development of extensive farming systems. Genetic selection on maternal attachment traits could therefore be advantageous to improve offspring survival and growth, and reduce labour, as suggested by Mignon-Grasteau et al. (2005). Genetic variations in maternal behaviour between breeds of sheep have been well documented (for review see: Dwyer, 2008; von Borstel, Moors, Schichowski, & Gauly, 2011) while little was known about within-breed genetic variability and even less about maternal reactivity traits. We hypothesized that maternal attachment to the litter has a genetic component in sheep, and we recently reported that as expected the maternal reactivity at lambing is a heritable trait (Hazard et al., 2020; Hazard et al., 2021).

Grazing behaviour is also important for animals raised in extensive production systems because it can support adaptability to changing environments. In particular, small ruminants reared in semi-extensive systems face many environmental and welfare challenges that are difficult to quantify. The evidence in the literature suggests that there are differences in grazing behaviour between and within breeds of sheep (Simm et al., 1996; Brand, 2000). The notion is that natural selection combined with subjective artificial selection have led to some animals being more adaptive to extensive conditions. In this regard, genetic variation may exist for key grazing behaviour traits (Simm et al., 1996; Dwyer et al., 2005), but relevant literature is scarce. During the SMARTER H2020 project, a study was performed on grazing behaviour of the indigenous Boutsko Greek mountainous sheep breed, which is reared semi-extensively. The results showed that duration of grazing and speed are heritable traits (Vouraki et al., 2023 – under review; SMARTER deliverable 2.4 – in preparation).

Acronyms used in these guidelines

AT Arena Test
CT Corridor Test
GPS Global Positioning System
LS Lambing Site
PCA Principal Component Analysis

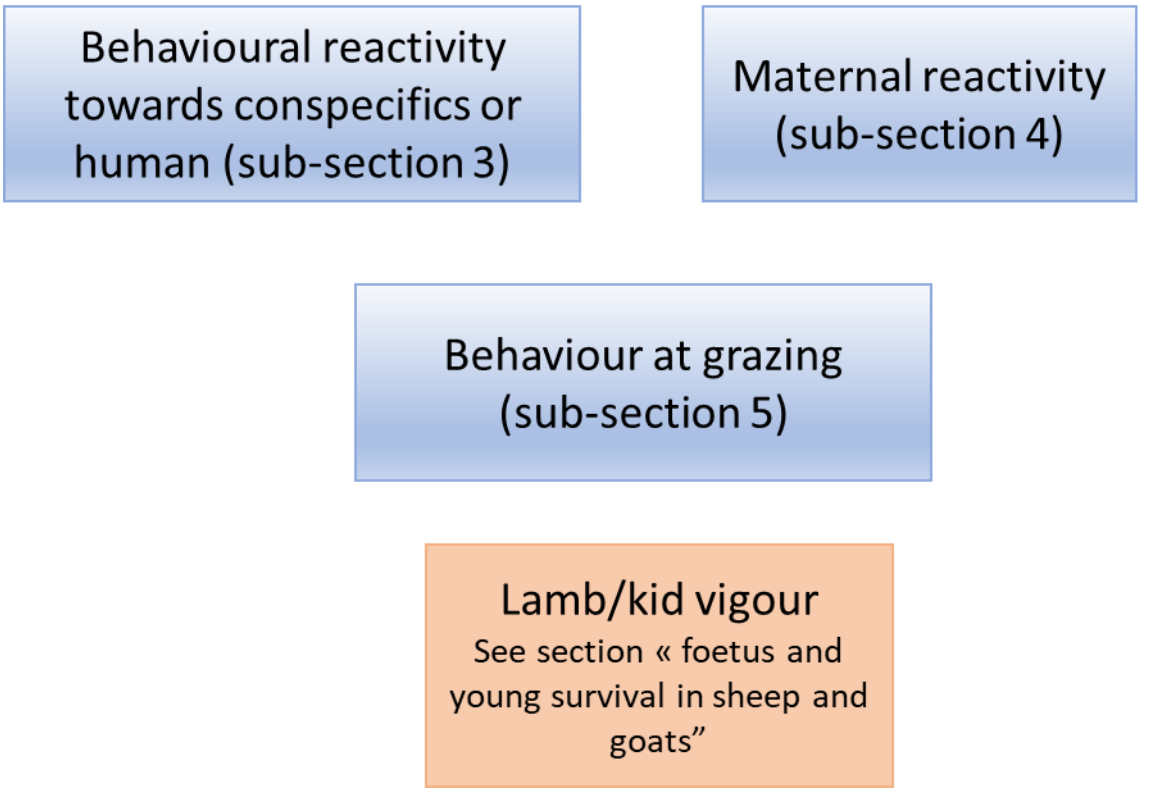
2 Scope

The aim of the present report is i) to define the behavioural traits of interest, ii) to describe approaches for behavioural measurements, iii) to describe their use for genetic analysis and evaluation.

To-date, the present guidelines describe 3 groups of traits related to behaviour:

- Behavioural reactivity towards conspecifics or humans
- Maternal reactivity
- Behaviour at grazing

Kid/lamb vigour is a relevant behavioural trait, but this trait is tackled within the section "foetus and young survival in sheep and goats" of the guidelines.



Most of the work undertaken on behaviour concerned sheep. This has been particularly the case in SMARTER. Most of the recommendations might be applied to goat as well. Nevertheless, we will use the ovine terms in the guidelines below.

3 Behavioural reactivity towards conspecifics or humans

3.1 Definition, terminology, rationale

- Behavioural reactivity towards conspecifics (i.e. sociability):

  - is the social motivation of the lambs to join their conspecifics in response to social isolation with or without presence of a motionless human. Expression of higher levels of a panel of behaviours, including vocalisations and locomotion, is hypothesised as an active way to maintain social link with conspecifics.

- Behavioural reactivity towards humans (i.e. docility):

  - is the reactivity of isolated lambs to a walking human. Higher flight distance between the lamb and a human indicates a lower docility toward a human.

Behavioural reactivity towards conspecifics and humans are measured in standardised behavioural tests (arena and corridor tests, described below).

Higher sociability and/or docility towards humans may improve adaptation of sheep to harsh environments through social facilitation (i.e. transmission of feeding preferences…), social cohesion (i.e. transhumance…) and reactivity to handling. Consequently, improving such behavioural traits may improve welfare, production, and labour of shepherd.

3.2 Data recording

3.2.1 Behavioural tests

The test described below have been implemented in France. It must be considered as a possible test, as others can be described later and enrich these guidelines.

Lambs must be individually exposed just after weaning (i.e. approximately 10 days after weaning) to two behavioural tests. The delay between weaning and behavioural tests must be sufficient for the change of social preferences of lambs for their dam to conspecifics.

