SMARTER

SMAll RuminanTs breeding for Efficiency and Resilience

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Report of novel phenotypes related to feed efficiency

Flavie Tortereau[1], Christel Marie-Etancelin[1], Hélène Larroque[1], Coralie Machefert[1], Isabelle Palhière[1], Florian Mosnier[2], Quentin Le Graverand[1], Florian Touitou[1], Arnaud Delpeuch[2], Sarah Teixeira[2], Marjorie Chassier[2], Gilles Laggrifoul[2], Georgios Arsenos[3], Sotiria Vouraki[3], Vanessa Fotiadou[3], Jette Jackobsen[4], Sebastian Mucha[5], Ignacio de Barbieri[6], Gabriel Ciappesoni[6] Beatriz Gutiérrez-Gil[7], Gonzalo Hervás[7], Pablo G. Toral[7], Aroa Suárez-Vega[7], Pilar de Frutos[7], Juan-José Arranz[7]

1- INRAE UMR GenPhySE

2- Idele

3- AUTH

4- NSG

5- SRUC

6- INIA-Uruguay

7- UNILEON

* Deliverable leader – Contact: Flavie.tortereau@inrae.fr

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About the SMARTER research project

SMARTER will develop and deploy innovative strategies to improve Resilience and Efficiency (R&E) related traits in sheep and goats. SMARTER will find these strategies by: i) generating and validating novel R&E related traits at a phenotypic and genetic level ii) improving and developing new genome-based solutions and tools relevant for the data structure and size of small ruminant populations, iii) establishing new breeding and selection strategies for various breeds and environments that consider R&E traits.

SMARTER with help from stakeholders chose several key R&E traits including feed efficiency, health (resistance to disease, survival) and welfare. Experimental populations will be used to identify and dissect new predictors of these R&E traits and the trade-off between animal ability to overcome external challenges. SMARTER will estimate the underlying genetic and genomic variability governing these R&E related traits. This variability will be related to performance in different environments including genotype-by-environment interactions (conventional, agro-ecological and organic systems) in commercial populations. The outcome will be accurate genomic predictions for R&E traits in different environments across different breeds and populations. SMARTER will also create a new cooperative European and international initiative that will use genomic selection across countries. This initiative will make selection for R&E traits faster and more efficient. SMARTER will also characterize the phenotype and genome of traditional and underutilized breeds. Finally, SMARTER will propose new breeding strategies that utilise R&E traits and trade-offs and balance economic, social and environmental challenges.

The overall impact of the multi-actor SMARTER project will be ready-to-use effective and efficient tools to make small ruminant production resilient through improved profitability and efficiency.

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Abbreviations:

ACF: Automated Concentrate Feeder
ADFI: Average Daily Feed Intake
ADG: Average Daily Gain
AFF: Automated Forage Feeder
BCS: Body Condition Score
BFT: BackFat Thickness
BW: Body Weight
DEG: Differentially Expressed Gene
E-BW: Body weight at the end of the control period
FC: Fat Content
FCR: Feed Conversion Ratio
FE: Feed Efficiency
FI: Feed Intake
MD: Muscle Depth
MIR: Mid infra-red
MY: Milk Yield
NEICMR: Net Energy Intake Corrected for Milk Ratio
NIRS: Next infra-red spectra
NMR: Nuclear Magnetic Resonance
OTUs: Operational Taxonomic Units
PC: Protein Content
PLS-DA: Partial Least Squares – Discriminant analysis
RFI: Residual Feed Intake
VIP: Variable Importance in Projection

1 Summary

Feed efficiency (FE) is a key trait to improve in small ruminants, from an environmental to an economic point of view. Different criteria exist to work on FE, the more popular being feed conversion ratio (FCR) and residual feed intake (RFI) that are considered the reference/gold standard. However, both require individual production (milk or body weight) and feed intake (FI) to be recorded. Different tools exist to record individual FI, but most of them cannot be used in commercial farms either because they do not comply with animal management (e.g., individual crates) or because they are too expensive (automatic feeders or individual troughs). Therefore, the objective of the presented work was to analyse the prediction performances of different proxies for FI and/or FE. These proxies have been defined at different levels:

- zootechnical traits that are routinely recorded (body weights, muscle depth and backfat thickness measured with ultrasound, milk yield, milk fat and protein contents, body condition scores, wool traits)
- zootechnical traits that are currently not routinely recorded (greenhouse gas emissions, morphological traits (shoulder height, chest depth and width)).
- Milk traits (untargeted metabolome with LC-MS, milk fatty acids composition with gas chromatography, milk fine composition with MIR spectra, milk total protein and lactose contents)
- Blood traits (untargeted approach with NMR on plasma, targeted metabolites contents (beta-hydroxybutrate, non-esterified fatty acids, total proteins and hormones), plasmatic ^{15}N natural abundancies)
- Faecal traits (NIR spectra on faeces)
- Ruminal traits (pH, ammonia content, microbiota description, untargeted metabolome with NMR, volatile fatty acids content)
- Milk molecular traits (transcriptomics and epigenetic marks in milk somatic cells)

A general table gathering all the considered proxies is given in a first part, along with the main conclusions. Those proxies have been evaluated in experimental designs as well as in commercial farms. Most of the experimental facilities could record individual FI so that reference FE criteria could be estimated. In commercial dairy farms, forage intake at the pen level was sometimes recorded, and concentrate intake was individually recorded in the milking parlour in some dairy farms. In meat sheep farms, no feed intake was recorded at the individual or the pen levels.

A total of 5,917 dairy sheep (from 7 breeds), 7,469 dairy goats (from 3 breeds), and 22,146 meat sheep (from 8 breeds) have been phenotyped to carry out this work. Reference FE criteria (i.e., estimated from individual FI) were estimated on 88 dairy sheep and 2,287 meat sheep. In dairy goats, concentrate intake was recorded in 1,289 individuals, but forage intake was not registered.

Numbers of individuals per partner are described in tables A, B and C for dairy sheep, dairy goats and meat sheep, respectively, and details per breed are provided.

We highlighted, at least in dairy sheep, that feed intake and milk yield were key parameters in the calculation of feed efficiency ratios: taking an average value for the individuals implies major re-ranking. Predicting feed intake can thus be an alternative to the direct prediction of feed efficiency. In meat sheep, the application of eigenvectors from principal component analysis of production traits (i.e., body weight, growth) and gas emissions turned to be helpful in the prediction of feed intake. Feed intake is predicted with higher accuracies than feed efficiency criteria.

Ruminal proxies, in univariate and multivariate analyses did not reach good prediction performances for feed efficiency. Multivariate analyses including proxies from different tissues were performed and highlighted that host traits return better predictions for feed intake, and to a less extent of feed efficiency than ruminal traits. The different proxies significantly associated with feed efficiency in small ruminants, included plasma and milk metabolites, milk fatty acids and milk somatic cell methylation marks and transcriptomic profile. The more promising proxies for feed efficiency that can be proposed to be recorded on a large scale are the ones measured in blood (plasma metabolites such as citrate

and amino acids) and in milk (fatty acids composition). The inclusion of fixed effects in the prediction models is of high importance in order to avoid any spurious correlations that might influence the prediction accuracy.

The next step is to perform genetic and genomic analyses (more than 6,200 individuals being genotyped on low to medium density SNP chips) of these considered proxies and analyse their genetic correlation with feed efficiency criteria.

2 Introduction

The overall aim of work package 1 is to identify novel traits to improve resource use efficiency. Feed efficiency (FE) is the ability of an animal to transform its feed into food edible by humans. FE can be assessed using indicators such as FCR (or its inverse commonly named feed efficiency) and RFI. However, these indicators require feed intake to be recorded. Different tools exist to record individual feed intake. Individuals can be placed in individual crates, but this does not comply with the gregarious behaviour of small ruminants, and it requires a lot of workloads, so they are only usable in experimental facilities. Automated feeders have been developed and mainly rely on electronic identification: one device, located in a given pen, can be used by several individuals. Although automatic feeders can be routinely used in experimental farms, very few are available in European commercial farms – mainly because of the cost.

Therefore, the objective is to identify proxies for feed intake and/or feed efficiency that can be easily and routinely measured on commercial farms to make it possible to include feed efficiency in selection indices. Proxies are mainly looked for in the main biological processes known to be influencing feed efficiency. In growing animals, it has been proposed that feed efficiency can be influenced by feed intake, digestion, host metabolism, activity level, and thermoregulation (Herd et al., 2004). Regarding small dairy ruminants, feed efficiency is mainly studied in lactating ewes, and the main biological basis of this trait is less documented than in growing animals. Nevertheless, feed intake, digestion, and host metabolism are also key factors influencing feed efficiency in lactating females, to which we can add body reserves variations along the production cycle.

In this report, part of proxies for feed intake and feed efficiency were considered at the level of the digestion process, which in ruminants, mainly takes place in the rumen. Ruminal microbiota, ruminal metabolomics, and methane emissions were analysed. At the animal level, different levels of proxies were considered:

- zootechnical traits that are routinely recorded (body weights, muscle depth and backfat thickness measured with ultrasound, milk yield, milk fat and protein contents, body condition scores, wool traits)
- zootechnical traits that are currently not routinely recorded (greenhouse gas emissions, morphological traits (shoulder height, chest depth and width)).
- Milk traits (untargeted metabolome with NMR, milk fatty acids composition with gas chromatography, milk fine composition with MIR spectra, milk total protein and lactose contents)

- Blood traits (untargeted approach with NMR on plasma, targeted metabolites contents (beta-hydroxybutrate, non-esterified fatty acids, total proteins and hormones), plasmatic ^{15}N natural abundancies)
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- Ruminal traits (pH, ammonia content, microbiota description, untargeted metabolome with NMR, volatile fatty acids content)
- Milk molecular traits (transcriptomics and epigenetic marks in milk somatic cells)

A general table gathering all the records per involved partner is provided for each production (dairy sheep (Table A), dairy goats (Table B) and meat sheep (Table C)), along with a description of deviations and delays from the DoA document.

Then, Table D summarises the main results, with the main conclusions about the ability to predict feed intake or feed efficiency, for all the small ruminant production.

Then, the different designs and methodologies are described for each small ruminant production (dairy sheep, dairy goats and meat sheep), followed by the results in details.

3 Datasets and deviations or delays from DoA

The three following tables (Table A, Table B and Table C) describe the dataset used for this D1.1, for each type of production (dairy sheep, dairy goats and meat sheep, respectively). In each table, the first lines refer to Table 1 of DoA which summarizes all the breeds and numbers of individuals per partner involved in SMARTER, not only for WP1. Then numbers of individuals (in brackets) and phenotypes considered in D1.1 are given for each trait.

The results included in this deliverable imply, in a fundamental way, the results of Task 1.1 and Task 1.2. These tasks are based on an intensive sampling of different small ruminant experimental (Task 1.1) and commercial (Task 1.2) populations. Since these species follow a seasonal reproduction process, one of the intensive sampling stages occurs from the winter and spring seasons. This period coincided with the COVID19 lockdown in Europe (from mid-March to June 2020), and it was impossible to sample the animals during this period.

The affected partners were:

- INRAE with three experimental populations, two sheep: Romane experimental population, Lacaune experimental population, and the Alpine goat experimental population;
- UNILEON, where sampling was affected by a considerable delay in collecting and analyzing samples in the ASSAF experimental population;
- NSG, there was a delay in collecting methane emission samples in the Norwegian White animals.
- INIA-UY, there have been slight delays in the sampling of limited scope.

The COVID lockdown has also affected this task focusing on sampling in commercial farms. During the lockdown, the technicians have not been able to visit commercial farms. An important part of this task focused on the technicians of the different breeders' associations to the commercial herds. As these visits could not occur during the lockdown periods, the data for this reproductive season has been considerably reduced or lost. In these cases, the sampling period for a complete reproductive season has been lost, equivalent to twelve months in those animals that follow the management pattern of one lambing per year. This fact has delayed the task considerably since the samples were collected about six months later.

Several mitigation measures have been put in place, including sampling animals in the following reproductive cycle (delay in experiments). In the case of commercial populations, where possible, sampling size has been increased in the next reproductive cycles to compensate for the lower number of data recorded during the lockdown. In any case, there is a delay in the analysis of certain experiments that have made it necessary to request an extension of the project.

3.1 Dairy sheep

Table A: number of records collected per partner for Dairy Sheep, the number of distinct individuals is given in brackets.

Group of traits	Trait	France- INRAE	Spain - UNILEON	Greece - AUTH	France Races de France
Breeds in DoA – Table 1	all	Lacaune (divergent lines)	Assaf (divergent lines)	Lacaune, Assaf, Frizarta, Chios	Lacaune, Manech Tête Rousse, Basco-Béarnaise
Numbers of individuals in DoA – Table 1	all	700 phenotyped and genotyped	48 phenotyped and genotyped	48 phenotyped and genotyped	20,000 phenotyped and 10,000 genotyped
Breeds in D1.1	all	Lacaune (divergent lines)	Assaf (divergent lines)	Lacaune, Assaf, Frizarta, Chios	Lacaune, Manech Tête Rousse, Manech Tête Noire and Basco-Béarnaise
Zootechnical	Body weights	177 (54)	80 (40)	300 (30 ewes; 8 Chios, 8 Frizarta, 7 Lacaune, 7 Assaf)	
Zootechnical	Muscle Depth (ultrasound)			300 (30 ewes; 8 Chios, 8	

Group of traits	Trait	France- INRAE	Spain - UNILEON	Greece - AUTH	France Races de France
				Frizarta, 7 Lacaune, 7 Assaf)	
Zootechnical	Backfat thickness (ultrasound)			300 (30 ewes; 8 Chios, 8 Frizarta, 7 Lacaune, 7 Assaf)	
Zootechnical	Milk yield	918 (795)	40 (40)	190 (38 Chios ewes)	10,870 (5,014: 4,105 Lacaune ewes, 355 Manech Tete Rousse, 229 Manech Tete Noire and 325 Basco-Bearnaise)
	Milk composition	918 (795)	40 (40)	190 (38 Chios ewes)	10,870 (5,014)
Zootechnical	Body Condition Score	177 (54)		490 (68 ewes: 46 Chios, 8 Frizarta, 7 Lacaune, 7 Assaf)	18,907 (5,064)
Zootechnical	Morphological traits				
Zootechnical	Average Daily Feed intake	144 (48)	40		14,931 (from pen intake) (3,577)
Ruminal microbiota	16S sequencing	918 (795)			/
Ruminal microbiota	18S sequencing				/
NMR	ruminal fluid	174 (54)			
NMR	plasma	174 (54)	40		
NMR	milk				
Metabolites	β-HB, NEFA, total proteins, and hormones	174 (54)		300 (30 ewes; 8 Chios, 8 Frizarta, 7 Lacaune, 7 Assaf)	

Group of traits	Trait	France- INRAE	Spain - UNILEON	Greece - AUTH	France Races de France
Gas chromatography	ruminal volatile fatty acids		40		
NIRS	faecal NIRS				
	15N natural abundancies				
ruminal parameters	pH, Ammonia,		40		
transcriptomics	RNAseq in milk cells		28		
epigenetics	Whole-genome bisulfite sequencing		28		
Genotypes	Illumina 50K	54			

For UNILEON deviations: The rescaling of the experiment (i.e., from 48 in DoA to 40) was due to budget constraints. The number of animals was reduced for analytical processes, such as WGBS and RNA-seq, with high individual costs. In addition, Table 1 of the DoA describes the complete size of the population, but a selection of the animals sampled for the different markers (epigenetic marks, DGE, etc.) was necessary to comply with the approved budget. In all cases, despite the limited budgetary availability, the objective was to maintain an experimental design that allowed reasonable statistical power, limited by budgetary availabilities.

Regarding AUTH partner: Forty-eight ewes were proposed to be used in different experimental scenarios (Table 1 of DoA) with a relative low risk regarding their implementation (section 1.3.5; WT5 Critical Implementation risks and mitigation actions). However, in section 3.4 (resources to be committed) and in particular 3.4.1 (Other direct costs) no budget was foreseen for either purchase of animals or feeds for partner AUTH that could facilitate the implementation of experiments as initially planned. Therefore, Partner AUTH had to improvise mitigation strategies and use its own resources to cater the needs of planned experiments to address the anticipated objectives. Hence, at the time of the commencement of the first experiment AUTH had available only 30 ewes to be used. Moreover, following the discussions between partners involved in WP1 during the first annual meeting in Edinburgh, AUTH proposed to undertake an extra experiment, not foreseen in the DoA, that would explore the possibility of identifying an easy and non-invasive indicator of feed efficiency. Specifically, the potential use of milk composition traits as predictors of feed efficiency in Chios dairy ewes was investigated. It should be noted here that the proposed experiment was done using existing animal and feed resources of partner AUTH since as stated in the DoA (section 3.4 resources to be committed, and in particular 3.4.1 Other direct costs) no budget was foreseen for either purchase of animals or feeds for partner AUTH. Feed efficiency phenotypes (feed intake and milk yield) were collected and analysed including milk composition traits exclusively to address the objectives set in SMARTER project.

3.2 Dairy Goats

Table B: number of records collected per partner for Dairy Goats, the number of distinct individuals is given in brackets.

Group of traits	Trait	France-INRAE	France – Capgènes	UK-SRUC
Breeds in DoA – Table 1	all	Alpine (divergent line)	Alpine and Saanen	Yorkshire composite
Numbers of individuals in DoA – Table 1	all	180 phenotyped and 180 genotyped	10,000 phenotyped and 1,500 genotyped	25,000 phenotyped and 13,000 genotyped
Breeds in D1.1	all	Alpine divergent line	Alpine and Saanen	Yorkshire composite
Zootechnical	Body weights			9,970 (1,146)
Zootechnical	Muscle Depth (ultrasound)			
Zootechnical	Backfat thickness (ultrasound)			
	Milk yield		30,117 (6,124: 2,582 Alpine 3,180 Saanen and 111 from other breeds)	9,970 (1,146)
	Milk composition		30,117 (6,124)	
Zootechnical	Body Condition Score	(199)		
Morphological traits	chest width		3,044 (3,044: 1,355 Alpine and 1,679 Saanen)	
Zootechnical	Average Daily Feed intake	(143)	29,437 (6,124: 2,582 Alpine and 3,180 Saanen, 111 other breeds)	9,970 (1,146)
Ruminal microbiota	16S sequencing			
Ruminal microbiota	18S sequencing			
NMR	ruminal fluid			
NMR	plasma			
Metabolites	β-HB,		2,080	

Group of traits	Trait	France-INRAE	France – Capgènes	UK-SRUC
			(534)	
Gas chromatography	ruminal volatile fatty acids			
NIRS	faecal NIRS			
	15N natural abundancies			
GHG emissions				
Genotypes	Illumina 50K	199	883	

Regarding INRAE deviations: A total of 199 dairy goats belonging to the high and low functional lines were produced with phenotyping of longevity. Out of the 199 dairy goats created, 80 were expected to be monitored for feed efficiency. However, we achieved to monitor 143 goats for feed efficiency in 3 years, with the useful phenotypes being: individual intake of concentrates, weight and milk production in order to analyse residual feed intake (RFI). This data will be analysed in the next periods.

3.3 Meat Sheep

Table C: number of records collected per partner for Meat Sheep, the number of distinct individuals is given in brackets. *: without samples from 2021 experiment.

Group of traits	Trait	France-INRAE	France – Races de France	INIA - Uruguay	NSG
Breeds in DoA – Table 1	all	Romane divergent lines	Vendéen, Rouge de l'Ouest, BMC, Charollais	Corriedale, Merino	Norwegian White Sheep
Numbers of individuals in DoA – Table 1	all	2,350 phenotyped and 1,000 genotyped	15,000 phenotyped and 50 genotyped	85,000 phenotyped and 220 genotyped	3,000 phenotyped
Breeds in D1.1	all	Romane (divergent line on RFI)	Vendéen, Rouge de l'Ouest, BMC	Corriedale, Merino and Dohne	Norwegian White Sheep
Zootechnical	Body weights	2,662 (616)	20,671 (13,919: 1,534 ewes + 2,798 lambs in Mouton Vendéen, 1,198 ewes + 56 lambs in Rouge de l'Ouest and 2,579 ewes + 5,754 lambs in BMC)	86,994 (1,611)	6,000 (6,000)
Zootechnical	Muscle Depth (ultrasound)	1,064 (616)	20,671 (5,311: 1,534 ewes in	1,611 (1,611)	

Group of traits	Trait	France-INRAE	France – Races de France	INIA - Uruguay	NSG
			Mouton Vendéen, 1,198 ewes in Rouge de l'Ouest and 2,579 ewes in BMC)		
Zootechnical	Backfat thickness (ultrasound)	1,064 (616)	20,671 (5,311)	1,611 (1,611)	
Zootechnical	Body Condition Score		20,671 (5,311)	1,611 (1,611)	
Zootechnical	Morphological traits		5,311 (5,311)	/	
Zootechnical	Average Daily Feed intake	783 (616)	/	1,611 (1,611)	
Ruminal microbiota	16S sequencing	581 (346)	/		
Ruminal microbiota	18S sequencing	387 (346)	/		
NMR	ruminal fluid	581 (346)	/		
NMR	plasma	581 (346)	/		
Gas chromatography	ruminal volatile fatty acids	581 (346)	/		
NIRS	faecal NIRS	430 (268)	/		
	15N natural abundancies	142 (71)	/		
GHG emissions			/	3,012 (1,506)	6,000 (6,000)
Genotypes	Illumina 50K	430	/	1,244	

For Races de France partner, 1,500 animals from 3 breeds were proposed, which numbers have been reached in Blanche du Massif Central (n=2,579 ewes + 5,754 lambs) and Mouton Vendéen (n=1,534 ewes + 2,798 lambs) and close to be reached in Rouge de l'Ouest (n=1,198 ewes + 56 lambs). Numbers were lower than expected in the Rouge de l'Ouest breed because of the COVID-19 crisis.

For NSG partner: 3,000 individuals were to be phenotyped for GHG with PAC. The aim was to measure them twice a fortnight apart. Measurements were however done in commercial flocks and not on a research farm, and returning to the same farm gathering the exact same animals two weeks after the first measurement was just not feasible practically. We therefore decided to measure 6,000 animals once instead of 3,000 animals twice yielding the same number of measurements.

4 Main results

From the Tables A, B and C datasets, different traits and biological samples have been analysed, with different statistical methodologies. Technical details about the experiments, data collection and methods are detailed in parts 5, 6 and 7 for dairy sheep, dairy goats and meat sheep respectively.

In Table D, we give the main results that were obtained.

Table D: Description of proxies studied in dairy sheep, dairy goats and meat sheep, and results about their potential to predict feed intake and/or fed efficiency.

Group of traits	Proxy for feed efficiency, recorded at the individual level	Evaluated in :			Importance and potential use as large scale measurable proxy
		dairy sheep	dairy goat	meat sheep	
Zootechnical (routinely recorded traits)	Body weights	X	X	X	Involved in RFI calculation (through metabolic weight and average daily gain) and can be considered as part of the proxies of feed intake and, to a less extent, of feed efficiency
	Muscle Depth and Backfat Thickness (ultrasound)	X		X	Can be used in the RFI calculation but its importance is low
	Milk yield	X	X		Involved in feed efficiency criteria (ratio and RFI) and can be considered as part of the proxies of feed efficiency
	Milk fat and protein contents	X	X		Involved in feed efficiency criteria (through energy corrected milk)
	Body Condition Score	X	X	X	Trajectory of BCS are correlated with feed efficiency ratios in dairy sheep
	Wool traits (Greasy Fleece Weight, Clean Fleece Weight, average Fibre Diameter, Coefficient of Variation of Fibre Diameter, and Staple Length)			X	Can be used in the RFI calculation but its importance is low
Zootechnical (not routinely recorded traits)	greenhouse gas (CH_4 and CO_2) emissions and O_2 consumption			X	Can be used along with body weights and growth as proxies for feed intake, but gas emissions recording is currently not doable on a large scale. New technnologies are required to record gas emissions on a large scale.
	Morphological traits (chest width, chest depth, shoulder height)	X	X	X	Chest width might be considered as a proxy for body weight
	Average Daily Feed intake	X	X	X	Mandatory trait to estimate all feed efficiency criteria, but not recordable on a large-scale. We are looking for proxies for this trait.
Milk traits (not routinely recorded)	metabolomics (NMR)	X			Milk fatty acids are proxies for feed efficiency traits (FCR and RFI). Milk fat and lactose contents are indicators of a more efficient use of energy intake. Milk metabolomics is indicator of the level of feed efficiency.
	milk fatty acids composition (gas chromatography)	X			
	milk protein, lactose	X			
	fine composition (MIR spectra)	X			
Blood traits (not routinely recorded traits)	plasma metabolomics (NMR)	X		X	Citrate, malate and amino acids can be considered as proxies for feed efficiency, but NMR is not doable routinely. New technnologies are required to record these metabolites on a large scale.
	targeted metabolites (β-HB)	X	X		These targeted metabolites still need to be analysed as potential proxies for feed efficiency.
	NEFA, total proteins, and hormones	X			
	plasmatic ^{15}N natural abundancies			X	^{15}N natural abundancies is a good proxy for FCR under a forage-based diet, but this trait is currently too expensive to be recordable on a large-scale
faecal traits	NIRS			X	Can be proposed as a proxy of feed intake or feed efficiency in integrative models.
Ruminal traits	pH, Ammonia,	X			These traits are recorded in order to understand the biology underlying feed efficiency. They can not be considered as proxies for feed efficiency because the sampling is not doable routinely and because of low accuracies of predictions.
	microbiota (16s and/ or 18S sequencing)	X		X	
	metabolomics (NMR)	X		X	
	volatile fatty acids (gas chromatography)	X		X	
Milk molecular traits (not routinely recorded)	RNAseq in milk cells	X			These traits are recorded in order to understand the biology underlying feed efficiency. They can not be considered as proxies for feed efficiency because the sampling is not doable routinely.
	Whole-genome bisulfite sequencing of milk cells	X			

Our objective is to identify proxies of feed efficiency indicators such as RFI and FCR. An alternative to the prediction of feed efficiency traits might be the prediction of one of the key components of FE, e.g. feed intake itself. In many dairy sheep and dairy goat experiments, only concentrate intake was recorded, with little (or no) knowledge of forage intakes. However, when considering average feed intake values in dairy sheep, we observed an important re-ranking of feed efficiency estimates. Therefore, feed intake is one of the most important factors to consider in feed efficiency traits calculations, along with milk production. We are therefore aiming at proposing proxies for feed efficiency traits, but also for feed intake.

Milk composition related traits are expected to be relevant to predict feed efficiency traits in lactating ewes: milk fat content as well as lactose content has been proposed as a good indicator of energy intake in lactating ewes, and milk fatty acids composition has been found to be associated with feed efficiency traits. Moreover, the prediction of feed efficiency traits from milk fatty acids composition reached good performances. **Body condition scores** have been the most recorded trait in dairy sheep and goats. No significant link has been found between time-point BCS and feed efficiency traits, but individuals with different body condition score dynamics along the lactation had different feed efficiency levels. In non-lactating ewes, variations in body composition traits have also been proposed as relevant proxies for negative energy balance status.

We report here the first studies of feed efficiency along the lactation stages in dairy goats. No proxies of feed efficiency neither of feed intake have already been proposed. Still, analyses of recorded traits such as beta-hydroxybutyrate in combination with classical recorded traits during lactation will be performed soon. Moreover, in dairy goats, it will be interesting to test the ability to predict feed efficiency from milk fatty acids composition, considering promising results obtained in dairy sheep.
The largest number of feed intake records and thus of feed efficiency estimates were obtained in meat sheep. Usually, feed intake control periods are performed on a 42-days basis. However, we demonstrated that shortening the period by a week has no impact on RFI estimates. Moreover, RFI can be calculated with the most parsimonious model that just considers feed intake, body weight, and average daily gain. Wool traits and body composition traits never reach significance in the models. Regarding proxies of feed intake, **the combination of greenhouse gas emissions with body weights and average daily gain** was found to be highly performant in the prediction of animals with extremely high or low feed intake levels. Multivariate analyses considering fixed effects and body weight also reached good levels of accuracy for the prediction of feed intake. The analyses of fine phenotypes (mainly omics data) showed that, when considered separately, ruminal variables seem to be less promising than **plasmatic variables** (citrate and amino acids mainly) to predict feed efficiency. The multi-omics integration of these datasets will be performed to highlight potential combinations of variables predicting feed intake or feed efficiency.
Finally, it should be noticed that ruminal variables (metabolites, microbiota) did not help in predicting feed intake nor feed efficiency. Prediction accuracies were higher for feed intake than for feed efficiency traits.

Future genetic analyses of the proposed proxies will be performed, and particular attention will be paid to genetic correlation with feed intake-related traits, including feed efficiency traits.

5 Dairy Sheep

5.1 Materials and Methods

5.1.1 Experimental designs

1. France (INRAE)

Two experimental designs were interconnected in the INRAE La Fage experimental farm: A- a large set of adult Lacaune ewes (n=795) belonging to MicroGenOL programme; B- a subset of 54 ewes with in-depth phenotyping during 3 successive lactations (feed intake recorded within the framework of the iSAGE programme).

A- From 2015 to 2019, 700 adult ewes belonging to 2 divergent lines (on Somatic Cell Score or on Milk Persistency) and 95 ewes from different genotypes at the SOCS2 mutation were genotyped and have their rumen microbiota quantified while indoor rearing. Animals were fed with 93% meadow hay and silage plus 7% of concentrate. On the one hand, two groups of ewes with extreme EBVs were created according to the log-transformed somatic cell count (SCC): difference between high-SCS line (SCS+) and low-SCS line (SCS-) reached 3.6 genetic standard deviation. On the second hand, two extreme groups of ewes were generated, one with high persistence (PERS+) and one with low persistence (PERS-) in the milk production curve; difference between these PERS lines reached 2.1 genetic standard deviation.

Blood was aliquoted for DNA extraction to perform:
- Genotyping (Illumina 54K SNP ovine chip).

Rumen fluid was aliquoted to perform:
- Microbiota analysis through the sequencing of V3-V4 regions of the 16S-rRNA gene.

At the nearest milk recording control, the fine milk composition was predicted from MIR spectra of milk.

- **SNP control** was done by defining a minimum call rate for individuals and loci and minor allele frequencies for the marker.
- **Microbiota analyses**: microbiota analyses were performed on all ewes phenotyped for a total of 795 animals. All sequences were analysed with the FROGS pipeline in the same batch. Microbiota counts obtained from FROGS were transformed in Centered Log Ratios (CLR) after the imputation of zeros by applying the Geometric Bayesian Method (GBM) (Martinez-Boggio et al., 2021) to consider their compositional nature. The lines effects (SCS and PERS) were estimated (Martinez-Boggio et al., 2021) and the work is in progress to evaluate the genetic links between rumen microbiota and milk compositions, and their microbiabilities.

B- Within the framework of iSAGE H2020 project, 54 Lacaune dairy ewes belonging to divergent lines for milk persistency only (PERS lines, with a genetic divergence of 2 s.d. on average), have been recorded for their bodyweight and the changes in their body reserves. Plasma measurements were performed for some metabolites and hormones monitored for two months at the beginning of lactation for three lactations (Lactation 1 to Lactation 3 from 2017 to 2019). Milk production, fat and protein contents, somatic cell count, and MIR spectra were collected at each official milk recoding control every three weeks. For half of these ewes, individual feed intake was also recorded to calculate individual energy and protein balance estimates.

The main iSAGE results show that the energy and nitrogen balances are different from one lactation to the next one: the ewes in 2nd lactation have these two balances the lowest on average. Despite the large inter-individual variability, the PERS+ line differs from the PERS- in terms of energy balance and plasmatic beta-hydroxybutyrate levels, but with no difference in nitrogen balance.

The SMARTER programme aims to deepen these results by adding new and finer phenotypes, such as quantifying rumen microbiota and metabolites (with no a priori) either in the rumen or blood. The objective was to identify other metabolites linked to variations in body reserves in dairy ewes and to make links between rumen microbiota composition and metabolites in the rumen and plasma. In parallel, we would like to characterize more precisely the fine metabolic response to selection on milk persistency and identify, if it exists, a "typical metabolism" for ewes that manage their energy deficit better. So each year, samples of blood and rumen fluid were collected when energy balances were the most contrasted (according to iSAGE results).

Blood was aliquoted to perform:

- Genotyping (Illumina 54K SNP ovine chip).
- Plasma is stored (after 10 minutes of centrifugation at 2400g) to perform metabolomics by NMR.

Rumen fluid was aliquoted to perform:

- Metabolomics by NMR
- Microbiota analysis through the sequencing of V3-V4 regions of the 16S-rRNA gene.

SNP genotyping was performed by Labogena company (Jouy-en-Josas, France) on Illumina 54K SNP ovine chips within the framework of MicroGenOL programme.

Laboratory analyses: protocols used to obtain NMR spectra from plasma and rumen juice and microbiota sequences are detailed in Appendix 1.

- **SNP control** was done by defining a minimum call rate for individuals and loci and minor allele frequencies for the marker.

- **Microbiota analyses**: microbiota analyses were performed on ewes phenotyped in 2017, 2018, and 2019. A total of 177 ruminal samples were collected and sequenced with RNA 16S. Samples collected in 2018 belonged to a previous program -named MicroGenOL- were sequenced in one run, and samples from 2017 and 2019 were sequenced in a second run. Among these samples, six ewes were only sequenced in their first lactation in 2017, 9 ewes on the two first lactations, and 51 ewes were phenotyped each of the 3 years. All sequences were analysed with the FROGS pipeline in the same batch.

Microbiota counts obtained from FROGS were analysed as previously detailed. Focusing on the 153 samples (51 ewes with the 3 years of samples) and after identifying the main fixed effects, the work evaluates the possible line effect and the repeatability of OTUs according to years.

- **Metabolomics analyses**: NMR analyses were performed on 174 blood samples and 174 rumen juice samples corresponding to 52 ewes with 3 years of sampling and 9 ewes with only 2 years of sampling. Analysis of these raw spectra with the ASICS (version 2.5.3) R package (Lefort et al., 2019) was done to quantify the abundances of main metabolomes.

2. Spain (UNILÉON-CISC)

All experimental procedures were approved (CSIC Approval No. OH-726-2018 / IGM Approval No. 100102/2018-1) by the Research Ethics Committee of the *Instituto de Ganadería de Montaña*, the Spanish National Research Council (CSIC), and the *Junta de Castilla y León* (Spain), following procedures described in Spanish and European Union legislation (R. D. 53/2013 and Council Directive 2010/63/EU).

Experimental population

The main objective of the UNILEON/CSIC experiment is to know if a nutritional challenge in the allometric stage of lamb growth influences the animal's feed efficiency during the milk production period.

In order to constitute a population of 40 adult pregnant ewes with synchronized lambing in the same period, a total of 76 ewe lambs from the Assaf breed were selected. The primary selection criterion was to choose contemporary animals (same lambing group), with similar lambs for both tails of the distribution of EBVs for milk production. Since lambs were purchased when they were only 1 month old, the average parent EBV provided by the breeders' association (ASSAFE) was used to estimate their EBV. Thus, 76 female lambs were selected (38 with high EBV and 38 with low EBV) and transferred to the experimental farm of the Instituto de Ganadería de Montaña (CSIC-UNILEON) at 2.5 months of age. Once they passed the veterinary control and adapted to the new facilities, they were separated into the Control group (C) and Nutritional Challenge group (NC), with a uniform distribution of EBV for MY in both groups. The lambs in the C group received the same diet as during the first month (standard feed for growth lambs), whereas the animals in the NC group received a diet with a 42.3% protein restriction compared with the C group. The nutritional challenge lasted approximately two months, between 3.5 and 5.5 months of age of the animals. Then, females were mated at around ten months of age (AI), and parturition occurred five months later. A schedule of the above is shown in Figure 1. During the lactation, different measurement periods were carried out as described below.

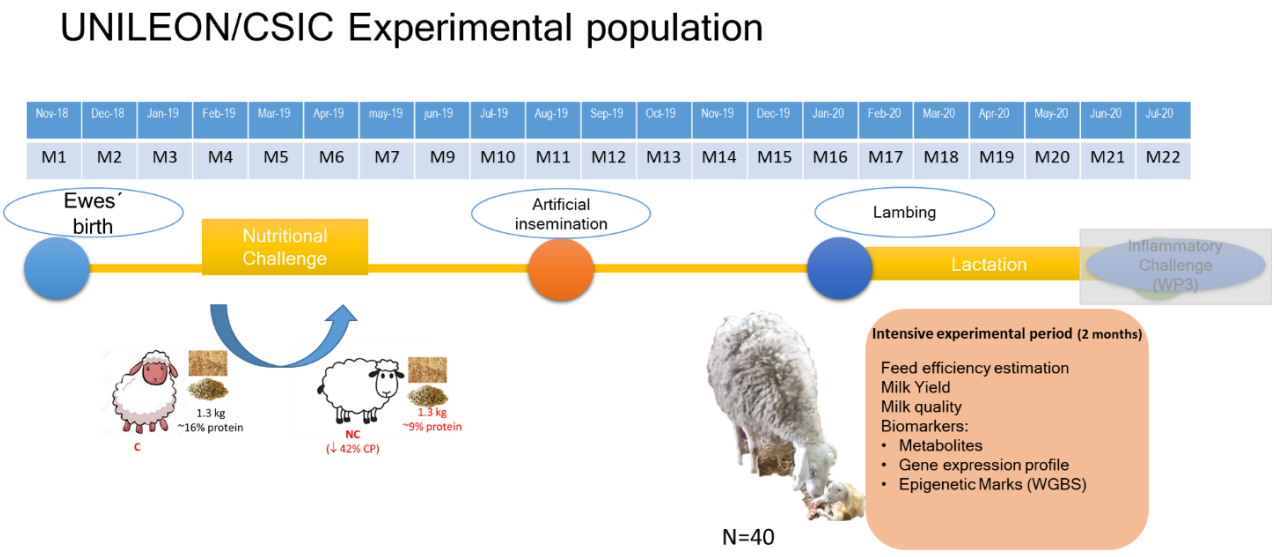


Figure 1*. Schematic representation of the experiment developed at UNILEON-CSIC. The upper timeline is divided into months of the experiment.*

Of the 76 animals initially purchased, six did not reach mating age, and 21 ewe-lambs did not become pregnant with AI flock and therefore could not be included in the intensive sampling period as they lambed one month after the main group. Finally, 49 animals were available for the intensive sampling period. As the economic readjustment allowed us to sample 40 animals, 20 nutritionally challenged (NC), and 20 controls (C) ewes were chosen. The EBV distribution for milk production allows us to make two groups: high EBV (H_EBV) with an average EBV of 86.5 ± 3.4, and low EBV with an average EBV of 37.4±3.5. The average EBV in Controls and NC was 58.7 ± 7.1 and 62.6 ± 6.1, respectively. A description of the basic statistics of the EVBs is provided in Appendix 1

Management in the intensive sampling period for Feed efficiency in adult ewes

Forty primiparous lactating Assaf ewes (BW = 63.6 ± 1.23 kg; DIM = 33 ± 1.3 at the beginning of the experiment; MY = 2.40 ± 0.098 kg/d and VF_150 62.1 ± 4.57) were used in this assay. Estrus had been synchronized and lambing concentrated in a few days to avoid differences due to the lactation stage.

Sheep were housed in individual tie stalls and fed ad libitum a TMR formulated from alfalfa hay (particle size > 4 cm) and concentrate (50:50) that they had been consuming since lambing. The diet included molasses to reduce the selection of ingredients, together with chemical composition, as shown in Table 1. Ewes were milked twice daily at approximately 08:30 and 18:30 h in a single-side milking parlor with 10 stalls (DeLaval, Madrid, Spain).

Table 1. *Formulation of the experimental diet (g/kg of fresh matter)*

	TMR
Dehydrated alfalfa hay (particle size >4 cm)	500
Whole corn grain	140
Whole barley grain	100
Soybean meal solvent 44% CP	150
Sugar beet pulp (pellets)	50
Molasses (liquid)	40
Vitamin-mineral supplement1	20

[1]*MACROFAC Rumiantes* (UP911755130; DSM Nutritional Products S.A., Madrid, Spain). Declared as containing: Ca (285 g/kg), Na (7.5 g/kg), Fe (3 g/kg), Mn (3 g/kg), Zn (2 g/kg), Mg (1 g/kg), P (910 mg/kg), Mo (100 mg/kg), Co (67 mg/kg), I (50 mg/kg), S (40 mg/kg), Se (7 mg/kg), vitamin A (200,000 IU/kg), vitamin D3 (40,000 IU/kg), vitamin E (667 mg/kg), ethoxyquin (12 mg/kg) and propyl gallate (2 mg/kg).

Measurements and Sampling Procedures

Diet. Feed intake was individual and daily measured over four weeks by weighting the diet dry matter (DM) offered and refused by each animal. Representative samples of the diet were collected (n = 3), stored at –30°C, and freeze-dried before chemical analysis.

Milk. Total milk produced by each animal, at morning and evening milkings, was also collected and weighed individually and daily for 4 weeks (5-8 of lactation) to calculate milk yield. Composite samples of the milk produced by each ewe were prepared daily according to individual yields in both milkings. One aliquot of that composite milk was preserved with bronopol (D&F Control Systems Inc., San Ramon, CA, USA) and stored at 4°C until analysis for fat, protein, and lactose concentrations by infrared spectrophotometry. Furthermore, on weeks 2, 3, and 4 of the experiment, aliquots of

composite milk (n = 4) from each ewe were collected and stored without preservative at −30ºC for FA analysis.

Furthermore, two milk samples per animal were collected for RNA extraction from milk somatic cells (MSC) on weeks 6-8 of lactation.

Body weight. The BW of each sheep was recorded at the beginning and the end of the 4-week period.

Rumen liquor. Rumen fluid samples from each sheep were collected to analyze fermentation parameters (pH, ammonia, lactate, and volatile fatty acids) and fatty acids (FA) profiles. The diet was offered, as usual, after the morning milking, but only for 1 h. Afterward, feed and water were removed and 3 h later, ewes were sampled using an oral stomach probe (Ramos-Morales et al., 2014).