The arena test (AT) consists 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 is performed indoors. The arena test pen consists in an unfamiliar enclosure virtually divided into 7 zones as described in detail by Ligout *et al*. (Ligout et al., 2011) (Figure 1). On one side of the enclosure (i.e. at the opposite of the entrance), a grid separates the tested lamb from another smaller pen containing 3 or 4 conspecifics. The first phase of the test (arena test phase 1, AT1) starts once the tested animal joins its flock-mates located behind a grid at the opposite side of the arena (time duration for joining: lower than 15 sec). No behavioural recording is performed during the joining. At

this time, an opaque panel is pulled down (from the outside of the pen) between the flock-mates and the tested lamb to prevent visual contact. After one minute the phase 1 stops and the panel is pulled up so the lamb can see its flock-mates again. Once the lamb has returned near to its flock-mates, or after 1 minute if the lamb did not do so, a non-familiar human slowly enters the arena through a door located near the pen of the flock-mates and stood 20 cm in front of the grid separating the arena from the lamb's flock-mates. The second phase (arena test phase 2, AT2) starts once the human is in place and lasts for a further 1 minute.

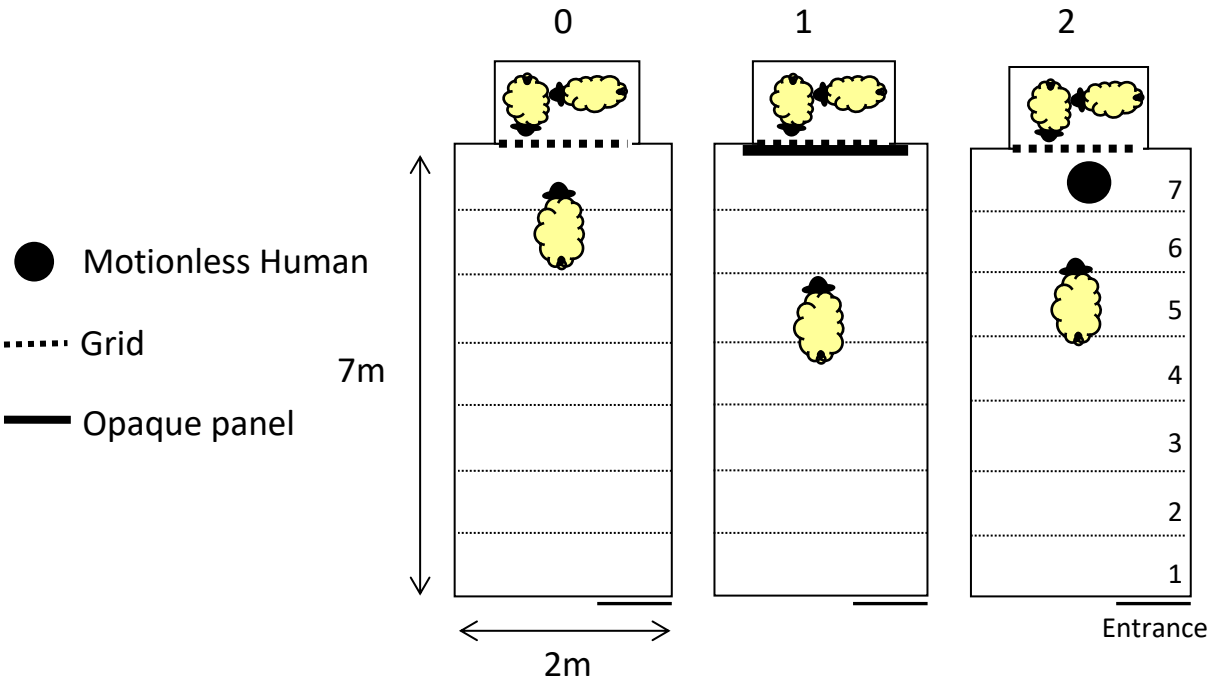


Figure 1: Experimental setup of the arena test for estimating the social reactivity of lambs. At the beginning of the test, animals can join their flock mates placed behind a grid barrier (social attraction, phase 0) and then were individually exposed to the social isolation (phase 1), and to the social attraction in presence of a motionless human (phase 2). (Adapted from Ligout et al., 2011)

The corridor test (CT) consists of two successive phases evaluating 1) reactivity to social isolation (CT1) and 2) reactivity to an approaching human (CT2). The test pen consists in a closed, wide rectangular circuit and has been described in detail by Boissy *et al*. (Boissy et al., 2005) (Figure 2). The first phase (corridor test phase 1, CT1) starts when the lamb enters the testing pen and lasts for 30 seconds. After that time a non-familiar human enters the testing pen and the second phase (corridor test phase 2, CT2) starts and lasts 1 minute. During this phase, the human walks at a regular speed through the corridor (the corridor is divided into 6 virtual zones and one zone is crossed every 5 seconds) until two complete tours has been achieved.