Plasma samples. Blood samples were collected for extraction of plasma on week 6 of lactation for later metabolomic analyses. Appropriated collection tubes with lithium heparin were used. After collection, blood samples were homogenized and centrifuged at 2500 rpm for 12 min. Then, plasma samples were frozen at −80°C.

Estimation of Feed Efficiency

To estimate the feed efficiency, residual feed intake (RFI) was calculated as the residuals of the following regression model using the GLM procedure of the SAS software package (version 9.4; SAS Institute Inc., Cary, NC, USA):

$$DMI = \mu + a \times ECM + b \times MBW + c \times \Delta BW_{BW} + RFI$$

where DMI represents the mean DM intake over the period (kg/day); μ is the intercept; ECM is the mean energy-corrected milk (kg/day); MBW is the mean metabolic body weight ($BW^{0.75}$; kg); ΔBW_{BW} is body weight change over the period adjusted for mean body weight (ΔBW × BW); RFI is the residuals; and *a*, *b*, and *c* are the regression coefficients. All variables included in the model were statistically significant.

The same procedure was used to estimate the residual energy intake (REI; Fischer et al., 2018) as the residuals of the following regression model:

$$NEI = \mu + a \times NE_L + b \times MBW + c \times \Delta BW_{BW} + REI$$

where NEI represents the mean net energy intake over the period (MJ/day); μ is the intercept; NE_L is the mean net energy requirements for lactation (MJ of net energy/day); MBW is the mean metabolic body weight ($BW^{0.75}$; kg); ΔBW_{BW} is body weight change over the period adjusted for mean body weight (ΔBW × BW); REI is the residuals; and *a*, *b*, and *c* are the regression coefficients.

Milk yield and milk composition data were used to estimate energy-corrected milk [ECM = kg/d of milk yield × [(0.0071 × g/kg of milk fat) + (0.0043 × g/kg of milk protein) + 0.2224], and net energy requirements for lactation (NE_L = 0.686 × ECM, and expressed as MJ of net energy/day), according to INRA (2018) equations for sheep.

In addition, the feed conversion ratio (FCR) was calculated as the relationship between mean DMI and ECM over the period.

Laboratory Analyses and bioinformatics pipelines: see appendix 1

Statistical analyses:
RNA-seq analyses

Whole-transcriptome analysis of milk somatic cell transcriptome was performed in animals with different feed efficiency. This experiment aims to contribute to WP1 in two aspects. First, it identifies differentially expressed genes in animals diverging in feed efficiency that could be used as biomarkers. Second, it contributes to moving forward the knowledge of the genetic basis of feed efficiency.

Data are analyzed to identify differentially expressed genes (DEGs) following a bioinformatic pipeline previously applied by our research group, described by Suarez-Vega et al. (2017). Additional enrichment analyses will help to identify the biological pathways and gene networks related to ovine sheep efficiency.
The DESeq2 package (Love et al., 2014), commonly used in differential expression studies, was used to focus on the differential analyses of low vs. high RFI animals.

To perform the differential expression analyses with DESeq2, we fitted two different models. The first one was Y=nutritional challenge+RFI, in which Y is the gene expression counts, Nutritional challenge classifies the animals in controls and NC, and the RFI variable classifies the animals in high- low-FE. The second model was fitted assuming an interaction between the nutritional challenge and the RFI (Y=nutritional challenge+RFI+ nutritional challenge:RFI). Differentially expressed genes were considered at a false discovery rate (FDR) < 5%.
The Weighted Gene Co-expression Network Analysis (WGCNA) (Langfelder and Horvath (2008) R package was used to build co-expression networks and identify groups of highly co-expressed genes. Individual analyses were conducted on each breed group (High and Low RFI, and C and NC animals). In order to evaluate the performance of transcriptomic data to predict FE, predictive models were built for RFI and FCR. As predictive features, we used gene expression values from two gene lists: (1) DEGs resulting from the contrast between consensus ewes for both indexes (1,017 genes; consensus-DEGs); (2) genes found in common between consensus-DEGs and a previous study evaluating FE in dairy Assaf ewes (45 genes; reduced-consensus-DEGs) (Suarez-Vega et al., 2023; Suarez-Vega et al., in preparation). Machine learning (ML) models were built based on Random Forest (RF) using the R package h2o (LeDell et al., 2020). A random discrete search composed by 5 folds cross-validation was performed during the hyperparameter tuning stage. In total, 100 models were built for each ML algorithm (2 traits x 2 lists of DEGs) through the random assignment of the transcriptomic data of 28 ewes, specifically we used 2/3 of the total dataset as train data (20 animals) and 1/3 as test data (8 animals) samples. For the 100 iterations of random assignments, none of the train and test sets generated were completely equal. For each model, we calculated the root mean squared error (RMSE) and the Spearman correlation (rho2) between the predicted and real values of RFI and FCR in the training stage.

Association of milk fatty acids with feed efficiency in Assaf sheep during lactation
After examining milk samples of the 40 lactating ewes by gas chromatography, a fatty acid (FA) profile was obtained for each animal. The constant sum of the concentrations of these FA suggests the common constrain that suffers data referred to as compositional data (Gloor et al., 2016). Therefore, FA measurements were transformed via centered log-ratio (CLR) for correcting sparse compositionality (Aitchison, 1982).
Following, to assess whether the milk FA composition significantly contributes to variation of the feed conversion ratio (FCR) and the residual feed intake (RFI), described above, we applied three quantitative analyses. We estimated the Spearman correlation and regression coefficient of each FA analysed with both feed efficiency parameters (FCR and RFI) to characterize the association level of the Milk FA CLR-transformed parameters with the feed efficiency indexes. Finally, we carried out an orthogonal partial least squares (OPLS) analysis and an orthogonal partial least squares discriminant analysis (OPLS-DA) over the FA CLR-transformed data using the R package ROPLS (Thévenot et al., 2015). These last analyses aimed to identify those FA useful in the prediction of feed efficiency indexes.

Association of milk metabolomics features with feed efficiency in Assaf ewes.

After milk processing, reversed-phase liquid chromatography-mass spectrometry (LC-MS) was performed to detect features ranging from polar to less polar compounds and lipids. Four high-resolution LC–MS datasets were obtained, and each dataset was processed using the R package XCMS (Smith et al., 2006). The processing steps(see Appendix 1 for a detailed description) included peak detection, peak grouping, peak alignments, and retention time shift correction. The resulting dataset included pairs of MS m/z and LC retention time (RT, min) for the detected compound features across all the samples within each dataset. This procedure revealed 3760 feature signals. Those for which less than 75% of the data were available from the ewes were filtered out, resulting in a final dataset composed of 3749 features. The ewes were classified into high, medium, and low FE groups for RFI (H-RFI, M-RFI, L-RFI) and FCR (H-FCR, M-FCR, L-FCR) based on the distribution of each metric. The mean of each group was compared using a t-test with R v.4.2.0, where the significant differences between groups were defined based on a p-value <0.05. Additionally, the mean DMI was compared between each group for RFI and FCR using the same approach. All p values were adjusted to a false discovery rate (FDR) threshold of 0.05. The R package mixOmics (Rohart et al., 2017) was used to perform a partial least squares discriminant analysis (PLS-DA) to evaluate the potential of selected features to discriminate the high, medium, and low FE groups for both RFI and FCR. The area under the curve (AUC) was calculated to estimate the discrimination potential of PLS-DA among the three FE groups. The first two principal components were plotted with centroids to define the groups identified by the PLS-DA using the plotIndiv function in the mixOmics package. Additionally, the performance of PLS-DA for the RFI and FCR groups was evaluated based on the mean error rate and Q2 for the first five principal components through cross-validation u lines 180-27sing 10 folds. Once the ewes were assigned to each group, the variable importance in the projection (VIP) was estimated to compute the influence on the features of every predictor in the PLS-DA model. Features with a VIP>2 were selected individually from the outputs of the discriminant analysis for RFI and FCR.

For the prediction of individual RFI and FCR using milk metabolomics features and ML algorithms, predictive models were built using the CLR-transformed values for each of all 3749 detected features (RFI_all and FCR_all) and for the subsets of features selected in the PLS-DA analysis for RFI and FCR with VIP>2 (RFI_VIP and FCR_VIP). The ML models were built based on a multilayer feedforward artificial neural network (deep) and random forest (RF) using the R package h2o (LeDell, et al., 2020), extreme gradient boosting (xgboost) using the R package xgboost (Chen and Guestrin, 2016), and support vector machine (SVM) using the caret R package (Kuhn, 2008). For the deep and RF algorithms, a random-discrete search composed of 5-fold cross-validation was performed during the hyperparameter tuning stage. The hyperparameter tuning stage for SVM and XGBoost was performed using an extensive search. The tuning stage for xgboost used 1,000 as maximum boosting interactions and 10 interactions as early stopping (stop if there is no improvement for 10 consecutive trees). SVM hyperparameter tuning was performed using the caret package with the “repeatedcv” method with 10 folds and 5 repeats. The grids explored in each ML algorithm are presented in Supplemental Table 1 (https://zenodo.org/record/8154651).

Performance of DMRs in milk somatic cells to predict feed efficiency in Assaf sheep

In the Assaf experimental population, DNA methylated marks in the genome of milk somatic cells were identified by whole genome bisulphite sequencing (see Appendix 1 for details). Differentially methylated regions (DMRs) were identified in divergent animals for residual feed intake using RFI (RFI, 7 H-RFI, and 7 L-RFI), FCR (FCR, 7 H-FCR, and 7 L-FCR), and a consensus between both metrics (Cons, 6 H-Cons and 6 L-Cons). These DMRs were used to assess the accuracy of prediction RFI and FCR using machine learning models. Predictive models were built using the mean methylation within each detected DMR as features. To estimate the predictive potential of these features DMRs identified in the

comparison between extreme RFI groups were used to predict RFI values (mRFI_RFI), DMRs identified in the comparison between extreme FCR groups were used to predict FCR values (mFCR_FCR), and DMRs identified in the comparison between extreme consensus groups were used to predict both RFI (mCons_RFI) and FCR (mCons_FCR) values. Additionally, the predictive performance of three machine learning (ML) models (xgboost, random forest (RF) and multi-layer feedforward artificial neural network (deeplearning)) was evaluated using the mean methylation level within the identified DMRs and genetic variants mapped within these regions. For the predictions, 100 steps of random-sampling of animals in the training and test sets were performed for each scenario. The average performance of each model was based on the root mean squared error (RMSE) and squared Spearman correlation (r^2).

3. Greece (Univ Thessaloniki)

First Greek experiment (AUTH)

The objective of the first experimental study was (i) to assess the potential use of body composition traits measured with ultrasonography as predictors of energy balance and (ii) to use blood biomarkers as predictors of fat and muscle reserves and their mobilization in dairy ewes.

Facilities, animals and study design

Forty-eight ewes were proposed to be used in different experimental scenarios covering metabolic, genetic and nutritional aspects (Table 1 of DoA) with a relative low risk regarding their implementation (section 1.3.5; WT5 Critical Implementation risks and mitigation actions). However, in section 3.4 (resources to be committed) and in particular 3.4.1 (Other direct costs) no budget was foreseen for either purchase of animals or feeds for partner AUTH that could facilitate the implementation of experiments as initially planned. Therefore, Partner AUTH had to improvise mitigation strategies and use its own resources to cater the needs of planned experiments to address the anticipated objectives. Hence, at the time of the commencement of the experiment AUTH had available only 30 ewes that were selected from four commercial flocks of Central Macedonia Region, Greece, and transferred to the Veterinary Faculty of Aristotle University of Thessaloniki farm facilities in Kolchiko Lagada. Selected ewes were purebred animals from four main dairy sheep breeds reared in Greece: Chios (n=8), Frizarta (n=8), Lacaune (n=7), and Assaf (n=7). The ewes (3-5 years of age) were clinically healthy, non-pregnant, non-lactating and in good body condition (BCS of 2.5-3.0).

Upon arrival, all ewes were clinically examined, individually weighted, body condition scored, and received an anthelmintic treatment. Initially, ewes were kept as one group in a wheat straw bedded pen and received a daily ration consisting of 0.3 kg Lucerne Hay, 0.2 kg wheat straw, and 0.5 kg of a pelleted concentrate mixture (corn grain 90%, soybean meal 8%, vitamins, and minerals 2%). Following this 3-week acclimatization period, ewes were allocated in four identical straw bedded pens based on their breed. Each pen was equipped with one feeder and two watering troughs that provided unlimited access to water throughout the day. Ewes were kept under the same feeding schedule for another week before the actual start of the experimental protocol with designated feeding treatments.

Ewes were subjected to specific feeding treatments to induce distinctive changes in body weight (BW) and BCS across the trial period (Figure 2). During the six weeks of the fattening period, ewes were offered a ration formulated to supply 160% and 150% of mean maintenance energy and protein requirements, respectively, at predicted dry matter intake within each breed group. At the end of the third week of the fattening period, the quantity of the ration was adjusted according to the new mean body weight in order to provide the same surplus amount of nutrients. Thereafter, ewes were fed the

ration they received during the acclimatization period for a two-week transition period. During the following four weeks of the experiment (fasting period), ewes were submitted to restricted feeding designed to supply 60% and 55% of mean maintenance energy and protein requirements, respectively, based on the BW at the onset of the fasting period.

Nutrient requirements were calculated using the INRA recommendations (UFL_main = $0.033 \times BW^{0.75}$; PDI _main = $2.5 \times BW^{0.75}$, INRA, 2007). The formulated rations were offered twice daily during the experiment (09:00 and 17:00). Nutrient requirements were adjusted according to changes of BW for the whole experimental period.

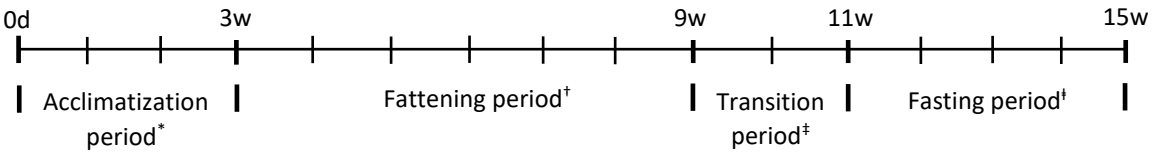


Figure 2. Experimental design.

*Acclimatization period: ewes were offered a daily ration consisting of 0.3 kg Lucerne Hay, 0.2 kg wheat straw and 0.5 kg of a pelleted concentrate mixture;

†Fattening period: ewes were offered a ration designed to supply 160% and 150% of mean maintenance energy and protein requirements, respectively;

‡Transition period: ewes were fed the ration they received during the acclimatization period;

‡Fasting period: ewes were offered a ration designed to supply 60% and 55% of mean maintenance energy and protein requirements, respectively.

Data collection

All animal measurements described below were performed at the end of each week during the fattening and fasting periods.

Live individual BW of ewes was measured before the morning feeding with a portable digital scale (accuracy 0.5 kg). BCS was assessed by palpation in the lumbar region by the same experienced evaluator using the 5-point scale (1-emaciated to 5-obese, in 0.25 point increments) suggested by Russel et al. (1969).

Backfat (BFT) and *longissimus dorsi* muscle (LDT) thickness were determined with ultrasonography. Each ewe was handled by two assistants, while the paralumbar area was shaved and cleaned with ethanol to move off debris. Measurements were performed with real-time B mode ultrasonographic equipment (Mindray 3300), using a 5.0 MHz, 55mm wide field of view linear transducer. Ultrasound gel was applied to the screening area to obtain adequate acoustic contact. The ultrasound probe was placed perpendicular to the vertebral column between the transverse processes of the 3rd and 4th lumbar vertebrae and slightly moved until a clear image was obtained. Images were frozen and immediately interpreted by the examiner. At each site, three measurements were performed at the same location in three consecutive scans, and the average value was recorded. All images were re-evaluated after the end of the experimental period. Inter- and intra-evaluation coefficient of variation was <3%. BFT measurements always included the skin layer. LDT was evaluated at the same site as the largest distance between the two muscular fasciae.

Finally, at the end of each week, a blood sample was collected from each ewe. Blood was collected from the jugular vein into 10 mL sterile glass vacuum tubes without anticoagulant (BD Vacutainer®, Plymouth, United Kingdom). After clotting, serum was obtained by low-speed centrifugation (2500 g × 15 min) and stored at -20°C pending analysis.

For each sample, concentrations of serum β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total proteins (TP), albumins (Alb), and urea nitrogen (BUN) were determined using an automated biochemical analyzer (Vitalab Flexor E, Vital Scientific N.V, The Netherlands), and commercially available kits (BHBA: Ben biochem. Enterprise, Milano, Italy; NEFA: Randox Laboratories Limited, UK; TP and ALB: Human, Wiesbaden, Germany; BUN: Thermo scientific, Middletown, VA, USA).

Data handling

The changes in BFT (ΔBFT), LDT (ΔLDT), and overall combined values of BFT and LDT (ΔTotal) were calculated by subtracting from each measurement the previous one in the same ewe. Moreover, negative energy balance status was defined as serum BHB ≥0.8 mmol/L or NEFA ≥0.7 mmol/L according to literature in dairy sheep (Panousis et al., 2018) and cows (Ospina et al., 2010; Moore and de Vries, 2020), respectively; due to lack of a research-based threshold for NEFA in dairy sheep, a stricter threshold set at ≥0.3 mmol/L was also defined.

Statistical analysis

The receiver operating characteristic (ROC) analysis was used to define thresholds for changes in body composition traits (ΔBFT, ΔLDT, and ΔTotal) to predict negative energy balance status (elevated BHB or NEFA status).

Mixed linear models were used to test the association of blood biomarkers (BHB, NEFA, TP, Alb and BUN) as potential predictors of fat and muscle reserves (BFT and LDT) and their mobilization (ΔBFT and ΔLDT). Specifically, the associations of BFT and ΔBFT with BHB and NEFA and of LDT and ΔLDT with each of the biochemical analytes were tested. Preliminary analyses were performed to identify significant effects on the studied traits. The effects of breed and period were tested; only the significant effect of the period was included in the final model:

$$Y_{ijn} = \mu + P_i + b1*BA + A_j + e_{ijn,}$$

Where:

Y_{ijn} = BFT or LDT or Δ_BFT or Δ_LDT;
μ = overall population mean;
P_j = fixed effect of period (two levels);
b1 = regression coefficient on each biochemical analyte (BA; BHB, NEFA, TP, Alb, BUN);
A_j = random effect of the animal (j=1-30 ewes);
e_{ijn} = random residual effect

All analyses were performed with IBM SPSS v.25 (Armonk, NY: IBM Corp.) The level of statistical significance was set at 0.05. However, for the association analyses of fat and muscle reserves and their mobilization with biochemical analytes, statistical significance had to account for multiple null hypotheses in order to minimize the true Type I and II errors. For this reason, the nominal P-value of 0.05 was adjusted by the total number of separate statistical analyses conducted. In the present study, we ran 14 separate analyses of as many hypotheses and a correction based on the Holm-Bonferroni method (Holm, 1979) was used to adjust for multiple testing. Based on the above, the new level of statistical significance was adjusted to P = 0.004.

Second Greek experiment (AUTH)

Following the discussions between partners involved in WP1 during the first annual meeting in Edinburgh, AUTH proposed to undertake an extra experiment, not foreseen in the DoA, that would explore the possibility of identifying an easy and non-invasive indicator of feed efficiency. Specifically, the objective of the second experimental study was to assess the potential use of milk composition traits as predictors of feed efficiency in Chios dairy ewes. It should be noted here that the proposed experiment was done using existing animal and feed resources of partner AUTH since as stated in the DoA (section 3.4 resources to be committed, and in particular 3.4.1 Other direct costs) no budget was foreseen for either purchase of animals or feeds for partner AUTH.

Facilities, animals and study design

The study was partially undertaken within the framework of the research project Legumes4Protein (Financed by the ERDF of EU and Greek funds through the Operational Program Competitiveness, Entrepreneurship and Innovation (RESEARCH-CREATE-INNOVATE: T1EΔK-04448) using animal and feed resources of that project which wasconducted in the facilities of the Veterinary Faculty of Aristotle University of Thessaloniki farm in Kolchiko Lagada, Greece. The duration was 60 days starting on the 1st of February 2021 and ending on the 30th of March 2021. A total of 40 dairy ewes, representatives of purebred Chios breed, were selected for the study. In the third month of their first to fifth lactation period, selected animals were housed in two different pens (n=20 animals per pen). The two groups (Group A and B) were balanced for milk production after weaning and for the number of lactation periods. However, two animals in Group A died during the study, and hence, the total number of studied ewes was reduced to 38 (Group A, n=18 and Group B, n=20).

Ewes were fed a pelleted concentrate diet (1.5 kg/animal day) together with Lucerne Hay (1.5 kg/animal/day) and wheat straw (0.3 kg/animal/day). The pelleted concentrate diets in Groups A and B had different physical compositions; however, they were equal in terms of energy and protein supply. The physical and chemical compositions of the diets are provided in Tables 2 and 3, respectively.

Table 2. Physical composition of the diets used in the study.

Feeds (kg/animal/day)	Group A	Group B
Lucerne Hay	1.50	1.50
Wheat straw	0.30	0.30
Corn	0.90	0.63
Wheat bran	0.35	0.37
Soybean	0.25	-
Lupin	-	0.125
Pea	-	0.125
Vetch	-	0.125
Fava bean	-	0.125

Table 3. Basic chemical composition of the diets used in the study.

Parameter	Lucerne Hay	Wheat straw	Pellet
DM (/kg)	0.85	0.88	0.87
UFL (/kg DM)	0.57	0.37	1
Crude protein (g/kg DM)	148	35	158.6

Data collection

Data collection was performed in five-time points corresponding to study days 0, 15, 30, 45, and 60. Prior to milking, the body condition of each goat was assessed by palpation of the dorsal lumbar region, and a score (BCS) was recorded based on the 5-point scale (1-emaciated to 5-obese, in 0.25 point increments) proposed by Russel et al. (1969). Assessment of BCS was always performed by the same qualified veterinarian to eliminate inter-classifier variability. Then, individual ewe milk yield was recorded using electronic milk recording and a milk sample was collected in 50 ml tubes to assess milk composition; fat, protein, lactose, and solids-non-fat (SNF) content. Milk samples were transported to the laboratory in 4^{o} C, and milk composition was determined with Near-Infrared Spectroscopy using a DA 7250 NIR analyser (PerkinElmer, Waltham, Massachusetts, USA). Finally, pellet and Lucerne Hay refusals from each group were collected, weighed, and recorded in a designated sheet, and the respective group feed intake was calculated. Individual feed intake was considered as the group feed intake divided by the number of ewes in the group. It should be noted here that data regarding feed efficiency phenotypes (feed intake and milk yield) were collected and analysed including milk composition traits exclusively to address the objectives set in the SMARTER project.

Data handling

Individual daily milk yield was calculated according to the official A4 method of the International Committee of Animal Recording (ICAR, 2016). Energy corrected milk yield (ECMY) for 6% fat was also calculated according to the following formula described by Tsiplakou et al. (2017):

ECMY = [0.28 + 0.12 + Fat (%)] × Milk yield (kg)

Moreover, pellet and Lucerne Hay group intakes were used to calculate the respective average individual animal intakes; an intake of 0.3 kg/day/ewe was assumed for wheat straw. Based on the above and the chemical composition of feeds provided in Table 3, individual energy intake was calculated. Then, individual feed efficiency was defined as the ECMY to energy intake ratio:

Feed efficiency = ECMY (kg) / Energy intake (UFL)

Statistical analysis

Preliminary analyses were performed to identify significant effects on feed efficiency. The effects of group, sampling number (data collection time points during study days), lactation rank, milk yield after weaning, and BCS were tested. The association of each milk composition trait with feed efficiency was assessed with mixed linear models, which included all significant effects from the preliminary analyses:

$$Y_{ijmn} = \mu + G_i + S_j + b_1*M + b_2*B + b_3*MC + A_m + e_{ijmn}$$

Where:

Y_{ijmn} = feed efficiency (natural log, n^{th} trait measurement on animal m);
μ = overall population mean;
G_i = fixed effect of group (two levels);
S_j = fixed effect of sampling number (five levels);
b_1 = regression coefficient on milk yield after weaning (M);
b_2 = regression coefficient on BCS (B);
b_3 = regression coefficient on each milk composition trait (MC; fat, protein lactose, SNF content);

A_m = random effect of the animal (m = 1-38 ewes);
e_{ijmn} = random residual effect.

All analyses were performed using the statistical package "lme4" in R programming language (Bates et al. 2015). The level of statistical significance was set at 0.05.

5.1.2 On-farm designs

1. France (Races de France)

In France, the monitoring of the zootechnical performances of dairy ewes was carried out on the same commercial farms during two dairy campaigns: 2019-2020 and 2020-2021 [from September 2019 to September 2021]. Data description and analysis for the 2020-2021 dairy year are in progress; therefore, this report will be based on data from 2019-2020.

Data were collected in 15 dairy sheep herds from 4 different breeds: 8 herds in Lacaune, 3 herds in Manech Tête Rousse, 2 flocks in Manech Tête Noire, and 2 flocks in Basco-Bearnaise.

In the Western Pyrenean breeds (including Manech Tête Rousse, Manech Tête Noire, and Basco-Bearnaise breeds), ewes in first or in the second lactation were phenotyped; whereas in the Lacaune breed, in the Roquefort area, all ewes were phenotyped whatever the lactation rank.

The Smarter protocol is reported in figure 3. This protocol includes individual phenotypic measurements on the ewes and the recording of environmental data from the farms. The overall number of ewes involved is described in table 4 by breed and parity.

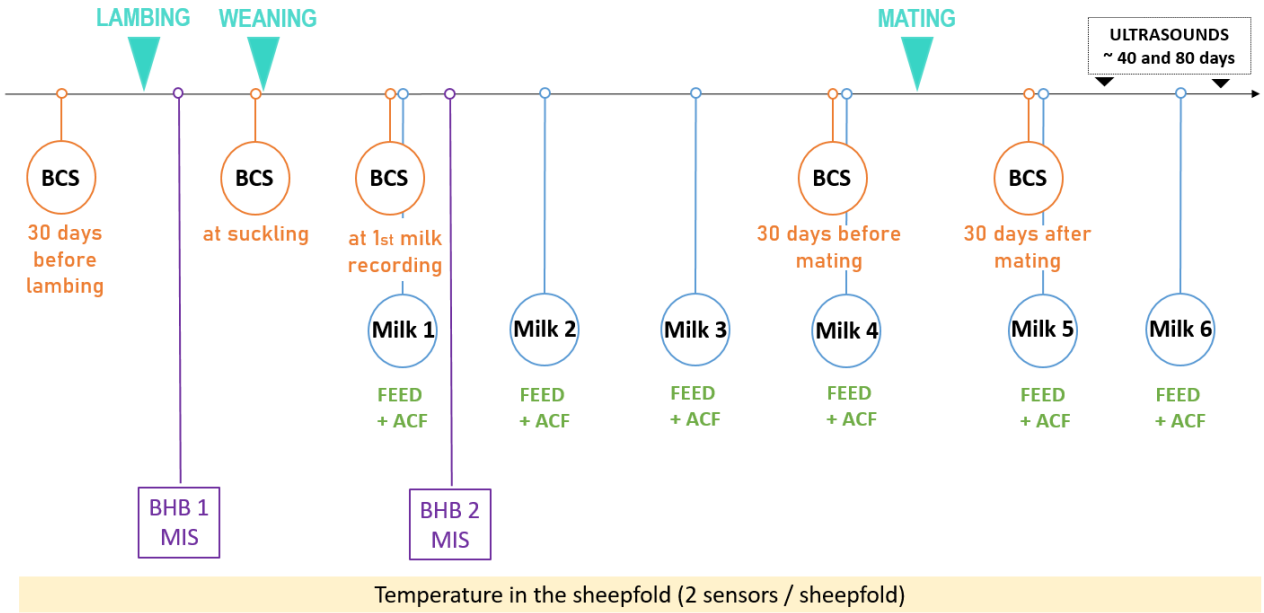


Figure 3: SMARTER experimental protocol in French dairy sheep farms - *BCS : body condition score, ACF : automatic concentrate feeder, BHB : beta-hydroxybutyrate, MIR : mid-infrared spectrum*

Table 4: Number of ewes by breed and parity included in the European SMARTER project

Parity / Breed	1	2	3	4 and more	Total
Lacaune	1384	951	752	1429	4516
Manech Tête Rousse	361	191	191	358	1101
Manech Tête Noire	293	132	107	259	791
Basco-Béarnaise	240	182	197	388	1007
Sum	2278	1456	1247	2434	7415

Body Condition Scores (BCS):

Five BCSs were measured on each ewe, at key physiological stages: 30 days before lambing, at the end of suckling (30 days after lambing), at the first milk test-day (60 days after lambing), 30 days before mating and 30 days after mating. A maximal recording interval of 30 days was defined before and after each key physiological stages, i.e. 60 days, except for the end of suckling for which it was of 30 days after lambing (Figure 4). The data recorded within these intervals were assigned to the corresponding physiological stages. Data outside of these intervals were removed (Table 5).

Moreover, ewes with no lambing and/or with reproductive abnormalities (e.g., no pregnant ewes or aborted ewes without lactation) and/or not mated ewes (which will be bred the following year) were removed from the dataset.

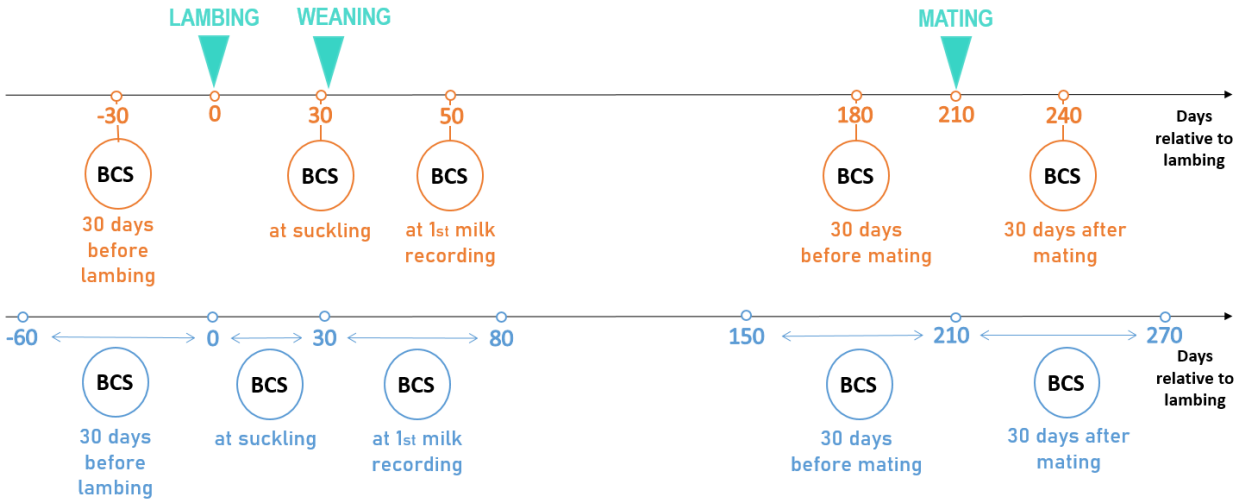


Figure 4: SMARTER experimental protocol: BCS target physiological stages and selected intervals – *in Orange = target physiological stages in the SMARTER protocol, in Blue = selected intervals around the target physiological stage*

Table 5: Number of ewes phenotyped for BCS per breed and physiological stage

Body score condition	**Target physiological stage** (in days to lambing)	**Intervals** (in days to lambing)	**Lacaune (LAC)**	**Manech Tête Rousse (MTR)**	**Manech Tête Noire (MTN)**	**Basco-Béarnaise (BB)**
BCS before lambing	-30	-60 à 0	3,585 [1]	334	192	266
BCS at suckling	30	0 à 30	3,149	208	141	162
BCS at first milk test-day	50	30 à 80	3,438	220	89	129
BCS before mating	180	150 à 210	3,386	310	167	105
BCS after mating	240	210 à 270	2,762	181	53	30
All BCS			2,293	39	21	19
Total number of different ewes			4,171	339	229	325

[1] 3585 Lacaune ewes have at least the BCS before lambing

A total of 7,415 ewes were measured, with 5,014 out of them having at least one BCS and milk record, and 3,577 out of them had at least one feed efficiency value calculated.

Two sets of data were defined for subsequent analyses. The first one consists of the 5,014 ewes (4,105 LAC; 355 MTR, 229 MTN, and 325 BB) with at least one BCS recorded in a previously defined time interval. The second sub-dataset consists of the 4,489 ewes (3,752 LAC; 338 MTR, 190 MTN, and 209 BB) having at least two BCS recorded in the defined intervals.

Imputation of the missing BCS was performed per farm for Western Pyrenean breeds and per farm and litter size (single/multiple) for Lacaune breed using the copyMean method.
Similarly, missing BWs were estimated by the technicians similar to the reference values available in the literature per breed (55 kg for MTN and MTR, 65 for BB) and parity in the case of Lacaune ewes (70 kg for primiparous and 75 kg for multiparous).

Milk control:

Dairy performances were individually measured with a target of a six-monthly test-day. The first milk test-day was performed approximately 50 days after lambing (Figure 3). For each test-day, milk yield protein content (PC), fat content (FC), somatic cell count and urea were recorded.

Feeding:

A feeding survey was conducted for each farm at each milk test-day in order to record the quality and quantities of feed distributed collectively in the herd (pasture, forage, concentrates) from the end of gestation to the end of exclusive milking. At each milk recording, the quality and quantities of concentrates distributed individually were recorded. Feed intakes were calculated from the feed distributed

in the collective feed (with a refusal rate of 10% and a correction for the growth of primiparous ewes) and the concentrates distributed individually in the milking parlour.
The energetic and protein values of each feed were known by carrying out forage analyses or by taking reference values from INRAE tables or from technical organizations. The percentage of dry matter (DM), net energy/kg DM, and PDI / kg DM (proteins digestible by the intestine) values of each feed are available.

Appendix 2 describes the feeding strategies encountered in the SMARTER herds.

Computation of feed efficiency-related traits:

Feed efficiency calculations were applied to the ewes having at least one BCS recorded. No individual total feed intake neither body weights were recorded, so RFI could not be calculated.

We calculated the Net Energy Intake Converted in Milk Ratio (NEICMR) that had been proposed (P. Hassoun personal communication) to take into account that the total energy intake is not only for production but also for maintenance requirements and that energy could be stored or mobilized in the body reserves.:

Net Energy Intake Converted in Milk Ratio (NEICMR)=

$$\frac{\text{Lactation requirements}}{\text{Feed intake} - \text{Maintenance requirements} + \text{variation in body condition}}$$

Lactation requirements: $=0.71 \times [MY \times (0.0071 \times FC + 0.0043 \times PC + 0.224)]$

Maintenance requirements: $=0.033 \times BW^{0.75}$

Variation in body condition: $=(-0.43 \times BW \times \Delta BCS / duration) \times 0.956$

With Δ BCS the difference between two BCS and duration being the time step between two BCS.

Feed intake: $=\sum_{i=1}^{n}(amount\ feed_i \times feed\ value_i)$

Feed value is expressed in net energy/g

For NEICMR at the first milk recording, the difference between BCS at suckling and BCS at first milk test-day was used. For the last milk test-day (6), the difference between BCS before mating and BCS after mating was used.

A NEICMR value lower than 1 means that the ewe is not efficient, the intake of the ration consumed for milk production (in net energy/d) is higher than the lactation needs of the animal (in net energy/d). Conversely, a value higher than 1 means that the ewe has not consumed more than necessary to cover her lactation requirements and/or mobilize her body reserves to produce milk.

The variables of daily milk production, protein content, fat content, and amount of dry matter intake were filtered to +/- 3 standard deviations from the mean to remove extreme values.

We also defined the coverage rate as the ratio between total intake of the ration (in net energy/day) divided by the total need of the animal (lactation, growth, and maintenance) (in net energy/day).

Three main limitations appear regarding NEICMR :

- Primiparous ewes growth requirements are difficult to estimate. For this purpose, 0.09 net energy/d and 5 PDI/d were added to the maintenance requirements of primiparous ewes. This corresponds to the difference in requirements between a 60 kg ewe (weight of a Lacaune ewe before lambing) and a 70 kg ewe (weight of an adult Lacaune ewe) (Bocquier *et al.* 2019).
- The farmers do not weigh distributed forages and refusals. Based on the expertise of technicians, a 10% refusal rate was applied to all amounts of hay, silage, and haylage feed distributed (Bocquier *et al.* 2019).
- Some data are missing, i.e., weights, forage intake, or are incomplete, i.e., BCS and milk yield. To compensate for these missing data, certain approximations can be made, but they must first be tested to evaluate the degree of imprecision generated.

Analysis of factors influencing BCS at each physiological stage

The BCSs of each physiological stage were analysed per breed according to model 1:

(Model 1) $Y_{ijklmn} = \mu + parity_i + herd_j + litter\ size_k + Day\ of\ measurement_l + month\ of\ lambing_m + sire_n + corrected\ milk\ yield_{ijklmn} + corrected\ PC_{ijklmn} + corrected\ FC_{jklmn} + e_{ijklmn}$

With Y_{ijklmn} is the BCS for the considered physiological stage; μ is the mean; the fixed effects in classes are the parity *i* (in 4 classes in Lacaune breed and 2 classes in Pyrenean breeds: 1/2), the herd *j*, the litter size *k* (single/multiple), the relative day of measurement *l* relative to lambing (3 classes of 20 days per interval of 60 d except for the BCS at suckling interval [0-30 d] with 3 classes of 10 days), the month of lambing *m*, the sire *n*. Effects of milk production (milk yield, FC, and PC) were added as covariates for the 3 BCS collected during lactation (at 1st milk recording, before and after mating). Finally, e is the residual error associated with each ijklmn observation. Daily MY, PC, and FC were previously corrected in a linear model for the effect of parity, litter size, month of lambing, days in milk, and the interaction between herd and milk recording date.

The analyses of variance were carried out with the "car" package of the R software. An effect is considered significant if the p-value is < 0.05.

Analysis of changes in body condition scores by physiological stage

In order to evaluate the effect of physiological stages, BCS for all physiological stages were analysed using a linear mixed model (model 2) with repeated measures:

(Model 2) $Y_{ijklma} = \mu + lactation\ rank_i + herd_j + litter\ size_k + physiological\ stage_l + month\ of\ lambing_m + duration\text{-}corrected\ milk_{ijklma} + adjusted\ standardized\ fat\ content_{ijklma} + adjusted\ standardized\ protein\ content_{ijklma} + animal_a + e_{ijklma}$

Where, Y_{ijklma} was the BCS of ewe at different physiological stages; μ is the mean; the fixed effects are defined in the same way as for model 1. The physiological stage *l* represents the BCS number in the SMARTER protocol (1/2/3/4/5). In order to take into account, milk production level for each physiological stage even outside the lactation period, the model included variables that accounted for

the overall level of production during lactation: duration-adjusted lactation milk was calculated from the following formula: (Lactation milk x (220 / Lactation duration + 60) x 1.3); adjusted standardized contents are the variables used for national genetic evaluation (Astruc, personal communication). The animal effect is the random effect of the ewe. The e is the residual error associated with each ijklmn observation.

The "lme4" and "lmerTest" packages of the R software were used to perform the mixed models on repeated measures. The use of the adjusted means estimated by the "emmeans" package and the Tukey adjustment will allow post hoc comparisons between groups, two by two, after adjusting the linear models.

Classification of individual body condition score profiles

The classification was carried out on ewes with at least two valid BCS. The objective was to categorize individual variability of BCS curves during a production cycle and characterize these curves. The R package "kml" for k-means longitudinal data (Genolini and Falissard, 2011; Genolini *et al.*, 2015) allowed partitioning of longitudinal data using the k-means method, with the objective to maximize the inter-cluster variance and minimize the intra-cluster variance.

Imputation methods to predict intermittent (in the middle of a trajectory) and monotonic (at the beginning or end of a trajectory) missing data are included in the kml package. The default method used was "copyMean", which combines trajectory-based (such as LOCF) and mean-based imputation methods. The kml method is iterative and allows for selecting a number of clusters (from 2 to 26). The optimal choice of the number of clusters to use was made based on three partitioning quality indices built on a scale from 0 to 1: the Calinsky-Hararasz3 index (Genolini *et al.*, 2015), the Ray and Turi index (Ray and Turi, 2000) and the Davies Bouldin index (Bossard and Guimier de Neff, 2012).

The repeatability and stability of clusters were tested by repeating the kml algorithm several times (~ 20 times). The classification was only dependent on the five BCS, and the environmental factors were used to interpret the BCS profile clusters. Cluster numbers were analyzed with the GLM function of R software through logistic regression to characterize the ewes of each cluster (model 3):

(Model 3) Y_{ij} = parity$_i$ + litter size$_j$ + duration-corrected milk$_{ij}$ + milk persistency$_{ij}$ + e_{ij}

Where, Y_{ij} is the cluster number; parity and litter size are fixed effects as defined in model 1; duration-corrected milk is a covariate as described below, and milk persistency is a covariate and was calculated individually from the coefficient of variation over the ewe's milk test-days.

In order to appreciate the importance of each factor in the partitioning of body condition profile classes, factors were removed one by one from the full GLM model, and the AICs (Akaike information criterion) were compared.

5.2 Results

1. France (INRAE)

A- On the Lacaune ewes from MicroGenOL:

- **SNP control**: the quality control parameters used for the selection of SNPs were a minimum call rate of 90% for individuals and 99% for loci. Marker loci with minor allele frequencies (MAFs) lower than 0.01. So, 45,923 SNPs were available for 795 phenotyped ewes.

- Microbiota: From 16S, 2 059 OTUs corresponding to 9 536 442 sequences were identified on ruminal fluids collected for the 795 ewes.

Discriminant analysis for both the SCS and PERS lines revealed very tiny differences, which suggested that genetic selection for udder health or milk production curves didn't modify the abundance of rumen bacteria and the animal's ability to digest their feed (Martinez-Boggio et al., 2021). Nevertheless, some abundant OTUs were linked with fine milk composition (figure 5).