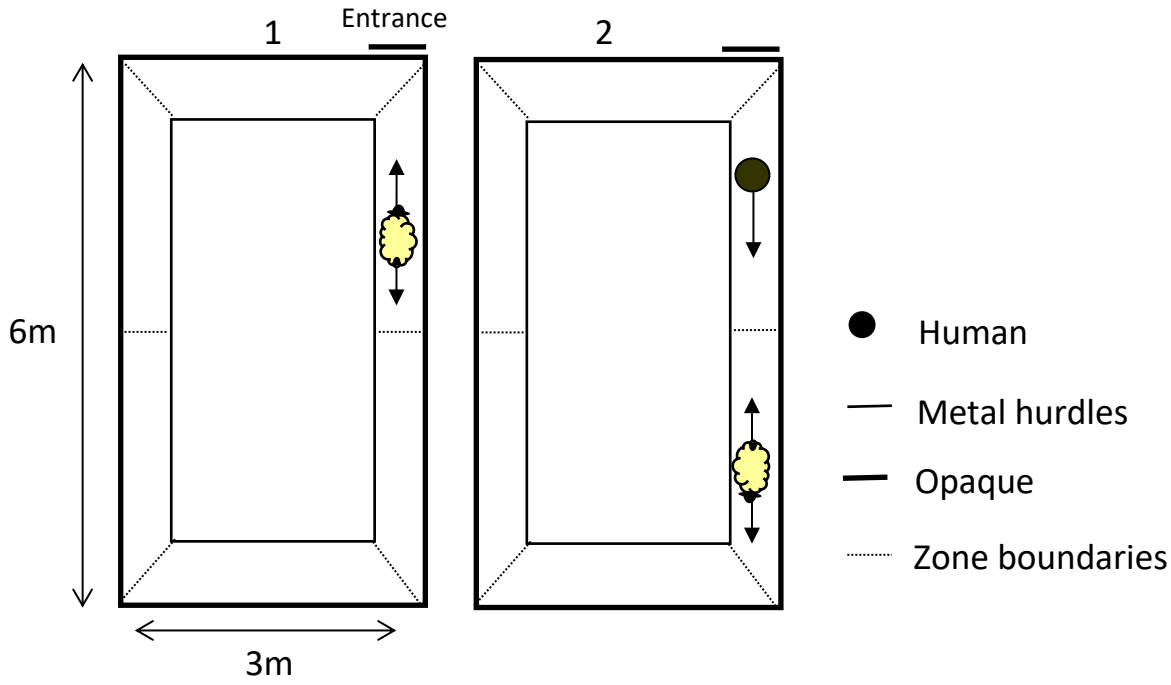


Figure 2. Experimental setup of the corridor test for estimating the emotional reactivity of lambs to social isolation in a new environment (phase 1) and then to an approaching human (phase 2) when the lamb is free to move in the whole pen. (Adapted from Boissy et al., 2005)

3.2.2 Behavioural traits

Several behaviours are measured during behavioural tests: vocalisations (i.e. frequency of high-pitched bleats), locomotion (i.e. number of virtual zones crossed), 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).

An investigator counts the lamb's vocalisations directly during the tests, from outside the pen using a laptop: number of times the animal bleats with an open mouth (high bleats, AT1/2-HBLEAT, CT1-HBLEAT). Locomotor activity is assessed by measuring the number of virtual zones crossed during arena test phases 1 and 2 (AT1/2-LOCOM) and corridor test phase 1 (CT1-LOCOM). This behaviour can be assessed using video recording or using infrared cells regularly positioned along the AT to detect displacement. The proximity to flock-mates and the human during AT2 is calculated by weighting of time spent in virtual zones (i.e. a high score indicated a high duration spent close to conspecifics and a human).

During CT2, every five seconds throughout this phase, an investigator records with a laptop the zones in which the human and the animal are located. In addition, the walking human records with a stopwatch the total duration during which the head of the lamb is visible. The mean flight distance (DIST) separating the human and the lamb (i.e. knowing the length of each virtual zone) and the time during which the human sees the lamb (SEEN) is measured in CT2.

3.3 Calculation of traits

Deviations from normality of row data must be tested using relevant statistical tests (e.g. the Kolmogorov–Smirnov test). Several raw measures must be transformed in order to minimise major deviations from the normal distribution. Square root transformation is applied to AT1/2-HBLEAT, CT1-HBLEAT. A multivariate analysis may be performed to take into account the multidimensional aspect of behavioural responses. Results of principal component analysis (PCA) indicate that the main principal components is structured mainly with similar behaviour (i.e. higher weight of similar behaviours for the different tests on the same component). Consequently, 3 synthetic variables may be constructed using PCA. Each PCA is performed for a set of similar behavioural variables across the behavioural tests. The first component of each PCA, explaining the largest part of total variance, is defined as a synthetic variable. Two synthetic variables are specific to the reactivity to social isolation: high bleats (HBLEAT, using AT1/2-HBLEAT and CT1-HBLEAT), locomotion (LOCOM, using AT1/2-LOCOM and CT1-LOCOM). One synthetic variable is specific to the reactivity to an approaching human: the tolerance to being approached when the lamb is free to flee (HUMAPPRO, using CT2-DIST and CT2-SEEN).

3.4 Use for genetic analysis and genetic evaluation

Genetic analyses and genetic evaluation can be performed on single traits and synthetic variables.

Genetic analyses (estimation of (co)variance components and of breeding values) for quantitative behavioural traits may be implemented with an animal mixed model. Random effects should include:

-a direct additive genetic effect of the animal (i.e. lamb),

-a maternal permanent environment effect (i.e dam), that describes lamb phenotypic variation caused by the environment of the ewe

-a litter permanent environment effect, that accounts for phenotypic variation caused by the environment of the litter.

All relevant fixed effects and interactions should be included in the model. Factors that could be considered include:

- a combination of the litter size at lambing and the number of lambs suckled with their dam
- sex of the lamb
- dam parity
- age of the dam nested within parity (if needed)
- contemporary group (e.g., depending on the data collection: flock-year-season…)

4 Maternal reactivity

4.1 Definition, terminology, rationale

- Behavioural reactivity at lambing (i.e. maternal reactivity):

- is the social motivation or attachment of the ewe for the litter expressed in response to an approaching human, or the withdrawal of the litter with or without presence of a human. Expression of higher levels of a panel of behaviours, including maternal behaviour scores, vocalisations and locomotion, is hypothesised as an active way to maintain social link with lambs.

Maternal reactivity is measured in standardised behavioural tests (a scoring test outdoor, an arena test indoor, described below).

Higher maternal reactivity may improve adaptation of sheep to harsh environments through a higher behavioural autonomy at lambing and a reducing dependency to the support providing by shepherds. Consequently, improving such behavioural traits may improve welfare, production, and labour of shepherd.

4.2 Data recording

4.2.1 Behavioural tests

The test described below have been implemented in France. It must be considered as a possible test, as others can be described later and enrich these guidelines. Ewes are individually exposed to two behavioural tests: a scoring test performed just after lambing, outside at the lambing site, and then an arena test performed indoor, one day after lambing. The second test is performed after the bonding period needed to establish the social link between ewes and lambs and which occurs generally within the first twelve hours after lambing (Keller et al, 2003).

Scoring test at lambing site: Maternal reactivity is assessed outside at the lambing site approximately 2 hours after lambing, only on ewes that lambed during daylight when the shepherd approaches the lambing ewes to catch lambs for weighing and identification. Scoring at lambing is not performed in the following situations: location of the lambing site does not allow applying the testing procedure (i.e., ewes isolated in a compact box-tree), perturbation of scoring by non-tested ewes, sanitary reasons that could affect behaviours (including difficult lambing, death of all lambs of a litter). Measurement of maternal reactivity at the lambing site (LS) consists of two successive phases: (1) when the shepherd approaches the lambs; and (2) the capture and displacement of the lambs by the shepherd. In the first phase (LS1), the shepherd stands approximately 15 meters away from the lambing spot and approaches the ewes and the lambs at a regular speed (1 m/s). In the second phase (LS2), the shepherd catches all the lambs at the same time and moves away from the lambing spot in the same direction as that of the approach, stopping at the starting point where he places the lambs back on the ground and then moves 15 meters away to allow the ewe to restore contact with her lambs. This second phase of the test is not applied to ewes that flee at the approach of the shepherd and do not return within 60 seconds after the end of LS1.