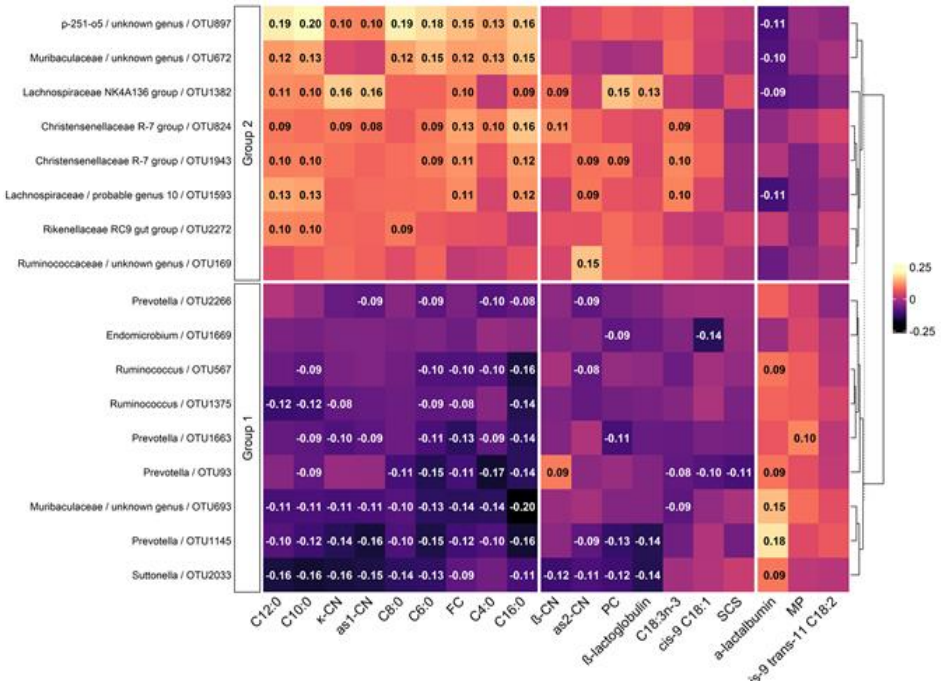


Figure 5: correlation matrix heatmap between bacterial taxa and milk traits

A first group of taxa, represented mostly by propionic acid and proteolytic bacteria such as *Prevotella*, *Suttonella*, and *Ruminococcus,* appeared negatively linked with milk fatty acids and caseins. In contrast, a second group positively correlated with milk composition was composed of OTUs belonging *Lachnospiraceae, Rikenellaceae* and *Christensenellaceae*, bacteria that can promote the production of short- and medium-chain saturated fatty acids SFAs via butyric and acetic acid.

B – On the subset of 54 Lacaune ewes:

- **Microbiota**: From 16S, 1 801 OTUs corresponding to 2 631 966 sequences were identified on ruminal fluids collected for the 51 ewes the 3 consecutive years, that is to say, on average 17 000 sequences by sample. In the first approach with PCA analysis (figure 6), we identified a main "year" effect, which is a combination of confounding effects such as year, lactation rank, and partly run of sequencing since samples from 2017 and 2019 in one side and samples from 2018 in other side were sequenced in 2 different runs.

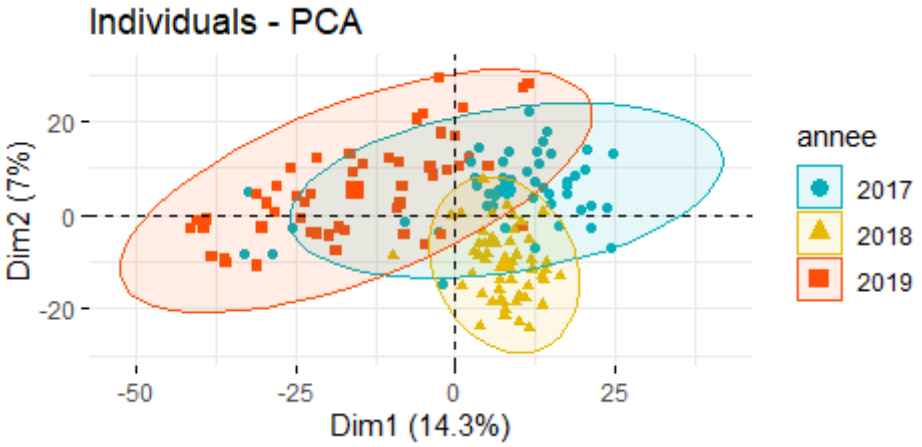


Figure 6: PCA of OTUs abundances – plot of the 153 samples (51 ewes x 3 times) according to the year of sampling

PCA didn't allow to visualize differences between the ewes' lines, but when looking at the alpha-diversity, PERS- line seem to have a more divers microbiota than PERS+ line (figure 7).

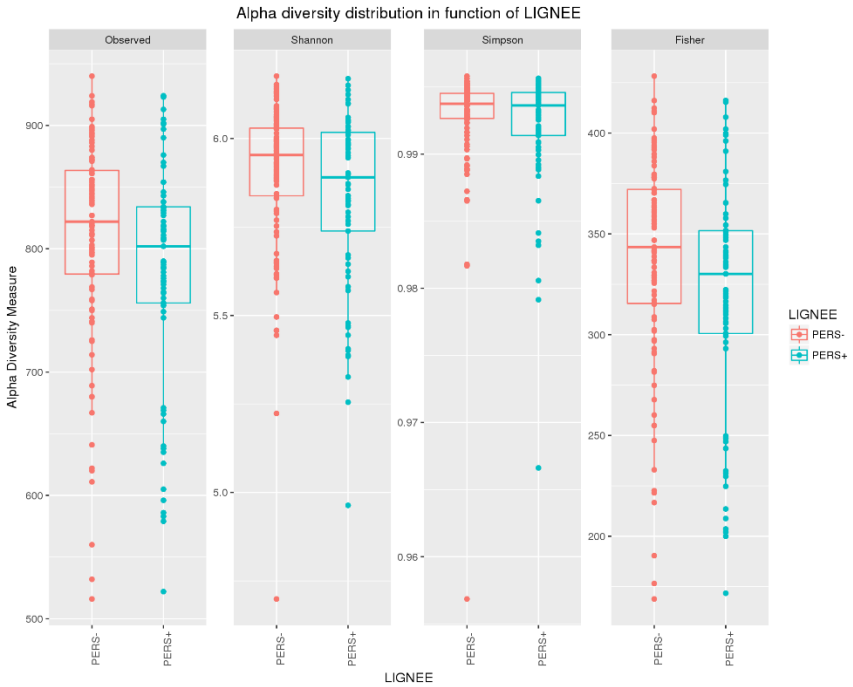


Figure 7: Boxplots of ewes' ruminal microbiota α-diversity according to the lines PERS- / PERS+

- **Metabolites**: 174 NMR spectra of blood plasma and 174 spectra of rumen juice were analysed. Over the 3 years of the trial, relative abundances of 29 metabolites from the rumen and 75 metabolites from the blood were quantified.

PCAs of metabolites abundances of blood plasma revealed a strong impact of the year of sampling, while no impact of year on the metabolite's abundances in the ruminal juice (Figure 8). PCAs never revealed an impact of the PERS lines, either in ruminal juice, nor in the plasma.

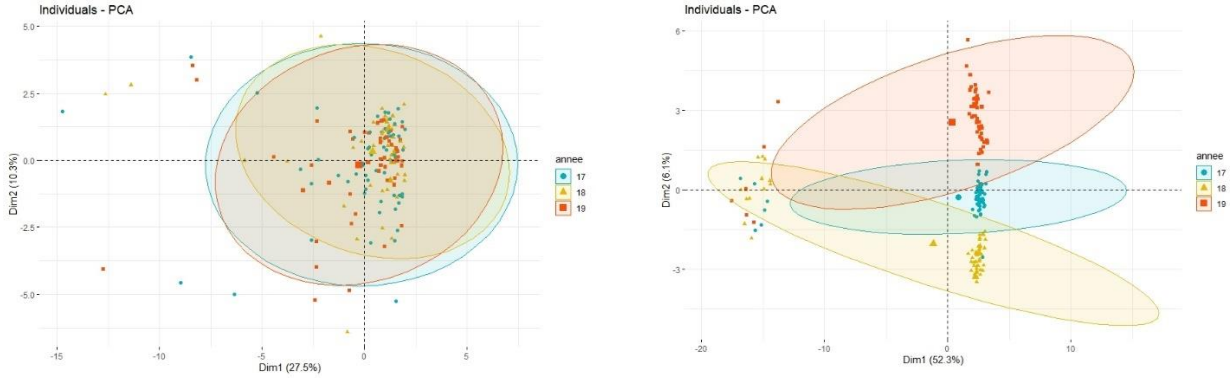


Figure 8: PCA of metabolites abundances (left from rumen juice – right from plasma) - plot of the 174 samples according to the year of sampling

With a variance analysis taking into account the year of sampling and the line as fixed effects, 7 metabolites were significantly different according to the line (Table 6): PERS- line were characterized by a higher abundances of DehydroAscorbicAcid in blood and IsovalericAcid and Succinate in rumen juice, while PERS+ line have a higher abundances of EthylmalonicAcid, Methylamine, L.Ornithine and Threitol in blood.

Table 6: significant PERS lines effect on metabolites abundances

	Proba	PERS- mean	PERS+ mean
Rumen juice			
IsovalericAcid	*	0.0334	0.0229
Succinate	*	0.0065	0.0041
Blood plasma			
DehydroAscorbicAcid	***	0.0787	0.0294
EthylmalonicAcid	**	0.0347	0.0438
Methylamine	*	0.0197	0.0231
L.Ornithine	*	0.1189	0.1451
Threitol	*	0.1529	0.1686

*: Proba <5%; **: Proba <1%; ***: Proba <0.1%

2. Spain (UNILÉON-CISC)

Feed efficiency experiment

Feed efficiency data in the lactation Assaf ewes experiment are given in Appendix 3. The distributions of RFI, REI, and FCR, generally conform to a normal distribution (figure 9). Regarding the basic statistics of FE parameters, the FCR show an average of 1.36 (SD =0.23), REI and RFI display an average of 0 (SD =0.91 for REI and SD=0.13 for RFI).

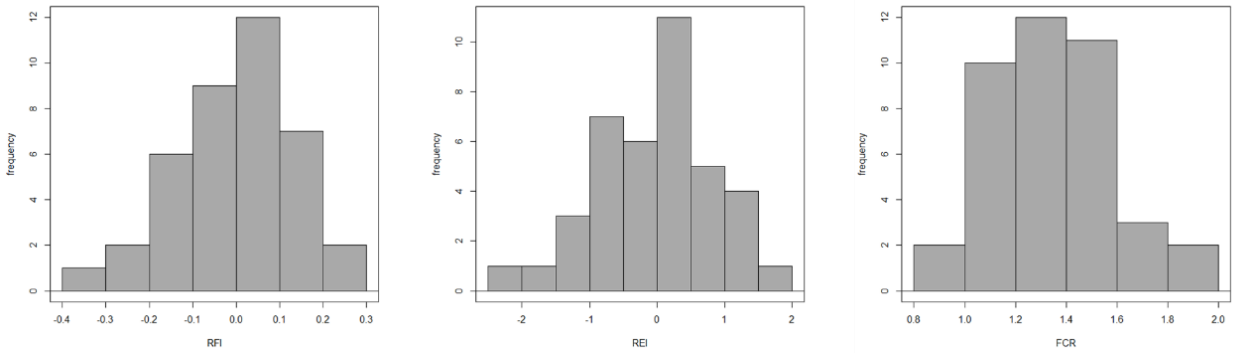


Figure 9: distribution of feed efficiency traits (RFI, REI and FCR) in Assaf ewes during lactation.

Milk Fatty Acids: Association analysis with feed efficiency

Spearman correlation coefficients were estimated between the milk FA and feed efficiency ratios (FCR and RFI). We identified 10 and 50 significant correlations (P-value < 0.05) between the FA included in this work and the RFI and FCR parameters, respectively (Tables 7 and 8). The significant correlations identified between the FA and the RFI parameters ranged from 0.323 to 0.481 (absolute values).

Table 7: Significant spearman correlation coefficients estimated between the FA analysed and the RFI parameter.

Fatty Acid	Coefficient	Pvalue
6:0	-0.348	3.01E-02
cis-9 10:1	-0.323	4.51E-02
cis-9 15:1	-0.342	3.31E-02
trans-9 16:1	-0.382	1.63E-02
10-oxo-18:0	-0.351	2.83E-02
cis-9 trans-12 18:2	-0.481	1.95E-03
cis-9 trans-13 18:2 (coelutes with an 18:2 isomer of indeterminate double bond position)	-0.360	2.43E-02
cis-9 trans-14 18:2 (coelutes with an unidentified component)	-0.424	7.16E-03
trans-9 cis-12 18:2	-0.330	4.01E-02
cis-9 trans-11 CLA	-0.349	2.96E-02

The significant correlations identified between the FA and the FCR parameter ranged from 0.318 to 0.704 (absolute values). Comparing the results of both feed efficiency parameters, we identified three FA significantly correlated with both FCR and RFI indexes (*"cis-9 trans-14 18:2 (coelutes with an unidentified component)", "trans-9 cis-12 18:2" and "trans-9 cis-11 CLA"*).

Table 8: Significant spearman correlation coefficients estimated between the FA analysed and the FCR parameter.

Fatty Acid	Coefficient	Pvalue	Fatty Acid	Coefficient	Pvalue
7:0	0.403	1.09E-02	*trans-10 18:1*	-0.595	6.48E-05
9:0	0.465	2.85E-03	*trans-12 18:1*	0.375	1.88E-02

10:0	0.436	5.48E-03	*trans-15 18:1*	0.466	2.78E-03
11:0	0.539	3.99E-04	*cis-11 cis-14 18:2*	-0.377	1.79E-02
12:0	0.553	2.58E-04	*trans-9 cis-12 18:2*	0.318	4.87E-02
cis-9 12:1	0.429	6.49E-03	*trans-11 cis-15 18:2*	-0.570	1.51E-04
trans-9 12:1	0.327	4.23E-02	*trans-12 cis-15 18:2*	-0.362	2.36E-02
cis-7 14:1	0.376	1.85E-02	*trans-10,trans-14 18:2*	0.366	2.19E-02
cis-12 14:1	0.418	8.03E-03	*trans-8 cis-10 CLA*	-0.471	2.50E-03
15:0	0.494	1.40E-03	*trans-9 cis-11 CLA*	-0.478	2.08E-03
anteiso 15:0	0.337	3.58E-02	*trans-11 trans-13 CLA*	0.479	2.05E-03
cis-13 16:1	0.639	1.17E-05	*18:3n-6*	0.345	3.15E-02
17:0	-0.585	9.11E-05	*18:3n-3*	0.458	3.35E-03
iso 17:0 (coelutes with cis-7 16:1)	0.505	1.05E-03	*trans-9 trans-12 cis-15 + cis-9 cis-12 trans-15 18:3*	0.527	5.68E-04
cis-9 17:1	-0.610	3.78E-05	*cis-9 trans-11 trans-15 CLnA*	0.365	2.23E-02
iso 18:0	-0.384	1.59E-02	*20:0*	0.610	3.76E-05
13-oxo-18:0	0.322	4.54E-02	*3,7,11,15-tetramethyl 16:0*	0.356	2.61E-02
16-oxo-18:0	-0.577	1.19E-04	*cis-11 20:1*	-0.492	1.48E-03
cis-11 18:1	-0.434	5.77E-03	*21:0*	0.695	9.07E-07
cis-12 18:1	0.521	6.75E-04	*20:5n-3*	0.393	1.33E-02
cis-13 18:1	-0.428	6.63E-03	*22:0*	0.687	1.34E-06
cis-16 18:1	0.630	1.74E-05	*23:0*	0.663	4.29E-06
trans-4 18:1	-0.435	5.60E-03	*24:0*	0.704	5.71E-07
iso 15:0 (contains trans-9 14:1 as a minor component)	0.399	1.19E-02	*cis-9 18:1 (+ trans-13 + 14 18:1 as minor components)*	-0.544	3.45E-04
cis-9 trans-14 18:2 (coelutes with an unidentified component)	0.354	2.68E-02	*cis-9 trans-13 18:2 (coelutes with an 18:2 isomer of indeterminate double bond position)*	0.364	2.27E-02

Furthermore, the regression analysis performed between the FA values and the feed efficiency parameters revealed a total of 13 and 38 significant associations (P-value < 0.05) between the FA included in this work and the RFI and FCR parameters, respectively. The R^2 Adjusted values identified between the FA and the RFI parameter ranged from 0.079 to 0.194 (Table 9). In contrast, the R^2 Adjusted values identified between the FA and the FCR parameter ranged from 0.076 to 0.313, slightly higher than the RFI results (Table 10).

Table 9. Significant regression coefficients estimated between the FA analysed and the RFI parameter.

Fatty Acid	R.Adj.	P-value
cis-7 14:1	*0.079*	*4.65E-02*
15:0	*0.146*	*9.44E-03*
anteiso 15:0	*0.080*	*4.51E-02*
cis-9 17:1	*0.085*	*4.03E-02*
10-oxo-18:0	*0.171*	*5.14E-03*
16-oxo-18:0	*0.079*	*4.56E-02*
cis-13 18:1	*0.194*	*2.94E-03*
trans-10 18:1	*0.148*	*8.96E-03*
cis-9 cis-12 18:2 (contains cis-9 cis-15 18:2 as minor component)	*0.092*	*3.42E-02*

trans-9 cis-11 CLA	*0.081*	*4.44E-02*
20:2n-6	*0.132*	*1.32E-02*
cis-13 22:1	*0.078*	*4.73E-02*
23:0	*0.107*	*2.37E-02*

The results of the regression analyses performed in both feed efficiency indexes revealed that seven out of the 101 FA analysed in this work were significantly associated with the RFI and the FCR parameters (*"cis-7 14:1", "15:0", "cis-9 17:1", "16-oxo-18:0", "cis-13 18:1", "trans-10 18:1" and "trans-9 cis-11 CLA"*). This last FA (*"trans-9 cis-11 CLA"*) was also significantly correlated with both indexes, which points out the relevance of this FA in predicting the value of feed efficiency.

Table 10. Significant regression coefficients estimated between the FA analysed and the FCR parameter.

Fatty Acid	R.Adj.	Pvalue	Fatty Acid	R.Adj.	P-value
7:0	*0.081*	*4.43E-02*	*cis-16 18:1*	*0.313*	*1.29E-04*
9:0	*0.119*	*1.79E-02*	*trans-4 18:1*	*0.183*	*3.89E-03*
11:0	*0.182*	*3.92E-03*	*trans-10 18:1*	*0.261*	*5.28E-04*
12:0	*0.153*	*7.90E-03*	*trans-12 18:1*	*0.129*	*1.42E-02*
cis-9 12:1	*0.128*	*1.47E-02*	*trans-15 18:1*	*0.258*	*5.70E-04*
cis-7 14:1	*0.084*	*4.06E-02*	*cis-9 trans-13 18:2 (coelutes with an 18:2 isomer of indeterminate double bond position)*	*0.124*	*1.59E-02*
cis-12 14:1	*0.113*	*2.09E-02*	*trans-11 cis-15 18:2*	*0.154*	*7.83E-03*
15:0	*0.172*	*5.09E-03*	*trans-10,trans-14 18:2*	*0.116*	*1.92E-02*
iso 15:0 (contains trans-9 14:1 as a minor component)	*0.105*	*2.50E-02*	*trans-9 cis-11 CLA*	*0.143*	*1.03E-02*
17:0	*0.194*	*2.96E-03*	*trans-11 trans-13 CLA*	*0.219*	*1.56E-03*
iso 17:0 (coelutes with cis-7 16:1)	*0.267*	*4.45E-04*	*18:3n-6*	*0.091*	*3.46E-02*
cis-9 17:1	*0.264*	*4.83E-04*	*18:3n-3*	*0.150*	*8.48E-03*
iso 18:0	*0.104*	*2.53E-02*	*trans-9 trans-12 cis-15 + cis-9 cis-12 trans-15 18:3*	*0.157*	*7.34E-03*
16-oxo-18:0	*0.303*	*1.70E-04*	*cis-9 trans-11 trans-15 CLnA*	*0.076*	*4.89E-02*
cis-9 18:1 (+ trans-13 + 14 18:1 as minor components)	*0.159*	*6.93E-03*	*cis-11 20:1*	*0.155*	*7.63E-03*
cis-11 18:1	*0.101*	*2.74E-02*	*20:5n-3*	*0.127*	*1.47E-02*
cis-12 18:1	*0.203*	*2.33E-03*	*22:0*	*0.277*	*3.43E-04*
cis-13 18:1	*0.117*	*1.87E-02*	*22:5n-3*	*0.132*	*1.31E-02*
cis-15 18:1	*0.081*	*4.42E-02*	*24:0*	*0.294*	*2.14E-04*

Finally, the OPLS analyses revealed that the FA analysed were not significant for the RFI prediction but achieved a predictive performance of 0.82 for the FCR index. The VIP scores and P-values obtained

from OPLS analysis were extracted to identify those FA relevant for the quantitative prediction of the FCR (Figure 10). A total of 40 FA achieved a VIP score higher than one and a P-value lower than 0.05 (Table 11).

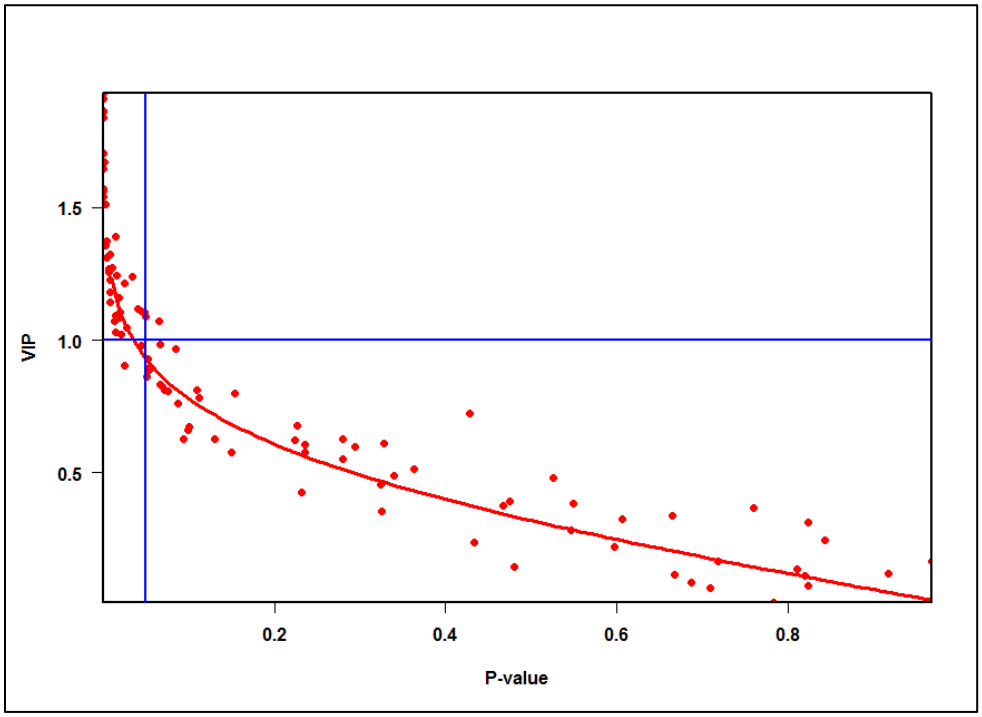


Figure 10: Representation of the variable importance in the projection (VIP) scores (Y-axis) and the p-values (X-axis) obtained from OPLS analysis for the 101 FA analysed in relation with the FCR parameter.

The total of 40 FA highlighted by the OPLS analysis is in agreement with the results presented by the Spearman correlation (Table 8) and regression analysis (Table 10) previously described with the FCR index (Table 11). These concordant results, especially between the regression analysis and the OPLS analysis, emphasize the role of the FA highlighted by both analyses in the prediction of the FCR index.

Table 11: Variable Importance in the projection (VIP) and P-values estimated from OPLS-DA between the FA and the FCR parameter.

Fatty Acid	VIP	P-value	Fatty Acid	VIP	P-value
9:0	*1.161*	*1.79E-02*	*trans-10 18:1*	*1.561*	*5.28E-04*
11:0	*1.375*	*3.92E-03*	*trans-12 18:1*	*1.392*	*1.42E-02*
12:0	*1.225*	*7.90E-03*	*trans-15 18:1*	*1.674*	*5.70E-04*
cis-9 12:1	*1.094*	*1.47E-02*	*cis-11 20:1*	*1.323*	*7.63E-03*
cis-7 14:1	*1.120*	*4.06E-02*	*trans-11 cis-15 18:2*	*1.145*	*7.83E-03*
cis-12 14:1	*1.022*	*2.09E-02*	*trans-10,trans-14 18:2*	*1.104*	*1.92E-02*
15:0	*1.314*	*5.09E-03*	*trans-9 cis-11 CLA*	*1.273*	*1.03E-02*
cis-13 16:1	*1.910*	*8.05E-05*	*trans-11 trans-13 CLA*	*1.673*	*1.56E-03*

17:0	*1.358*	*2.96E-03*	*18:3n-6*	*1.241*	*3.46E-02*
iso 17:0 (coelutes with cis-7 16:1)	*1.706*	*4.45E-04*	*18:3n-3*	*1.183*	*8.48E-03*
cis-9 17:1	*1.646*	*4.83E-04*	*trans-9 trans-12 cis-15 + cis-9 cis-12 trans-15 18:3*	*1.255*	*7.34E-03*
iso 18:0	*1.217*	*2.53E-02*	*cis-9 trans-11 trans-15 CLnA*	*1.104*	*4.89E-02*
16-oxo-18:0	*1.542*	*1.70E-04*	*20:0*	*1.931*	*8.44E-05*
cis-9 18:1 (+ trans-13 + 14 18:1 as minor components)	*1.271*	*6.93E-03*	*cis-9 trans-13 18:2 (coelutes with an 18:2 isomer of indeterminate double bond position)*	*1.245*	*1.59E-02*
cis-11 18:1	*1.045*	*2.74E-02*	*21:0*	*1.864*	*4.06E-06*
cis-12 18:1	*1.514*	*2.33E-03*	*20:5n-3*	*1.032*	*1.47E-02*
cis-13 18:1	*1.084*	*1.87E-02*	*22:0*	*1.569*	*3.43E-04*
cis-15 18:1	*1.108*	*4.42E-02*	*22:5n-3*	*1.071*	*1.31E-02*
cis-16 18:1	*1.862*	*1.29E-04*	*23:0*	*1.838*	*2.30E-05*
trans-4 18:1	*1.310*	*3.89E-03*	*24:0*	*1.542*	*2.14E-04*

Lastly, the ten most efficient and least efficient animals related to the RFI and FCR indexes were included in the OPLS-DA to identify variables useful for discriminating both classes. The predictive performance of the OPLS-DA analyses was 0.99 for the RFI index and 0.90 for the FCR index. The results of these analyses, depicted in Figure 11, strongly support the possibility of discriminating between these two groups (most and least feed efficient animals) using the 101 FA measured in this study, reinforcing the results obtained by the previous analyses.

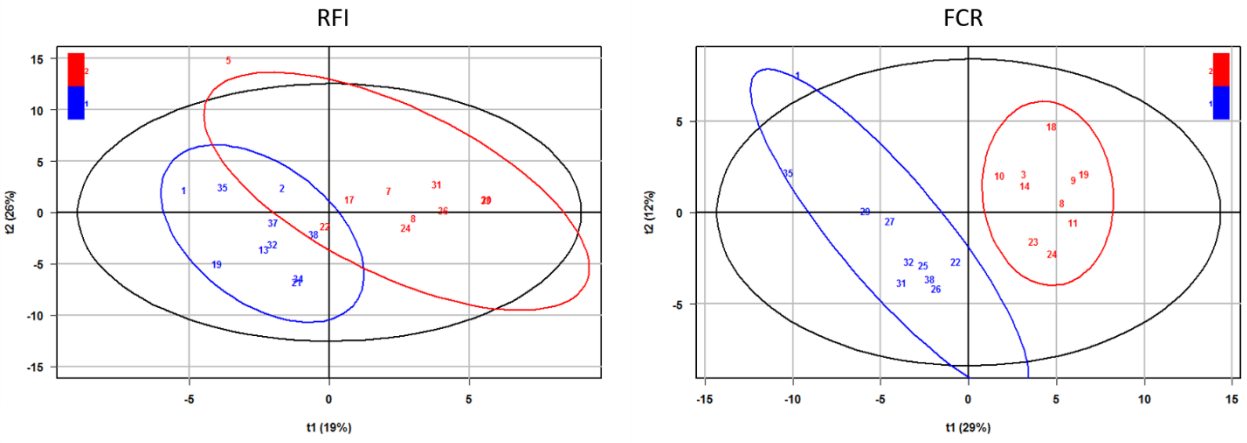


Figure 11. Classification scores corresponding to the most (coloured in blue: 1) and least (coloured in red: 2) efficient animals included in the OPLS-DA concerning the RFI (left) and FCR (right) indexes studied in this work**.**

Analysis of milk somatic cells transcriptome by RNA-seq ant its association with feed efficiency in Assaf sheep

To determine differences in gene expression between the different groups considered (high *vs*. low-feed efficiency (FE), and Nutritional Challenged (NC) *vs*. controls ewes) we performed RNASeq analyses on the milk somatic cell transcriptome from 28 animals from the whole population of 40 ewes used in the trial. RNA samples were selected based on the RIN quality (RIN > 7) and balanced between groups. The phenotypic values for the RFI, FCR of the ewes whose RNA was sequencing are summarized in appendix 4.

After massive parallel RNA sequencing of the studied samples, an average of 34 million pair-reads was obtained for each animal. In the alignment stage, and considering the Oar_rambouillet_v2.0 reference genome, about 95% of the reads per sample aligned to unique locations of the ovine genome.

***Differential expression analysis using RFI as the phenotype for feed efficiency*.**

For the differential expression analyses using RFI as a phenotype for FE, we selected 9 high-FE ewes (RFI=-0,12 (SD=0,07); 5 controls and 4 NC) and 10 low-FE ewes (RFI=0,1 (SD=0,08); 4 controls and 6 NC) from the extremes of the distribution. This selection excluded animals discordantly classified by RFI and FCR indexes. Based on the gene quantification results, a generalized principal component analysis (GLM-PCA) evaluated the distribution of the analyzed transcriptome samples. In this case, the first dimension (dim1) separated the high and low RFI groups almost perfectly, while samples seemed to be not grouped according to the nutritional challenge (Figure 12).

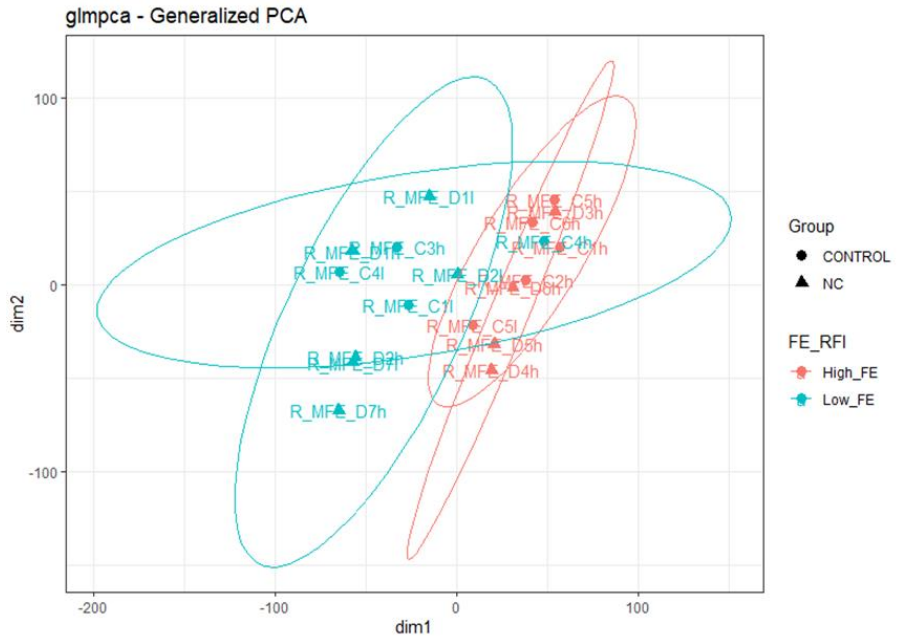


Figure 12: Graphic representation of the generalized principal component analysis (GLM-PCA), based on the gene expression quantification analysis for the 19 RNA-Seq samples, was finally considered in the differential gene expression analysis based on RFI index for feed efficiency.

In the differential expression analysis with DESeq2 and Model 1, 2 differentially expressed genes (DEGs) were identified between NC and control ewes. These genes were lncRNA (*LOC114109620*) and

KLK12. *KLK12* encodes for Kallikrein-12, and the latter gene was previously found differentially expressed between two dairy sheep breeds, Churra and Assaf, showing differences in milk yield and milk composition (Suarez-Vega et al., 2015).

In the comparison between high and low-FE animals, we found 1,938 DEGs (FDR <0.05). Of these, 952 showed a higher expression in the high-FE group, and 986 showed a higher expression in the low-FE. After performing a functional enrichment analysis using WebGstalt software (Liao et al., 2019), one of the most biologically relevant GO terms enriched in the high-FE ewes was *GO:0048732*: *gland development* (35 genes; FDR=0.04) which includes, among others, relevant genes linked to milk protein and fatty acid synthesis (Table 12).

Table 12: List of DEG enriched in the GO term GO:0048732: Mammary Gland Development.

ID	Gene Symbol	ID	Gene Symbol
AACS	acetoacetyl-CoA synthetase	*LRP5*	LDL receptor related protein 5
ACO2	aconitase 2	*MGMT*	O-6-methylguanine-DNA methyltransferase
AK4	adenylate kinase 4	*MPST*	mercaptopyruvate sulfurtransferase
ASS1	argininosuccinate synthase 1	*MTX1*	metaxin 1
CDO1	cysteine dioxygenase type 1	*NOTCH4*	notch receptor 4
CITED2	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2	*PBX1*	PBX homeobox 1
CSN2	casein beta	*PITX1*	paired like homeodomain 1
CSN3	casein kappa	*RTN4*	reticulon 4
CTC1	CST telomere replication complex component 1	*SCRIB*	scribble planar cell polarity protein
DBP	D-box binding PAR bZIP transcription factor	*SEMA3A*	semaphorin 3A
ELF5	E74 like ETS transcription factor 5	*SIX4*	SIX homeobox 4
ESR1	estrogen receptor 1	*STAT5A*	signal transducer and activator of transcription 5A
FASN	fatty acid synthase	*SULF2*	sulfatase 2
FPGS	folylpolyglutamate synthase	*THRA*	thyroid hormone receptor alpha
GOT2	glutamic-oxaloacetic transaminase 2	*THRB*	thyroid hormone receptor beta
GPAT4	glycerol-3-phosphate acyltransferase 4	*VDR*	vitamin D receptor
HPN	hepsin	*XDH*	xanthine dehydrogenase
IGFBP5	insulin like growth factor binding protein 5		

In the comparisons performed using model 2 (*Y=nutritional challenge+RFI+ nutritional challenge:RFI*), we found 17 DEGs between high-FE and low-FE control ewes, 597 DEGs between high-FE and low-FE ewes from the nutritional challenge, 12 DEGs between NC and control high-FE sheep, and no differentially expressed genes between NC and control for the low-FE sheep. In Figure 13 the Venn Diagram shows the common genes found differentially expressed between the different comparisons performed with model 1 and model 2 where it is worthy of highlighting the DEGs found in common by three different comparisons. *MPHOSPH10* gene, coding for M-Phase Phosphoprotein 10, was found differentially expressed in comparing high and low-FE animals for model 1 and high and low-FE animals subjected to the NC for model 2 with the DEGs found between NC and control for high-FE animals. This

gene has been found upregulated in mice subjected to time-restricted feeding, which exhibits protection from excessive weight gain and metabolic diseases (Chaix et al., 2019).

Six genes (*CNTFR*, *COPS9*, *LOC101107700*, *LOC114116088*, *PIK3CB,* and *TMEM238*) were found differentially expressed in common for high vs. low-FE animals in model 1 and high vs. low-controls and high vs. low-NC animals in model 2. Among these DEGs, mutations in *CNTFR* have been selected to be included in a small SNP panel for FE in beef cattle (Abo-Ismail et al., 2018). In addition, functional variants in *PIK3CB* gene have been proposed for FE in dairy cattle (Lam et al., 2021). Lastly, *COPS9* transcription factor has been highlighted as a central regulator in a study for FE in pigs (Ramayo-Caldas et al., 2019).

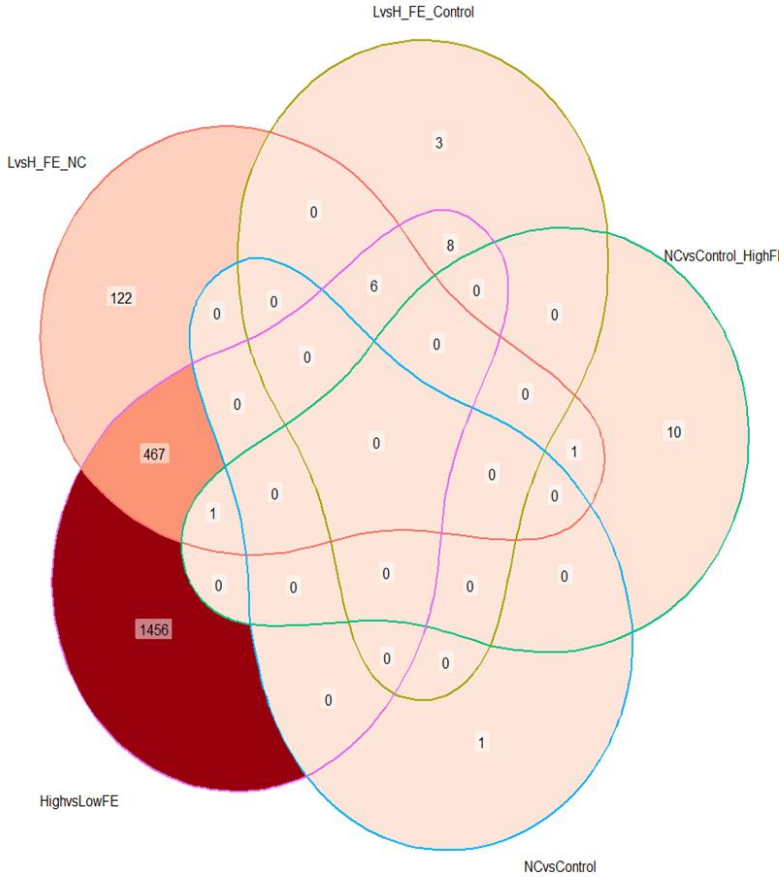


Figure 13: Venn Diagram showing the differentially expressed genes found in the comparisons for model 1 (High *vs.* Low feed efficiency (HighvsLowFE) and Nutritional Challenge (NC) *vs.* Control (NC vsControl)) and model 2 (in the NC animals High *vs.* Low feed efficiency (LvsH_FE_NC), in the controls High *vs.* Low feed efficiency (LvsH_FE_Control, and in the high-feed efficiency animals, NC *vs.* Control (NCvsControl_HighFE).

Differential expression analysis using FCR index as the phenotype for feed efficiency.

For the differential expression analysis using FCR index as phenotype for FE we used 8 high-FE ewes (FCR=1.14 (SD=0,1); 4 controls and 4 NC) and 11 low-FE ewes (FCR=1.52 (SD=0,17); 5 controls and 6 NC) from the extremes of the distribution. As for RFI analyses, this selection excluded animals discordantly classified by RFI and FCR indexes. The distribution of the RNA-Seq samples is shown in the GLM-PCA represented in Figure 14. As could be appreciated, the first dimension differentiates high and low-FE ewes. At the same time, the controls and NC are not well differentiated, having NC animals a higher variability.

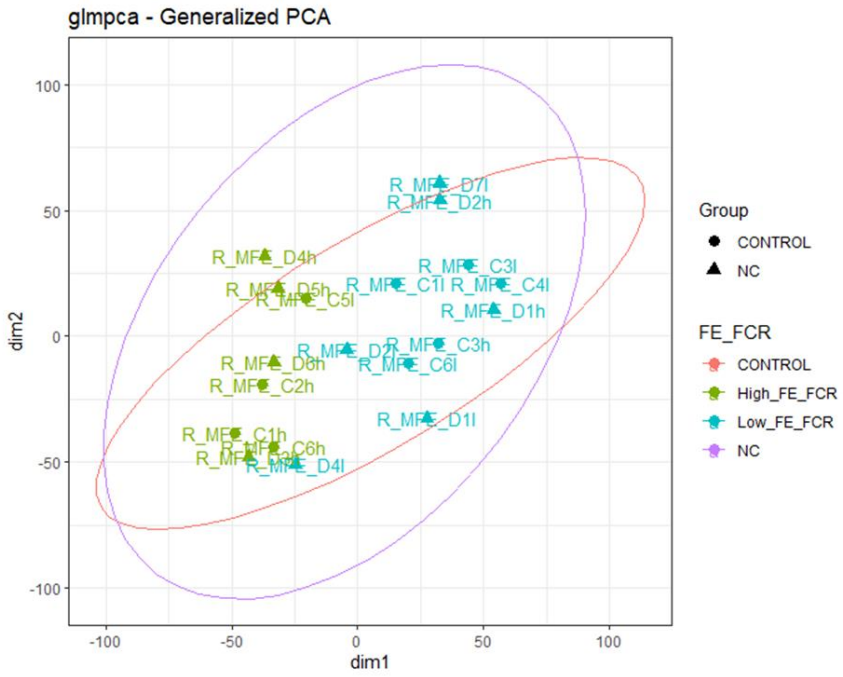


Figure 14: Graphic representation of the generalized principal component analysis (GLM-PCA), based on the gene expression quantification analysis for the 19 RNA-Seq samples, was finally considered in the differential gene expression analysis based on the FCR index for feed efficiency.