Arena test: After lambing, all the ewes and lambs (both day and night births) are transferred to a shelter close to the place of lambing and penned individually for few hours. They are then moved to a collective pen until the next day when they are tested in the arena test (24h ± 6h after lambing). The arena test (AT) is performed indoors and adapted from the original test developed by Boissy and colleagues (2005) to investigate social attachment in sheep (Ligout et al., 2011). In the present study, the test consists of three successive phases evaluating the ewe's 1) attraction to her litter, 2) reactivity to social separation from her litter, and 3) reactivity to a conflict between social attraction to her litter

and avoidance of a motionless human. The test pen consists of an unfamiliar enclosure virtually divided into 7 zones (zone 7 being the zone nearest to the litter). On one side of the enclosure, a grid separates the tested ewe from another smaller pen containing her lamb(s). The first phase of the test (AT1) starts when the tested ewe enters the arena and lasts for 30 s. Then, a remotely controlled opaque panel is pulled down in front of the grid to prevent visual contact between the tested ewe and her lambs. The second phase (AT2), during which the tested ewe is separated from her lambs, lasts 1 min. Finally, the panel is raised so the tested ewe can see her lamb(s) again. Once the ewe has returned near to her lamb(s), a non-familiar shepherd slowly enters the arena through a door located near the grid separating the arena from the litter, and stands 20 cm in front of the grid. The third phase of the test (AT3) starts once the shepherd is in place and lasts for 1 min.

4.2.2 Behavioural traits

Scoring test at lambing site: A scoring system, close to those defined by O'Connor et al. (1985), was developed for each of the two phases to evaluate maternal reactivity. In LS1, a maternal behaviour score (LS1-MBS) is recorded on a 5-point scale as follows: 1 - ewe flees and does not 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 behaviour score (LS2-MBS) is 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).

Arena test: Locomotor activity and localisation are analysed from the video footage or infrared cells (as described above). Locomotor activity is assessed by measuring the number of zones crossed during the 3 phases (AT1/2/3-LOCOM). The time spent in each zone is recorded. The ewe's proximity to the litter and/or the human during phases 1 and 3 (AT1/3-PROX) is calculated using the following formula: proximity score = $\sum_{i=1}^{n}(time\ spent\ in\ zone_i)\left(\frac{i-1}{6}\right)$, with n = 7. Two types of vocalisations are recorded manually during the test with an electronic device: number of high-pitched bleats are recorded when the animal bleats with an open mouth (AT1/2/3-HBLEAT) and number of low-pitched bleats are recorded when the animal bleats with a closed mouth (AT1/2/3-LBLEAT).

4.3 Calculation of traits

Logarithmic transformation is applied to AT1/2/3-LBLEAT to minimise major deviations from the normal distribution. All other elementary variables described above are directly used for genetic analyses.

4.4 Use for genetic analysis / genetic evaluation

The (co)variance components for quantitative behavioural traits can be estimated by restricted maximum likelihood methodology applied to an animal. The (co)variance components for categorical behaviours can be estimated by MCMC and Gibbs sampling methods using a threshold.

All analyses assume a repeatability linear model with behaviour measured across productive cycles considered to be the same trait with a constant variance. Random effects included a direct additive genetic effect of the animal (i.e., ewe) and a permanent environmental effect of the animal.

All relevant fixed effects and interactions should be included in the model. Factors that could be considered include:

- the litter size at lambing
- dam parity
- age of the dam nested within parity (if needed)
- contemporary group (e.g., depending on the data collection: flock-year-season effect…)

5 Behaviour at grazing

5.1 Definition, terminology, rationale

Grazing behaviour is a complex combination of various movements and activities of animals in different spatial-temporal scales (Andriamandroso et al, 2016). Indicative traits related to grazing behaviour include:

- Duration of grazing
- Distance walked
- Speed
- Altitude difference
- Elevation gain/loss
- Energy expenditure at grazing

A better understanding of the phenotypic and genetic background of grazing behaviour traits could help towards the development of appropriate breeding programmes to increase adaptation to extensive rearing conditions. However, recording of such traits is challenging. The use of new technologies such as global positioning systems (GPS) could help towards efficiently monitoring grazing behaviour (Homburger et al., 2014; Feldt and Schlecht, 2016).

5.2 Data recording

The following guidelines for recording grazing behaviour traits of sheep are based on a study implemented in Greece (Vouraki et al., 2023 – under review; SMARTER deliverable 2.4 – in preparation). Specifically, in the latter study, grazing behaviour of Boutsko sheep reared semi-extensively in mountainous regions was monitored using GPS technology. Moreover, phenotypic and genetic parameters for key grazing behaviour traits were estimated. These guidelines could be enriched in the future based on other relevant studies.

Monitoring of sheep grazing behaviour is performed using appropriate GPS devices attached on designated collars (Figure 3). Rotational monitoring of animals can be applied to reduce the number of devices needed. Selected GPS devices should be of low weight in order to be accepted by the animals without any obvious irritation. Batteries with extended life should be used to provide sufficient energy for GPS tracking for as many as possible consecutive days. In the aforementioned study, “Tractive GPS”

devices (Tractive, Pasching, Austria) were used that weighted 28 grams. GPS tracking of each animal was performed for 4-10 days at 2-60 minutes intervals; number of tracking days and intervals were based on available signal and animal movement.

GPS generated data of each animal for the total tracking period are exported in .gpx format. In the case of "Tractive GPS", the location history function of MyTractive web app (https://my.tractive.com/#/) is used to export recorded data. Then, the exported files are split by date using a designated software such as GPSBabel (version 1.8.0). For each animal, daily routes and corresponding GPS data can be visualized and extracted using appropriate software such as Viking GPS data editor and analyser (version 2.0).

Recorded grazing behaviour traits include duration of daily grazing, distance, speed, minimum and maximum altitude, and total elevation gain. Other useful metrics including number and average distance between tracking points, tracking duration and route followed by the animals should also be extracted to be used in ensuing analyses.

Figure 3. GPS device attached on designated collar.

5.3 Calculation of traits

Based on minimum and maximum altitude, altitude difference is calculated. Moreover, energy expenditure for walking can be estimated 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).

Quality control of GPS generated phenotypes is performed for extreme values and errors. Specifically, limits are set for minimum and maximum altitudes to reflect the real altitude of the studied regions. Tracking points beyond these limits are then removed from the corresponding .gpx files and data are recalculated. Moreover, daily records for which GPS tracking of animals stopped before returning to their shed must be excluded. Finally, if needed, grazing behaviour traits should be logarithmically transformed to ensure normality of distribution.