We found 3 DEGs between NC and control ewes using model 1. As in the differential expression analysis using RFI, KLK12 was one of the genes differentially expressed. In addition, we found differentially expressed another kallikrein protein (*KLK6*), suggesting a relevant role of these proteins for the NC, and the hemoglobin beta gene (*HBB*), which has been previously related to FE in cattle (Paradis et al., 2015).

For the differential expression analysis between high and low-FE ewes, we found 3,008 DEGs (1,426 upregulated in high-FE and 1,582 upregulated in low-FE; FDR<0.05) 1,561 DEGs in common with the previous analyses in which RFI index was used as phenotype for FE. Among the DEGs between high and low-FE ewes using FCR as the feed efficiency phenotype, it is worthy of highlighting that the highest enriched GO term in the low-FE animals was *GO:0009408: response to heat (*37 DEGs; FDR=2.6551e-9), figure 15 shows the differences in expression between high and low-FE animals for 6 of these proteins. A study in pigs has proposed that less efficient animals can have a greater heat production related to physical activity and basal metabolic rate (Barea et al., 2010), and other studies in the same

species found positive correlations between correlated modules of genes and RFI and FCR traits suggesting that less efficient animals have higher expression of heat-related proteins (Ramayo-Caldas et al, 2018).

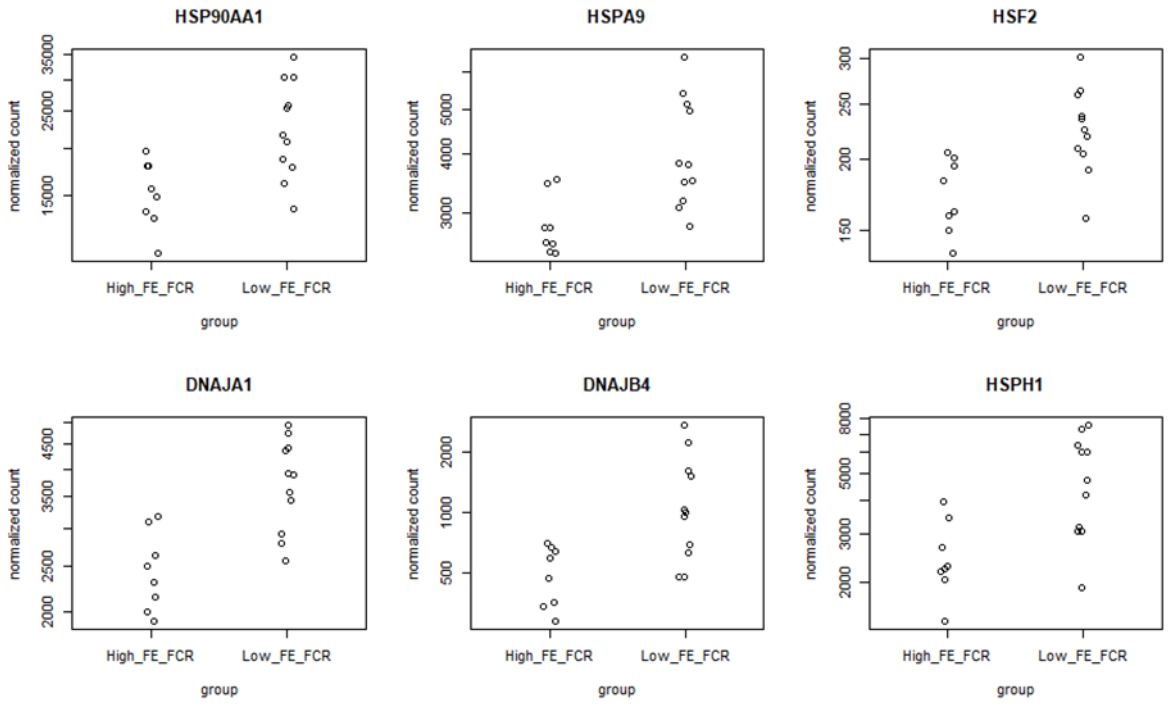


Figure 15: Plot showing gene expression for 6 differentially expressed genes linked to GO:0009408: response to heat found upregulated in low-feed efficiency ewes using RFI index as a phenotype for feed efficiency.

When using model 2 (*Y=nutritional challenge+RFI+ nutritional challenge:RFI*) for differential expression analyses, we found 1,016 DEGs between high and low-FE animals for controls, 439 DEGs between high and low-FE animals for NC, 5 DEGs between control and NC for high-FE ewes and non DEG for the comparison between control and NC for low-FE ewes. In total, 125 DEGs were found in common when high and low-FE control and high and low-FE NC ewes were compared; these genes were also found differentially expressed for FE with model 1. Among these DEGs, it is worthy of highlighting a list of 24 genes upregulated in the high-FE ewes and grouped under the GO term *GO:0044281:small molecule metabolic process* (24 DEGs; FDR=0.02; Table 13). Among them, there are genes related to fat and protein synthesis, suggesting that high-FE animals upregulated genes have an important implication in milk production.

Table 13: List of DEG enriched in the GO term GO:0044281:small molecule metabolic process.

ID	Gene Symbol	ID	Gene Symbol
SCD	stearoyl-CoA desaturase	RORC	RAR related orphan receptor C
IDH1	isocitrate dehydrogenase	GMDS	GDP-mannose 4,6-dehydratase
PEMT	phosphatidylethanolamine N-methyltransferase	TKFC	triokinase and FMN cyclase
FOLR2	folate receptor beta	PYCR1	pyrroline-5-carboxylate reductase 1
XDH	xanthine dehydrogenase	PYCR3	pyrroline-5-carboxylate reductase 3
GCAT	glycine C-acetyltransferase	COMT	catechol-O-methyltransferase
MACROD1	mono-ADP ribosylhydrolase 1	LRP5	LDL receptor related protein 5
LDHD	lactate dehydrogenase D	NDUFS6	NADH:ubiquinone oxidoreductase subunit S6
AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1	NDUFB10	NADH:ubiquinone oxidoreductase subunit B10
GPT2	glutamic--pyruvic transaminase 2	TKT	transketolase
ALDH2	aldehyde dehydrogenase 2 family member	SIRT5	sirtuin 5
GAMT	guanidinoacetate N-methyltransferase	SUCLG1	succinate-CoA ligase alpha subunit

Performance of transcriptomic data to prediction RFI and FCR indexes using Random Forest

Once detected the DEGs, we evaluated the potential of gene expression to predict FE traits. The predictive models were constructed using two lists as features for the prediction: on one side, the 1,017 consensus-DEGs obtained in the differential expression analyses and, on the other side, the 45 genes in common with a previous study evaluating FE using the milk transcriptome (reduced-consensus-DEGs). In general, better performance was shown for the reduced-consensus-DEGs (median SC of 0.37 (FCR) and 0.22 (RFI), and RMSE of 0.16 and 0.11 for FCR and RFI, respectively) than for the whole set of consensus-DEGs (median SC of 0.27 (FCR) and 0.09 (RFI), and RMSE of 0.18 and 0.08 for FCR and RFI, respectively) (Figure 16)

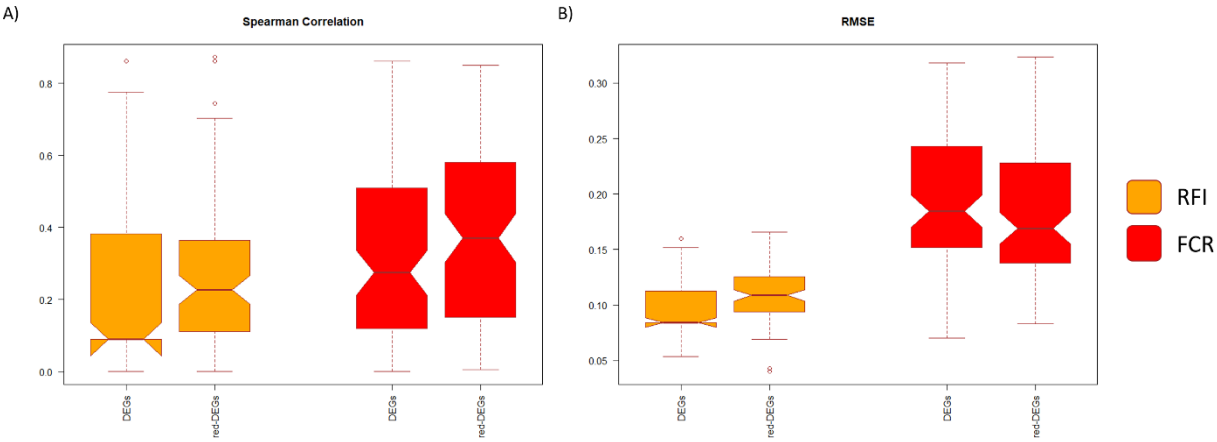


Figure 16. Boxplots representing the Spearman correlation (A) and RMSE (B) values obtained in the prediction of individual RFI (orange) and FCR (red) indexes using transcriptomic features and the Random Forest algorithm. The X-axis shows the different features used: total differentially expressed genes (DEGs) or reduced consensus genes (redGEGs).

Association of milk metabolomics with FE in Assaf sheep

The PLS-DA analysis displays a high potential of the raw milk metabolomic signature to discriminate the high, medium, and low FE animals for both RFI and FCR metrics (Figure 16). The AUCs obtained for the discrimination among the H-RFI, L-RFI, and M-RFI groups and the other groups were 0.969, 0.951, and 0.992, respectively. In addition, for FCR, the AUCs obtained for the discrimination among H-FCR, L-FCR, and M-FCR groups and the other groups were 0.976, 0.987, and 0.981, respectively. The mean error rate and Q2 metrics for the RFI groups were 0.621 and 0.379, respectively, while those for the FCR groups were 0.426 and 0.574, respectively. Therefore, all three groups were well discriminated for RFI and FCR. For the reduced set of features, 41 and 26 features with VIP>2 were selected for RFI and FCR, respectively (Supplemental Table 4, https://zenodo.org/record/8154651).

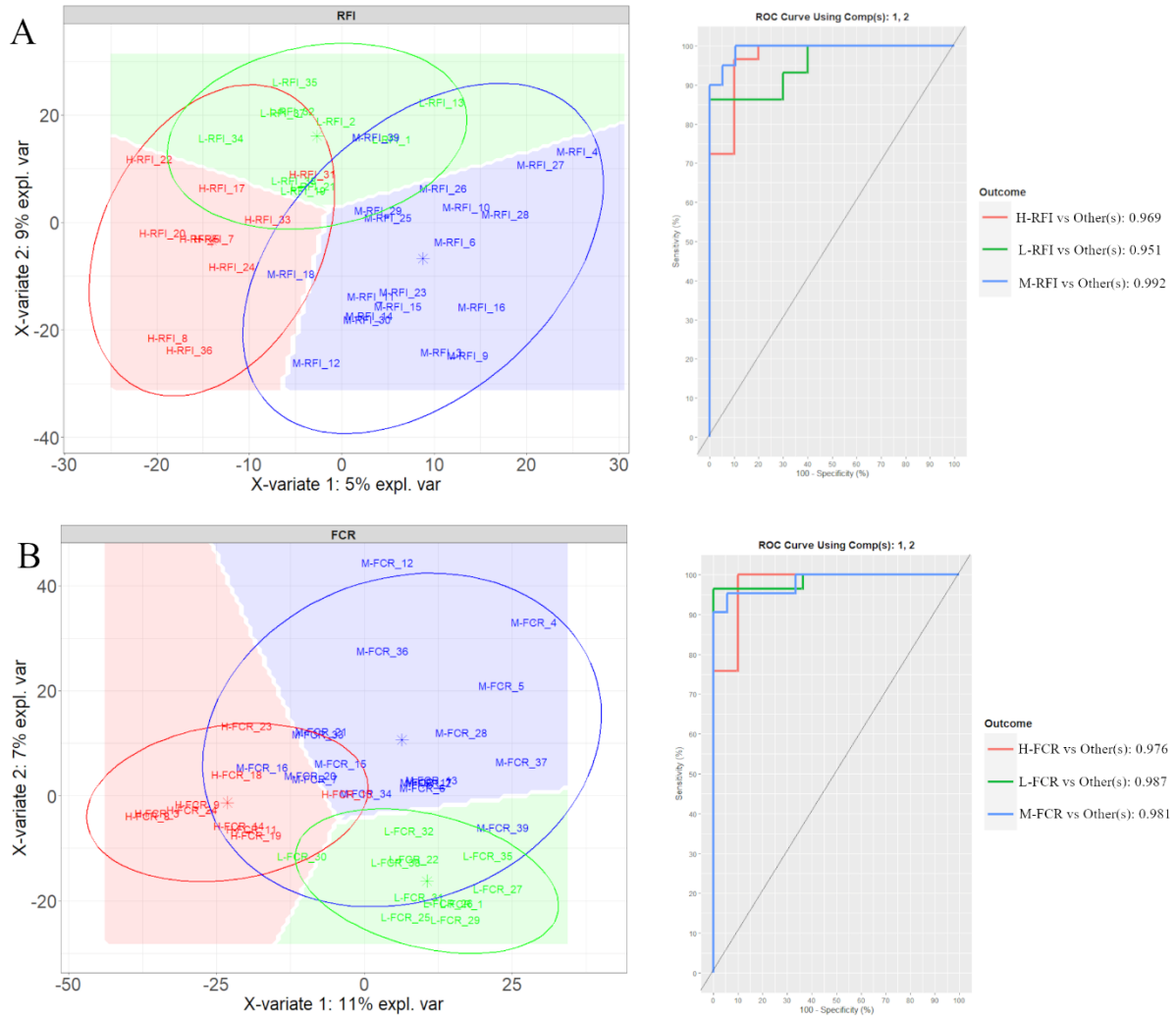


Figure 17. Clustering performance of high, medium, and low groups for RFI (A) and FCR (B) obtained by applying partial least squares discriminant analysis (PLS-DA). The ROC curves show the area under the curve (AUC) obtained for discriminating between one group and the others using the first two principal components.

Performance of milk metabolomics to predict individual FE

In general, the predictions for FCR outperformed the predictions for RFI. Higher means of r^2 (Pearson correlation) and CCC were observed for FCR predictions in all scenarios (Table 14). On the other hand, a larger mean RMSE was obtained for FCR in all scenarios. However, it is important to highlight that RMSE should not be directly compared between the metrics, as RFI and FCR have different distributions and scales. In the scenario where all the features were used for prediction, the highest r^2 for FCR was obtained using OPLS (0.62±0.06), while that for RFI was obtained using RF (0.08±0.10). Regarding RMSE, the smallest mean for FCR was obtained using SVM (0.14±0.03) and that for RFI using xgboost (0.14±0.02). An interesting result was obtained for the predictions performed using the subset of features selected during the PLS-DA analysis for RFI and FCR. Regarding the CCC (concordance correlation coefficient) values, in all the scenarios where all the features were used for RFI prediction, means close to zero were obtained, while in the RFI predictions using the 41 features selected based on VIP>2 resulted in substantially higher CCC means (Table 14). The exception was the scenario where OPLS was used to predict RFI with the subset of 41 features, which also resulted in a mean close to zero (-0.04). The 41 features selected for RFI (VIP>2) provided better predictions (higher r2) for RFI in all the models, excluding OPLS. The highest r^2 mean for RFI using the 41 features was obtained using the deep model (0.18±0.14). Similarly, for FCR, only SVM was not outperformed by the predictions performed using the 26 features selected in the PLS-DA analysis for FCR groups. The mean r2 and CCC for all features using SVM were 0.58±0.15 and 0.67±0.10, while using the 26 features, the r2 and CCC means were 0.51±0.17 and 0.61±0.11, respectively. It is important to highlight that for the predictions performed using OPLS, the reduced dataset performed exactly (for r2, CCC, and RMSE) as the raw milk metabolomic signature. The highest r^2 means obtained using ML models for FCR predictions based on the 26 features was with the xgboost algorithm (0.55±0.15). On the other hand, higher CCC means were obtained for RF and OPLS, 0.68±0.11 and 0.68±0.10, respectively. These results summarized before are detailed in a manuscript (Marina et al., under review).

Table 14: Summary statistics (mean±standard deviation (sd) for the squared Pearson correlation (r^2), concordance correlation coefficient (CCC), and root mean squared error (RMSE) values obtained for the predictions of residual feed intake (RFI) and feed conversion ratio (FCR) using the raw milk metabolomic signature (all) and the reduced feature datasets selected based on Variable Importance in the Projection (VIP) obtained in the PLS-DA analysis.

Model	r^2		CCC		RMSE	
	RFI Mean±sd	FCR Mean±sd	RFI Mean±sd	FCR Mean±sd	RFI Mean±sd	FCR Mean±sd
Deep_all	0.08±0.11	0.43±0.18	-0.05±0.20	0.54±0.17	0.17±0.04	0.18±0.04
RF_all	0.08±0.10	0.48±0.20	0.01±0.14	0.53±0.14	0.15±0.02	0.17±0.04
SVM_all	0.06±0.06	0.58±0.15	-0.01±0.15	0.67±0.10	0.16±0.02	0.15±0.03
xgboost_all	0.07±0.08	0.43±0.20	0.01±0.15	0.51±0.17	0.14±0.02	0.18±0.04
OPLS_all	0.04±0.06	0.62±0.06	-0.04±0.14	0.68±0.10	0.16±0.02	0.14±0.03
Deep_VIP	0.18±0.14	0.46±0.16	0.28±0.20	0.58±0.14	0.14±0.02	0.19±0.03
RF_VIP	0.11±0.15	0.52±0.18	0.17±0.17	0.68±0.11	0.14±0.02	0.16±0.03
SVM_VIP	0.16±0.14	0.51±0.17	0.30±0.20	0.61±0.11	0.15±0.03	0.18±0.03
xgboost_VIP	0.17±0-15	0.55±0.15	0.24±0.19	0.67±0.10	0.13±0.02	0.16±0.02
OPLS_VIP	0.04±0.14	0.62±0.15	-0.04±0.14	0.68±0.10	0.16±0.02	0,14±0.03

ML models: Deep: Multi-layer feedforward artificial neural network, RF: Random Forest, SVM: Support Vector Machine, xgboost: Extreme Gradient Boosting, OPLS: Orthogonal Partial Least Square.

Predictive accuracy of DMRs between high and low FE animals using ML algorithms

The predictive performance of DMRs to predict individual RFI and FCR was tested using three ML algorithms. For this purpose, 100 rounds of random assignment of animals in the training and test datasets were compared. Similar performance was obtained across ML algorithms and FE datasets (Table 15). The smallest mean RMSE (0.17) was obtained for mRFI_RFI, mCons_RFI, and mRFI_RFI + VARs using *RF* and mRFI_RFI + VARs using *xgboost*. Regarding the mean r^2, the largest observed value (0.20) was obtained for mCons_RFI + VARs and mCons_FCR + VARs using *deeplearning*. The highest ratios were obtained for mCons_RFI + VARs (RMSE=0.07, r^2=0.62) using RF and mRFI_RFI using *xgboost* (RMSE=0.10, r^2=0.86). In addition, the highest correlation between the actual and predicted values was obtained in the mCons_FCR scenario using xgboost (r^2=0.93). On the other hand, the lowest correlation in the best model was obtained in the mCons_RFI scenario using deeplearning (r^2=0.37). These results are being compiled in a manuscript (Fonseca et al., in preparation).

Table 15: Mean (±standard deviation) for the root mean squared error (RMSE) and mean squared Pearson correlation (r^2) obtained in the 100 rounds of random assignment of ewes in training and test datasets for each machine learning model to predict residual feed intake (RFI) and feed conversion ratio (FCR).

Prediction*	*deepleaning*		*RF*		*xgboost*	
	RMSE	r^2	RMSE	r^2	RMSE	r^2
mRFI_RFI	0.20±0.05	0.18±0.22	0.17±0.03	0.14±0.16	0.18±0.03	0.16±0.20
mFCR_FCR	0.35±0.10	0.14±0.18	0.31±0.07	0.15±0.17	0.35±0.07	0.14±0.17
mCons_RFI	0.19±0.08	0.16±0.19	0.17±0.03	0.17±0.21	0.18±0.02	0.16±0.16
mCons_FCR	0.35±0.11	0.18±0.20	0.31±0.08	0.14±0.17	0.34±0.08	0.17±0.21
mRFI_RFI + VARs	0.25±0.15	0.18±0.22	0.17±0.03	0.15±0.16	0.17± 0.03	0.11±0.15
mFCR_FCR + VARs	0.59±0.41	0.19±0.20	0.31±0.09	0.14±0.14	0.35± 0.07	0.18±0.20
mCons_RFI + VARs	0.24±0.08	0.20±0.23	0.18±0.04	0.17±0.19	0.18± 0.03	0.18±0.20
mCons_FCR + VARs	0.44±0.13	0.20±0.22	0.30±0.08	0.13±0.15	0.33± 0.08	0.15±0.20

*mRFI_RFI: Mean methylation within DRMs identified comparing RFI groups used to predict RFI; mFCR_FCR: Mean methylation within DRMs identified comparing FCR groups used to predict FCR; mCons_RFI: Mean methylation within DRMs identified comparing Consensus groups used to predict RFI; mCons_FCR: Mean methylation within DRMs identified comparing Consensus groups used to predict FCR; mRFI_RFI + SNPs: Mean methylation within DRMs identified comparing RFI groups plus SNPs within the same DMRs used to predict RFI; mFCR_FCR + SNPs: Mean methylation within DRMs identified comparing FCR groups plus SNPs within the same DMRs used to predict FCR; mCons_RFI + SNPs: Mean methylation within DRMs identified comparing Consensus groups plus SNPs within the same DMRs used to predict RFI; mCons_FCR + SNPs: Mean methylation within DRMs identified comparing Consensus groups plus SNPs within the same DMRs used to predict FCR.

3. Greece (Univ Thessaloniki)

First experiment

Association of body composition traits measured with ultrasonography as potential predictors of negative energy balance

Significant (P<0.05) optimal cutoffs for changes in body composition traits as predictors of negative energy balance status are presented in Table 16. Results showed that a NEFA status of more than 0.3

mmol/L could be predicted by a decrease in BFT and the sum of BFT and LDT of more than 0.075 mm and 0.350 mm, respectively. Likewise, a NEFA status of more than 0.7 mmol/L could be predicted by a decrease in BFT in LDT and their total sum of more than 0.15 mm, 0.065 mm, and 0.350 mm, respectively. No significant (P>0.05) cutoffs for predicting elevated BHB status were reported.

Table 16: Optimal cutoffs for body composition traits measured with ultrasonography to predict negative energy balance status (elevated non-esterified fatty acids (NEFA) status).

NEFA status	Trait	Cutoff	AUC	95% CI	P-value	Se	Sp
NEFA (≥0.3 mmol/L)	ΔBFT (mm)	-0.075	0.664	0.591-0.736	<0.001	81.3	52.3
	ΔTotal (mm)	-0.350	0.622	0.550-0.694	0.001	75.0	45.8
NEFA (≥0.7 mmol/L)	ΔBFT (mm)	-0.150	0.649	0.544-0.753	0.003	80.9	51.2
	ΔLDT (mm)	-0.065	0.611	0.514-0.708	0.021	61.3	63.4
	ΔTotal (mm)	-0.350	0.643	0.547-0.739	0.004	70.1	56.1

AUC=area under the curve; Se=sensitivity; Sp=specificity; ΔBFT=change in back fat thickness; ΔLDT=change in *longissimus dorsi* muscle thickness; ΔTotal=change in the overall combined values of back fat and *longissimus dorsi* muscle thickness.

Association of blood biomarkers as potential predictors of fat and muscle reserves and their mobilization

Estimates on the association of studied blood biomarkers with fat and muscle reserves and their mobilization are presented in Table 17. A nominally significant (P<0.05) positive association of serum albumins with LDT was reported. Specifically, a change of serum albumins by 1 g/dL was associated with a change in LDT by 1.27 mm. Such an association could be explained by the fact that serum albumins and LDT are both deposits of amino acids in the muscle tissues. However, this association did not maintain significance after Holm-Bonferroni correction (P>0.004).

Table 17: Association of blood biomarkers with fat and muscle reserves and their mobilization.

Trait	Blood biomarker	Estimate (β)	SE	P-value
BFT (mm)	NEFA (mmol/L)	-0.089	0.105	0.402
	BHB (mmol/L)	-0.013	0.586	0.819
ΔBFT (mm)	NEFA (mmol/L)	-0.186	0.127	0.142
	BHB (mmol/L)	-0.041	0.047	0.391
LDT (mm)	NEFA (mmol/L)	0.641	0.504	0.205
	BHB (mmol/L)	0.216	0.216	0.321
	TP (g/dL)	-0.309	0.158	0.052
	Alb (g/dL)	1.271	0.501	0.012
	BUN (mg/dL)	0.015	0.018	0.403
ΔLDT (mm)	NEFA (mmol/L)	0.538	0.546	0.326
	BHB (mmol/L)	-0.102	0.273	0.711
	TP (g/dL)	-0.067	0.117	0.567
	Alb (g/dL)	-0.053	0.280	0.851
	BUN (mg/dL)	0.014	0.016	0.395

BFT= back fat thickness; ΔBFT= change in back fat thickness; LDT= *longissimus dorsi* muscle thickness; ΔLDT= change in *longissimus dorsi* muscle thickness; NEFA= non-esterified fatty acids; BHB= β-hydroxybutyrate; TP= total proteins; Alb= albumins; BUN= urea nitrogen.

Conclusions

Results obtained in the present study suggest that changes in body composition traits measured with ultrasonography, specifically the decrease in backfat and *longissimus dorsi* muscle thickness, could be used to predict negative energy balance status in non-lactating dairy ewes. Moreover, there was an association between the concentration of serum albumins and muscle reserves, although it was not statistically significant (after Holm-Bonferroni correction). Further research with a higher sample size could help to further investigate the latter association.

Second experiment (AUTH)

Quality control of production records from the second experiment was implemented based on biological limits set to reflect the real productive profile and capacity of the studied breeds. These limits set 6 records of milk protein content (protein content less than 3%) and 9 records of daily milk yield (less than 0.2 kg according to ICAR recommendations) as missing values. Moreover, 18 records of milk composition traits were not estimable and were also set as missing values. Finally, given that feed efficiency records were significantly skewed, the trait was logarithmically transformed (natural log), and two outliers were removed to ensure normality of distribution. Descriptive statistics of the edited data are shown in Table 18.

Table 18: Descriptive statistics of recorded data after quality control.

Trait	N	Mean	SD	min	max
Daily milk yield (kg)	183	1.2	0.53	0.2	3.54
Energy corrected milk (kg)	181	1.28	0.55	0.21	3.29
Fat content (%)	181	6.59	1.38	3.02	10.8
Protein content (%)	175	5.35	0.81	3.06	7.52
Lactose content (%)	181	4.67	0.35	3.61	5.4
SNF content (%)	181	11.62	0.98	6.17	13.88
BCS (1-5)	190	2.89	0.22	2.25	3.5
Daily pellet intake (kg)	190	1.34	0.13	1.13	1.5
Daily hay intake (kg)	190	1.13	0.23	0.83	1.73
Daily straw intake (kg)	190	0.3	0	0.3	0.3
Energy intake (UFL)	190	1.82	0.18	1.59	2.24
Feed efficiency	181	0.71	0.31	0.12	1.97
Feed efficiency (ln)	179	-0.43	0.44	-1.64	0.68

SNF= solids-non-fat; BCS= body condition score

Estimates on the association of milk composition traits with feed efficiency are presented in Table 19. Statistically significant positive associations ($P < 0.05$) were reported with fat and lactose content. Specifically, one unit increase of fat and lactose content was associated with an increase in feed efficiency by 8.7% and 21.7%, respectively.

Table 19. Association of milk composition traits with feed efficiency on the natural log scale.

Trait	Estimate (β)	SE	P-value
Fat content	0.083	0.020	0.0004
Protein content	0.069	0.050	0.1679

Lactose content	0.196	0.080	0.0146
SNF content	0.0001	0.027	0.9965

SNF= solids-non-fat

Conclusions

Results obtained in the present study suggest that Chios dairy ewes with higher milk fat and lactose contents utilize energy intake more efficiently. In this regard, these milk composition traits could be used by farmers as an easy, non-invasive way for selecting towards increased feed efficiency. Further, research with higher sample size and individual animal housing for precise feed intake recording could help to further support the findings of our study.

4. France (Races de France)

Main results regarding the Lacaune breed has been published in Machefert et al., 2023.

Zootechnical performances

The average zootechnical performances (milk and mating performances), as well as the feeding, are summarized in Table 20 per breed, distinguishing between primiparous (L1) and multiparous in 2nd lactation (L2). On average, 3.9 milk test-days were recorded for ewes from the Western Pyrenean breeds and 5.2 for Lacaune ewes, with most Lacaune ewes having 6 milk recordings.

Table 20: Average and variability of zootechnical performances according to breed for dairy ewes in parity 1 (L1) and 2 (L2) phenotyped in Smarter in 2019-2020

	Lacaune		**Manech Tête Rousse**		**Manech Tête Noire**		**Basco-Béarnaise**	
Parity	L1	L2	L1	L2	L1	L2	L1	L2
Number of ewes	1,119	892	203	152	125	104	197	128
% of the herd	31	21	33	17	37	17	24	18
Total milk production of the lactation (L)	309	383	238	253	196	197	127	199
Milk production at the fisrt milk recording (L)	2.45	2.97	1.98	2.31	1.77	2.07	1.97	2.19
Lactation length (d)	170	187	179	182	155	147	106	148
Fertility at animal insemination (%)	76	75	84	76	47	50	75	53
Prolificity at animal insemination	1.50	1.55	1.46	1.39	1.29	1.53	1.23	1.35
Dry matter intake at the first milk recording (kg)	2.76	2.76	2.30	2.30	2.32	2.33	2.23	2.23

Feed effi-ciency by net energy intake con-verted in milk ratio

Because some data are missing, we evaluated the ability to approximate different components of feed efficiency: BCS, forage intake, and standardized milk production. From our tests (Appendix 5), we identified that the phenotypes contributing the most to the calculation of individual feed efficiency

were, in decreasing order of importance: 1) standardized milk production, 2) forage intake, 3) body condition scores, and 4) weights.

The results of feed efficiency estimated by the NEICMR were on average higher in the Roquefort area, i.e., for the Lacaune breed. Results are detailed in Appendix 6 according to the stage of lactation.

In the Lacaune breed, most animals have a result of feed efficiency lower than 1 (Figure 18). Feed efficiency seems to decrease during lactation. Two phases can be observed: the first one from lactation stage 2 to 5 where feed efficiency results are mainly between 0.75 to 1.13, then from lactation stage 6 where results decrease more from 0.6 to 0.75. The low feed efficiency results from lactation stage 6 onwards could be explained by the integration of grazing in the rations for all Lacaune farms. Grazing may start earlier in lactation, but the ewes will be grazing from the 6th month of lactation. Also, the greater variability of results above 1.25 could be explained by the integration of grazing in the ration, which introduces an additional bias in estimating individual feed consumption on pasture.

In the Pyrenean breed, the majority of females have a feed efficiency value lower than 1. In the Manech Tête Rousse breed, we observe the opposite phenomenon to the Lacaune ewes, where feed efficiency seems to increase during lactation. The low numbers must be taken into account when interpreting the results for BB and MTN breeds.

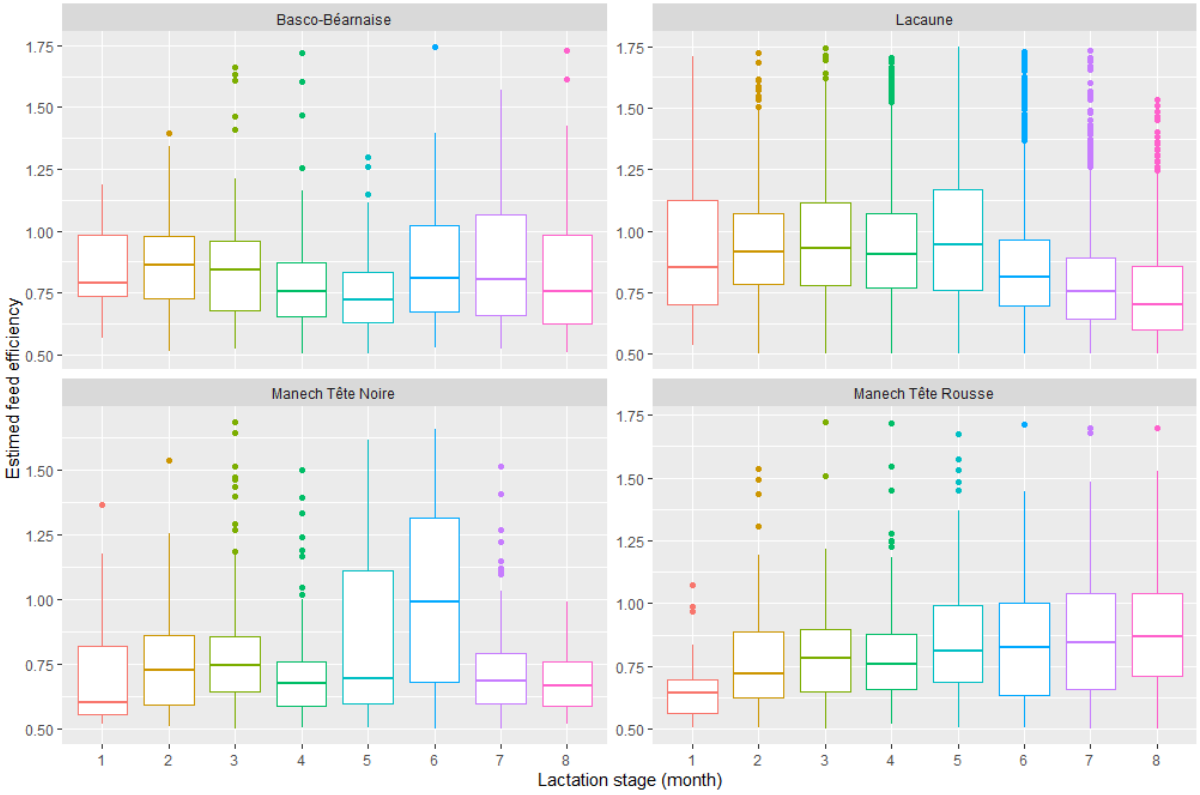


Figure 18: Estimated feed efficiency according to the stage of lactation (months since lambing) and according to the breed of dairy ewes

Correlations between two successive lactation stages range from 0.24 to 0.60 for Lacaune ewes and 0.17 to 0.63 for Western Pyrenean ewes (Table 21). Correlations are weaker between feed efficiency scores from more distant stages of lactation. However, we observe the strongest correlations between lactation stages 3, 4, 5, and 6 in the Lacaune breed and lactation stages 2, 3, 4, and 5 in the Pyrenean breeds.

Table 21: Correlation between feed efficiency results calculated from the net energy intake converted in milk ratio, between stages of lactation (in months) for Lacaune ewes (above the diagonal) and Western Pyrenean area (under the diagonal)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8
Month 1		0.36	0.31	NS	NS	NS	NS	.
Month 2	0.63		0.42	0.28	0.20	0.24	0.28	0.42
Month 3	0.40	0.50		0.60	0.51	0.51	0.09	0.15
Month 4	NS	0.40	0.24		0.44	0.50	0.12	NS
Month 5	0.37	0.35	0.31	0.44		0.47	0.10	NS
Month 6	0.58	0.21	0.22	NS	0.47		0.24	0.16
Month 7	NS	NS	0.14	0.21	0.17	0.17		0.37
Month 8	.	NS	NS	NS	NS	NS	0.26	

NS : Not Significant

Correlations between the estimated feed efficiency results and the milk performances (daily milk production, lactation milk yield, fat content, protein content, somatic cell count, urea, lactation length and persistence) for the Lacaune ewes are low (from -0.18 to 0.26) except for the daily milk production where the correlation is 0.5.

The Pearson correlation between the net energy coverage rate per day and the estimated feed efficiency is -0.93. The relationship between both variables shows that less efficient ewes tend to have a coverage rate above 100%. These ewes would ingest more feed than necessary to cover their maintenance and lactation needs. Ewes that can be qualified as efficient have a coverage rate lower than 100%, so the energy intake of the ration is insufficient to cover their needs. A ewe with a feed efficiency of 1 will have a coverage rate of 100%. The range of coverage data for a ewe with low efficiency is higher than for ewes with an efficiency of more than 1. However, it should be taken into account that there are fewer ewes with an efficiency greater than 1.

BCS

The variability of BCS is described in Table 22. BCS in Lacaune breed ranged from 2 to 4 with similar standard deviations between physiological stages. The variability of BCS was lower in the Pyrenean breeds than in Lacaune, with a lower level of body condition (-0.25 to -0.5 BCS points). The same observations were made for all Pyrenean breeds.

Table 22: Mean, standard deviation (sd), minimum, maximum, and coefficient of variation (CV) of BCS by area

Breeds	Variables	BCS before lambing	BCS at suckling	BCS at first milk test-day	BCS before mating	BCS after mating
Lacaune	Mean	3.08	2.76	2.79	2.88	2.94
	Sd	0.28	0.31	0.34	0.25	0.26
	Minimum	2	2	2	2	2
	Maximum	4	3.75	3.75	3.75	3.5
	CV	9	11	12	9	9
Pyrenean breeds	Mean	2.45	2.45	2.4	2.57	2.63
	Sd	0.22	0.22	0.21	0.21	0.21
	Minimum	1.75	1.75	2	1.75	2.25
	Maximum	3	3	3	3	3
	CV	9	9	9	8	8

Correlations between successive BCS were positive and high between the first three BCSs collected at the beginning of lactation (from -30 to +80 days) and between the last two BCSs (± 1 month around the mating time) (Figure 19). The lower correlation between the BCS at first milk test-day and the one before mating was related to the higher time interval (134 days on average) between these two time-points. The closer the BCS measurements are in time, the less possibility of BCS evolution.

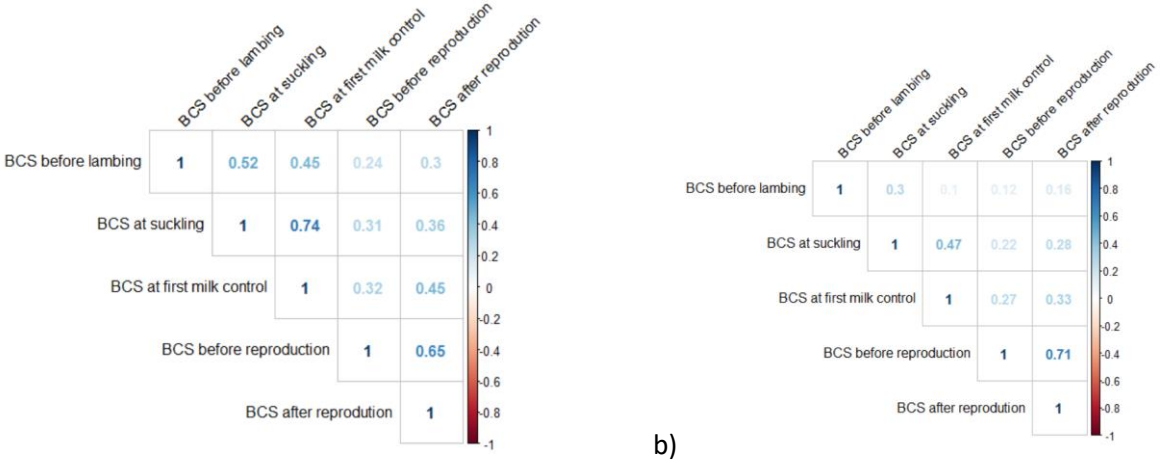


Figure 19: Correlations between body condition scores collected in the 2019-2020 dairy year in Lacaune breed (a) and Pyrenean dairy sheep breeds (b)

Factors influencing BCS at each physiological stage

Factors influencing body condition scores are reported in Table 23. The models perform better in the Lacaune and Manech Tête Rousse breeds. The coefficients of determination of the five linear models are high (R^2 = 0.46 to 0.77 for the Lacaune breed and 0.50 to 0.67 for the Manech Tête Rousse breed).

Looking digressively at the coefficient of determination of each model, the two factors that seem to have the most influence on the body score condition are the sire and the farm.

Table 23: Factors affecting BCS at each physiological stage in Lacaune breed and Manech Tête Rousse (MTR) breeds

Variables	BCS before lambing model		BCS at suckling model		BCS at first milk control model		BCS before mating model		BCS after mating model	
	Lacaune	MTR	Lacaune	MTR	Lacaune	MTR	Lacaune	MTR	Lacaune	MTR
Farm	*	**	***	NS	***	.	***	***	***	***
Lactation rank	***	NS	.	**	NS	**	***	.	***	NS
Litter size	.	*	***	**	***	***	***	NS	***	**
Day of measurement	*	NS	***	NS	NS	NS	***	*	NS	*
Month of lambing	***	*	***	NS	**	NS	***	***	NS	NS
Sire	NS	NS	***	NS	***	NS	***	***	***	NS
Corrected milk yield					***	**	***	***	***	***
Corrected FC					***	NS	***	NS	***	NS
Corrected PC					NS	NS	NS	NS	NS	NS

**** : p-value ≤ 0.001 ; ** : p-value ≤ 0.01 ; * : p-value ≤ 0.05 ; NS : Not Significant*

BCS: Body condition score ; MTR: Manech Tête Rousse ; FC: fat content ; PC: protein content

Lacaune dairy ewes with multiple lambs had significantly lower BCS at each stage of the production cycle. These differences were of the order of 0.2 BCS points between singles and multiples. The largest differences of BCS were found in the 2nd BCS measurement when ewes were about to start milk production (e.i. when the lambs were suckling), and at the peak of lactation at the first milk recording. The same tendency was observed for the Pyrenean breeds; however, the differences in BCS were lower and were only significant for the first three BCS.

Parity had a significant influence on the BCS throughout the production cycle of the dairy ewes. In the Lacaune breed, primiparous ewes have a higher BCS than multiparous ewes before lambing and have a lower BCS at the end of lactation, near mating, than multiparous ewes. At the beginning of lactation, there was no significant difference in the BCS of the multiparous animals between parity. However, there were significant differences in BCS between ewes in 2nd and 4th lactations and more before lambing and around mating time. For the Pyrenean breeds, ewes in 2nd lactation also have a significantly lower body condition than primiparous ewes before lambing for all three breeds, at the beginning of lactation for MTR and BB ewes, before mating for MTN, and after mating for MTR ewes.

The day of the measurement had a significant effect on the BCS at suckling and before mating in the Lacaune breed. The closer to lambing (day 0), the higher the BCS at suckling ([0; 10 d]: BCS = 3.18; [20; 30 d], BCS = 3). For the BCS before mating, the closer to mating (210 d), the higher it was ([150 ; 170 d] : BCS = 3.21 ; [190 ; 210 d], BCS = 3.27). For the three breeds of Pyrenean dairy ewes, the day of the measurement did not significantly affect BCS during the production cycle.

Effect of sire on BCS:
The correlations between successive BCS solutions for sire are significantly positive and high (Table 24). The father thus seems to have the same impact on two successive BCS.

Table 24: Pearson correlations between sire effect solutions for successive BCSs for Lacaune breed.

	BCS 1 – BCS 2	**BCS 2 – BCS 3**	**BCS 3 – BCS 4**	**BCS 4 – BCS 5**
Pearson correlation	0.54	0.82	0.20	0.64

BCS 1 = BCS before lambing, BCS 2 = BCS at suckling, BCS 3 = BCS at 1st milk control, BCS 4 = BCS before mating, BCS 5 = BCS after mating. Correlations are significant (p-value < 0.05)

Changes in body condition scores by physiological stage
Model 2, highlights a significant effect of physiological stage on BCS (p-value < 0.05) (Table 25). After Tukey's adjustment, we observe that physiological stage conditions are significantly different for Lacaune ewes. The contrast between BCSs is significantly different between successive and non-successive BCS.

For the Pyrenean breeds, some BCS are not significantly different: in Manech Tête Rousse, the difference of BCS is not significant between the BCS at suckling and the BCS at 1st milk control, also between the BCS before lambing and the BCS before mating. In the Basco-Béarnaise breed, the first three BCS are not significantly different and the last two BCS around mating. In the Manech Tête Noire breed, the first three BCS are not significantly different.

For the other variables in the model, all fixed effects are significant (p-value < 0.01 for the adjusted standardized fat content and p-value < 0.001 for other factors), except for the adjusted standardized protein content that is not significant.

Table 25: Value of estimated adjusted mean body condition scores by physiological stage and breed (emmeans package from R)

	BCS before lambing model	BCS at suckling model	BCS at first milk control model	BCS before mating model	BCS after mating model
Lacaune	3.01	2.70	2.74	2.83	2.90
Manech Tête Rousse	2.66	2.50	2.46	2.68	2.74
Manech Tête Noire	2.35	2.35	2.40	2.60	2.76
Basco-Béarnaise	2.35	2.31	2.34	2.59	2.68

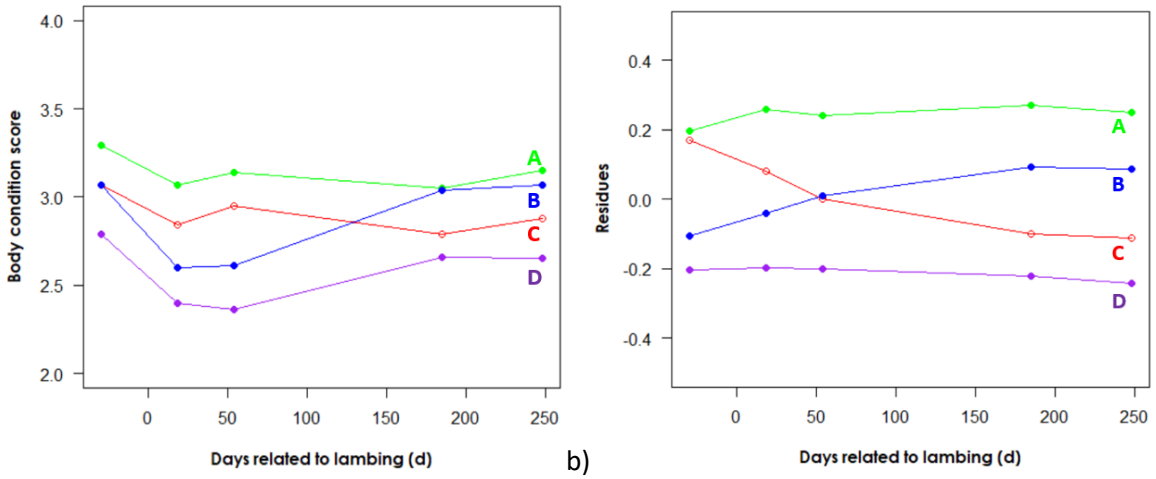
The animal variance (V_A = 0.01) is lower than the residual variance (V_R = 0.05). The $V_A/(V_A + V_R)$ ratio (0.2) represents the repeatability of the BCS i.e, a maximum limit for the heritability for this trait.

Typology of body condition profiles

The BCS profiles were studied within each breed. The results of the typology is presented here for the Lacaune breed. Based on the evolution of the three partitioning quality criteria presented in the kml package, four clusters were chosen to characterize the BCS profiles of Lacaune dairy ewes.

Figure 20a presents the average BCS profile of each cluster. BCS curve A (n=1,011), has the highest and most consistent BCS level over time. The curves B (n=743) and C (n=1,123) start with a pre-lambing BCS of 3, and then a 0.25 BCS point gap widens in early lactation between both curves. After suckling, ewes in profile B rebuild their body reserves until they reach the same BCS as profile A, and ewes in profile C decrease in BCS. Profile D (n=720) is close to profile B with a lower body condition level.

These four body condition kinetics in the Lacaune breed are mostly characterized by the herd. The two main groups of profiles (A/C and B/D) distinguished the two Lacaune breeding organizations. Thus we decided to correct the BCS for herd effect. Kml analysis based on these corrected BCS also reveals four BCS profile clusters (Figure 20b). Similar kinetics to the initial one is observed with two profiles (A, D) showing a strong difference in BCS level as well as a profile of ewes that replenish (B) or mobilize (C) their body reserves.



a) b)

Figure 20: Partitioning of body condition profiles of dairy ewes into four clusters based on initial BCS (a) and BCS corrected for herd effect (b) (Lacaune breed)

The correction for herd effect had an impact on the classification of ewes within clusters. About 20% of the ewes initially in clusters A (highest level of BCS) and B (reconstitution profile) remained in these same clusters. Cluster C (mobilization profile) underwent the most reclassification (91%), in contrast to cluster D (lowest level of BCS) where 55% of the ewes came from the initial cluster D and none from the opposite cluster (A).

In order to characterize each cluster, a logistic regression (model 3) was performed with the corrected cluster identification as the dependant variable. Each effect of the model was found to be significant in explaining the typology of BCS profiles in the different clusters: Parity was the factor with the largest impact on the partitioning of BCS profiles, followed by milk persistency and duration corrected milk yield. Litter size has the least impact on the classification.

Cluster A is mostly composed of mature ewes and, more specifically, of ewes having completed 3 or more lactations (68%) with single litter size (61%) and lower milk performance (lower average milk production and milk persistency qualified as low to medium) (Table 26). Cluster B is mostly composed of mature ewes (88%), more dairy (295 L on average), but with a milk persistency that can be limited. The ewes in cluster C are mostly primiparous, with on average a high level of milk production and of milk persistency. Cluster D is composed mostly of ewes with multiple lambs (74%) and a higher level of milk production (309 L).

Table 26: Composition of cluster based on BCS corrected for the herd effect, with the percentage of intra-cluster modalities (Lacaune breed)

Factors	Conditions	A	B	C	D
Lactation rank	L1	15 %	12 %	48 %	24 %
	L2	17 %	30 %	14 %	27 %
	L3	25 %	21 %	14 %	14 %
	L4 and more	43 %	35 %	24 %	36 %
Litter size	Single	61 %	46 %	50 %	26 %
	Multiple	39 %	54 %	50 %	74 %
Dairy performances	Duration corrected milk yield (L)	276	295	295	309
	Persistency	-		+	

Ewes from clusters A and D had significantly different feed efficiency levels with average values of 0.86 and 0.93 UFL/d, respectively. The average feed efficiency of ewes from clusters B and C were similar 0.89 UFL/d). Almost no significant correlation between feed efficiency and BCS was obtained, and the highest correlation (0.20) was observed after mating.

5.3 Main results in dairy sheep

About 160 dairy ewes have been phenotyped in experimental facilities, and more than 5,000 dairy ewes have been phenotyped in commercial farms.

Total individual feed intake was available for 88 lactating ewes from experimental designs. In commercial farms, only partial knowledge of feed intake was available: in some farms, individual concentrate intake was recorded and forage intake at the pen level. Because the group of feed intake masks individual variability in feed intake, the main results must be confirmed with individual records.

However, the study performed in Lacaune ewes highlighted that milk production and forage intake were the two factors influencing feed efficiency the most: considering average values for both traits led to major reclassifications of the ewes. In Chios sheep, based on group feed intake, they suggest that milk fat and lactose contents are indicators of energy intake efficiency, with lactating ewes having higher values being more efficient. In the Assaf experimental population, milk fatty acids composition has been significantly associated with feed efficiency criteria (FCR and RFI), and they have been highly performant in predicting extreme feed efficiency indexes. Body condition scores have been the most recorded traits in dairy sheep. If no significant link has been highlighted with feed efficiency traits, it is worth noticing that feed efficiency levels were different in different clusters of ewes defined based on BCS. Variations in body composition traits assessed by ultrasound are proposed to be considered as a

proxy for negative energy balance status in non-lactating ewes. These results have to be confirmed, particularly in lactating ewes.

In conclusion, in dairy sheep, feed efficiency proxies are likely to be linked with milk fatty acid composition and ewes body composition and body composition scores.

6 Dairy Goats

6.1 Materials and Methods

6.1.1 Experimental designs

1. France (INRAE)

In Bourges INRAE experimental farm, the protocol to phenotype feed intake related traits (including proxies), body condition traits, and milk-related traits in goats belonging to divergent lines on longevity has been designed for Alpine goats.

A total of 199 dairy goats belonging to the high and low functional lines were produced with phenotyping of longevity. The analysis of survival in the full design (including also previously created animals) showed that the overall survival of high_longevity goats was significantly better than low_longevity goats (hazard ratio of culling/death = 0.63, confidence interval = 0.47; 0.86). Results were published in Ithurbide et al., J. Dairy Sci. 2022, in the frame of SMARTER. So the total number of 180 goat expected is achieved.

Out of the 199 dairy goats created, 80 were expected to undergo challenges in WP3 in first lactation (as described in DoA) and the same animals were expected to be monitored for feed efficiency. However, we achieved to monitor 143 goats for feed efficiency in 3 years, with the useful phenotypes being: individual intake of concentrates, weight and milk production in order to analyse residual feed intake (RFI).

In 2019, 49 goats from these lines were born in January and mated in august 2019, and they were lambing in January 2020. In 2020 and 2021, two cohorts of primiparous goats (N=47 and 47 in 2020 and 2021, respectively) belonging to longevity divergent lines were created.

Goats born in 2019, 2020 and 2021 were monitored daily for individual concentrate intake using the INRAE feeder, for feed intake-related traits (including proxies), for body condition traits, and for milk-related traits on several periods during their first lactation.

6.1.2 On-farm designs

1. France (CapGènes)

Animals and phenotypes

The experiment was performed in 14 commercial farms and in the INRAE Experimental Farm of La Sapinière, between 2019 and 2021. The data sampling is not finished yet for campaign 2. A total of

6,124 (2,582 Alpine and 3,180 Saanen) dairy goats were phenotyped for feed efficiency and feeding behaviour traits (Table 27).

Table 27: Status of data sampling in the 13 commercials farms and La Sapiniere during the two campaigns of sampling, and the actual number of animals recorded. Farms were classified into four main breeding/feeding systemsC: conventional, O: organic, P: pasture/indoor, G: green feeding. Feed intakes were differently recorded: individual concentrate intakes from automatic feeder (AF) or Manually delivered (M) in milking parlour, or at the level of the batch (B)B.

		Number of animals collected per breed	
Farm	Feed recording	Alpine	Saanen
Farm 1 (C)	AF	66	123
Farm 2 (C)	B	965	0
Farm 3 (C)	B	0	876
Farm 4 (C)	B	0	545
Farm 5 (C)	M	0	528
Farm 6 (C)	B	524	0
Farm 7 (C)	B	144	0
Farm 8 (C)	B	0	268
Farm 9 (O/G)	M	0	742
Farm 10 (P)	AF	276	0
Farm 11 (C)	M	95	10
Farm 12 (C)	B	232	88
Farm 13 (C)	M	48	0
Farm 14 (G)	AF	111	0
La Sapinière (C)	AF	121	0
Total		2,582	3,180

Feed intake was usually recorded four times during the lactation (Figure 21): at the beginning of the lactation (feed intake 1, between 0 and 60 days in milk (DIM) and feed intake 2, between 60 and 90 DIM), around reproduction (feed intake 3, between 210 and 260 DIM) and at the end of the lactation (feed intake 4, between 240 and 280 DIM). A total of 29,437 records (12,250 and 17,187 for Alpine and Saanen, respectively) were recorded.

Animals were fed with different forages and concentrates, depending on the breeder. On each test day, feed intake was determined by weighing the total ration distributed and that wasted by trained staff from the milk recording organisms. The forage quantity was measured by weighing all the offered forage, with a scale, at the batch or farm level (not individually). For concentrates, the quantity was measured either individually with automatic feeders or manually in the milking parlour or at the batch level by weighing all the offered concentrates, depending on the farm. Thus, for farms without individual distribution of concentrates, the individual feed intake was the average feed intake of the

batch to which the animal belongs (83% of the dataset). For farms with individual distribution of concentrate, the individual feed intake was the average feed intake of the batch to which the animal belongs for forage, plus the individual intake of concentrate (17%). Dry matter intake (DMI) was thus estimated from the information indicated on the concentrate labels and from forage analysis, for each animal and each test day. Energy Intake (EI) was estimated by multiplying DIM and energy concentration. Nutritional feed quality was recorded for each forage and each concentrate, and energy content was given by INRAE (Agabriel, 2010). Test day milk recording data (milk yield, fat, and protein contents) were also measured at the same time as the feed intake control.

The chest width (CW) was used as a proxy of the body weight and was measured one time during the lactation (about 150 DIM), on primiparous goats during campaign 1 and on the primiparous and multiparous goats during campaign 2.

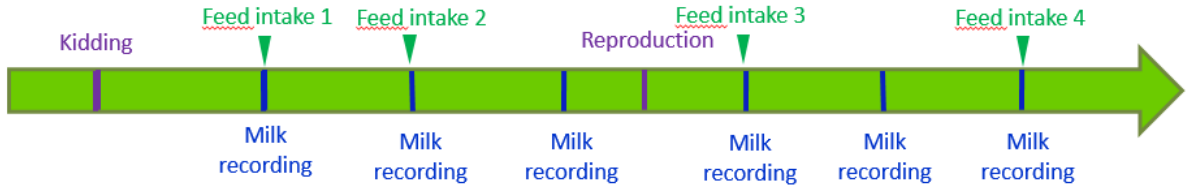


Figure 21: Sampling of food intake during a complete lactation.

Blood β-hydroxybutyrate (BHB) measurements were performed in one commercial farm during campaign 1 (n=415 measurements) and in three other commercial farms during campaign 2 (n=1,180 measurements), on primiparous goats only. Additional 485 measurements were also performed in the INRAE La Sapiniere farm. A total of 2,080 measurements were obtained from 534 primiparous goats of Alpine and Saanen breeds.Blood BHB is a proxy to monitor extensive mobilization of body reserves and intense ketone body synthesis leading to metabolic disorders (ketosis). The gold standard measure is a laboratory dosage of serum after blood centrifugation. This time-consuming method prevents large-scale measurement and genetic parameter estimation. Data from INRAE experimental farms were used to compare a new method using a blood sugar/ketone meter (Abbott Freestyle Optium NeoH) with gold-standard laboratory measurements. Results provided good correlations in meat sheep (r=0.76; N=48) and dairy sheep (r=0.58; N=48) between online and laboratory methods. A protocol was drafted and sent to French partners (Capgenes, Race De France) to implement large-scale monitoring on-farm with the ketone meter for genetic analyses. This BHB measurement protocol is presented in appendix 7.

For all the farms, the cartilage or blood sampling was complete: 1,768 samples were collected. A total of 1,464 goats have been genotyped with the 50K chip (1,082 funded by the SMARTER project).

The selection of the goats to be genotyped was made with the following criteria: primiparous, pure breed, known parents, with valid and complete phenotypes (milk recording, feed intake, chest width).

Database constitution

Some filtration criteria were applied to the initial database to have a clean database (Table 28):

- Breed: goats had to be purebred of Alpine or Saanen breeds,
- CW: goats had to have a chest width measurement,
- CaL: the difference between the day of feed intake measure and test day milk had to be inferior or equal to 7 days,
- LATOCA: filtration on extreme values for milk, fat and protein yields, fat and protein contents,
- DM: 2 kg <Dry matter intake (DM) <3.85 kg, goats with higher refused quantity were eliminated,
- LL: goats in long lactation were eliminated.

Table 28: Number of animals per farm after each filter criteria.

Filters:	No filter	Breed	CW	CaL	LATOCA	DM	LL
Farm 1	245	183	52	51	49	43	41
Farm 2	930	928	665	664	649	649	621
Farm 3	876	876	405	405	405	405	367
Farm 4	574	563	386	370	369	369	355
Farm 5	573	568	215	210	208	208	182
Farm 6	524	524	298	298	295	295	280
Farm 7	145	144	46	45	45	45	40
Farm 8	227	226	72	72	71	71	44
Farm 9	763	733	259	259	256	256	127
Farm 10	275	275	157	155	155	155	155
Farm 11	120	105	42	41	41	41	32
Farm 12	336	320	108	108	108	108	80
Farm 13	48	48	46	46	46	46	46
Farm 14	112	111	31	31	30	30	29
La Sap.	66	65	63	63	62	0	0
Total	5,814	5,669	2,845	2,818	2,789	2,721	2,399

After filtration, 2,399 animals were retained, which is 41% of the initial data. An animal was recorded several times per campaign, maximum 4 times/per campaign, during each feed intake recording. The final database contains 5 880 lines of animal*feed intake records, with an average of 2.45 feed intake records per goat.

Calculation methods for feed efficiency

Feed efficiency was estimated by two methods:

- **Ratio** between product (outcome) and feed intake (expenses):

$$\text{Feed efficiency} = \frac{UFL_{lait}}{Quantité\ Énergie\ Ingérée - UFL_{ent} - UFL_{crois}}$$

According to this model, efficient animals were those with positive values.

- **Residual energy intake (REI)** was estimated as the residual of a linear regression model:

$$DEI\ (UFL) = \beta_0 + \beta_1 \times MY + \beta_2 \times FC + \beta_3 * PC + \beta_4 \times CW + \boldsymbol{REI}$$

Where, DEI is the daily energy intake (expressed in Unité Fourragère Lait unit (UFL)), β_0 is the intercept, β_1 is the regression coefficient for MY (milk yield), β_2 and β_3 are the regression coefficients of FC and PC (fat and protein contents) and β_4 is the regression coefficient for chest width (CW). REI has been estimated for two breeds in commercial flocks: Saanen and Alpine.
According to this model, efficient animals were those with negative REI values.

We classified the animals in 3 groups of REI, using standard deviation (sd_REI): inefficient (REI >0.5 x sd_REI), intermediate (-05 x sd_REI ≤ REI≤ 0.5 X sd_REI), efficient (REI<-0.5 x sd_REI).

2. UK (Yorkshire dairy goats)

A total of 1,146 dairy goats were milked three times per day in the first stage of lactation, which was reduced to twice a day when milk yields decreased. During the first lactation, females were fed ad libitum for the first 150 days, at which point feed was restricted according to milk yield. Animals were fed a digestible fibre-based blended feed. Animals entered a rotary feeding station where their ID tag was read, and feed was allocated. Subsequently leftover feed was weighed and the difference between feed dispensed and leftover was calculated. Only concentrate intake was recorded whereas hay was fed ad libitum and was not recorded. Body weight data was recorded for each animal after every milking using an automated scale.

A univariate, random regression animal model was used with second-order Legendre polynomials for random animal and permanent environment effects. Fixed effects were: year-season of kidding, age at kidding, test day, feeding regime (ad libitum vs restricted feeding), and fixed lactation curves using third-order Legendre polynomials. Each trait was included as a fixed effect for all other traits in the univariate analyses, but not in bivariate analyses used to calculate genetic correlations. An approximation of feed efficiency (FE) was obtained by including MY and BW as covariables.

6.2 Results

6.2.1 INRAE experimental design

Concentrate intakes of dairy goats were recorded through automatic feeders. Results do not show any difference in feed intake raw phenotypes (**Table 29**), number of visits, intake per visit, intake per day between the high and low longevity lines.

Table 29: Average Feed intakes in primiparous dairy goats from two cohorts monitored in 3 different periods with a daily ration of 0.9 Kg concentrate/goat/day.

	Cohort 1 (FI recording in September 2020 during 8 days)		**Cohort 2 (FI recording in March/April 2021 during 50 days)**		**Cohort 2 (FI recording in August 2021 during 50 days)**	
	LGV-	**LGV+**	**LGV-**	**LGV+**	**LGV-**	**LGV+**
N	18	29	20	27	20	27

Nb visits / day	15.8 ±4.1	17.4±7.3	19.9±5.00	19.5±3.4	17.7±3.32	17.4±3.41
Intake per visit (kg)	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01
Intake per day (kg)	0.640±0.12	0.630±0.14	0.820±0.034	0.822±0.028	0.849±0.08	0.865±0.01
Intake duration per day (s)	551±170	678±508	738±170	775±145	641±133	688±132
Intake speed per day (kg/hrs)	4.72±0.75	4.40±1.24	4.74±0.80	4.62±0.53	5.24±0.99	5.17±0.73

FI: Feed Intake; LGV-: divergent line for lower longevity; LGV+: divergent line for longer longevity.

6.2.2 France (on farm design)

Ratio

The feed efficiency values (ratio) ranged from 0.236 to 2.249, with a mean value of 0.91 and a variation coefficient of 25.92% for Alpine breed; and from 0.119 to 2.126, with a mean value of 0.75 and a variation coefficient of 27.38% for the Saanen breed (Table 30).

Table 30: Numbers (n), minimums (Min), means, standard deviations (SD), variation coefficient (CV), and maximums (Max) of feed efficiency (ratio) for both Alpine and Saanen breeds.

Breed	n	Min	E1	Q1	Median	Mean	SD	CV	Q3	E3	Max
Alpine	2919	0.236	0.203	0.754	0.888	0.91	0.24	25.92%	1.041	1.621	2.249
Saanen	2961	0.119	0.134	0.608	0.739	0.75	0.21	27.38%	0.881	1.372	2.126

Feed efficiency (ratio) seems to be decreasing during lactation (Figure 22).

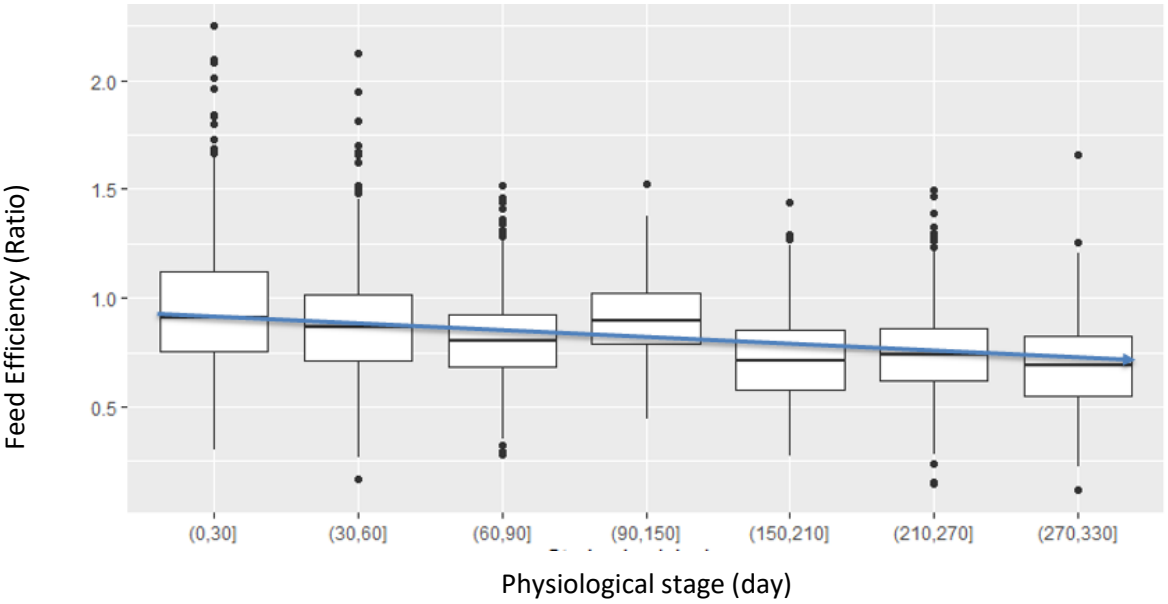


Figure 22: Evolution of feed efficiency (ratio) according to the physiological stage during lactation.

In both breeds, an important variability between farms was observed for feed efficiency (ratio) (Figure 23).

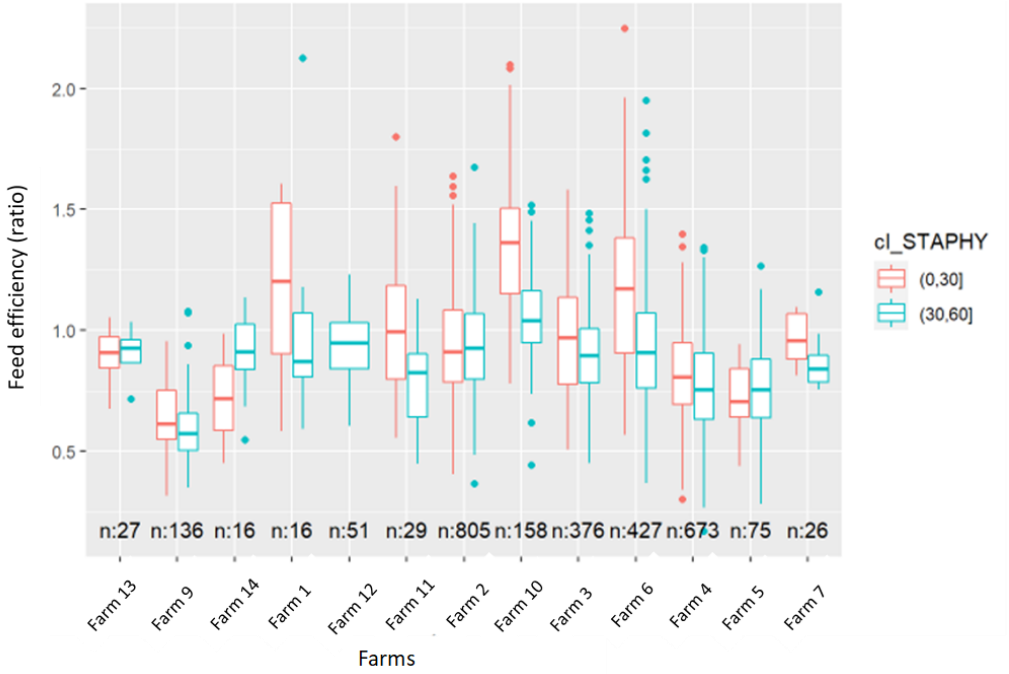


Figure 23: Evolution of feed efficiency (ratio) during the 2 months following kidding (red: (0,30] days and blue: (30-60] days), per farm.

REI

The REI values ranged from -1.04 to 0.95, and from -1.10 to 1.07, for Alpine and Saanen breed respectively, and with a mean value of 0 as they are residuals of multiple linear regression for both breeds (Table 31 and figure 24).

Table 31: Numbers (n), minimums (Min), means, standard deviations (SD), and maximums (Max) of REI for both Alpine and Saanen breeds.

Breed	n	Min	E1	Q1	Median	Mean	SD	Q3	E3	Max
Alpine	2919	-1.04	-0.86	-0.16	0.01	0	0.29	0.17	0.86	0.95
Saanen	2961	-1.10	-0.78	-0.14	-0.01	0	0.26	0.16	0.78	1.07

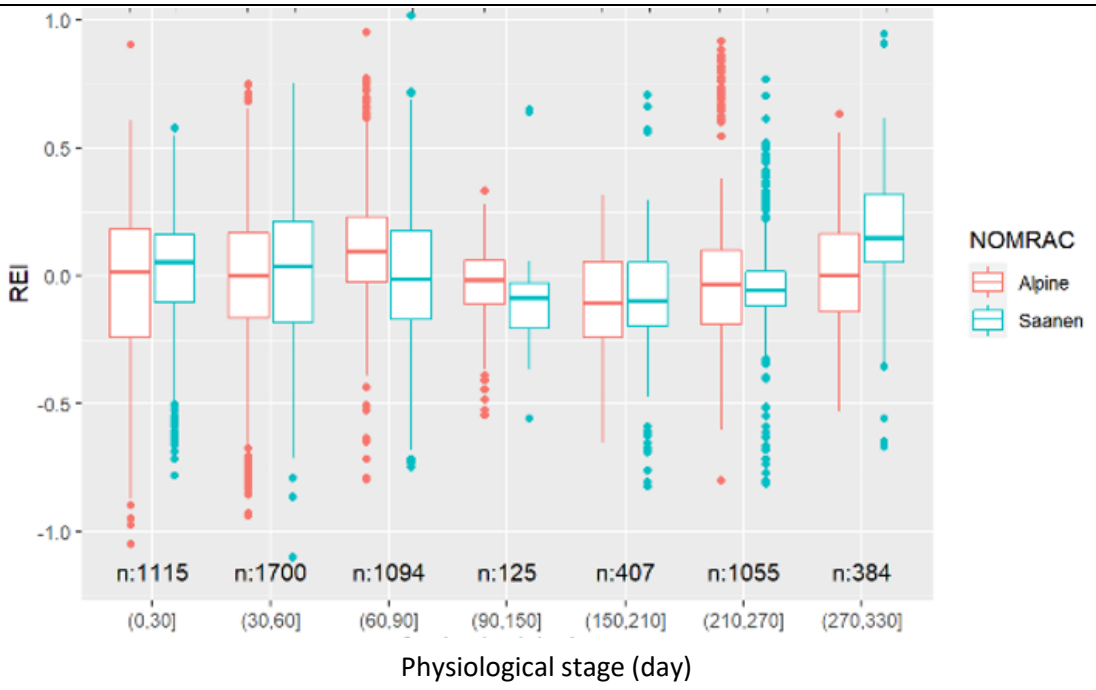


Figure 24: Evolution of REI according to the physiological stage during lactation for Alpine and Saanen breeds.

An important variability between farms was observed for REI in both breeds (Figure 25).

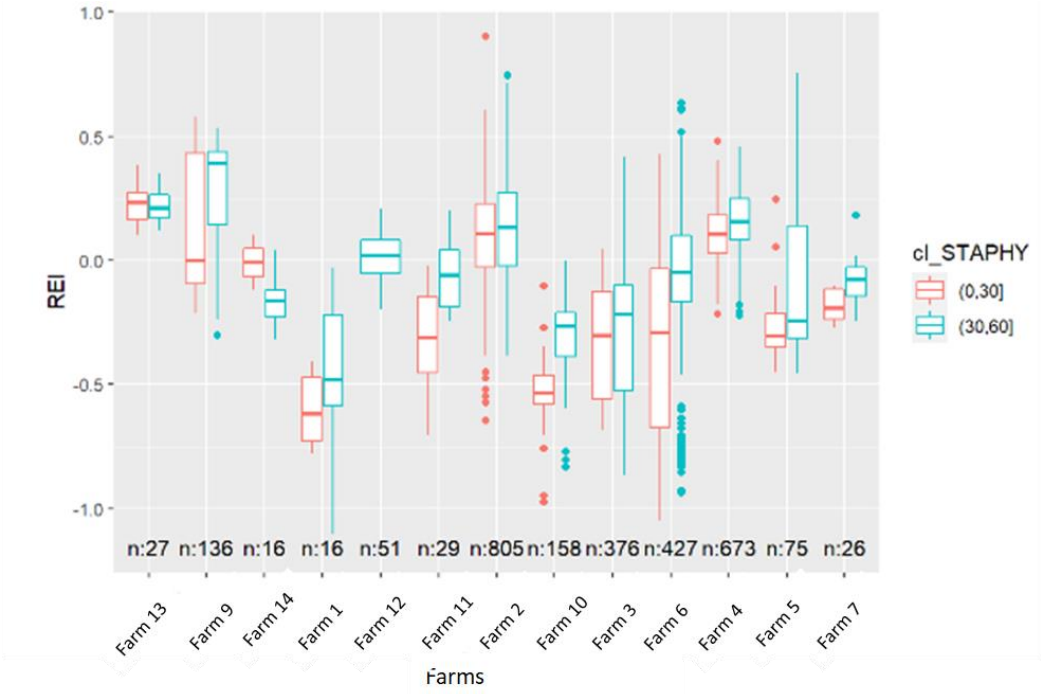


Figure 25: Evolution of REI during the 2 months following kidding (red: (0, 30] days and blue: (30, 60] days), per farm.

Goats were divided into 3 REI groups: efficient, intermediate, and inefficient (Table 32). The efficient group has lower dry matter intake (DMI) and net energy intake (NEI) values than the inefficient group. No difference was observed between the 3 groups for milk yield (MY), fat (FC), and protein (PC) contents.

Table 32: Number of goats, means of dry matter intake (DMI), net energy intake (NEI), REI, milk yield (MY), fat (FC) and protein (PC) contents, milk yield at day 35 (MY_{35}), chest width (CW), the proportion of concentrates (PCO) and feed efficiency ratio for efficient, intermediate and inefficient REI groups.

		REI groups			
		Efficient	Intermediate	Inefficient	SD
Number of goats		969	1,433	1,222	
Mean	DMI (kgMS)	2.59	2.85	3.08	0.30
	NEI (UFL)	2.34	2.68	2.97	0.31
	REI (UFL)	-0.33	0.00	0.32	0.27
	MY (kg)	3.60	3.62	3.52	1.06
	FC (g/L)	39.36	39.48	39.79	5.56
	PC (g/L)	34.03	34.02	34.17	3.19
	MY_{35} (kg)	3.87	3.91	3.81	1.10
	CW (cm)	89.83	90.11	89.47	5.32
	PCO	37.61	40.08	40.12	8.68
	Feed efficiency (ratio)	1.01	0.82	0.69	0.24
Number of farms		12	14	12	
% primiparous		82.77	79.00	74.55	
Number of feed intake controls		1.60	1.84	1.39	

Comparison between Ratio and REI

Feed efficiency ratio is positively correlated with milk yield production (r=0.71), unlike REI due to the regression linear model construction (Figures 26 and 27). The more efficient goats are the more productive milk.

The correlation between REI and feed efficiency ratio is negative: -0.58 (Figure 26b).

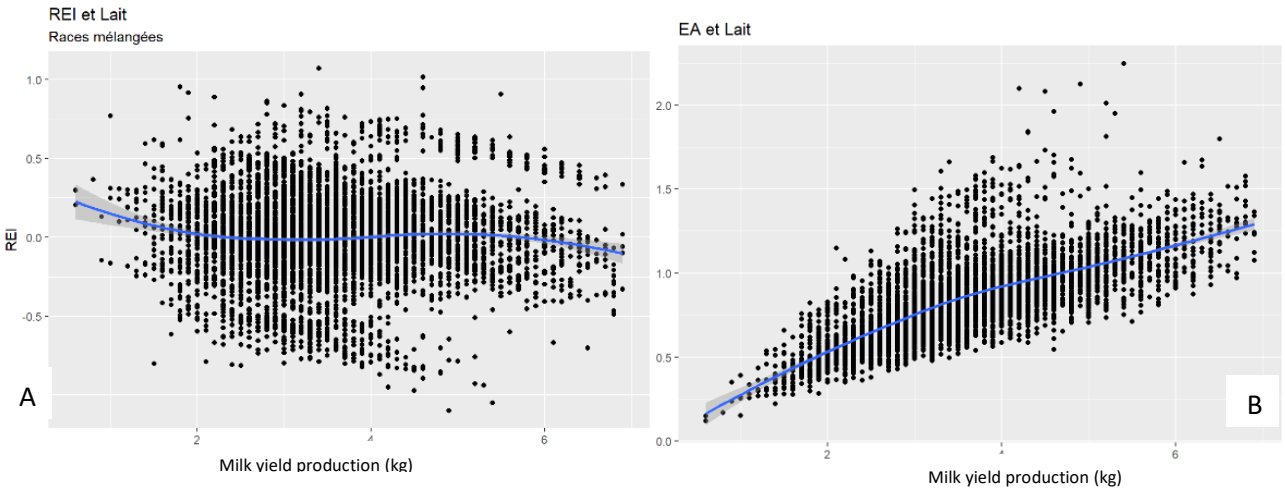


Figure 26: Relationship between REI and milk yield production (A) and relationship between feed efficiency ratio and milk yield production (B).

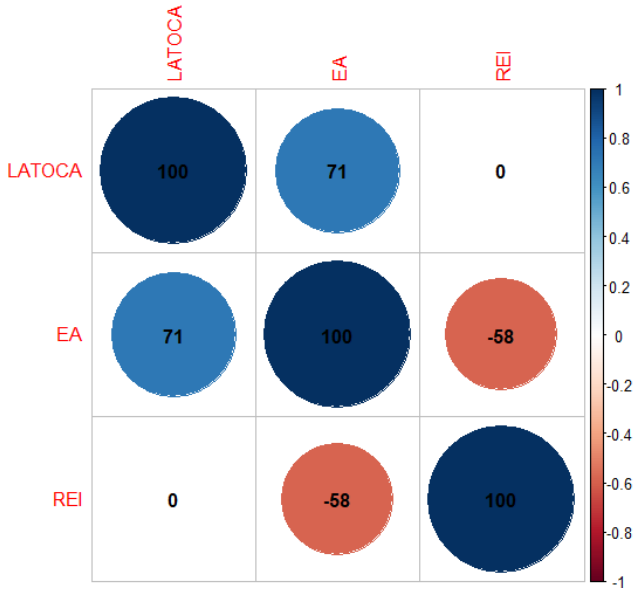


Figure 27: Correlations between milk yield production (LATOCA), feed efficiency ratio (EA) and REI.

For the blood β-HB concentration, we obtained 3.9 measurements per lactation, on average, for 534 primiparous goats. The average blood β-HB concentration was 0.48 mmol/L and ranged from 0.10 to 1.70. The factors of variation were identified with an ANOVA: the farm, the lactation stage, the milk yield, the breed. Genetic parameters were estimated using a linear repeatability model. The heritability was estimated at 11% and the repeatability at 28%.

6.2.3 UK (Yorkshire dairy goats)

Initial analysis of feed intake in dairy goats was focused on the estimation of heritability and genetic correlations between feed intake (FI), body weight (BW), and milk yield (MY). The data consisted of 9,970 test day records for FI (kg), MY (kg), and BW (kg) relating to 1,146 mixed-breed dairy goats (Table 33). Animals were the progeny of 64 sires and 909 dams, and the pedigree contained 6,644 animals.

Table 33: Descriptive statistics for feed intake, milk yield, and body weights in Yorkshire Dairy Goats

Trait	Min	Max	Mean	SD
Feed intake (kg)	0.14	3.89	1.57	0.48
Milk yield (kg)	0.03	8.85	4.32	1.23
Body weight (kg)	35.50	118.60	73.05	12.31

Genetic parameters were obtained under a random regression model with second order Legendre polynomials for random animal and permanent environment effects. Heritability for FI, BW, and MY ranged from 0.04 (520 DIM) to 0.32 (124 DIM), 0.33 (37 DIM) to 0.68 (520 DIM), and 0.19 (513 DIM) to 0.36 (97 DIM), respectively. Genetic correlations at the start and end of lactation were as follows: FI and MY 0.52 (s.e. 0.25) to 0.91 (s.e. 0.05); FI and BW 0.08 (s.e. 0.21) to 0.47 (s.e. 0.21); BW and MY -0.24 (s.e. 0.27) to -0.25 (s.e. 0.22).

6.3 Main results in dairy goats

About 6,960 dairy goats have been phenotyped under commercial conditions.

Total individual feed intake was not available but concentrate intake was recorded individually for the 1,146 UK dairy goats and part of the French Alpine and Saanen. Based on these partial feed intake records, it has been observed that the feed efficiency ratio decreased throughout lactation (which might be directly linked to the decrease in milk yield throughout lactation). In contrast, the REI evolution was not affected by the lactation stage, and the correlation between both feed efficiency criteria was found to be negative.

When clustering goats from French commercial farms in three groups based on their REI levels, no significant differences were observed for classical milk production traits (milk yield, fat, and protein contents). At the genetic level, in Yorkshire goats, significant positive genetic correlations (>0.52) have been identified between feed efficiency and milk yield throughout lactation.

7 Meat Sheep

7.1 Materials and Methods

7.1.1 Experimental designs

1. FRANCE (INRAE)

Novel phenotypes are being collected on experimental Romane individuals belonging to two lines divergently selected on RFI. The divergent selection started in 2014, and animals phenotyped for novel traits, in the frame of the Smarter project, were born in 2018, 2019, 2020 and 2021 and belong to the 2nd, 3rd and 4th generations of selection.

Each year, around 100 male lambs born from planned matings are controlled from 90 to 140 days of age under a concentrate diet (as presented in (Tortereau et al., 2020)).

During this 6-week period of control, lambs are weighed at the beginning and at the end of the control period, and ultrasounds are performed at the end of the control to measure BFT-US and MD-US. During all the control periods, concentrate intake is recorded through ACF. Male lambs are then controlled under a total mixed diet (2/3 forage + 1/3 concentrate), with similar phenotypes being recorded: feed intake, body weights, and body composition traits (BFT and MD at the end of the control period). Because fewer individuals can access AFF than ACF (Weisbecker et al., 2020), male lambs were split into two groups for this control under a total mixed diet. The first group was tested during 6 weeks

from 5 to 7 months old, and the second group was tested during 6 other weeks, from 7 to 9 months old.