5.4 Use for genetic analysis / genetic evaluation

(Co)variance components of grazing behaviour phenotypes and relevant breeding values (EBVs) can be estimated by restricted maximum likelihood methodology applied to an animal mixed model that should include the following random and fixed effects:

Random effects: additive genetic effect of the animal and permanent environmental effect.

The relevant fixed effects may include:

- Farm
- Number of GPS tracking points
- Tracking duration
- Distance between tracking points
- Climatic parameters (e.g. temperature-humidity index)
- Sampling time

6 Acknowledgements

We gratefully acknowledge the contributions to these recording of behaviour guidelines by the following people:

- Dominique Hazard, INRAE, France
- Angeliki Argyriadou, University of Thessaloniki, Greece
- Georgios Arsenos, University of Thessaloniki, Greece
- Alain Boissy, INRAE, France
- Vasileia Fotiadou, University of Thessaloniki, Greece
- Sotiria Vouraki, University of Thessaloniki, Greece

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

7 References

Alderman, G., Cottrill, B. Energy and Protein Requirements of Ruminants. In An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients; CAB International: Wallingford, UK, 1993.

Andriamandroso, A., J. Bindelle, B. Mercatoris, F. Lebeau (2016). A review on the use of sensors to monitor cattle jaw movements and behavior when grazing. Biotechnologie, Agronomie, Société et Environnement, 20.

Beausoleil, N. J., D. Blache, K. J. Stafford, D. J. Mellor, and A. D. L. Noble. 2012. Selection for temperament in sheep: Domain-general and context-specific traits. Appl. Anim. Behav. Sci. 139:74–85.

Boissy, A., Bouix, J., Orgeur, P., Poindron, P., Bibe, B., & Le Neindre, P. (2005). Genetic analysis of emotional reactivity in sheep: effects of the genotypes of the lambs and of their dams. *Genetics Selection Evolution, 37*, 381-401. doi:10.1051/gse:2005007

Brand, T. S. (2000). Grazing behaviour and diet selection by Dorper sheep. Small Ruminant Research, 36(2), 147-158.

Brien, F. D., Cloete, S. W. P., Fogarty, N. M., Greeff, J. C., Hebart, M. L., Hiendleder, S., . . . Miller, D. R. (2014). A review of the genetic and epigenetic factors affecting lamb survival. Animal Production Science, 54, 667-693. doi:10.1071/an13140

Cloete, S. W. P., Burger, M., Scholtz, A. J., Cloete, J. J. E., Kruger, A. C. M., & Dzama, K. (2020). Arena behaviour of Merino weaners is heritable and affected by divergent selection for number of lambs weaned per ewe mated. Applied Animal Behaviour Science, 233. doi:10.1016/j.applanim.2020.105152

Dwyer, C. M., Lawrence, A. B. (2005). A review of the behavioural and physiological adaptations of hill and lowland breeds of sheep that favour lamb survival. Applied animal behaviour science, 92(3), 235-260.

Dwyer, C. M. (2008). Genetic and physiological determinants of maternal behavior and lamb survival: Implications for low-input sheep management. Journal of Animal Science, 86, E246-E258. doi:10.2527/jas.2007-0404

Dwyer, C. M. (2014). Maternal behaviour and lamb survival: from neuroendocrinology to practical application. animal, 8, 102-112. doi:doi:10.1017/S1751731113001614

Feldt, T., Schlecht, E. (2016). Analysis of GPS trajectories to assess spatio-temporal differences in grazing patterns and land use preferences of domestic livestock in southwestern Madagascar. Pastoralism, 6(1), 1-17.

Gilmour, A. R., Gogel, B. J., Cullis, B. R., & Thompson, R. (2009). ASReml User Guide Release 3.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. www.vsni.co.uk.

Hazard, D., Moreno, C., Foulquié, D., Delval, E., François, D., Bouix, J., Boissy, A. (2014). Identification of QTLs for behavioral reactivity to social separation and humans in sheep using the OvineSNP50 BeadChip. *BMC Genomics, 15*, 778. doi:10.1186/1471-2164-15-778

Hazard, D., Bouix, J., Chassier, M., Delval, E., Foulquie, D., Fassier, T., Boissy, A. (2016). Genotype by environment interactions for behavioral reactivity in sheep. *Journal of Animal Science, 94*, 1459-1471. doi:10.2527/jas2015-0277

Hazard, D., Macé, T., Kempeneers, A., Delval, E., Foulquié, D., Bouix, J., & Boissy, A. (2020). Genetic parameters estimates for ewes' behavioural reactivity towards their litter after lambing. *Journal of Animal Breeding and Genetics, n/a*. doi:10.1111/jbg.12474

Hazard, D., Kempeneers, A., Delval, E., Bouix, J., Foulquie, D., & Boissy, A. (2021). Maternal reactivity of ewes at lambing is genetically linked to their behavioural reactivity in an arena test. Journal of Animal Breeding and Genetics, 139, 193-203. doi:10.1111/jbg.12656

Hazard, D., E. Delval, S. Douls, C. Durand, G. Bonnafe, D. Foulquié, D. Marcon, C. Allain, S. Parisot, A. Boissy (2022). Divergent genetic selections for social attractiveness or tolerance toward humans in sheep. WCGALP 2022

Homburger, H., Schneider, M. K., Hilfiker, S., Lüscher, A. (2014). Inferring behavioral states of grazing livestock from high-frequency position data alone. *PLoS One*, *9*(12), e114522.

Jamrozik, J., Schaeffer, L. R., Jansen, G. B. (2000). Approximate accuracies of prediction from random regression models. Livestock Production Science, *66*(1), 85-92.

Keller, M., Meurisse, M., Poindron, P., Nowak, R., Ferreira, G., Shayit, M., & Levy, F. (2003). Maternal experience influences the establishment of visual/auditory, but not olfactory recognition of the newborn lamb by ewes at parturition. Developmental Psychobiology, 43, 167-176. doi:10.1002/dev.10130

Ligout, S., Foulquie, D., Sebe, F., Bouix, J., & Boissy, A. (2011). Assessment of sociability in farm animals: the use of arena test in lambs. Applied Animal Behaviour Science, 135, 57-62. doi:10.1016/j.applanim.2011.09.004

Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J.-M., Fisher, A. D., Hinch, G. N., . . . Beaumont, C. (2005). Genetics of adaptation and domestication in livestock. *Livestock Production Science, 93*, 3-14. doi:10.1016/j.livprodsci.2004.11.001

O'Connor, C. E., Jay, N. P., Nicol, A. M., & Beatson, P. R. (1985). Ewe maternal behaviour score and lamb survival. Proceedings of the New Zealand Society of Animal Production, 45 159–162.

Simm, G., Conington, J., Bishop, S. C., Dwyer, C. M., Pattinson, S. (1996). Genetic selection for extensive conditions. Applied Animal Behaviour Science, 49(1), 47-59.

SMARTER deliverable D2.4. New prototype and report for industry on GPS-generated phenotypes for behavioural adaptation to extensive grazing systems; artificial rearing adaptation phenotypes; lamb vigour scores linked to lamb survival; new foetal and neonatal survival phenotypes (in preparation).

von Borstel, U. K., Moors, E., Schichowski, C., & Gauly, M. (2011). Breed differences in maternal behaviour in relation to lamb (Ovis orientalis aries) productivity. Livestock Science, 137, 42-48. doi:10.1016/j.livsci.2010.09.028

Vouraki, S., Papanikolopoulou, V., Argyriadou, A., Fotiadou, V., Tsartsianidou, V., Triantafyllidis, A., Banos, G., Arsenos, G. Genetic parameters for grazing behaviour traits of Boutsko sheep. In 74th Annual Meeting of the

European Federation of Animal Science (EAAP 2023), Lyon, France, 26 August - 1 September 2023 (under review).

Recommendations on recording lifetime resilience in small ruminants

SMARTER – Deliverable D6.3

Version 1.0 – 12 May 2023

Table des matières

Change Summary

Date of change	Nature of Change
January 2023	First draft
May 2023	Final version for SMARTER deliverable 6.3

1 Introduction

Lifetime resilience is often tackled through longevity and aspects of productive longevity. Longevity is an indirect global trait to quantify productive lifespan of livestock, and for increasing durability and profitability of farms. In dairy ruminants, longevity definitions include: (i) true longevity (all culling reasons, including milk productivity); and (ii) functional longevity (all culling reasons, except sought productivity, such as milk productivity or growth). Functional longevity (corrected for production level – milk, growth) reflects the animals' accumulated ability to overcome health and nutritional challenges. It is an indirect global approach to quantify adaptive capacity to various production environments. Different indicators may be calculated. One indicator is the length of productive life which is computed as the time interval (in days) between first lambing/kidding and culling. Longevity is linked with various predictors, such as fertility, udder health and conformation, resistance to disease, body condition score changes across ewe/doe lifetime. These predictors may be used in in breeding program to get an earlier breeding value of longevity and may help monitor lifetime resilience at the farmer level.

2 Scope

To define approaches for the definition of longevity as well as the traits that can be calculated, and the downstream analyses that can be set up (including the use of early predictors to enhance longevity in the evaluation process).

To propose a grid for setting up an observatory of the culling causes.

3 Longevity

3.1 Definition, terminology, rationale

The notion of longevity can cover several meanings. Longevity can be understood as the true longevity, i.e. the ability of the animal to live as long as possible, whatever its production level and its functional characteristics. But the animal longevity also depends on the replacement rate which is a choice of the breeders. As the production level is tackled by specific traits such as milk production or growth or fat/muscle depth, leading to chosen culling, what is often

sought is the functional longevity, which is an ability independent from the production level, but which is related to functional abilities, whose failure may lead to undesired culling.

Undesired culling may be due to:

- udder health problem (clinical, subclinical, chronic mastitis),
- lack of resistance to disease such as parasites,
- problem of footrot,
- unfavourable shape of the udder (lack of adaptation to machine milking or to suckling)
- unfavourable general conformation
- undesired behaviour (temperament in the milking parlour)
- infertility or any problem of reproduction
- problem of feet or legs, lameness
- lack or excess of body tissue mobilisation
- any other undesired cause of culling

Even if some of these sought abilities may be considered per se in the selection process by phenotyping and evaluating related traits (for example resistance to mastitis, resistance to gastro-intestinal parasite, fertility, udder morphology), it is not possible to account for all of them. If properly modelled, functional longevity may be considered as a global and composite approach, allowing to assess the sustainability of the population in selection and of the practiced selection.

For this, different traits may be considered, quite often easy to compute with data usually already existing in the genetic database (ex. length of productive life, which can be calculated as the culling date minus the date of the first lambing). There is no additional recording to set up. The difficulties in handling functional longevity are related to the modelling of the trait, given that the trait is fully known when the animal is culled. When not yet culled, the model to set up are quite complex.

Even though there is no need to identify/know the cause of culling, the knowledge of the cause of culling might be a relevant observatory of the hierarchy of the culling cause, which may lead to put an emphasis on some issue. For example, if we observe an increase in some culling causes (let's say parasitism) this should lead to a specific program to assess resistance to parasite.

One drawback of the functional longevity trait is its lack of precocity. As stated above, it is necessary to have the date of culling or to have accumulated enough lactation to compute the trait. And an appropriate model (e.g. survival analysis) can only partially disentangle this difficulty. It is possible to address this issue by running a multi trait genetic evaluation model combining the longevity trait and some other proxy traits (such as udder morphology, udder health, etc). Genomic selection is a complementary way to generate early genetic prediction, provided a good accuracy of the EBVs of animals in the reference population.

3.2 Data recording

3.2.1 Longevity traits

The table 1 presents some criteria commonly used in small ruminants to measure longevity. Here, the criteria deal with true longevity, the only one measurable in herd/flocks. Functional longevity will be estimated later, at the statistical analysis step. The table 1 also shows the data required for calculating the longevity criteria. For example, the length of productive life is calculated using only two variables: the birth date and the culling date (or death date), as the difference between both. It is important to notice that the culling date, which is rarely recorded by the farmers, can be replaced by the date of the last event registered for the animal (for example, date of the last performance recording, or of the last reproduction event).

Table 1. Definition of some commonly used longevity criteria

Longevity criteria	**Raw data required**	**Calculation**
Length of total lifespan (LTL)	Birth date (BD) Culling or death date (CD)	LTL= CD - BD in days (or months or years)
Length of productive life (LPL)	First lambing/kidding date (FKD) Culling or death date (CD)	LPL = CD – FKD in days (or months or years)
Total number of days in production (NDL)	Days in milk per lactation (DIM) or Lambing/kidding date + dry off date for each lactation	NDL = ∑ DIM
Number of lactations (NLACT)	Each lambing/kidding event (KE)	NLACT = ∑ KE
Number of lambs or kids during lifetime (NLAMB)	Prolificacy at each lambing/kidding (PR)	NLAMB = ∑ PR

The length of total lifespan can also be estimated easily, with only two variables usually registered by farmers. The difference with the length of productive life is that it considers the period before the first lambing/kidding and the lambing/kidding interval. If the age at the first lambing/kidding and the lambing/kidding interval are similar between animals, the length of total lifespan will be very close, in terms of signification, to the length of productive life.

The total number of days in production only covers the “useful” life of the females because it doesn’t include the unproductive periods (such as dry off or large lambing/kidding interval after reproduction failure), compared to length of productive life. But the number of variables necessary to compute it is larger.