Under a concentrate diet, the individual RFI was estimated as the residual of the multiple linear regression of ADFI on ADG, E-BFT, and E-MD to account for production requirements and on the metabolic BW at the end of the test ((E-W)0.75) to account for maintenance requirements (proc reg; SAS Institute Inc., Cary, NC, USA). The year was added as a fixed effect, and FCR was calculated as the ratio ADFI/ADG.

Under a forage diet, the individual RFI was estimated with a similar model, including an additional fixed effect of the control period within each year.

At the end of each 6-week period of control, different biological samples are stored: blood sampled at the jugular vein, rumen fluid (through an oesophageal probe), and faeces.

Blood is aliquoted to perform:

- genotyping (Illumina 50K SNP chip).
- plasma is stored (after 10 minutes of centrifugation at 2400g) to perform metabolomics (NMR) and ^{15}N isotopic analysis.
-

Rumen fluid is aliquoted to perform:

- Metabolomics (NMR)
- Long-chain fatty acids (gas chromatography)
- Volatile fatty acids (gas chromatography)
- Microbiota analysis through the sequencing of V4-V5 regions of the 16S rRNA gene.

SNP genotyping was performed by Labogena company (Jouy-en-Josas, France) on Illumina 50K SNP chips. A total of 430 animals (born and phenotyped from 2018 to 2021) was genotyped.

Laboratory analyses: The protocols used to obtain NMR spectra from plasma, and rumen juice, microbiota sequences, long-chain fatty acids, volatile fatty acids, Faecal NIRS, and 15N natural abundancies are detailed in Appendix 1.

Statistical analyses:

The effect of divergent selection on RFI was analysed in males only, on traits such as body weights, growth, body composition, feed intake, and residual feed intake, collected under the concentrate diet, with analysis of variances taking into account the following fixed effects when significant (p-value < 0.05): divergent line (two levels), year (six levels), generation (three levels), pen (seven levels) and an integrative indicator variable (six levels), summarising the early life conditions of lambs: litter size (1, 2, 3 or 4 and more), suckling method (maternal or artificial feeding) and rearing method (1, 2 or 3 and more lambs reared per dam). Body weight at the beginning of the test was included as a covariate.

Similar analyses were performed under the forage-based diet, taking into account the following fixed effects: divergent line (two levels), year (three levels), period in the year (two levels), generation (two levels), pen (two levels)

The effects of divergent selection on microbiota and metabolomics were studied through multivariate analyses. Discriminant analyses were performed to estimate the predictability of traits from either metabolomics or ruminal microbiota data.

Microbiota statistical analyses: microbiota analyses were performed on lambs phenotyped for RFI in 2018, 2019, and 2020 (samples collected in 2021 are still being processed). If the OTUs identification was performed jointly for concentrate and total mixed diet in order to have similar OTUs affiliations under both diets, the statistical analyses were performed separately for each diet. Microbiota datasets obtained through sequencing are compositional datasets. Therefore, for each diet, OTUs were transformed in Centered Log Ratios (CLR) after the imputation of zeros by applying the Geometric Bayesian Method (GBM). On these CLR-OTUs, analyses of variance were performed, including main technical effects as fixed effects (year, run of sequencing, pen, sequencing depth). Residuals were retrieved and analysed through multivariate approaches. PLS-DA and sPLS-DA were performed with MixOmics R work package (Rohart et al., 2017), discriminant analyses being performed considering the divergent lines or groups based on feed intake or phenotypic RFI. Cross-validations were performed to estimate the error rate. Differential analyses (Analysis of compositions of microbiomes with bias correction (ANCOM-BC)) were performed to identify OTUs that have significantly different abundancies in animals grouped based on their feed efficiency or feed intake. These analyses were performed for 16S OTUs dataset and 18S OTUs dataset separately.

Metabolomics statistical analyses: Only NMR analyses from lambs phenotyped for RFI in 2018 and 2020 are presented here (samples collected in 2021 are still being processed). For each diet (concentrate or forage-based) x biological fluid (plasma or ruminal fluid) combination, metabolite relative quantifications were corrected using a linear model for the following fixed effects: year (2 levels) and pen (11 levels nested in year levels) for the concentrate diet, and with additional effects of adding period (2 levels) for the forage-based diet. Partial Least-Square-Discriminant Analysis (PLS-DA) from R package mixOmics (Rohart et al., 2017) were applied on residuals obtained from the linear models to dig into the links between genetic lines and metabolites in the rumen or plasma under each diet. The area under the ROC curve was used to select the number of components to take into account in the discriminant analyses. Then loadings and Variable Importance in Projection (VIP) were used to determine which variables were the most explanatory. ANOVA, using the previously quoted fixed effects and the line, was used as a univariate way to compare the lines for all metabolites highlighted by PLS-DA.

Faecal NIRS analyses: PLS were performed on NIRS spectra (after a pre-correction known as "SNV + Detrending" (Barnes et al., 1989) a first-derivative treatment), separately for each diet, and jointly for both diets. Cross-validation (6-fold) was performed to validate the models. PLS were performed to predict feed intake of the last 2, 5, or 15 days before faecal sampling and to predict RFI or FCR.

^{15}N : Nitrogen isotopic discrimination (Δ^{15}Nanimal-diet) was calculated as the difference between δ^{15}N in plasma and that measure in the corresponding diet, the latter calculated by weighting the contribution of each ingredient to total dietary N. The Δ^{15}Nanimal-diet values were regressed by simple linear regression on the residual feed intake values to assess their relationship. In addition, ANOVA was conducted to determine whether Δ^{15}Nanimal-diet values differed ($P<0.05$) across the two divergent genetic RFI lines.

Genotypes: VanRaden's first genomic relationship matrix (VanRaden, 2008) was computed from curated SNPs with the R package AGHmatrix (Amadeu et al., 2016). A PCA was then performed on this genomic relationship matrix with the mixOmics R package (version 6.18.1) (Rohart et al., 2017).

Data integration: Details about data integration are provided in Le Graverand et al., 2023 (a). To summarize: fixed effects and body weights were integrated with ruminal microbiota data in order to check whether ruminal microbiota data provided added information for prediction than routinely collected information. Sparse Partial Least Squares Regressions (sPLSR) were performed to assess how well feed efficiency related traits could be predicted with microbiota and zootechnical traits data. sPLSR were carried out with the mixOmics R package (version 6.18.1) (Rohart et al., 2017) on the CLR values adjusted for sequencing effects. Five-fold cross-validations were performed in order to tune hyperparameters and to calculate prediction accuracies (Pearson correlations calculated between true value and predicted value on the testing set, unseen during training of the models). three sets of predictors were considered: (i) The first set included either 16S- or 18S-adjusted CLR values. (ii) The second set included systematic effects: suckling method (concentrate diet only), phenotyping period (mixed diet only), year, pen, age and final BW. (iii) Finally, the third set of predictors included the systematic effects and CLR values of 16S or 18S OTUs (Le Graverand et al., 2023a).

A second integration strategy involved more omics datasets: multivariate analyses were performed with six distinct 'blocks' of predictors: fixed effects and covariates (FC), genotypes (SNPs), plasma NMR spectra (NMR), ruminal volatile fatty acids (VFAs), long-chain fatty acids (LFAs), bacteria and archaea abundances (16S amplicon sequencing). We modified a Partial Least Square regression approach (PLS) to account for the three batches while selecting biomarkers of feed efficiency (Rohart et al., 2017). Cross-validation was repeated to fit one model per block on our training data (60% of the samples). Then, predictions for the validation set (30% of the samples) were obtained by using a weighted aggregation – based on the performance on each validation set. Testing data (10%) were independently used to assess the overall prediction accuracy based on Pearson correlations. (Le Graverand et al., 2023b)

2. Uruguay (INIA-u):

We evaluated 1,611 lambs born from 2017 to 2020 in two research stations: Glencoe from INIA and CIEDAG from the Uruguayan wool secretariat. The lambs belong to the information nucleus of the breeds Merino, Dohne, and Corriedale, which are part of the Uruguayan Genetic Evaluation of each breed. Traits related to growth, muscularity, fat, wool production, health, behaviour, feed intake, feed efficiency, and methane emissions were measured:

- Wool traits (Ramos et al., 2021): Greasy Fleece Weight (GFW), Clean Fleece Weight (CFW), average Fibre Diameter (FD), Coefficient of Variation of Fibre Diameter (CVFD), and Staple Length (SL). These traits were recorded at first shearing, which was between 12 and 14 months of age.
- Growth traits: Weaning Weight (WWT) measured at 4-5 months of age, and Yearling Body Weight (YWT) recorded at first shearing. It is also systematically measured during the feed intake test.
- Muscularity and fat: Rib eye area and backfat thickness were measured at the end (last day of the test) of each feed intake test by ultrasonography (Ramos et al., 2020).
- Body condition score following Russel et al. (1969) after shearing.
- Worm resistance: measured as Fecal worm Egg Count (FEC). Animals were recorded twice during their first year of life under natural mixed-species challenge, according to the protocol

to evaluate the genetic resistance to gastrointestinal parasites in Uruguay (Goldberg et al., 2011).

In parallel, a sample of blood of each animal was taken, and DNA was subsequently extracted (Sambrok et al., 1989) and genotyped (not all animals) (Table 34).

Table 34: Number of animals evaluated per breed, trait, and period of time (fully detailed data is presented in Table 36 in the Results section).

	Merino	Dohne	Corriedale	Total 2021	2022
Feed intake (kg/a/d)	854	237	290	1611	2159
Body weight (kg)	854	237	290	1611	2159
Wool data	854	237	290	1611	2159
Rib eye area (cm^2)	854	237	290	1611	2159
Backfat depth (mm)	854	237	290	1611	2159
Body condition score	854	237	290	1611	2159
Fecal egg count	854	237	290	1611	2159
Methane (g/a/d)	823	230	223	1506	2050
DNA	854	237	290	1611	2159
Genotype (50 K)	854	0	290	1244	1662

Feed intake tests (Ferreira et al., 2021; Amarilho et al., submitted to Livestock Science). Eighteen feed intake tests have been performed from 2018 until October 2021, in different periods of the years, from February to December. These tests were conducted at La Magnolia Experiment Unit (INIA), Tacuarembó, Uruguay. All protocols applied were approved by INIA Animal Ethics Committee (INIA 2018.2). The following references were considered to adapt the feed intake trial to our conditions: Cammack et al. (2005), Cockrum et al. (2013), Paganoni et al. (2017), Redden et al. (2013).

After 7 days of group acclimatisation to the feed, the animals were drenched and allocated to one of five automated feeding systems in accordance with the bodyweight (BW), sex, type of birth, and sire, during a 49-day test period (7 extra days for acclimatization and 42 days for measurements). Animals were fed *ad libitum* with Lucerne haylage (crude protein, 22%; NDF, 35%; ADF, 283%). All animals were tagged with RFID tags. Each pen had five individual automated feeders and two weighing platforms, which were equipped with an electronic tag reader, precision scale, and connected to a central computer (Figure 27); this allowed the control of feed intake and BW of the animals in a daily basis.

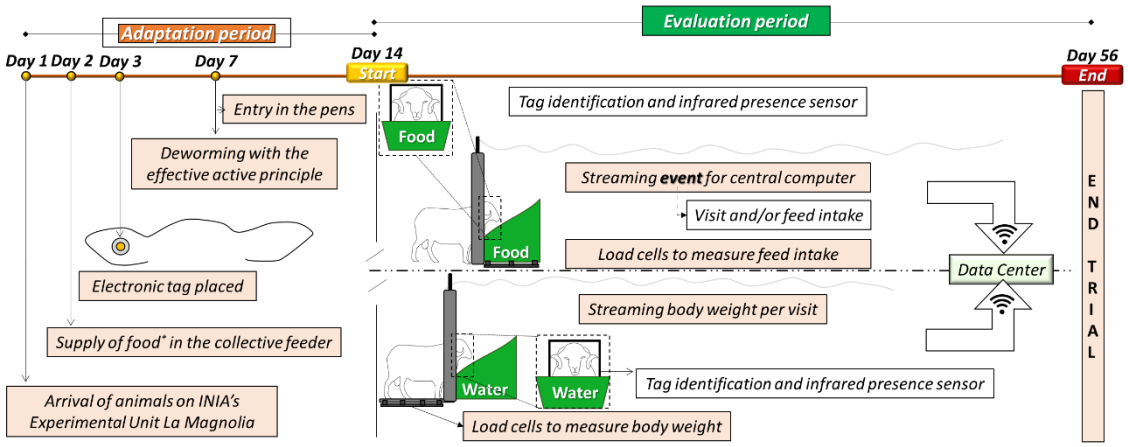


Figure 28: Diagram of the feed intake test.

The RFID tag allows for identification of a specific animal at a feed bin and, therefore, allows for the feeding system to record visits of individual animals with specific start and end times and, consequently, their intake based on the difference in weight before and after the visit.

The body weight of the animals was registered each time they accessed the water bins.

After each feed bin and BW platform visit, the system documented the visit events by recording the animal's identification tag, bin, and platform number. These data were continuously registered throughout the control.

Methane emissions were estimated following the portable accumulation chambers protocol described by Goopy et al. (2011; 2016), Paganoni et al. (2017), and Robisnon et al. (2014). In brief, two estimates per animal were performed during the last weeks of the feed intake test. The traits evaluated were methane emissions, CO_2 emissions, and O_2 consumption. In the week of measures, 1 pen per day was measured in two consecutive runs of 10 animals; therefore, 20 animals per day were measured, and by the end of the week, 100 animals. If the feed intake test considers more than 100 animals, an extra run per day was performed if necessary. In accordance with Robinson et al. (2020), animals were on feed until the moment of the measure. At 20 to 30 and 40 to 50 minutes later, estimates of the concentration of CH_4, CO_2, and O_2 were performed; in parallel, estimates of temperature, atmospheric pressure, and gases concentration were done. Gases measures were done with Eagle 2 equipment (RKI instruments, Union City, CA, USA). The Eagle 2 and chambers were checked between measure weeks and the Eagle 2 was calibrated periodically in accordance with the specifications provided by RKI instruments.

Statistical analyses

Basic data edition and calculations of ADG and RFI: Firstly, animals with any sanitary problem were removed from the study. In addition, BW measures smaller than 15 kg and greater than 80 kg were considered biologically unlikely and later excluded. In addition, BW was deleted if were considered outliers by the Student residuals with < -3 SD and > +3 SD using PROC GLM of software SAS program version 9.4 for Windows (Copyright © 2012 SAS Inst., Cary, NC).

The model of the ADG estimate for the linear regression equation corresponded to:

$$Y = \beta_0 + \beta_1 X$$

where Y = daily BW (kg); β_0 = regression intercept; β_1 = ADG (kg/day); and X = experimental day.

For feed intake, the data considered as biologically unlikely was excluded (feed intake < 0 kg, feed intake > 2 kg, feed intake > 1 kg with visit duration less 3 minutes or visit with more than one hour long), furthermore, days where a malfunctioning in one feeder was detected the data from all feeders in that pen were also excluded. The average data of fresh feed intake was used for estimating the average intake in test periods. The dry matter feed intake was obtained by multiplying the fresh feed intake data by the percentage of dry matter (after drying it in a < 60 ºC air force oven for 72 h, and then multiplying the fresh food for the analytical dry matter) of the feed.

The basic model to calculate RFI was similar to the methodology proposed by Koch et al. (1963); average FI and ADG estimates were used for linear regressions:

$$Y = \mu + S * P * T + BW^{0.75} + ADG + \mathcal{E}$$

where Y = observed individual daily average feed intake (total food intake into day) express in dry matter; μ = is an all animals constant referred to average daily feed intake; S x P ×* T = is composed for sex, pen, trial/test; $BW^{0.75}$ = is the metabolic body weight (MBW) (mid-test body weight elevated to 0.75 as covariable); ADG = is the daily body-weight gain (g/day, covariable); and Ɛ = the residual error as RFI (difference between the observed and expected DMI).

Study of including alternative fixed effects on models of RFI: This study aimed to compare different models for the estimation of RFI in Merino lambs, based on data of 577 animals, born in 2018 and 2019, sired by 16 rams. Models were compared using the Akaike Information Criterion (AIC). A first comparison considered the basic model presented above, and then we evaluated the inclusion of the following effects: birth type, lambing batch-year, age, rib-eye area, fat thickness, and two estimates of wool growth during the test, Trial Clean Fleece Growth and Trial Staple Length Growth, calculated using the Wool Production Potential principles. Another analysis was conducted using only 2019 progeny when Staple Length Growth (SLG) was recorded during the test. The model was as described above but, in this case, estimates of wool growth were SLG, estimated greasy fleece growth based on SLG and an average daily gain that did not consider the weight of the wool were included.

Study of linear versus weekly models for ADG and RFI and different lengths of the feed intake trial: For this study 3 (out of 17) feed intake (FI) trials were considered. The objective was to evaluate linear versus weekly models to calculate ADG and RFI and to explore if it would be possible to accurately estimate RFI and ADG with FI trials shorter than 42 days. Linear ADG was estimated as described before. For the weekly ADG, the calculation was done through the difference in the BW of week Y+1 minus the BW in week Y, divided by seven. Thus, the ADG estimate by linear regression generated only one gain value per animal, while weekly inferences generated six values. The FI and BW measurements were made with 42, 35, 28, and 21 days on trial. We used the average values of FI and BW for each respective period and 6, 5, 4, and 3 average values by week measurements. ADG estimates on 42, 35, 28, and 21 days on trial were estimated by linear regression and calculated 6, 5, 4, and 3 ADG measures by weekly models.

Two models were used to calculate residual feed intake (RFI). Model 1 was already described. Model 2, considered the average FI and ADG estimations by week:

$$Y_{rep} = \mu + P * T + PER + BW^{0.75}{}_{rep} + ADG_{rep} + \mathcal{E}$$

where Yrep = observed week average individual daily feed intake; μ = is an all animals constant referred to average weekly feed intake; P * T = is composed for pen per trial; PER = weeks as covariable; $BW^{0.75}$rep = is the mid-week body weight elevated to 0.75 as covariable); ADGrep = is the daily body-weight gain by week (g/day, covariable); and Ɛ = the residual error as RFI (difference between the observed and expected DMI).To estimate RFI in Model 1, a general linear model (PROC GLM) was used, to Model 2, the PROC MIXED was performed in SAS software. The R-squares were plotted with radarchart function, using the R Package 'fmsb'. The dominance analysis method was used to compare the relative importance of predictors (covariables) in multiple regression model that compose the RFI models, using the R Package 'dominanceanalysis', the results were plotted using the R Package 'ggplot2'. The FI, ADG and the output of RFI models were submitted of Pearson and Spearman correlation analysis, using the R Package 'corrplot' of software R version 3.5.3.

Methane: Data from each bach of methane measurements was transformed considering, BW of the animal, the time between measures and start of measurement, gas concentration inside and outside of the chamber, temperature and atmospheric pressure, following the procedure described by Jonker et al. (2018). Pen, batch, and trial were considered in the models as fixed effects, in addition to other fixed effects like RFI group, the breed that will be detailed when necessary.

Study of contrasting groups for RFI: Within each breed, animals were classified based on percentiles from RFI into three RFI groups: High efficiency (>25%), Medium (50%), and Low efficiency (<25%). This class was included as a fixed effect in a model that also includes the age of the animal, type of birth, sex*test*pen. The effect of RFI group was tested on: Feed intake (FI, kg/d), RFI (by breed), Feed conversion ratio (FI/ADG and MEintake/ADG), N° of meals, Staple Length (SL, cm), Backfat depth (mm), Ln FEC1, Mid FI test BW (kg), Metabolic BW, SL trial (mm), Yearling BW (kg), Greasy Fleece Weight (kg), Clean Fleece Weight (kg), Fiber diameter (µ), Staple Length (cm), FAMACHA, Temperament, BCS at FEC1.

To estimate the effect of RFI group on gas emissions a repeatability mixed model was adjusted (animal effect included as random effect), fixed effects of age of the animal, sex*test*pen, and RFI group were included. Additionally, the random effects of PAC and day*time of record were also included.

Study of phenotypic correlations between feed intake and other traits and of potential proxies for feed intake: Phenotypic correlations between traits that were significantly affected by RFI group in at least one breed and gas emissions traits (i.e., FI, RFI, FCR, N°meals, SL, Backfat) were calculated. In addition, gas emissions traits were also included in the analyses.

To study potential additional proxies to estimate FI, a PCA was performed. Only 784 Merino animals were included in the dataset. The traits involved in these analyses were: Feed intake (kgDM/d), Metabolic Body Weight ($kg^{0.75}$), Average Daily Gain (ADG, kg), Methane, CO_2, and O_2 emissions (g/day). Following these analyses, a validation by using the data from Dohne and Corriedale breeds was performed. Finally, animals were classified into three classes (high, medium, and low intake), and this classification was compared to the observed feed intake classification.

7.1.2 On-farm designs

1. FRANCE (idele/races de france)

In France, three meat sheep breeds are involved in the recording of new resilience and Efficiency traits: Blanche du Massif Central (n=5 farms), Mouton Vendéen (n=5 farms), and Rouge de l'Ouest (n=5 farms). The collection of phenotypes started in 2019 on ewes and lambs. The objective was to phenotype a total of 1,500 ewes per breed and their progeny over two production cycles.

Phenotypes are presented in figure 29 for ewes (green boxes) and lambs (orange boxes):

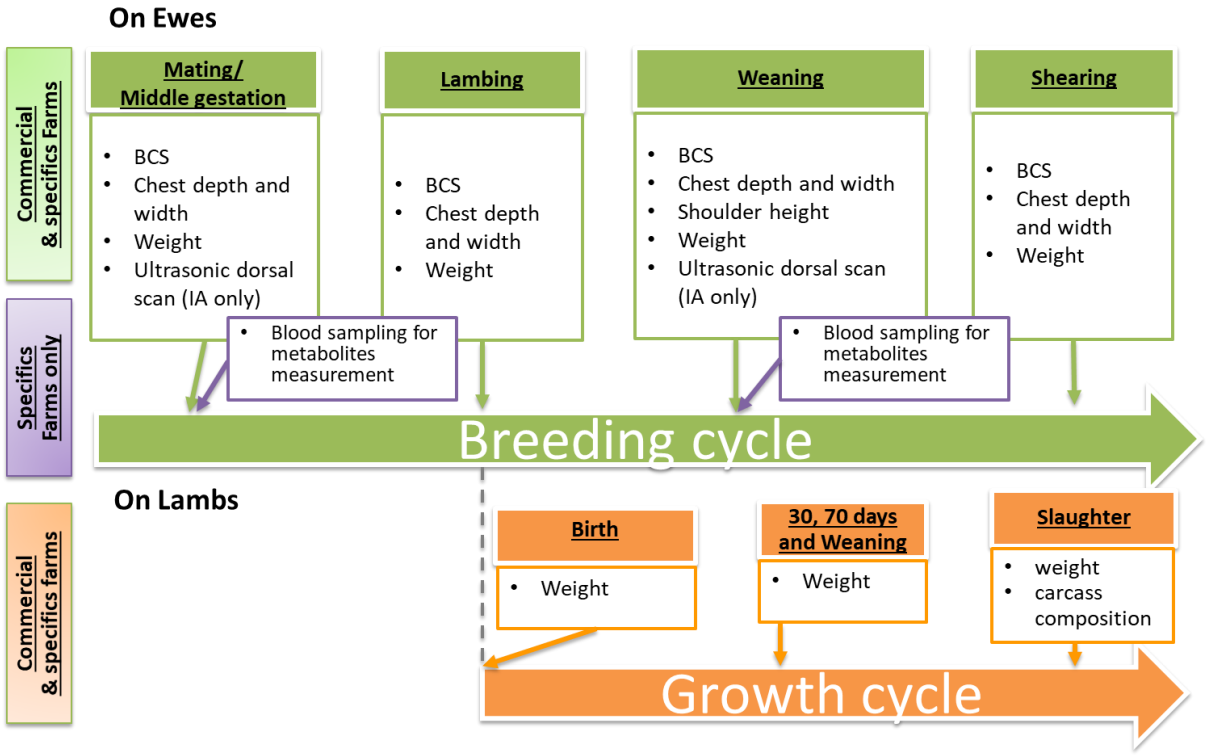


Figure 29: On-farm phenotyping protocol on ewes and lambs in three French meat sheep breeds

Most of the collected traits on ewes are body weights, BCS, body composition traits assessed through ultra-sound and body measurements at the chest level. From chest width and depth, an approximation of chest circumference was calculated. From this chest circumference, we computed a new variable (chest_BW ratio) defined as the ratio of chest circumference divided by the body weight. On this on-farm protocol, metabolite measurements were restricted to beta-hydroxybutyrate measured in two specific farms (Ciirpo and Fedatest). Blood samples were analysed directly after sampling with the Freestyle Optium Neo H (Abbot company) equipped with Freestyle Optium H β-ketone electrodes (Abbot company). These data were not yet all available for analysis, so results are not available for this deliverable.

On lambs, data consisted mainly in body weights recorded at birth, 30 days and 70 days of age and at weaning. At birth, they were also scored for their birth assistance (from 0 to 4, with 4 indicating major troubles for lambing), their level of activity (from 0 to 3 with 3 indicating a lamb not intending to move), and their suckling assistance (from 0 to 3, with 3 indicating a lamb that was not able to suckle by its own). Scores given in this study were adapted from the grid proposed by Matheson et al. (2012)., and already presented in Tortereau et al. (2018).

No intake has been registered, even at the pen level. Therefore, no direct link with feed intake nor feed efficiency will be established. However, each ewe had phenotypes (body weights and BCS) recorded at key points in their production cycle. The objective was to classify ewes based on their phenotypes, then to classify lambs based on their own phenotypes (body weights mainly and vigour scores), and finally to cross those clusters of ewes and lambs to identify links between ewes and lambs status. Each breed was analysed separately.

The first step was to correct raw data with environmental factors. For ewes' traits, residuals were retrieved from analyses of variance, including fixed effects of herd/group, the lambing rank, the period of the year, litter size, and for BCS-related traits, the BCS operator. For lambs' traits, residuals were retrieved from analyses of variance, including fixed effects of herd/group, period of the year, sex, litter size, rearing mode, and rearing type, and, for traits recorded in the abattoir, a fixed effect of the abattoir was included.

Multivariate analyses were performed on the residuals, separately, within each breed, for ewes (with Principal Component Analyses (PCA)) and lambs (with Factor Analyses of Mixed Data (FAMD)) datasets. The *FactoMineR* and *factoextra* R packages were used, and particularly the PCA() and FAMD functions. The principal components from those analyses were then used as variables for the classification: we applied the Hierarchical Classification on Principal Components with the HCPC() function.

The final analysis consisted in joining ewe and lamb clusters to identify potential links between some clusters.

2. Norway (NSG):

The aim of the Norwegian part of WP1 was to measure methane (CH_4) and carbon dioxide (CO_2) emissions as well as oxygen (O_2) consumption of Norwegian White sheep using portable accumulation chambers (PAC) and correlate emissions to other traits in the breeding goal.

Just over 6,000 Norwegian White sheep from 57 commercial ram circle flocks were measured using PAC equipment. Flocks from ram circles were chosen as they circulate rams for natural mating within the ram circle and in addition are required to use some artificial insemination. The use of common rams thus results in genetic connectedness between flocks (Kuehn *et al.*, 2008).

Ten PAC chambers were placed in the box of a truck, and ten animals were forming a lot measured simultaneously. Animals of the same lot were fed the same diet (silage/pasture) for at least 3 days prior to measurement. In addition, animals were off feed for at least one and a maximum of four hours prior to measurement. The latter ensures stable emissions.

All measurements were on breeding females where most have lambed at least once or were expected to lamb for the first time at the next lambing season. Prior to PAC measurement, animals were weighed.

Fifty-minute CH_4 concentration was converted to CH_4 g/hr, taking into account the size of the PAC chamber, weight of the animal prior to PAC measurement, as well as temperature and air pressure outside the chamber. Both CH_4 g/hr and ewe weights were scaled, they were divided by the mean of the lot and multiplied by the mean of all observations.

Two different methane traits were defined:

1) Methane corrected for weight at measurement
2) Methane corrected for proxy feed intake, where proxy feed intake was defined as sum moles of CH_4 and CO_2

Variance Components Estimation

The methane dataset was merged with ten years of growth and carcass data of traits currently included in our routine genetic evaluations from the 57 flocks with PAC measurements. Pedigree was traced as far back as possible on animals with data. Variance components were obtained using the Apex linear models software licensed by GHPC PTY. LTD., Armidale, Australia.

Breeding value prediction

The variance components for methane emission and other traits in the breeding goal were utilized to predict EBVs for all animals using ssGBLUP.

7.2 Results

7.2.1 France experimental design

Divergent lines were initiated in 2014. The first generation of selection has been produced in 2015 and 2016, the second generation of selection in 2017-2018, and the third generation of selection in 2019-2020. The first animals from the fourth generation of selection were produced and phenotyped in 2021.

The fine-phenotyping was performed in the years 2018 to 2021. In table 35 are listed all the traits available.

Zootechnical traits and RFI calculation:

Table 35: Description of traits recorded under concentrate and forage-based diets for Romane male lambs belonging to divergent lines on RFI.

Diet	trait	N	Mean ± sd	LS Means RFI+	LS Means RFI-	p-value Divergent line
Concentrate	E-BW (kg)	562	54.28±7.18	54.33	54.56	0.33
	ADG (g/d)	562	345.9±61.7	342.9	347.6	0.36
	MD-US (cm)	562	2.76±0.24	2.76	2.76	0.84
	BFT-US (mm)	562	6.05±1.11	6.07	6.25	0.0023
	ADFI (g/d)	562	2025±287	2077	1967	<0.0001
	RFI (g/d)	562	2.84±133.70	59.76	-52.46	<0.0001
	FCR	562	5.99±1.13	6.13	5.81	<0.0001
Forage	E-BW (kg)	167	65.4±6.0	65.3	65.0	0.37
	ADG (g/d)	167	124.6±64.2	125	118	0.36
	MD-US (cm)	167	2.71±0.24	2.7	26.9	0.31
	BFT-US (mm)	167	4.52±0.77	4.40	4.60	0.05
	total ADFI (g/d)	167	1828±263	1839	1799	0.17
	Forage daily intake (g/d)	167	1218±226	1234	1195	0.13
	Concentrate daily intake (g/d)	167	642±86			
	RFI (from ADFI) (g/d)	167	0±168	16.16	-15.89	0.22

Diet	trait	N	Mean ± sd	LS Means RFI+	LS Means RFI-	p-value Divergent line
	RFI (from forage intake) (g/d)	167	0±152	15.93	-15.47	0.19
	FCR (from ADFI)	167	22.8±60.1	21.8	22.6	0.93
	FCR (from forage intake)	167	15.3±40.9	14.8	15.0	0.98

The effect of the divergent selection was tested on the different performance traits recorded under a concentrate or a forage-base diet (Table 35). Under a concentrate diet, main significant differences were observed for ADFI, RFI, and FCR. A significant difference was also observed for BFT-US, with efficient (i.e., RFI-) lambs having close to 0.18 mm BFT-US more than less efficient animals. Body weights, growth, and MD-US were not significantly between RFI+ and RFI- lambs under a concentrate diet. Under a forage-based diet, only BFT-US was significantly different between lambs from both divergent lines, with efficient lambs having a higher BFT-US (0.20 mm more than less efficient lambs).

Correlations between traits within each diet are presented in table 36.

Under both diets, body weights, growth, body composition traits, and average daily feed intake were all positively correlated, with correlations ranging from 0.16 (between ADG and BFT-US under a concentrate diet) to 0.85 (between E-BW and ADFI under a concentrate diet). By construction, RFI was only significantly correlated with feed intake. Under a forage diet, FCR was only significantly correlated with ADG (-0.26), whereas, under a concentrate diet, it was also significantly correlated with all the other traits except BFT-US.

Table 36: Phenotypic correlations between traits recorded under concentrate (above the diagonal) and forage-based diets (under the diagonal) for Romane male lambs belonging to divergent lines on RFI.

	E-BW (kg)	ADG (g/d)	MD-US (cm)	BFT-US (mm)	total ADFI (g/d)	Forage daily intake (g/d)	RFI (from total ADFI) (g/d)	RFI (from forage intake) (g/d)	FCR (from total ADFI)
E-BW (kg)		0.30	0.59	0.35	0.85		*0.04*		0.34
ADG (g/d)	0.29		0.16	0.28	0.42		*-0.007*		-0.68
MD-US (cm)	0.51	0.20		0.30	0.47		*0.0025*		0.18
BFT-US (mm)	0.40	0.21	0.31		0.27		*0.02*		*-0.06*
total ADFI (g/d)	0.63	0.41	0.52	0.36			0.49		0.33
Forage daily intake (g/d)	0.59	0.28	0.41	0.34	0.94				
RFI (from total ADFI) (g/d)	*0.00*	*0.00*	*0.00*	*0.00*	0.64	0.63			0.38
RFI (from forage intake) (g/d)	*0.00*	*0.00*	*0.00*	*0.00*	0.60	0.68	0.93		

FCR (from ADFI)	*-0.03*	-0.26	*-0.05*	*-0.08*	*-0.07*	*-0.03*	*-0.04*	*-0.05*	
FCR (from forage intake)	*-0.02*	-0.26	*-0.05*	*-0.07*	*-0.06*	*-0.01*	*-0.03*	*-0.03*	0.99

In italics are the correlations that were not significantly different from 0.

Genotypes: The PCA performed on the genomic relatedness matrix highlighted a clear structuration of our experimental population, which was expected (figure 30). RFI divergent lines were associated to the first PCs when PCAs were fitted on genomic relatedness matrices (11% of explained variance).

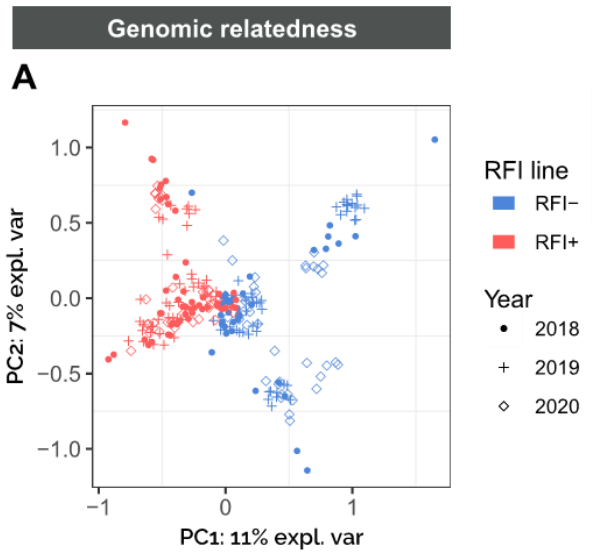


Figure 30: Principal component analyses of lamb genomic relatedness matrices

SNPs are assumed to predict very accurately residual feed intake because of the experimental design which rely on families developed over 3-4 generations. Prediction of residual feed intake with SNPs on this design reached an accuracy of 0.44 (0.11). We assume that this accuracy is largely over-estimated and not reasonably transposable to other populations.

Ruminal microbiota: Ruminal samples were available for 273 animals under a concentrate diet and on 167 out of them under a forage-based diet.

From 16S sequencing: 994 OTUs and 1,328 OTUs were identified on ruminal fluids collected after the concentrate and forage-based periods, respectively. OTUs from the 16S sequencing belong to bacteria (97% and 93% under a concentrate or forage-based diet, respectively) and archaea.

From 18S sequencing: 213 OTUs and 224 OTUs were identified from ruminal fluids collected after concentrate and forage-based diets, respectively.

Main environmental factors affecting OTU abundancies are technical factors such as sequencing depth and the run of sequencing.

Under a concentrate diet, OTUs identified with 16s and 18s sequencing best discriminate animals on their ADFI level and their phenotypic RFI level, respectively (table 37 and figures 31A and 31B).

Under a forage-based diet, OTUs identified with 16s and 18s sequencing best discriminate animals on their phenotypic RFI level and on their genetic line (table 37 and figures 31C and 31D).

However, the best discriminant analysis reaches a BER of 38.3%, which is too high to predict feed intake for genetic predictions correctly.

Table 37: Balanced Error Rates (BER) from sPLS-DA on 16s and 18s OTUs, with discriminant factors being the divergent line, the phenotypic ADFI level, phenotypic RFI level or genetic RFI level.

Diet	**Concentrate based-diet**				**Forage based-diet**		
Sequencing	Lines	phenotypic ADFI	Phenotypic RFI	RFI EBVs	Lines	phenotypic ADFI	Phenotypic RFI
16S	45.1%	38.3%	42.1%	41.0%	46.1%	46.3%	45.6%
18S	45.9%	43.2%	39.6%	48.5%	40.4%	48.7%	45.7%

ADFI: Average Daily Feed Intake; RFI: Residual Feed Intake; EBV: Estimated Breeding Value.

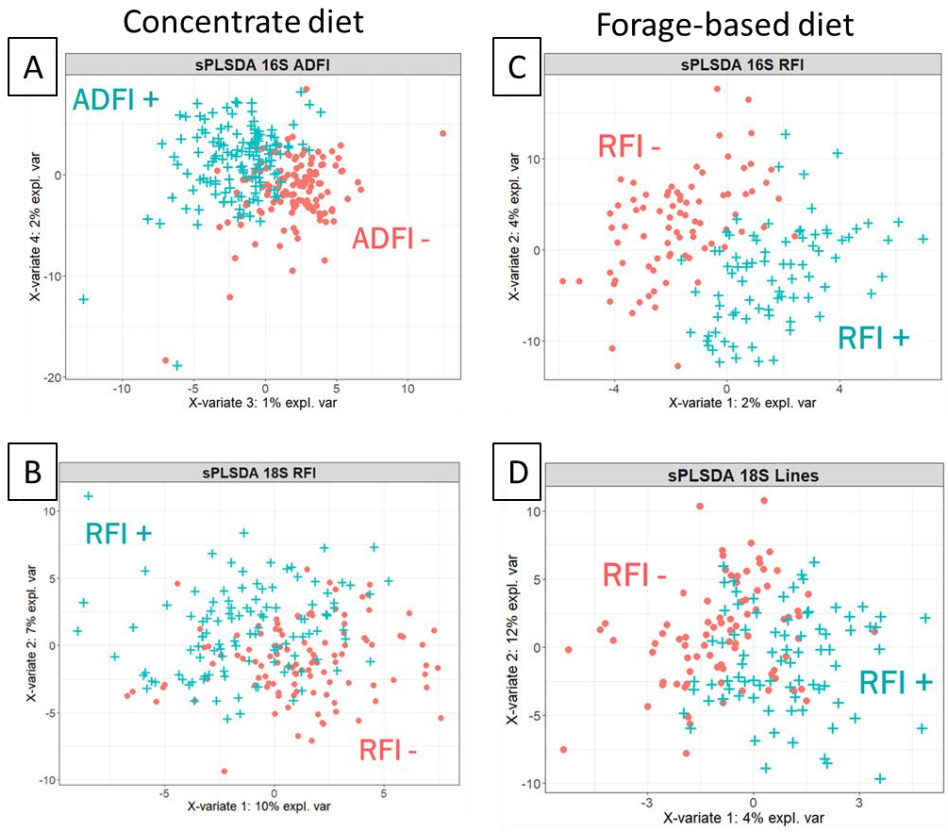


Figure 31: Components 1 and 2 from sPLS-DA of 16s (A and C) and 18S (B and D) sequencing of ruminal microbiota from Romane male lambs fed with concentrate (A and B) or with a forage-based diet (C and D). Only the most discriminant factors are represented: Average Daily feed Intake (A), phenotypic RFI (B and C), or divergent line (D).

NMR: a total of 440 plasmatic and 440 ruminal samples from 2018, 2019, and 2020 were analysed for this report. Both fluids were sampled from 273 animals under a concentrate diet and from 167 animals (out of the 273) under a concentrate-based diet.

Raw spectra were analysed with the ASICS (version 2.5.3) R package (Lefort et al., 2019). Under a concentrate diet, 34 and 44 metabolites were detected in plasmatic and ruminal samples respectively, and assigned relative concentrations. Under a forage-based diet, 23 and 19 metabolites were detected in plasmatic and ruminal samples, respectively, and assigned relative concentrations.

Under a concentrate diet, citrate and malate were the two metabolites with a VIP value higher than 1.5 when 8 components were retained. Under a forage-based diet, only citrate had a VIP value higher than 1.5 when 6 components were retained (figure 32).