For the total number of lambs or kids during lifetime, it is possible to target to live-born lambs/kids only or those reared to weaning, if these data are routinely recorded.

3.3 Calculation of traits

The last column of the table 1 indicates how to calculate the different longevity criteria, from the raw variables.

The length of total lifespan and the length of productive life are estimated as differences in days between two dates: i) the culling date and ii) the birth date or the first lambing/kidding date, respectively.

The total number of days in production corresponds to the sum of the days in milk of each lactation of the female.

For the last two criteria (number of lactations or number of lambs/kids), the estimation corresponds to cumulative performances across lifetime.

Instead of waiting for the end of the animal's life to calculate the longevity criterion (which is sometimes long), one solution deals with limiting the animal career to a maximum number of years or lactations. For example, the length of productive life can be calculated only on the first 6 lactations. Subsequently, the length of productive life will be defined as the total number of days between the first lambing/kidding and the end of the 6th lactation. In the same way, the total number of lambs/kids can be estimated at a fixed age, 8 years old for example.

3.4 Use for genetic analysis / genetic evaluation.

3.4.1 Models

The genetic ability for longevity is evaluated via the functional longevity, i.e. the true longevity corrected for production traits. Functional longevity is defined at this step, by integrating the level of production as fixed effect in the analysis of longevity criteria described in the table 1.

Different methods are used for the genetic evaluation of longevity traits.

The first method is based on linear models. The main advantage of these models is their ease of implementation because they are used for most of the traits under selection. But they have different drawbacks regarding longevity:

-they do not fit well longevity because longevity indicators do not follow a normal distribution

-they consider only animals that have finished their productive life. This has two consequences: the longevity data are skewed if living animals are ignored; the breeding value is available lately in the life of the animals. This is notably the case for males for whom most of their offspring must be culled to be evaluated.

-they are not able to include time-dependant variables (e.g. parity, lactation stage). Time dependant variables are useful to take into account the changes in breeding conditions that occur during the life of the animal, and thus to better model longevity data.

The second method is based on proportional hazard model or survival analysis. This type of model counterbalances all the drawbacks of linear models and thus, are the best ones to estimate breeding values for functional longevity. Nevertheless, they are complicated to implement in a routine genetic evaluation process, few software exist for genetic survival

analyses (Survival kit, Ducrocq et all, 2005), and an evaluation based on an animal model is not feasible in large dataset, leading to use sire-maternal grand-sire models or sire models. Under this assumption, ewes/does EBVs are not available (Ducrocq, 2001).

A third method, less widespread, considers the first three lactations as separate traits in a multiple trait animal linear model. Each lactation is assigned to 1 (instead of 0) once the female reaches the next lactation.

3.4.2 Factors of variation

The main factors of variation of longevity data are:

-herd/flock

-year

-kidding/lambing season

-birth season

-age at first lambing/kidding

-breed

-herd/flock size and herd/flock size variation

-lactation stage, parity (if survival analysis model)

-within herd/flock production level: this factor of variation is essential to integrate to estimate the functional longevity. Usually, it is the within herd/flock level of production (and not the absolute level of production) that is considered because it explains the decision of the breeder to cull the animal.

3.4.3 Heritabilities of functional longevity

Heritabilities range between 5% and 17%, indicating that this trait has a low to moderate genetic background. This might be due to the composite signification of longevity, which represents a synthesis of various abilities (see § on predictors).

However, the genetic variation coefficients are moderate suggesting that a genetic variability may be exploited to set up a selection.

3.4.4 Genetic correlations

The genetic correlations between functional longevity and other traits are:

-close to 0 for milk production traits. This results from the model, in which longevity is corrected for level of production.

-from 0 to 0.40 for udder type traits. The rear udder attachment and the udder floor position are the most correlated to functional longevity.

-from 0.20 to 0.50 for general conformation

-from 0.01 to 0.15 for reproduction traits (kidding interval, age at first kidding, artificial insemination fertility)

-from -0.15 and -0.40 for somatic cell counts

3.4.5 EBVs and reliabilities

Because of the low accuracy of breeding values, only males (and especially artificial insemination males) evaluated on longevity of their daughters, have EBVs that can be used for selection. A minimum number of daughters culled per sire is required to reach a sufficient accuracy. The consequence is that the AI males get their first longevity EBV quite late in their life. Survival analysis models, because they consider censored data (living daughters), allow to have a better accuracy and thus, an earlier EBV for AI males.

Other strategies are possible to increase the accuracy of functional longevity EBVs:

-introduce genomic information in the genetic evaluation

-use a multiple trait model, including both functional longevity and other traits considered as predictors of longevity.

Predictors of longevity

Given the low heritability of survival traits and its late knowledge (the traits become accurate enough when enough information on culling or reproduction/lactation is known), it is necessary to enhance direct evaluations by indirect information coming from early predictors. Some relevant predictors are listed below:

Morphological traits, such as general conformation or udder morphology (especially in dairy species).

Reproduction traits (fertility, lambing/kidding interval, age at first lambing/kidding, pregnancy scan results, …).

Udder health, and particularly milk somatic cell count.

Resistance to disease such as resistance to parasites or to footrot.

Traits related to feet and legs, such as lameness or twisted or bowed legs, closed or opened hocks.

Change in body condition score.

Serum immunoglobulin concentration in the early life (Ithurbide et al, 2022a)

Maturity (dairy species) that can be defined as the ability to maintain a good level of production over the parities, independently of the level of production on the whole lifetime (equivalent of a persistency, but over the lactations and not over the test-days).

Milk metabolites (Ithurbide et al, 2022b)

Body tissue mobilisation (Conington, 2023). It was demonstrated that ewe tissue mobilisation was genetically associated with ewe fertility and productive longevity (such as pregnancy scan result, foetal loss from scan to lambing, lamb loss from lambing to weaning, number of lambs weaned). It is made possible by collecting body condition score (BCS) data throughout the

reproductive cycle (e.g. pre mating, pregnancy scan, pre lambing, mid lactation, weaning) and calculating gain or loss of BCS between physiological stage.

These predictors are linked to longevity traits. An unfavourable udder shape, reproduction disorders, a susceptibility to a given disease or a low maturity may lead to a undesired culling and therefore a low longevity of the animal. Few genetic correlations have been published but correlations between EBVs show favourable correlations between these predictors and longevity.

Longevity traits, once evaluated, either in linear or survival analysis model, may be combined with the longevity traits in a multi-trait evaluation, to incorporate the information from early predictors.

A full multiple trait evaluation is not feasible in large datasets. Therefore, approximate strategies must be used, such as considering records adjusted for all non genetic effects in linear models (yield deviation or daughter yield deviation, other type of pseudo records).

3.5 Culling causes

Even though the knowledge of the causes of culling is not necessary to generate a phenotype of longevity and an EBV of functional longevity, the knowledge of the causes of culling, through an observatory based on a sufficient panel of flocks/herds, and repeated each year, may give relevant information on the hierarchisation and the evolution of the culling causes. It may also allow to better understand the strategies of culling of the farmers and thus better model the functional longevity.

The culling causes may be collected with different level of precision, from a general group of causes to a precise cause, through an intermediate information.

In sheep as in goat, the following group of culling causes may be collected:

- Udder health (mastitis)
- Udder morphology
- Production ability
- Respiratory disorders
- Reproduction disorders
- Digestive disorders
- Nervous disorders
- Musculoskeletal disorders
- Skin disorders
- Conformation
- General condition
- Age
- Behaviour

- Accident
- Other ailments (e.g. sudden death, brucellosis, intoxication, fever …)
- Voluntary culling

Each group may be completed with sub-group or precise cause. Below are two examples, first for udder health (table 2), second for reproduction disorders (table 3).

Table 2. Detailed categorisation of udder health culling causes

Group	Sub-group	Specific cause
Udder health (mastitis)	Gangrenous mastitis	Gangrenous mastitis
		Brief mastitis
	Characteristic symptoms	Mastitis
		Clinical mastitis
		Mastitis during suckling
		Coliform mastitis
		Listeria mastitis
		Mastitis before lambing/kidding
		Agalactia mastitis
	Functional symptoms	Blood in the milk
	Chronic mastitis, palpation	induration of the udder
		Bumps in the udder
		Nodules
		Mammary abcess
		Saggy udder
		Visna mastitis
	Unbalanced udder	Milk in one side
		Unbalanced udder
	Subclinical	Subclinical mastitis
		Somatic cell count – CMT
	Other	Other

Table 3. Detailed categorisation of reproduction disorders culling causes

Group	Sub-group	Specific cause
Reproduction disorders	Fecundity	Open + infertile
		Lately fertile, out of season
		Ram infertile
	Gestation	Abortion
		Vagina or rectal prolapse
		Pregnancy toxaemia
		Difficult gestation
		Early abortion
		Late abortion
	Lambing/kidding	Difficult lambing/kidding
		Caesarean
		Uterus inversion
		Infection during lambing/kidding
		Vagina or rectal prolapse
		non deliverance
		Acute metritis
		Chronic metritis
	Miscellaneous	Reproduction disorders
		Vaginal sponge infection
		Hermaphrodite
		Various
	Male: testicles	1 testicle
		Small testicles
		Abscess
		Contagious epididymitis
	Male: penis	Urinary gravel
		Wound
		Phimosis

4 Acknowledgements

We gratefully acknowledge the contributions to these lifetime resilience guidelines by the following people:

- Joanne Conington, SRUC, the UK
- Isabelle Palhière, INRAE, France
- Jean-Michel Astruc, IDELE, France
- Carolina Pineda Quiroga, NEIKER, France

This work received funding from the European Unions' Horizon 2020 Research & Innovation program under grant agreement N°772787—SMARTER.

5 References

Arnal M., M. Chassier, V. Clément and I. Palhière, Maturity, a heritable trait in Saanen French dairy goat. 74th Annual Meeting of the European Federation of Animal Science (EAAP), Aug 2023, Lyon, France

Buisson D., J.M. Astruc, L. Doutre, I. Palhière. Toward a genetic evaluation for functional longevity in French dairy sheep breeds. Proc 12th WCGALP, 2022

Castañeda-Bustos, V. J., Montaldo, H. H., Torres-Hernández, G., Pérez-Elizalde, S., Valencia-Posadas, M., Hernández-Mendo, O., & Shepard, L. (2014). Estimation of genetic parameters for productive life, reproduction, and milk-production traits in US dairy goats. Journal of Dairy Science, 97(4), 2462-2473.

Castañeda-Bustos, V. J., Montaldo, H. H., Valencia-Posadas, M., Shepard, L., Pérez-Elizalde, S., Hernández-Mendo, O., & Torres-Hernández, G. (2017). Linear and nonlinear genetic relationships between type traits and productive life in US dairy goats. Journal of Dairy Science, 100(2), 1232-1245.

Conington, J., Bishop, S. C., Grundy, B., Waterhouse, A., & Simm, G. (2001). Multi-trait selection indexes for sustainable UK hill sheep production. Animal Science, 73(3), 413-423.

Conington, J. (2023). New breeding goals for lifetime resilience for maternal sheep breeding programmes. SMARTER deliverable D2.2.

Ducrocq, V. 2001. A Two-Step Procedure to get Animal Model Solutions in Weibull Survival Models Used for Genetic Evaluations on Length of Productive Life. Interbull Bulletin, vol.27, pp.147-152

Ducrocq, V. (2005). An improved model for the French genetic evaluation of dairy bulls on length of productive life of their daughters. Animal Science, 80(3), 249-256.

Geddes, L., Desire, S., Mucha, S., Coffey, M., Mrode, R. and Conington, J. 2018. Genetic parameters for longevity traits in UK dairy goats. IN: Proceedings of the World Congress on Genetics Applied to Livestock Production, Volume Species - Caprine: 547.

Ithurbide, M., Huau, C., Palhière, I., Fassier, T., Friggens, N. C., & Rupp, R. (2022a). Selection on functional longevity in a commercial population of dairy goats translates into significant differences in longevity in a common farm environment. Journal of Dairy Science, 105(5), 4289-4300.

Ithurbide, M., Wang, H., Huau, C., Palhière, I., Fassier, T., Pires, J. & Rupp, R. (2022b). Milk metabolite profiles in goats selected for longevity support link between resource allocation and resilience. In Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) pp. 276-279

Palhière I., C. Oget, R. Rupp, Functional longevity is heritable and controlled by a major gene in French dairy goats, 11th WCGALP, Auckland, Nouvelle-Zelande, 11-16 février 2018

Pineda-Quiroga, C., Ugarte, E. 2022. An approach to functional longevity in Latxa dairy sheep. Livestock Science 263, 105003

Sasaki, O. (2013). Estimation of genetic parameters for longevity traits in dairy cattle: A review with focus on the characteristics of analytical models. Animal Science Journal, 84(6), 449-460.

SMARTER Deliverable 2.2 - “New breeding goals for lifetime resilience for maternal sheep breeding programmes”

Talouarn E., V. Loywyck, Flavie Tortereau. Ewes’ functional longevity: analysis and modelling in French suckling sheep breeds. 69th Annual Meeting of the European Federation of Animal Science (EAAP), Aug 2018, Dubrovnik, Croatia.