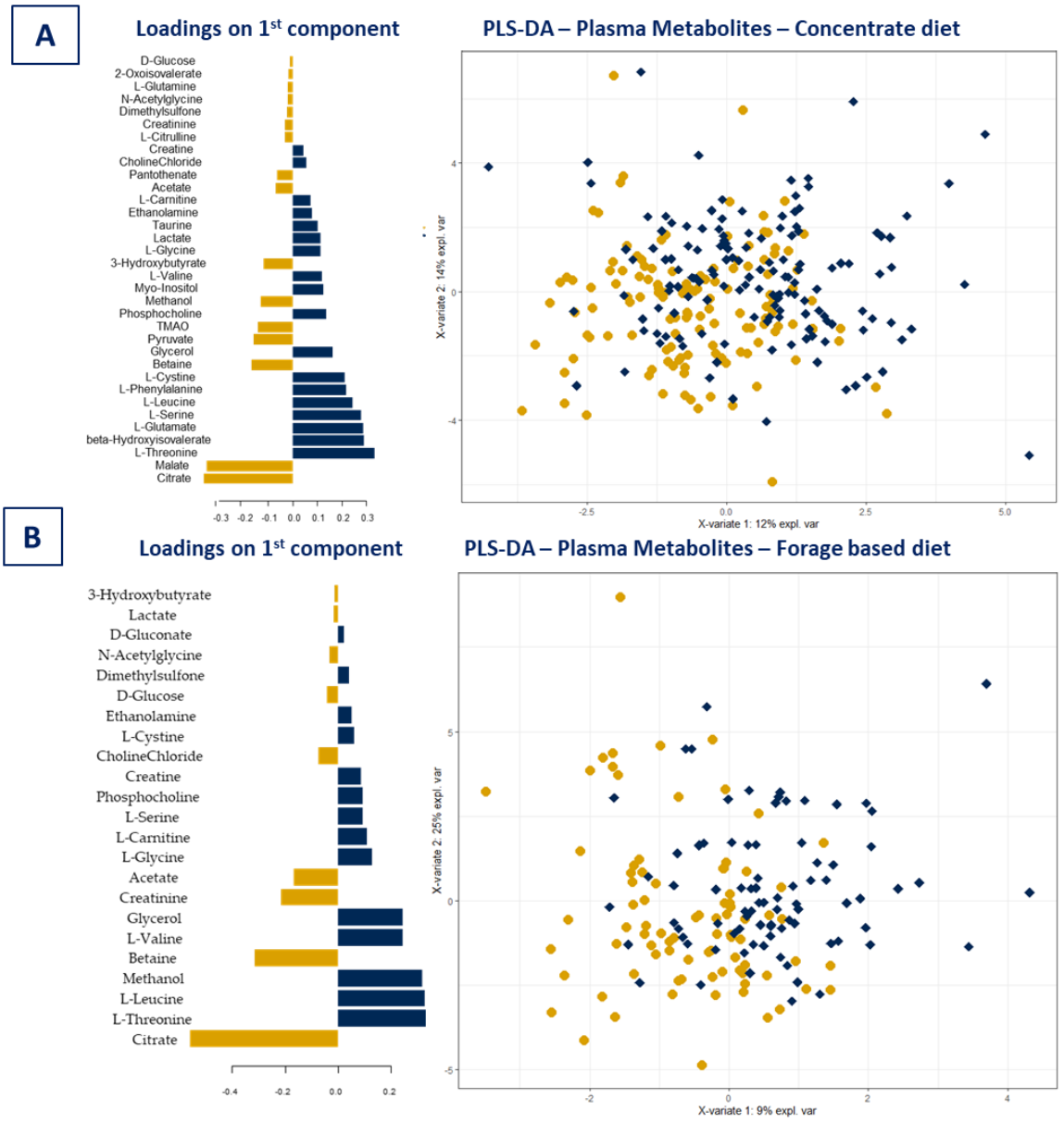


Figure 32: Partial least squares – discriminant analyses for plasmatic metabolites in Romane male lambs fed concentrate (A) or forage-based (B) diets. Efficient and less efficient individuals are presented in yellow dots and blue diamonds, respectively.

No metabolite could be identified as significantly different between lambs from both divergent lines, from the ruminal fluid, whatever the diet.

SPIR on faecal samples: The prediction of feed efficiency (RFI or FCR) was only performed on 2018 animals, whereas prediction of feed intake was performed on 2018, 2019 and 2020 animals. From this subset of data, the prediction returned low R^2CV (table 38) or both traits.

Feed intake was well predicted (R^2CV = 0.72) when both diets were considered jointly.

Table 38: Calibration and validation statistics of PLS on faecal NIRS to predict feed intake (FI) (g/kg Metabolic Weight(MW) or g/kg live weight) , RFI and FCR of Romane male lambs divergently selected on RFI, considering separately or jointly two periods of control (under a concentrate diet or a forage-based diet).

	Predicted trait	N	SEC	R^2C	SECV	R^2CV
Concentrate diet	RFI	91	129.79	0.11	137.61	0.02
	FCR	91	0.92	0.12	0.96	0.04
	FI 15 days (g/kg MW)	261	9.37	0.27	9.73	0.21
	FI 5 days (g/kg MW)	257	10.63	0.22	11.06	0.20
	FI 2 days (g/kg MW)	244	12.58	0.34	13.07	0.29
	FI 15 days (g/day)	262	263	0.16	270	0.12
	FI 5 days (g/day)	268	300	0.16	304	0.14
	FI 2 days (g/day)	249	307	0.20	314	0.16
Forage-based diet	FCR	47	9.58	0.17	9.70	0.15
	FI 15 days (g/kg MW)	161	7.54	0.36	8.71	0.19
	FI 5 days (g/kg MW)	163	8.91	0.42	9.50	0.34
	FI 2 days (g/kg MW)	163	10.67	0.51	11.48	0.42
	FI 15 days (g/day)	164	227	0.33	248	0.19
	FI 5 days (g/day)	164	231	0.46	256	0.34
	FI 2 days (g/day)	164	265	0.55	303	0.40
Both diets	FCR	141	3.59	0.62	3.98	0.61
	FI 15 days (g/kg MW)	423	8.78	0.74	9.14	0.72
	FI 5 days (g/kg MW)	423	9.73	0.75	10.42	0.72

	FI 2 days (g/kg MW)	413	12.47	0.67	13.07	0.63
	FI 15 days (g/day)	429	266	0.40	272	0.37
	FI 5 days (g/day)	430	279	0.47	286	0.46
	FI 2 days (g/day)	412	284	0.52	298	0.47

N = number of samples; SEC = standard error of calibration; R^2C = determination coefficient of calibration ; SECV = standard error of cross-validation; R^2CV = determination coefficient of cross-validation.

15N on plasma: The $\Delta^{15}N$ biomarker, which is the difference between the natural abundancy of ^{15}N measured in plasma and the natural abundancy of ^{15}N measured in the feed has been calculated in a subset of 71 Romane male lambs born in 2019 and fed successively with concentrate and a forage-based diet. The $\Delta^{15}N$ biomarker was not correlated with RFI, whatever the diet. However, it was correlated under a forage diet with FCE, which is the ratio between ADG and feed intake (figure 33).

The correlations between $\Delta^{15}N$ biomarker and feed intake were equal to 0.35 (under a concentrate diet) and to 0.33 (considering the quantity of forage intake under a forage-based diet).

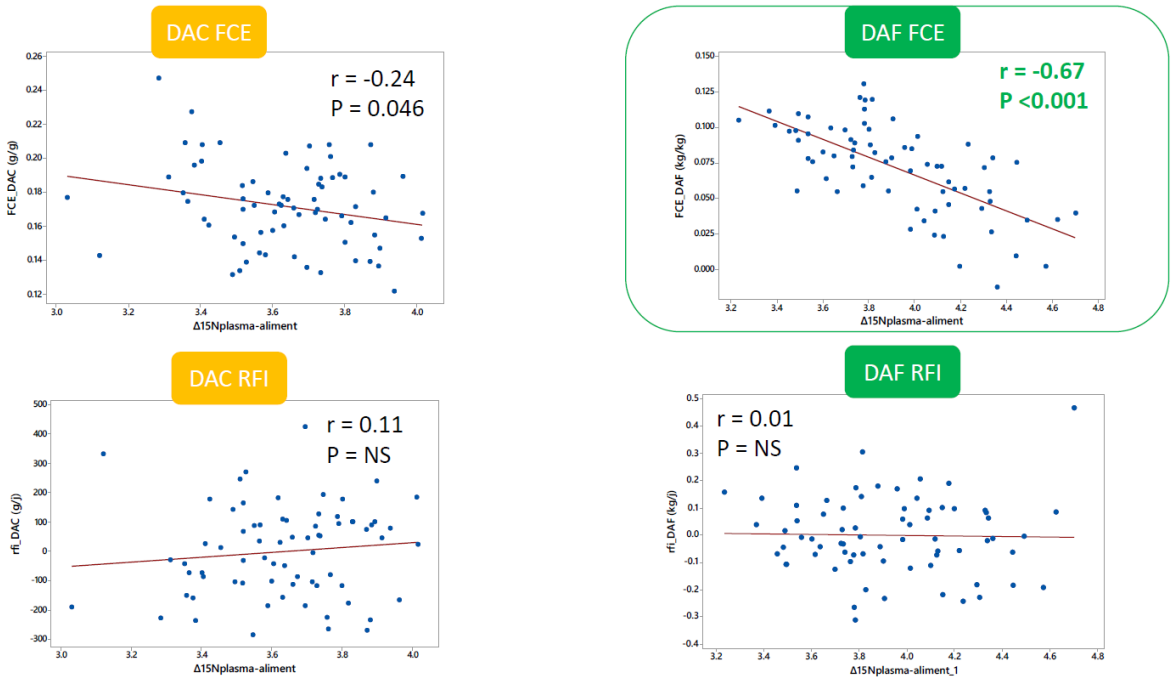


Figure 33: Feed efficiency traits (FCE on the top and RFI at the bottom) according to $\Delta^{15}N$ (plasma – food) under two diets (100% concentrate and 1/3 concentrate + 2/3 forage, on the left and right respectively).

Data integration: Integration of fixed effects, body weight and transformed microbiota abundances has been published in Le Graverand et al., 2023. To summarize:

- under a concentrate diet, when host feed efficiency was predicted from microbiota data only, the average correlations between actual traits and predictions were almost null for REI (0.11 for 16S compared to 0.06 for 18S data) or low to moderate for FCR (0.35 for 16S, 0.16 for 18S). Under a mixed diet, predicting REI led to low average correlations (0.35 with 16S, 0.17 with 18S).
- Predicting energy intakes with 16S or 18S data led to a large range of correlations: from 0.05 with 18S under a concentrate diet to 0.56 with 16S under a mixed diet.
- Regardless of diet and trait, average correlations for predictions from fixed effects and body weight ranged from 0.31 to 0.84. Furthermore, correlations were significantly higher than predictions derived from microbiota data only.
- Finally, combining microbiota data with fixed effects and final BW never significantly improved correlations compared to predictions from fixed effects and BW. Most of the correlations were not significantly different.

Results of the integration of 6 different omics and non-omics datasets are presented in Le Graverand et al., 2023 (b). When RFI was predicted with our approach combining different omics, accuracy increased and reached an average of 0.55 (0.11). Based on weights attributed to blocks of predictors, we were able to rank the most predictive blocks to explain RFI: SNPs, fixed effets (and covariate), plasma NMR, ruminal 16S abudances, long chain fatty acids and volatile fatty acids. Furthermore, within each block we identified variables that were highly associated with feed efficiency RFI, including β-hydroxyisovaleric acid and a SNP located on the chromosome 3. To conclude, blending models is useful to integrate heterogeneous omics data: from predicting efficiency, to identifying associations between multi-omics predictors.

7.2.2 Norway (NSG):

Phenotypic results are presented in Table 39.

Table 39 - Phenotypic average (SD) of CH4, body weight and CH4 / (CH4 + CO2)

Trait	Average (SD)
CH4 gr/hr	1.34 (0.27)
Body weight, kg	83.08 (9.87)
CH4 mol / (CH4 + CO2) mol	0.05 (0.01)

Heritability estimates for weight corrected methane emission and for feed intake proxy corrected methane emission are shown below in Table 40, and genetic correlations between methane emission and traits currently included in the Total Merit Index are shown in Table 41.

Table 40 - Heritability of weight corrected methane emission, and for feed intake proxy corrected methane emission

Trait	Heritability
Weight corrected methane emission	0.18
Feed intake proxy corrected methane emission	0.34

Table 41 - Genetic correlations between methane traits and traits in the breeding goal

Trait	Birth Weight	Carcass Weight	EUROP Carcass Classifi-cation	EUROP Fat Grading	Birth Weight (mat.)	42-day Weight (mat.)	Carcass Weight (mat.)	Fleece Weight	Fleece Grade
Weight corrected methane	-0.11	0.02	-0.08	-0.27	0.23	0.32	0.25	0.04	0.07
Feed intake proxy corrected methane	-0.08	-0.18	0.04	0.00	0.00	0.16	0.18	0.05	0.05

Preliminary estimates show heritabilities of 18% for gram methane per hour corrected for body weight, and 34% for mol methane corrected for feed intake proxy (sum moles methane and carbon dioxide). Genetic correlations were positive and antagonistic between weight corrected methane emission and maternal genetic effects of birth weight, 42-day weight, and carcass weight, indicating proper weighting needs to be applied if including weight corrected methane emission in the total merit index not to get adverse response in these traits when selecting for reduced methane emission. On the contrary, genetic correlations between feed intake proxy corrected methane emission and traits in the breeding goal were close to zero indicating that it should be possible to include feed intake proxy corrected methane emission in the total merit index without antagonistic response in traits already included in the breeding goal.

The studied gas traits look promising, but more work is needed to explore additional gas trait definitions and the expected response in traits already included in the breeding goal for Norwegian White Sheep.

In summary, the mechanics is in place to compute breeding values for methane emission. Implementation in the breeding goal however requires continued phenotyping in years to come.

7.2.3 INIA-Uruguay

Descriptive statistics

Descriptive statistics of the traits recorded during feed intake trials are presented in table 42. Corriedales were the youngest animals at the moment of the test, while Dohnes were the oldest and Merinos intermediate. While only females are evaluated for Corriedale and Dohne, in Merino, both

sexes were considered. Independently of the age and BW at the moment of the test, the three breeds presented a feed intake from 3 to 3.5% of BW and ADG between 160 to 200 g/d.

Table 42: Descriptive statistics (number of animals, mean, standard deviation) for traits related to efficiency by breed (INIA progeny 2018-2020 and CIEDAG 2017 for Corriedale).

	Corriedale			Dohne			Merino		
Label	**N**	**Mean**	**sd**	**N**	**Mean**	**sd**	**N**	**Mean**	**sd**
age at test (days)	281	222	61	214	421	9	811	288	45
age at weaning (days)	149	112	8	214	132	27	578	136	12
Rib eye area (cm^2)	281	6.62	1.47	214	9.94	2.02	810	7.55	1.48
Backfat depth (mm)	281	2.38	1.15	214	2.81	0.88	810	2.10	0.70
N° of meals	281	55.07	17.15	214	82.19	14.87	811	61.38	17.83
ADG (kg/d)	281	0.166	0.046	214	0.181	0.050	811	0.202	0.068
R^2 ADG	281	0.91	0.08	214	0.87	0.08	811	0.90	0.08
Feed intake (kgDM/d)	281	1.13	0.27	214	1.54	0.24	811	1.36	0.25
Feed Intake (MjME/d)	281	2.71	0.73	214	3.81	0.68	811	3.32	0.64
Feed conversion ratio (FI/ADG)	281	7.12	2.02	214	9.02	2.39	811	7.29	2.11
Feed conversion ratio (ME/ADG)	281	17.00	4.91	214	22.22	5.89	811	17.76	4.95
Mid FI test BW (kg)	281	33.57	5.11	214	49.99	5.62	811	40.77	6.20
Metabolic BW (kg)	281	13.92	1.60	214	18.78	1.59	811	16.10	1.83
BW gain on trial (kg)	280	6.74	2.09	210	7.96	2.30	805	8.06	2.74
Methane (g/d)	218	16.44	4.85	208	28.15	5.74	784	23.36	5.45
CO_2 (g/d)	218	829	183.8	208	1484	344.0	784	1086	233
O_2 (g/d)	218	847	160.0	208	1322	325.5	784	1000	190
Methane intensity (g/kgBW-gain)	218	0.43	0.11	208	0.54	0.10	784	0.54	0.10
Methane yield (g/kgDM24h)	218	11.36	2.56	208	15.11	3.67	784	14.33	2.95
Staple length trial (mm)	147	19.43	5.85	128	18.31	2.51	546	18.47	6.88
RFI (breed)	281	0.00	0.14	214	-0.01	0.13	811	-0.01	0.13
age at Shearing (days)	149	398	7	212	396	7	578	412	9
FEC 1	176	1718	2214	206	2013	2233	557	2696	1969
Ln FEC1	176	6.75	1.35	206	6.97	1.34	557	7.67	0.80
Yearling BW (kg)	193	35.45	4.73	213	42.68	5.18	575	49.30	11.55
Greasy Fleece Weight (kg)	194	3.25	0.48	213	2.62	0.39	575	4.12	0.70
Clean Fleece Weight (kg)	192	2.43	0.36	210	1.98	0.31	572	3.08	0.55
Fiber diameter (µ)	192	23.23	1.72	210	18.28	1.35	577	14.87	0.92
Staple length (cm)	192	12.46	1.77	210	10.07	1.36	577	10.96	1.30
FAMACHA 1	147	1.6	0.6	213	2.1	0.7	576	2.2	0.9
Temperament	148	42.68	24.76	213	67.65	30.62	569	59.91	30.11
Body condition score at FEC1	149	3.0	0.3	213	3.1	0.4	576	2.9	0.4

Models

The results indicated that the basic model (sex-pen-trial, ADG, MW) is the most parsimonious for both analyses; the other fixed effects, body composition, and fleece growth traits were not significant ($p>0.05$) (AIC difference >2). Furthermore, RFI values estimated with the basic and alternative models were highly correlated ($r=0.99$). In conclusion, it might not be necessary to include estimations of wool growth during 42-day tests in RFI models when evaluating Merino sheep (Marques et al., 2021).

Pearson and Spearman correlations between RFI linear and weekly 42-days models were 0.93 and 0.92, respectively. The 35-days length models (linear and weekly) presented Pearson and Spearman correlations greater than 0.98 with the 42-days models. The RFI models with 35 days allowed to decrease seven days the FI test maintaining accuracy and explaining 75.3% and 63.6% of the FI by the linear and weekly models, respectively (Amarilho et al., submitted).

RFI Contrasting groups

The association between Predicted and Observed Feed Intake (kg DM/day) by breed is presented in figure 34. High, medium, and low RFI animals are present at any observed intakes (low, medium, or high).

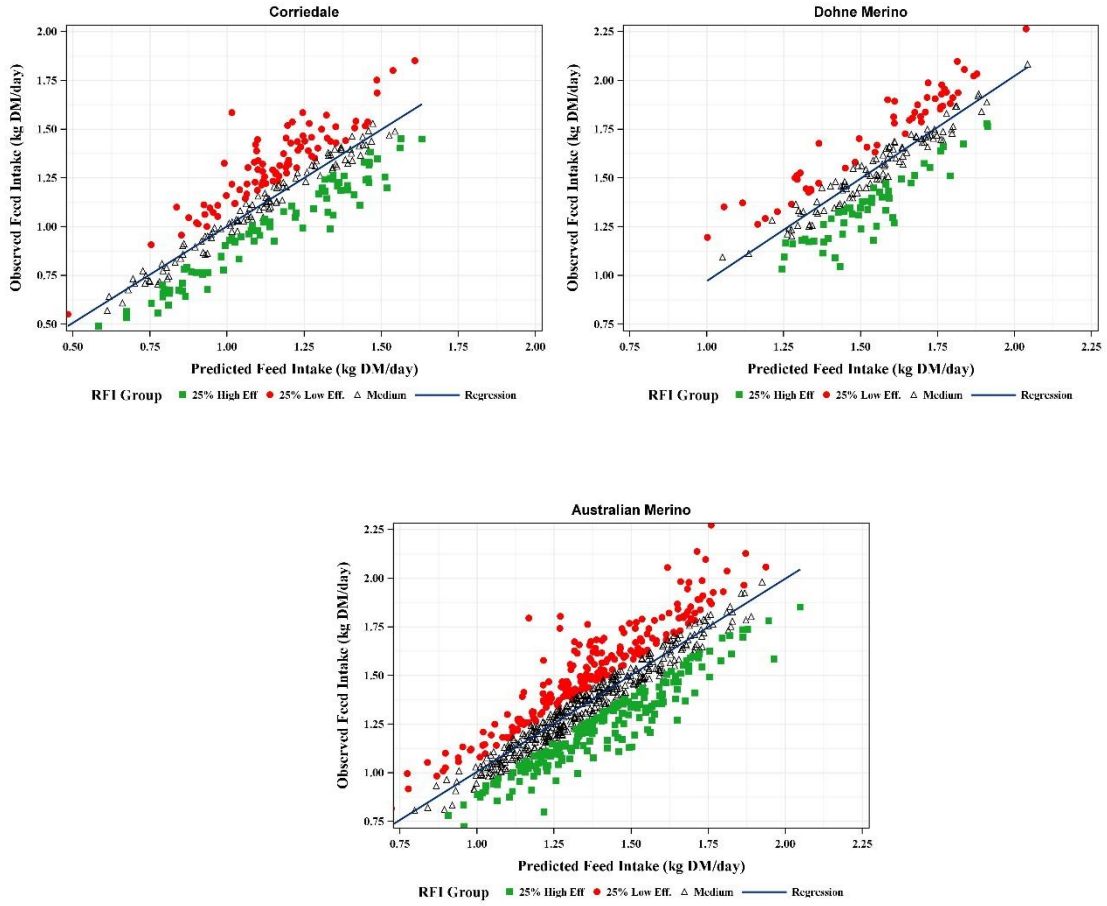


Figure 34: Predicted Feed Intake vs Observed Feed Intake (kgDM/day) by RFI group for Corriedale, Merino and Dohne breeds.

The RFI group had a significant effect ($p<0.05$) on FI, FCR, RFI, and the number of meals, regardless of the breed (Table 43).

Table 43: RFI group effect on feed intake, feed conversion ratio, RFI and behaviour for all breeds (all $p<0.05$)(means±sd)

Breed		**Corriedale**			**Dohne**			**Merino**	
RFI group	High	Medium	Low	High	Medium	Low	High	Medium	Low

Breed	Corriedale			Dohne			Merino		
FI (kgDM)	0.97±0.02	1.12±0.01	1.27±0.02	1.34±0.02	1.52±0.01	1.70±0.02	1.18±0.01	1.33±0.01	1.50±0.01
FCR (MJEM)	14.8±0.54	16.4±0.47	19.7±0.62	19.9±0.79	22.2±0.55	24.5±0.79	15.5±0.27	18.0±0.18	20.7±0.26
FCR (kgDM)	6.2±0.23	6.9±0.20	8.3±0.26	8.1±0.32	9.1±0.22	10.0±0.32	6.4±0.11	7.4±0.08	8.5±0.11
RFI	-0.14±0.01	0.00±0.01	0.16±0.01	-0.18±0.01	-0.01±0.01	0.16±0.01	-0.16±0.00	-0.01±0.00	0.15±0.00
N° Meals	44.1±1.65	56.3±1.46	66.9±1.91	73.9±1.90	81.6±1.33	91.5±1.92	52.9±1.00	60.4±0.69	72.5±0.98

In terms of GHG emissions (table 44), the RFI group affected the total methane only in the Merino breed ($p<0.05$), where the less efficient animals emitted 6.6% more methane. Alternatively, the RFI group affected the methane emission corrected by feed intake in the model (methane g/d FI 24-48-72 hours) or as precorrection (methane g/DMI 24hs). In all cases, the more efficient animals had higher methane yields than less efficient ones. Only in the Merino breed were observed a tendency in methane intensity ($p=0.0538$) affected by the RFI group. More efficient animals tended to emit 10% less methane by kg of body weight gain (21 days before each methane record). In the case of CO_2, more efficient animals had lower emissions in Dohne and Merino breeds ($p<0.05$), 6.8 and 4.8%, respectively.

Table 44: RFI group effect on GHG emissions reported as total methane, methane yield, methane intensity, and CO_2 for Corriedale, Dohne, and Merino breeds

Breed	Corriedale			Dohne			Merino		
RFI group	High	Medium	Low	High	Medium	Low	High	Medium	Low
Methane (g/d)	16.3[a]	16.2[a]	16.7[a]	26.6[a]	28.2[a]	27.8[a]	22.6	22.9	24.1
Methane yield (g/d) (by FI 24-48-72hs)	17.2	16.2	15.7	28.7	28.5	26.9	24.8	23.3	22.6
Methane yield (g/kgDM 24hs)	5.7	5.0	4.3	7.5	6.8	6.0	7.0	6.4	5.9
Methane intensity (g/kgBWG)	4.6[a]	4.4[a]	4.9[a]	9.6[a]	8.8[a]	9.2[a]	6.9	7.1	7.5
CO_2 (g/d)	830.9[a]	831.7[a]	851.2[a]	1354.8	1438.4	1446.5	1056.7	1063.6	1107.1

In the Corriedale breed, the RFI group also significantly affected the staple length where the less efficient animals have longer wool. In Merino, more efficient animals were slightly leaner than the low efficient group (table 45).

Table 45: Contrast by RFI group for Corriedale and Merino breeds

	RFI group				
	High	Medium	Low	Breed	P
Staple length (cm)	11.9±0.37	12.8±0.33	12.8±0.34	Corriedale	<0.05
Staple length (cm)	10.8±0.10	10.9±0.07	11.1±0.09	Merino	0.0801
Backfat (g/kgBWG)	2.01±0.04	2.08±0.03	2.19±0.04	Merino	<0.05
Fiber diameter (μ)	22.4±0.41	23.1±0.36	23.4±0.37	Corriedale	0.535

Phenotypic correlations

Moderate correlations were estimated between FI and RFI in the three studied breeds (tables 46, 47 and 48). The correlation between GHG emissions and FI also presented moderated correlations, being CO_2 the trait with higher correlations with FI within each breed. In relation to RFI, the number of meals and CH4 yield were the most correlated traits, again within each breed.

Table 46: Phenotypic correlations for traits related to efficiency for the Corriedale breed.

Corriedale	RFI	FCR	Nmeals	SL	Backfat	CH_4	CO_2	O_2	CH_4 intensity	CH_4 yield
Feed intake (kg/d)	0.74	0.23	0.55	0.40	0.16	0.60	0.65	0.56	0.35	-0.39
RFI (breed)		0.42	0.53	0.28	-0.03	0.28	0.22	0.15	0.27	-0.52
Feed CR (FI/ADG)			0.25	0.16	0.01	0.11	-0.03	-0.15	0.08	-0.12
N° of meals				0.20	0.05	0.32	0.43	0.41	0.25	-0.23
Staple Length (cm)					-0.06	0.43	0.27	0.27	0.39	-0.11
Backfat depth (mm)						-0.08	0.20	0.11	-0.25	-0.04
Methane (g/d)							0.72	0.61	0.88	0.23
CO_2 (g/d)								0.84	0.49	0.11
O_2 (g/d)									0.42	0.11
CH_4 intensity (g/kgBW)										0.26

Table 47: Phenotypic correlations for traits related to efficiency for the Dohne breed.

Dohne	RFI	FCR	Nmeals	SL	Backfat	CH_4	CO_2	O_2	CH_4 intensity	CH_4 yield
Feed intake (kg/d)	0.61	0.02	0.38	-0.09	0.39	0.38	0.70	0.64	-0.01	-0.43
RFI (breed)		0.30	0.44	0.03	0.09	-0.02	0.16	0.15	-0.04	-0.51
Feed CR (FI/ADG)			-0.13	-0.05	-0.05	-0.05	-0.04	-0.03	-0.03	0.03
N° of meals				0.13	0.09	0.02	0.08	0.08	0.00	-0.31
Staple Length (cm)					-0.20	-0.02	-0.31	-0.34	0.06	0.11
Backfat depth (mm)						0.07	0.45	0.45	-0.19	-0.24
Methane (g/d)							0.52	0.42	0.85	0.49
CO_2 (g/d)								0.96	0.13	-0.16
O_2 (g/d)									0.05	-0.20
CH_4 intensity (g/kgBW)										0.63

Table 48: Phenotypic correlations for traits related to efficiency for the Merino breed.

Merino	RFI	FCR	Nmeals	SL	Backfat	CH_4	CO_2	O_2	CH_4 intensity	CH_4 yield
Feed intake (kg/d)	0.57	-0.22	0.29	0.32	0.24	0.64	0.80	0.74	0.19	-0.23
RFI (breed)		0.20	0.30	0.13	-0.02	0.18	0.28	0.22	0.21	-0.42
Feed CR (FI/ADG)			0.24	-0.19	0.07	-0.15	-0.34	-0.35	-0.15	0.09
N° of meals				-0.02	0.17	0.23	0.13	0.11	0.03	-0.04
Staple Length (cm)					-0.07	0.25	0.30	0.35	0.16	-0.05
Backfat depth (mm)						0.16	0.17	0.11	-0.16	0.06
Methane (g/d)							0.71	0.66	0.75	0.41
CO_2 (g/d)								0.93	0.37	-0.03

Merino	RFI	FCR	Nmeals	SL	Backfat	CH_4	CO_2	O_2	CH_4 intensity	CH_4 yield
O_2 (g/d)									0.32	-0.01
CH_4 intensity (g/kgBW)										0.43

Principal component analysis for Feed Intake

Descriptive statistics of the traits involved in the PCA are presented in Table 49.

Table 49: Descriptive Statistics for the traits analysed for PCA by breed.

Breed	Trait	N	Mean	sd	min	max
Corriedale	Metabolic BW (kg)	281	13.9	1.6	9.6	18.3
	ADG (kg)	281	0.166	0.046	0.030	0.274
	Methane (g/d)	218	16.4	4.8	5.7	28.5
	CO_2 (g/d)	218	828.9	183.8	404.3	1447.7
	O_2 (g/d)	218	847.0	160.0	445.8	1315.8
Dohne	Metabolic BW (kg)	214	18.8	1.6	13.8	22.9
	ADG (kg)	214	0.181	0.050	0.079	0.325
	Methane (g/d)	208	28.1	5.7	15.7	50.6
	CO_2 (g/d)	208	1483.6	344.0	850.2	2537.8
	O_2 (g/d)	208	1321.5	325.5	774.1	2734.0
Merino	Metabolic BW (kg)	811	16.1	1.8	11.7	23.2
	ADG (kg)	811	0.202	0.068	0.055	0.469
	Methane (g/d)	784	23.4	5.4	8.9	44.7
	CO_2 (g/d)	784	1086.2	232.5	533.9	2269.6
	O_2 (g/d)	784	1000.3	189.4	560.9	2114.7

The PCA 1 explains 68.54% of the total variance. The correlation between PCA 1 and the observed FI was 0.82 for Australian Merino (figure 35). Therefore, with two estimates of GHG emission in portable accumulation chambers, in addition to measures of BW and ADG, it would be possible to have an estimation of feed intake.

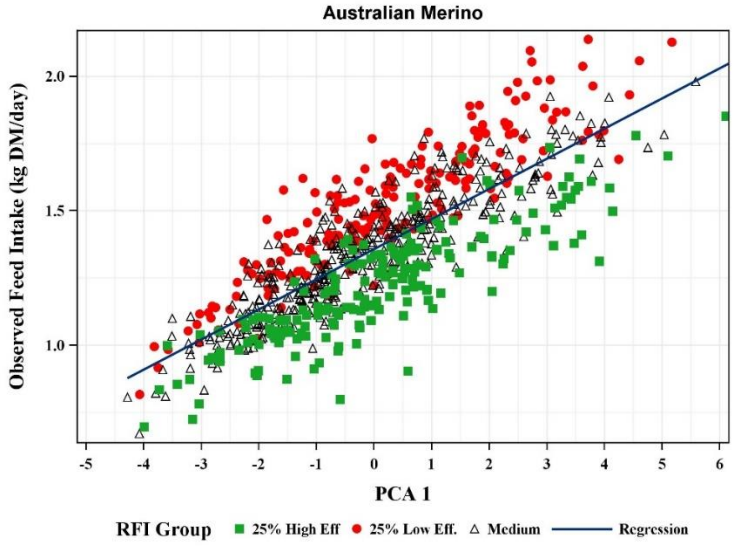


Figure 35: PCA 1 vs. observed feed intake for Australian Merino (r= 0.82).

Table 50: Eigenvectors for the five principal components.

	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5
Metabolic BW	0.405	0.594	-0.360	0.594	0.023
ADG (kg)	0.396	-0.644	0.321	0.569	-0.037
Methane (g/d)	0.421	0.419	0.764	-0.245	0.064
CO_2 (g/d)	0.505	-0.112	-0.275	-0.371	-0.720
O_2 (g/d)	0.497	-0.211	-0.330	-0.354	0.689

To validate the results obtained with Merino, the eigenvectors of PCA 1 obtained (Table 50) were multiplied by the standardized values of the five analysed traits of Corriedale and Dohne breeds to calculate a PCA 1 estimated for each breed. The correlation between these estimated PCA 1 vs observed feed intake was 0.7341 and 0.7338 for Corriedale, Dohne, and Merino, respectively (figure 36).

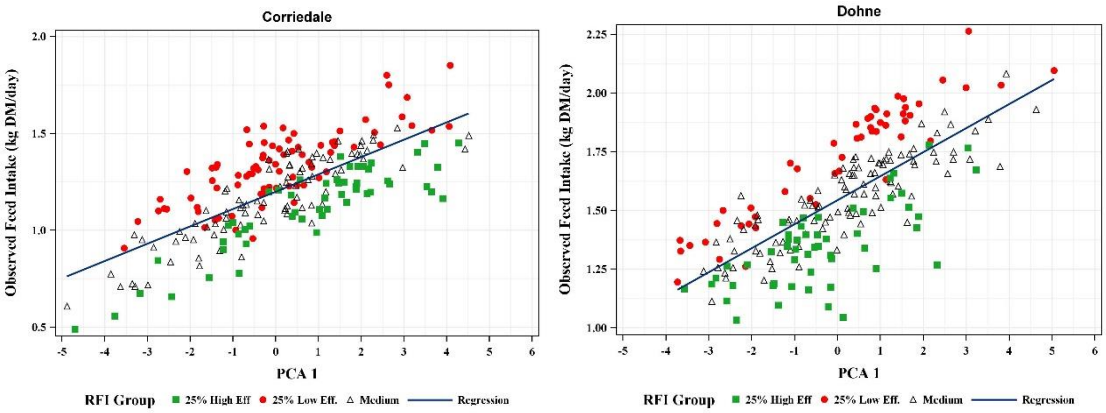


Figure 36: PCA 1 estimated from Merino data vs. observed feed intake for Corriedale and Dohne breeds.

Animals were classified based on percentiles from PCA 1 (from PCA analysis for Merino and estimated for Corriedale and Dohne) into three classes: High (>25%), Medium, and Low PCA (<25). A confusion matrix between these classes and observed FI is presented in table 51. In conclusion, the PCA estimated class can help to predict if an animal has high or low feed intake based on traits that can be recorded under field conditions. The false-negative and positive rates were almost zero.

Table 51 - Confusion matrix: percentage of animal classified by observed feed intake and PCA

		1. High PCA	**2. Medium PCA**	**3. Low PCA**
Corriedale n=218	**1. High FI**	53.7	46.3	0.0
	2. Medium FI	22.9	61.5	15.6
	3. Low FI	0.0	32.7	67.3
Dohne n=208	**1. High FI**	61.5	38.5	0.0
	2. Medium FI	18.3	62.5	19.2
	3. Low FI	1.9	36.5	61.5
Merino n=784	**1. High FI**	73.5	26.0	0.5
	2. Medium FI	13.3	73.2	13.5
	3. Low FI	0.0	27.6	72.5

7.2.4 France (on-farm)

Three breeds collected phenotypes on ewes and lambs. By the end of August 2021, more than 5,300 ewes and 8,600 lambs had been phenotyped (table 52). Few data were retrieved from the slaughterhouses, particularly for the Mouton Vendéen and Rouge de l'Ouest breeds.

Table 52: Number of individuals (ewes and lambs) phenotyped on-farm in three French meat sheep breeds.

Filtering	**Blanche du Massif Central**	**Mouton Vendéen**	**Rouge de l'Ouest**
EWES			
no filtering	2,579	1,534	1,198
with data before and after one lambing	650	753	489
LAMBS			
no filtering	5,754	2,798	56
with carcass data	1,222	9	0
Pairs of ewes and lambs with complete datasets			
no filtering	572 ewes with 932 lambs	532 ewes with 823 lambs	27 ewes with 45 lambs

The description of phenotypes in ewes and lambs is given in tables 53 and 54, respectively. For ewes, this description was done over all stages of the production cycle.

Table 53: descriptive statistics of traits recorded on ewes in three French meat sheep breeds. N gives the number of records.

Ewe Traits	Blanche du Massif Central		Mouton Vendéen		Rouge de l'Ouest	
	n	mean±sd [min;max]	n	mean±sd [min;max]	n	mean±sd [min;max]
Body weight (kg)	8,867	68.34±11.26 [34.00;125.00]	3,902	61.14±11.45 [33.20;103.00]	2,692	67.55±14.22 [31.00;113.00]
BCS	10,544	2.43±0.70 [1.00;5.00]	6,591	2.98±0.84 [1.00;5.00]	2,723	3.20±0.77 [1.00;5.00]
Chest width (cm)	15,777	25.45±2.49 [14.00;54.00]	6,794	25.64±3.12 [17.00;39.0]	2,746	27.06±3.57 [16.70;40.00]
Chest depth (cm)	15,777	32.14±2.53 [19.40;62.80]	6,790	32.88±2.88 [16.50;69.00]	2,747	34.06±3.24 [21.00;77.60]
Shoulder height (cm)	11,332	60.56±3.78 [49.50;73.00]	5,603	59.28±3.81 [45.00;71.00]	2,234	62.96±3.68 [50.90;74.00]
BFT-US (mm)	2,700	6.27±1.92 [2.40;16.00]	851	4.62±2.03 [1.90;13.30]	840	4.76±1.45 [1.70;11.70]
MD-US (mm)	2,700	26.65±3.12 [16.85;35.6]	851	25.14±3.95 [14.15;39.95]	840	26.61±3.42 [15.50;35.05]
Chest circumference (cm)	15,777	182.26±13.98 [114.90;299.99]	6,790	185.51±16.38 [125.14;319.18]	2,746	193.51±19.23 [135.42;369.44]
chest/BW ratio (cm/kg)	8,867	2.74±0.36 [1.78;5.80]	3,850	3.10±0.46 [1.84;7.80]	2,691	2.95±0.48 [1.84;5.69]

Table 54: descriptive statistics of traits recorded on lambs in three French meat sheep breeds. N gives the number of records. TW30d = typical weight at 30 days.

Lamb Traits	Blanche du Massif Central		Mouton Vendéen		Rouge de l'Ouest	
	n	mean±sd [min;max]	n	mean±sd [min;max]	n	mean±sd [min;max]
Birth weight (kg)	6,044	4.77±1.01 [1.30;8.20]	2,741	4.18±0.94 [1.20;7.70]	58	4.83±1.13 [2.50;7.50]
TW30d (kg)	5,483	12.57±2.70 [3.90;23.00]	2,644	11.35±2.81 [3.50;19.90]	59	13.16±2.47 [8.00;19.00]
ADG 0-30d g/d)	5,363	257.33±74.25 [-83.33;550.00]	2,481	239.14±79.83 [10.00;506.67]	57	275.50±75.60 [100.00;446.67]

On the subset of ewes having data before and after one lambing, a PCA was performed on the residuals from the linear model, taking into account main environmental factors. No multivariate was performed on the Rouge de l'Ouest breed because not enough data were available by the analysis.

In the Blanche du Massif Central breed, the first two axes accounted for 59.3% of the total variance. The main contributing traits to the first axis were the body weights (before and after lambing), the chest/BW ratio (before and after lambing), and the chest circumference before lambing. The second

axis was mainly characterised by the evolution of traits before and after lambing: the difference in body weights, the difference in chest/BW ratio and the difference in BCS.

In the Mouton Vendéen breed, the first two axes of the PCA accounted for 55% of the total phenotypic variance. The main contributing traits to the first axis were mainly the ones recorded before lambing: body weight, chest/BW ratio, and the chest circumference, as well as the body weight recorded after lambing. The most contributing variables to the second axis were the difference in body weight, BCS and chest/BW ratio before and after lambing, and the traits recorded after lambing: body weight, BCS and chest/BW ratio.

A FAMD analysis was performed on the lambs dataset. In the Mouton Vendéen and Blanche du Massif Central breeds, the first two axes accounted for 19.4% of the total variance (with axis 1 accounting for 10.8% and 12.2% for the Mouton Vendéen and Blanche du Massif Central breed, respectively). The first axis was mainly affected by body weights (at birth and at 30 days of age) and ADG between birth and 30 days, whereas the second axis was mainly affected by vigour traits.

For the Blanche du Massif Central breed, the HCPC returned 4 and 3 clusters for ewes and lambs, respectively. In the Mouton Vendéen breed, the HCPC returned 3 clusters for ewes and lambs.

Regarding lambs, the three clusters had similar characteristics whatever the breed with:

- Cluster A (1,918 Blanche du Massif Central and 1,139 Mouton Vendéen lambs): lambs with lower body weights and growth performances than the breed average
- Cluster B (274 Blanche du Massif Central and 7 Mouton Vendéen lambs): lambs with body weights and growth performances equal to the breed average
- Cluster C (2,722 Blanche du Massif Central and 1,166 Mouton Vendéen lambs): lambs with higher body weights and growth performances than the breed average

Regarding ewes, two clusters were similar between both breeds:

- Cluster A (197 Blanche du Massif Central and 159 Mouton Vendéen ewes): ewes with BCS, body weight, and chest circumference before lambing close to the average of the breed, but with BCS, body weight and chest circumference after lambing higher than the average of the breed. In this cluster, the difference between traits recorded before and after lambing is higher than the average of the breed. Ewes in this cluster also have a higher shoulder height than the average of the breed.
- Cluster B (102 Blanche du Massif Central and 186 Mouton Vendéen ewes): ewes with BCS, body weight, and chest circumference before lambing higher than the average of the breed, and with BCS body weight and chest circumference after lambing close to the average of the breed. In this cluster, the difference between traits recorded before and after lambing is lower than the average of the breed. Ewes in this cluster also have a shoulder height close to the average of the breed.
- Cluster C (144 Blanche du Massif Central ewes): ewes with BCS body weight and chest circumference before lambing close to the average of the breed, but with BCS, body weight, and chest circumference after lambing lower than the average of the breed. In this cluster, the difference between traits recorded before and after lambing is lower than the

average of the breed. Ewes in this cluster have a shoulder height lower than the average of the breed.

- Cluster D (222 Mouton Vendéen ewes): ewes with BCS, body weight, and chest circumference before and after lambing lower than the average of the breed. In this cluster, the difference between traits recorded before and after lambing is lower than the average of the breed. Ewes in this cluster have a shoulder height lower than the average of the breed.
- Cluster E (214 Blanche du Massif Central ewes): ewes with BCS, body weight, and chest circumference before lambing lower than the average of the breed, but with BCS, body weight, and chest circumference after lambing and shoulder height close to the average of the breed. In this cluster, the difference between traits recorded before and after lambing is higher than the average of the breed.

Finally, when lambs and ewes' clusters were analysed jointly, we did not observe clear links between them in any of both breeds (tables 55 and 56).

Table 55: Distribution of ewes and lambs from the Blanche du Massif Central Breed among the different clusters defined based on the data.

	Ewes cluster A (n=267)	Ewes cluster B (n=150)	Ewes cluster C (n=210)	Ewes cluster E (n=302)
Lambs cluster A (n=364)	38% of ewes from cluster A 27% of lambs from cluster A	38% of ewes from cluster B 16% of lambs from cluster A	43% of ewes from cluster C 25% of lambs from cluster A	38% of ewes from cluster E 32% of lambs from cluster A
Lambs cluster B (n=58)	7% of ewes from cluster A 31% of lambs from cluster B	7% of ewes from cluster B 17% of lambs from cluster B	5% of ewes from cluster C 19% of lambs from cluster B	6% of ewes from cluster E 33% of lambs from cluster B
Lambs cluster C (n=507)	55% of ewes from cluster A 30% of lambs from cluster C	55% of ewes from cluster B 16% of lambs from cluster C	52% of ewes from cluster C 21% of lambs from cluster C	56% of ewes from cluster E 33% of lambs from cluster C

Table 56: Distribution of ewes and lambs from the Mouton Vendéen breed among the different clusters defined based on the data.

	Ewes cluster A (n=207)	Ewes cluster B (n=295	Ewes cluster D (n=323)
Lambs cluster A (n=364)	43% of ewes from cluster A 25% of lambs from cluster A	36% of ewes from cluster B 29% of lambs from cluster A	52% of ewes from cluster D 46% of lambs from cluster A
Lambs cluster C (n=461)	57% of ewes from cluster A	64% of ewes from cluster B	48% of ewes from cluster D

	Ewes cluster A (n=207)	Ewes cluster B (n=295	Ewes cluster D (n=323)
	25% of lambs from cluster C	41% of lambs from cluster C	34% of lambs from cluster C

From the clustering analysis, we cannot highlight any trend between the evolution of ewes' conditions (from their BCS and BW) and the performances of their lambs (until 30 days of age).

Regarding beta-hydroxybutyrate records with the Abbot kit, a total of 756 measures were recorded on ewes: 368 during gestation and 388 at weaning of their lambs.

7.3 Main results in meat sheep

A total of 2,227 meat sheep have been analysed in experimental farms, and 19,919 meat sheep (ewes and lambs) were analysed in commercial farms. Body weights and body composition traits were the main recorded traits.

In commercial farms, no feed intake was recorded, but in experimental farms (including test stations for individual control of performances), total feed intakes were recorded with different devices. Therefore, feed efficiency traits and the quality of proxies were assessed on these experimental individuals. Regarding RFI calculation, it has been proposed to decrease by one week the length of feed intake recording, with no impact on the RFI calculation. Moreover, no significant impact of wool traits neither of body composition traits has been found on feed efficiency traits, which makes the more parsimonious model requiring only feed intakes and body weights to be recorded.

From the first results, it appears that ruminal variables (microbiota, metabolomics) cannot be proposed as proxies for feed intake neither for feed efficiency when they are considered separately. However, data collected at the level of the host (plasmatic metabolites such as citrate or some amino acids and ^{15}N natural abundancies) are more likely to be proposed as potential proxies of feed efficiency or feed intake. The combination of gas emissions and classical recorded traits (ADG and BW) were found to well predict has high or low feed intake.

Feed intake is predicted with higher accuracies than feed efficiency traits.

First multi-omic integration analyses in Romane meat divergent lines highlighted that the best proxies for residual feed intake were fixed effets (and covariate) and plasma NMR.

Genetic analyses and particularly the estimation of genetic correlation between the proposed proxies and feed intake or feed efficiency traits, will provide additional information to better propose valuable proxies to be more largely recorded.

In commercial farms, the clustering of ewes and lambs based mainly on body weights and body condition scores did not highlight any significant link between the status of the ewes and the performances (early body weights and growth) of their lambs.

8 Communications related to feed efficiency and its proxies

Results presented in this report have been communicated in international conferences:

2020

Esteban C, Toral PG, Fernández-Díez C, Suarez-Vega A, Gutiérrez-Gil B, González Recio O, Hervás G, Marina H, Frutos P and Arranz J-J (2020) Study of rumen microbiota in dairy sheep with different feed efficiency using nanopore sequencing. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.) Virtual Meeting 1-4 December 2020.

De Barbieri I, Navajas EA, Giorello D, Velazco JI, Banchero G, Rodríguez B, Rovira F and Ciappesoni G (2020) Association between feed efficiency and methane emissions, performance and health in Merino sheep. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.) Virtual Meeting 1-4 December 2020.

Della Badia A, Hervás G, Toral PG, Amor J, Belenguer A, Fernández-Díez C and Frutos P (2020) The paradox of using residual feed intake or conversion ratios to study feed efficiency in dairy ewe. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.) Virtual Meeting 1-4 December 2020.

Franco M, Toral PG, Esteban C, Gutiérrez-Gil B, Hervás G, Arranz J-J, Frutos P and Suárez-Vega A. (2020) Milk transcriptome analysis identifies genes and pathways affecting feed efficiency in dairy ewes. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.) Virtual Meeting 1-4 December 2020.

Touitou F, Marie-Etancelin C, Weisbecker J-L, Marcon D, François D, Bessa R, Meynadier A and Tortereau F. (2020). Divergent selection on residual feed intake in Romane meat sheep breed to dissect biological processes underlying feed efficiency. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.) Virtual Meeting 1-4 December 2020.

Weisbecker J-L, Huau C, BompaJ-F, Marcon D, Estivalet L, Marie-Etancelin C, Tortereau F, Heirman T, Laperruque F, Francois D and Ricard E. (2020). High-throughput phenotyping of intakes in small ruminants. Book of Abstracts of the 71st Annual Meeting of the European Federation of Animal Science.) Virtual Meeting 1-4 December 2020.

2021

Marques, C.B.; Ciappesoni, G; Velazco, J.I.; Navajas, E.A.; Ferreira, G.F.; Ramos, Z.; Rovira, F.; De Barbieri, I. (2021) Evaluation of different models to define a more suitable Residual Feed Intake estimation in Merino sheep. Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science.

Le Graverand Q, Tortereau F, Meynadier A, Marcon D and Marie-Etancelin C, (2021) The rumen microbiota is modified in lambs divergently selected for residual feed intake. Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science.

Marie-Etancelin C, Weisbecker J-L, Marcon D and Tortereau F (2021): Impact of RFI selection on lambs' feeding behaviour. Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science.

Tortereau F, Marie-Etancelin C, Frutos P, Conington J, Arsenos G, De Barbieri I, Jakobsen JH, Moreno-Romieux C, Arranz J-J. (2021) SMARTER – Which novel traits to improve feed efficiency? Proceedings of the 44th ICAR Annual Conference virtually held from Leeuwarden, NL, 26-30 April 2021

Touitou F, Meynadier A, Marty-Gasset N, Vialaneix N, Lefort G and Tortereau F. (2021). Plasmatic and ruminal metabolomes of lambs divergently selected on residual feed intake. Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science.

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11 Appendix

11.1 Appendix 1 – Detailed traits description, laboratory protocols and bioinformatics pipelines

Partner	Analysis	Protocol
UNILEON	Basisc statistics of analytical groups	The following table shows the basic statistics of the animals selected for the intensive sampling population. The table shows the average, standard deviation (SD), standard error of the mean (SEM), maximum (Max), and minimum (Min) EBV values in each of the groups considered. The distribution of control (C) and nutritional challenge (NC)

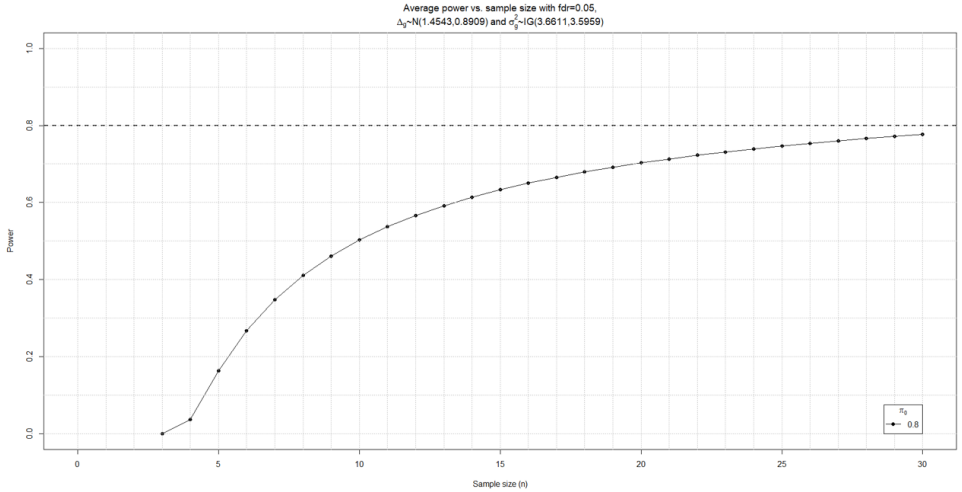
<table>
<tr><td></td><td></td><td>animals are equal in the two groups (high vs. low) of estimated breeding values (EBV) for milk production.
<table>
<tr><th>Group</th><th>N</th><th>Average</th><th>SD</th><th>SEM</th><th>Max</th><th>Min</th></tr>
<tr><td>High_EBV</td><td>20</td><td>86.45</td><td>15.19</td><td>3.40</td><td>109.5</td><td>60.5</td></tr>
<tr><td>Low_EBV</td><td>20</td><td>37.38</td><td>15.45</td><td>3.46</td><td>57.5</td><td>5.5</td></tr>
<tr><td>C</td><td>20</td><td>58.66</td><td>31.68</td><td>7.08</td><td>109.5</td><td>5.5</td></tr>
<tr><td>NC</td><td>20</td><td>62.63</td><td>27.07</td><td>6.05</td><td>109.5</td><td>9.0</td></tr>
</table></td></tr>
<tr><td>UNILEON</td><td>Feed composition</td><td>Feed samples were prepared (ISO 6498:2012) and analyzed for DM (ISO 6496:1999), ash (ISO 5984:2002), and CP (ISO 5983-2:2009). The NDF, ADF and ADL concentrations were sequentially determined using an Ankom^{2000} fiber analyzer (Ankom Technology Methods 13, 12, and 8, respectively; Ankom Technology Corp., Macedon, NY, USA); the former was assayed with sodium sulfite and α-amylase, and both NDF and ADF were expressed with residual ash. Starch content was analyzed by a total starch assay kit obtained from Megazyme (K-TSTA; Megazyme Intl. Ireland Ltd., Wicklow, Ireland). The FAME of lipid in freeze-dried TMR samples were prepared in a 1-step extraction-transesterification procedure (Shingfield et al., 2003), adding 1 mg of cis-12 13:1 (10-1301-9, Larodan Fine Chemicals AB, Solna, Sweden) as an internal standard. The methyl esters were separated and quantified using a gas chromatograph (Agilent 7890A GC System, Santa Clara, CA, USA) equipped with a flame ionization detector and a 100-m fused silica capillary column (0.25 mm i.d., 0.2-µm film thickness; CP-SIL 88, CP7489, Varian Ibérica S.A., Madrid, Spain), and hydrogen as fuel and carrier gas (207 kPa, 2.1 mL/min). Total FAME profile in a 2-µL sample volume at a split ratio of 1:50 was determined using a temperature gradient program described in Shingfield et al. (2003). Peaks were identified based on retention time comparisons with commercially available standards (from Nu-Chek Prep, Elysian, MN, USA; and Sigma-Aldrich, Madrid, Spain).</td></tr>
<tr><td>UNILEON</td><td>Milk sampling</td><td>Following the protocol by Suárez-Vega et al. (2015), the collection was performed approximately 1 h after milking and 10 min after the injection of oxytocin (5 IU/ewe; Facilpart, Laboratorios SYVA, León, Spain) to maximize the concentration of mammary epithelial cells. Udders were cleaned with water and soap and then disinfected with povidone-iodine; nipples were also washed with RNaseZap (Ambion, Austin, TX, USA). Individual samples were obtained by hand-milking each half of the mammary gland into an RNase-free 50-mL tube (2 samples/ewe) that was covered with sterile gauze to filter the milk. Samples were kept in ice and immediately transferred to the laboratory for RNA extraction.</td></tr>
<tr><td>UNILEON</td><td>Milk composition</td><td>Fat, protein, and lactose concentration were determined by infrared spectrophotometry (ISO 9622:1999) using a MilkoScan FT6000 (Foss, Hillerød, Denmark). Lipid in 1 mL of milk was extracted and converted to FAME by base-catalyzed transesterification (Shingfield et al., 2003). Total FAME profile was determined using the same chromatograph and temperature gradient program applied for the analysis of feed, but isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield et al., 2003). All peaks were identified based on retention time comparisons with commercially available standards (from Larodan Fine Chemicals AB, Nu-Chek Prep, and Sigma-Aldrich), cross-referencing with chromatograms reported in the literature (e.g., Shingfield et al., 2003), and comparison with reference samples for which the FA composition was determined based on GC analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivatives (Bichi et al., 2013; Toral et al., 2017).</td></tr>
</table>

UNILEON	Milk DNA and RNA extractions	Milk samples were kept on ice and immediately transferred to the laboratory to obtain the milk somatic cell pellet through washing and centrifugation procedure with PBS solution. Then, cell pellets were diluted in 0.5 ml of RNAlater (Ambion, Austin, TX, USA) and stored at -80ºC till RNA-extraction. RNA extraction was performed following a standard Trizol protocol as detailed by Suarez-Vega et al. (2016). In addition, milk samples were also collected for DNA extraction on week 8 of the lactation to assess epigenetic marks by whole-genome bisulfite sequencing (WGBS). The extraction protocol was an adapted version of MasterPure™ DNA Purification Kit for Blood Version II (Lucigen, USA), described by Murphy et al. (2002). After extraction, DNA samples were kept at 4ºC, whereas RNA samples were stored at −80ºC.
UNILEON	Rumen fluid management	Immediately after collection, the ruminal fluid was filtered with a 400 µm nylon mesh (Fisher Scientific S.L., Madrid, Spain). Then, 4 mL of each sample were acidified with 4 mL of 0.2 *M* HCl for ammonia analysis and further 4 and 0.8 mL aliquots were taken, respectively, for the analysis of lactic acid and VFA (the latter deproteinized with 0.5 mL of 0.5 *M* HCl solution containing 0.25 *M* metaphosphoric acid and 6.9 m*M* crotonic acid). These samples were stored at −30°C until analysis. The remaining fluid sample was immediately frozen at −80°C, freeze-dried, and stored again at −80°C until analyzed for FA composition.
UNILEON	Rumen fluid composition	Ammonia and lactic acid concentrations were determined by colorimetric methods (Reardon et al., 1966; Taylor, 1996; respectively) and VFA by GC, using crotonic acid as an internal standard (Ottenstein and Bartley, 1971), in centrifuged samples. Lipid in 200 mg of freeze-dried rumen liquor was extracted twice using 4 mL hexane:isopropanol (3:2, v/v, pH 2; Shingfield et al., 2003), and adding *cis*-12 13:1 as an internal standard. Organic extracts were combined and dried at 45°C with a continuous flow of nitrogen, and FA were converted to methyl esters using a sequential base-acid catalyzed transesterification (Toral et al., 2017). Then, the FA profile was determined using the same procedures applied for the analysis of milk.
UNILEON	Gene expression profile by RNA-Seq.	The RNA integrity (RIN value) was measured for all the milk RNA samples using an Agilent 2100 Bioanalyzer device (Agilent Technologies). Due to budget limitations, of the 40 ewes involved in the feed efficiency experiment, a total of 28 animals were selected for transcriptome analysis. The selection criteria were to choose 14 animals from each point of the RFI distribution (high and low efficiency, based on its RFI). We ensure a balanced representation of animals from the C and NC groups and high and low EBV in each of the two combinations of higher and lower efficiency animals. The cDNA library construction and sequencing were conducted at NOVOGENE in Cambridge (UK). The construction of the cDNA library takes place using the UltraTM RNA Library Prep Kit (NEBNext®). With that, 28 stranded paired-end libraries were prepared. According to the manufacturer's instructions, cDNA libraries were sequenced to a minimum depth of 30 million paired-end reads on a Novaseq 6000 (Illumina), generating stranded paired-end reads of 150 bp. ***Differential expression analysis*** The main steps in RNAseq analysis are (i) Quality Control using FASTQC and Trimmomatic to remove those sequences that presented low quality or inadequate length and cut out those sequences that have errors as residues of the adapters used in sequencing. (ii) Alignment against the reference genome (Oar_rambouillet_v1.0) using STAR v2.4.0 software (Dobin et al., 2013) and quantifications of transcripts for the different samples using RSEM software (v.1.3.0) (Li and Dewey, 2011) using the annotation from Ensembl database.

Once tables with the quantified reads for each gene and sample had been obtained, the differential expression analysis was performed. This type of analysis aims to identify those genes that are significantly differentially expressed (DEGs) between the groups or conditions analyzed, particularly between the High-RFI versus the Low-RFI.

Power analysis

The Estimation of statistical power in RNA-seq was performed using the R package ssizeRNA (Bi and Liu, 2016), taking into account the dispersion and analysis parameters used in our experiment. Briefly, during the simulation procedure, we contemplated a genome of 20,000 genes with an expected proportion of DEG of 10% and FDR of 0.05. The sample size in cases and controls was 14 in each group. With these data, we obtained an average power of 0.656 considering that not all genes share the same fold change; in our case, we considered that fold-change comes from a log-normal distribution (log − Normal(log(2), 0.5 ∗ log(2)). The following figure plots the average power for different sample sizes. With the same parameters but fixing the fold-change in ≥ 2, the statistical power reaches 0.76 for a sample size of 14 (data not shown).



Functional enrichment analysis (GO term enrichment analysis)

Once DEGs had been identified between the two conditions studied, subsequent analyses were proposed to identify biological functions and metabolic pathways significantly enriched between the over-expressed or under-expressed genes in the animals with high and low feed efficiency using ToppGene software (Cincinnati Children's Hospital Medical Center, 2020). The final objective of this analysis is to know if the biological and functional information linked to the DEGs identified between the two experimental groups allows us to explain the difference in RFI between both conditions, determining the biological processes and metabolic pathways enriched in the set of DEGs.

Finally, a network analysis was carried out to identify those genes whose proteins presented protein-protein correlations both direct (physical interactions) and indirect (functional interactions) with the STRING program (Szklarczyk et al., 2018). Once the protein-protein interactions network has been built, the STRING tool allows different types of functional enrichment analysis to be carried out against various databases, allowing the identification of gene ontology (GO) terms, metabolic pathways (KEGG), and Reactome Pathway (Kanehisa and Goto, 2000; Slenter et al., 2018).

		Variant calling and annotation After the alignment, Samtools (Li et al, 2009) was used to convert *sam* files to bam files and then to sort and merge the bam files from the same animal at different time-points. Then, Picard (Wysoker et al, 2010) was used to add read groups and mark duplicated reads on the merged bam files. SNP and Indel calling was performed using the BCFtools software (Li, 2011). To obtain high-quality variants, strict filter conditions were applied using SnpSift (Cingolani et al, 2012) (Variation Quality (QUAL) >30, Mapping Quality (MQ) >40, Quality By Depth. (QD) >5, Fisher Strand (FS) <60 and a minimum Depth of coverage (DP) >5 in all the samples). SnpEff (Cingolani et al, 2012) were used to predict the functional consequences of the detected variants. ***Weighted Gene Coexpression Network Analysis (WGCNA)*** The Weighted Gene Co-expression Network Analysis (WGCNA) (Langfelder and Horvath (2008) R package was used to build co-expression networks and identify groups of highly co-expressed genes. Individual analyses were conducted on each breed group (High and Low RFI, and C and NC animals). First, the low count genes and outliers were filtered by leaving only genes with at least 1 count per million in 90% of each group. The gene expression counts were normalized using the default procedure from the DESeq2 package by correcting for effect not analyzed (C, vs NC or HEBV vs. LEBV) to reduce potential effects from the given factor. The final dataset expression values were subjected to the WGCNA using the WGCNA Bioconductor package in R (Langfelder et al., 2008). Pairwise Pearson's correlations among all genes were calculated to create a similarity matrix. The similarity matrix was used to calculate the Topological Overlap Matrix (TOM). Modules of co-expressed genes were identified by using the dynamic tree cut algorithm. The modules were constructed with a minimum module size of 30 genes. The module eigengenes were computed for each module using the first principal component to capture the variation in gene expression within each module. The eigengene sign was chosen to have a positive correlation with average module gene expression. The correlation between module eigengene and RFI or treatment diet was evaluated to select modules associated with the respective traits (p-value < 0.05). In addition, FDR were computed using Benjamini–Hochberg (BH). To interpret the functional and biological significance of the co-expressed gene network modules significantly associated to RFI and diet, ToppGene software (Cincinnati Children's Hospital Medical Center, 2020) was used to identify significantly enriched GO terms and KEGG metabolic pathways
UNILEON	Epigenetic marks (DNA-methylation) by WGBS (Whole-genome bisulfite sequencing)	The animals chosen to analyze epigenetic marks were the same 28 selected in the case of the RNA-seq experiment detailed above. The quality and concentration of DNA samples obtained from milk somatic cells were assessed with a Qubit 2.0 fluorometer. Library construction and sequencing were conducted at NOVOGENE in Cambridge (UK). The library construction involved DNA fragmentation, methylation of cytosines, and bisulfite treatment. Sequencing was performed for different libraries according to the concentration and the demand of data amount on a Novaseq 6000 (Illumina) sequencer generating paired-end reads of 150 pb with a minimum depth of 30 million paired-end reads per sample. ***Identification of differentially methylated regions (DMRs) and genes related to DMRs*** A preliminary version of the bioinformatic workflow analysis for detecting differentially methylated regions (DMRs) between the groups to be compared (NC vs C; HighEBV vs

		LowEBV) has been recently presented by Suárez-Vega et al. (2021). Quality control assessments were performed using FASTQC and Trimmomatic . High-quality sequences were mapped to the ovine reference genome (Oar_rambouillet_v1.0) using BISMARK (Krueger & Andrews 2011). Then, the same software was used to deduplicate mapping files and perform the methylation calling using *deduplicate_bismark* and *bismark_methylation_extractor* packages, respectively (Krueger & Andrews 2011). DMRs were determined using the R package DSS (dispersion shrinkage for sequencing data) (Feng et al. 2014). We identified DMRs using the coverage files from BISMARK and the *calldmr* function from DSS package with a smoothing window of 200 bp, p-value threshold of 1e-05, and a percentage of CG sites with significant p-values in the DMR greater than 0.5. To be considered significant, a DMR was required to contain at least three CpG sites (default parameter). Once identified, genes and QTLs within the DMRs were annotated using GALLO package in R (Fonseca et al., 2020). Finally, functional enrichment analyses was performed in the genes related to DMRs to identify and interpret the biological processes involved in the groups compared based on GO terms and KEGG pathways using ToppGene software (Cincinnati Children's Hospital Medical Center, 2020).
UNILEON	Metabolomic analyses	*Sample processing* A 50 mL milk sample was collected from each of the 39 ewes for untargeted metabolomics on the last day of the trial. Each sample was aliquoted into 200 µL portions and stored at -80 °C. Each aliquot was mixed with 1 mL of methanol-chloroform (3:1) in an Eppendorf tube. The mixture was vortexed at 3000 rpm for 1 min and then sonicated in an ice-water bath for 2 mins. Following this, centrifugal precipitation was performed at 21,000 g and 5 °C for 10 minutes. The resulting transparent supernatant was collected for further processing. *Reversed-phase liquid chromatography-mass spectrometry (LC-MS)* Reversed-phase LC–MS was performed to detect features ranging from polar to less polar compounds and lipids. For each sample, 200 µL of the supernatant was transferred to an LC injection vial. The column flow rate was set to 400 µL/min, and the column temperature was maintained at 55 °C. Each sample underwent two rounds of sample solution injections, one for positive ion detection and another for negative ion detection. For compound detection and relative quantitation, the MS instrument was operated in the Fourier transform MS detection mode at a mass resolution of 60,000 FWHM @ m/z (mass-to-charge ratio) 400 and within a mass range of m/z 50 to 1800. To ensure the quality of LC–MS detection during data acquisition, a quality control (QC) sample was prepared by pooling samples from 10 randomly selected test samples. The QC sample was injected in parallel with the test samples in completely random order during data acquisition. *Hydrophilic interaction LC–MS (HILIC-MS)* HILIC-MS was performed to detect highly polar features. For each sample, 600 µL of the supernatant was mixed with 150 µL of water and 200 µL of chloroform. Subsequently, 5 µL aliquots from each sample solution were injected into a Waters HILIC column. The high-resolution LC–MS analysis was conducted using the same equipment and a similar procedure as described above. In this case, the column flow rate was set to 300 µL/min, and the column temperature was maintained at 40 °C. The mass detection range for this analysis was m/z 50 to 1000. *Data processing* Four high-resolution LC–MS datasets were obtained, and each dataset was processed using the R package XCMS (Smith et al., 2006). The processing steps included peak detection,

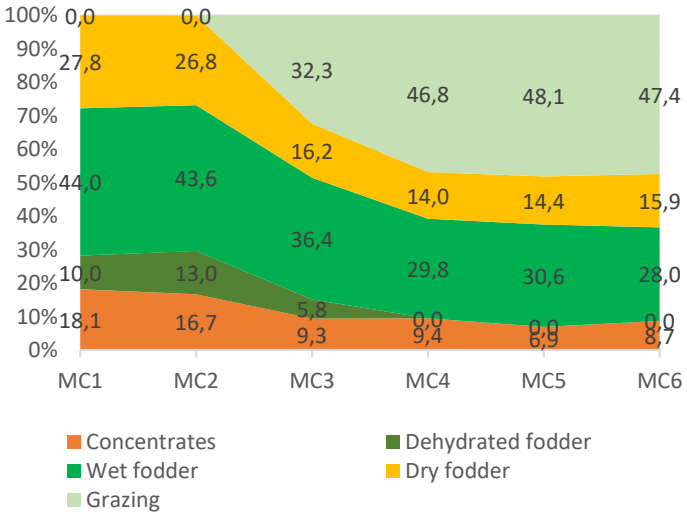
		peak grouping, peak alignments, and retention time shift correction. The resulting dataset included pairs of MS m/z and LC retention time (RT, min) for the detected compound features across all the samples within each dataset. This procedure revealed 3760 feature signals. Those for which less than 75% of the data were available from the ewes were filtered out, resulting in a final dataset composed of 3749 features. As untargeted metabolomics data are compositional in nature (Sisk-Hackworth and Kelley, 2020), the feature signals were normalized using a centered log-ratio (CLR) transformation. The processed data were implemented in subsequent statistical analyses. *Analysis of the discrimination between high and low FE groups and selection of features with better discriminating profiles* The ewes were classified into high, medium, and low FE groups for RFI (H-RFI, M-RFI, L-RFI) and FCR (H-FCR, M-FCR, L-FCR) based on the distribution of each metric. The R package mixOmics (Rohart et al., 2017) was used to perform a partial least squares discriminant analysis (PLS-DA) to evaluate the potential of selected features to discriminate the high, medium, and low FE groups for both RFI and FCR. The area under the curve (AUC) was calculated to estimate the discrimination potential of PLS-DA among the three FE groups. The first two principal components were plotted with centroids to define the groups identified by the PLS-DA using the plotIndiv function in the mixOmics package. Additionally, the performance of PLS-DA for the RFI and FCR groups was evaluated based on the mean error rate and Q2 for the first five principal components through cross-validation u lines 180-27sing 10 folds. Once the ewes were assigned to each group, the variable importance in the projection (VIP) was estimated to compute the influence on the features of every predictor in the PLS-DA model. Features with a VIP>2 were selected individually from the outputs of the discriminant analysis for RFI and FCR. *Prediction of RFI and FCR using machine learning algorithms* Predictive models were built using the CLR-transformed values for each of all 3749 detected features (RFI_all and FCR_all) and for the subsets of features selected in the PLS-DA analysis for RFI and FCR with VIP>2 (RFI_VIP and FCR_VIP). The ML models were built based on a multilayer feedforward artificial neural network (deep) and random forest (RF) using the R package h2o (Candel, Arno, Viraj Parmar, Erin LeDell, 2016), extreme gradient boosting (xgboost) using the R package xgboost (Chen and Guestrin, 2016), and support vector machine (SVM) using the caret R package (Kuhn, 2008).
INRAE	Dairy performances	The recording of milk yield and milk content was carried out by applying the standard AC milk-recording design. At each test-day, morning milk yield was measured, and protein content (PC), fat content (FC), somatic cell count and urea were quantified on a sample of this milking. The milk yield of the day was estimated by corrected the morning milk yield for the evening and morning differences using the ratio between the total volumes of milk produced by the whole flock at two milkings.
INRAE	Metabolomic analyses (plasma and rumen fluid)	Plasma samples were centrifuged at 3,000 g for 5 min at 4°C then 200 µL of the supernatant was transferred to a microtube containing 500 µL of phosphate buffer (pH= 7.0) with 17.2 mg/mL of Trimethylsilylpropanoic acid (TSP) used as a reference for chemical shift and centrifuged for 15 min at 4,190g and 4°C. Finally 600 µL of the supernatant were transferred to NMR tubes. As previously explained for plasma samples, rumen fluid samples were centrifuged, transferred to a microtube containing phosphate buffer and TSP except that instead of one centrifugation for 15 minutes at 4,190g and 4°C, the supernatant was transferred to

		another microtube and centrifuged again in the same conditions before 600 µL of the supernatant being transferred to NMR tubes. Samples were kept at 300K while spectra were acquired using the cpmgpr1D Bruker pulse program on a Bruker AVANCE III HD 600 MHz NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at 600.13 MHz for 1H resonance frequency using an inverse detection 5 mm 1H-13C-15N-31P cryoprobe attached to a cryoplatform (the preamplifier cooling unit) with the following parameters : 512 transient and 16 "dummy" scans using a relaxation time of 2.0s and an acquisition time of 1.36s resulting in the acquisition of 32k data points. Spectra issued from the analysis were aligned using the TSP peak (or glucose peaks when TSP had been complexed with residual proteins). Pre-processing of the spectra (zero order phase correction, shift referencing on TSP in rumen spectra and on D-Glucose doublet at 5.24ppm when proteins were complexed with TSP making it unavailable for referencing, baseline correction) was performed using the TopSpin® software. Pre-processed spectra were imported in ASICS (version 2.5.3) package from R software which was used to normalize areas under the spectra to constant sum before removing the solvent (water) signals i.e between 4.5 to 5.1 ppm. Parameters were the following: noise.thres was set to 0.015 in plasma samples and 0.075 in rumen samples in accordance with noise baseline observed in spectra, max.shift was set to 0.01 in all spectra and clean.thres was set to 50 to keep only the metabolites that were present in at least 50% of the samples in each matrix*diet.
INRAE	microbiota sequencing	Microbial DNA was extracted and 16sRNA was amplified using V4-V5 probes. With this pair of probes, not only bacteria but also archea's DNA was amplified. The 18sRNA was also amplified, to study protozoa and fungi. A second amplification was performed to fix tags on the first amplified sequences, and tagged PCR products were loaded on the Illumina MiSeq cartridge (Illumina, San Diego, CA, USA) at the Get-PlaGe platform of INRAE (Toulouse, France). Raw reads were treated with FROGS pipeline (Escudié et al., 2018) and finally, OTUs were affiliated with the Silva database (version 138) (Quast et al., 2013)
INRAE	Long-chain fatty acids	Rumen fluid was processed as described in (Alves et al., 2013). Briefly, rumen samples were freeze-dried and then transesterified into fatty acid methyl esters (see details in (Jenkins, 2010)). These fatty acids methyl esters were identified by electron impact and chemical ionization mass spectrometry.Spectra were not analysed yet.
INRAE	Volatile fatty acids	Rumen fluid is centrifuged for 20 minutes at 2,880g and 4°C, and 1mL of supernatant is transferred into a microtube containing 200mL of metaphosphoric acid (concentration?). This microtube is centrifuged for 15 minutes at 20,000g and 4°C and 100 µL of supernatant is transferred to a microtube containing 75 µL of methylvaleric acid and 900 µL of ultra-pure water. This final mix is analysed through gas chromatography coupled with a flame ionization detector.
INRAE	NIRS on faecal samples	faecal samples were dried at 60°C during 72 hours, grounded at 1mm, placed in a 50 mm diameter ring cup and scanned in reflectance mode at 2 nm intervals from 400 to 2,500 nm using a Foss NIRSystems model 6,500 scanning VIS/NIR spectrometer (Foss NIRSystems, Silver Spring, MD, USA). Spectra were recorded with the ISIcan version 2.21 software (Infrasoft International, State College, PA, USA). Spectral data treatment was performed using WinISI II version 1.60 (Infrasoft International,Port Matilda, PA, USA). The standard normal variate and detrend (SNVD) scatter correction procedures were applied to the raw data (Barnes et al., 1989). Spectra were then transformed using a mathematical first-order gap derivation.

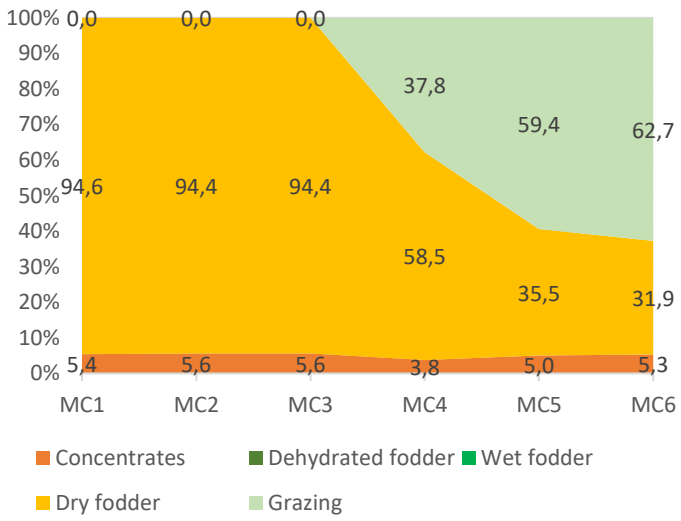
INRAE	Natural ^{15}N abundances	Natural ^{15}N abundances ($\delta^{15}N$) in plasma and feed samples from lambs fed concentrate or diets rich in forage were analyzed using an isotope-ratio mass spectrometer (Isoprime Vision; Elementar France) coupled to an elemental analyser (Vario cube; Elementar France). Analysis were performed at INRAE (https://www6.clermont.inrae.fr/plateforme_exploration_metabolisme). For plasma preparation, 10µL were pipetted into a small tin capsule, evaporated at room temperature for 24h and then the capsule closed before being loaded in the analyzer. Each plasma sample was analyzed in triplicate. For dried and ground feed, between 4 and 6 mg were weighted (relative to their N content) and also analyzed in triplicate. In-house standards (glutamic acid) were included in each run every 10 samples to correct for possible time-variations in the analysis. Results were expressed using the delta notation (δ15N).

11.2 Appendix 2 – feeding systems in the French dairy farms involved in the data collection

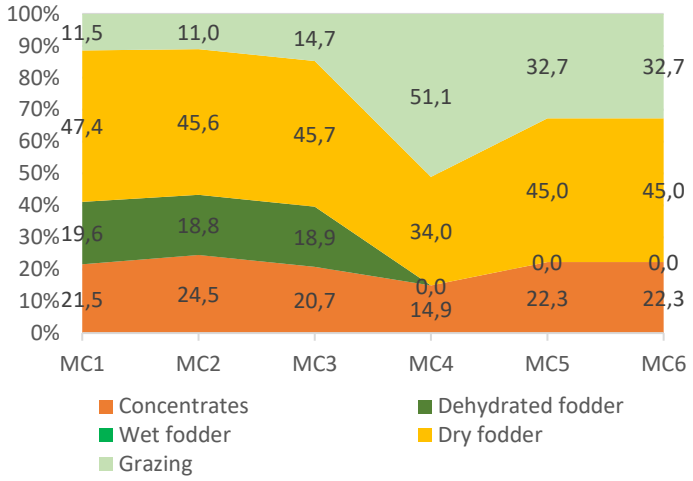
The figure below shows examples of feeding strategies encountered on the farms. On a wet forage feed, the proportion of concentrates is higher (13% on average). In the figure, the share of concentrates is distributed collectively in the sheepfold but also individually at the automatic concentrate feeder in the milking parlour. Concentrates represent ~20% of the total feed. These strategies are in link with the requirements of PDO cheeses described above. Grazing is present in all SMARTER farms and can start at the beginning or in the middle of lactation. In the Pyrenean farms, dehydrated feed is used more often.



a)



b)



c)

Figure: Average share of feed (dry, wet or dehydrated forages, grazing and concentrates) in the collective feed (in % of the distribution of each feed category in the total ration) at each milk test-day (MTD). Figure 6a: an example of a feed based on wet fodder (silage, silage wrap) distributed to the Lacaune breed, figure 6b: example of a feed based on dry fodder distributed to the Lacaune breed. Figure 6c: example of a feed distributed in the Western Pyrenean area.

11.3 Appendix 3 - Feed efficiency values in Assaf animals of the UNILEON-CSIC experiment.

Residual Feed Intake (RFI), Residual Energy intake (REI), and Feed Conversion Ratio (ECR) indexes. EBV indicates the Estimated Breeding Value for milk yield.

Animal	Nutritional Challenge*	RFI	REI	FCR
1	**C**	-0.25	-1.72	1.09
2	**C**	-0.20	-1.39	1.25
3	**C**	0.04	0.25	1.92
4	**NC**	0.08	0.52	1.11
5	**C**	0.15	1.01	1.36
6	**C**	0.02	0.15	1.27
7	**C**	0.14	0.95	1.42
8	**NC**	0.22	1.49	1.57
9	**C**	0.00	-0.02	1.67
10	**NC**	0.05	0.34	1.64
11	**C**	-0.01	-0.05	1.57
12	**C**	0.00	-0.03	1.38
13	**C**	-0.10	-0.69	1.30
14	**C**	0.01	0.08	1.53
15	**NC**	0.02	0.12	1.46

16	**NC**	0.07	0.46	1.24
17	**NC**	0.14	0.95	1.43
18	**NC**	0.00	0.02	1.87
19	**NC**	-0.14	-0.93	1.56
20	**NC**	0.19	1.33	1.33
21	**C**	-0.14	-0.97	1.42
22	**NC**	0.12	0.84	1.18
23	**C**	0.06	0.44	1.53
24	**NC**	0.11	0.77	1.68
25	**NC**	0.05	0.35	1.18
26	**NC**	-0.02	-0.16	1.11
27	**C**	-0.04	-0.27	0.99
28	**C**	-0.34	-2.35	1.13
29	**C**	0.03	0.19	1.20
30	**C**	-0.09	-0.65	1.52
31	**C**	0.17	1.14	1.12
32	**NC**	-0.13	-0.87	1.20
33	**NC**	0.07	0.50	1.52
34	**NC**	-0.09	-0.59	1.23
35	**NC**	-0.18	-1.21	1.02
36	**NC**	0.28	1.90	1.32
37	**C**	-0.07	-0.50	1.24
38	**NC**	-0.16	-1.08	1.10
39	**NC**	-0.04	-0.27	1.30
40	**C**	NA	NA	0.88

*C: Control; NC: Nutritional Challenge. NA Data non-available.

11.4 Appendix 4 - Phenotypes for Residual Feed Intake (RFI), and Feed Conversion Ratio (FCR) for the ewes using in RNA-Sequencing work.

Sample_id	**Group**	**animal**	**RFI**	**FCR**
R_MFE_C6h	CONTROL	61522	-0.04	0.99
R_MFE_D6h	NC	61536	-0.18	1.02
R_MFE_C1h	CONTROL	61472	-0.25	1.09
R_MFE_D3h	NC	61521	-0.02	1.11
R_MFE_C7l	CONTROL	61528	0.17	1.12
R_MFE_D6l	NC	61520	0.05	1.18
R_MFE_D3l	NC	61509	0.12	1.18
R_MFE_D4h	NC	61530	-0.13	1.2
R_MFE_C7h	CONTROL	61526	0.03	1.2

R_MFE_D5h	NC	61534	-0.09	1.23
R_MFE_C5l	CONTROL	61540	-0.07	1.24
R_MFE_D7h	NC	61501	0.07	1.24
R_MFE_C2h	CONTROL	61473	-0.2	1.25
R_MFE_C4h	CONTROL	61483	0.02	1.27
R_MFE_C5h	CONTROL	61493	-0.1	1.3
R_MFE_D7l	NC	61539	0.28	1.32
R_MFE_C3h	CONTROL	61480	0.15	1.36
R_MFE_C3l	CONTROL	61490	0	1.38
R_MFE_C1l	CONTROL	61484	0.14	1.42
R_MFE_D2l	NC	61502	0.14	1.43
R_MFE_D1l	NC	61499	0.02	1.46
R_MFE_C4l	CONTROL	61498	0.01	1.53
R_MFE_D5l	NC	61504	-0.14	1.56
R_MFE_C2l	CONTROL	61489	-0.01	1.57
R_MFE_D1h	NC	61488	0.05	1.64
R_MFE_C6l	CONTROL	61486	0	1.67
R_MFE_D2h	NC	61518	0.11	1.68
R_MFE_D4l	NC	61503	0	1.87

11.5 Appendix 5 – Test of approximation of variables contributing to the definition of feed efficiency

The first step consisted in validating the approximations using data from a precise individual food monitoring (weighing) carried out at the experimental unit of INRAE: Domaine de La Fage, in the framework of the European iSAGE project. Thus, it was possible to test different hypotheses of "simplification" allowing to measure the sensitivity and robustness of calculation method of feed efficiency retained for SMARTER farms.

Within the framework of the iSAGE project, 48 Lacaune ewes were individually monitored for their dairy performances, weights, body conditions (BCS) and feed intakes (weighing of forages and concentrates distributed and refused in individual troughs). All these measurements were performed individually, weighing of feed quantities and refusals were performed daily over a period of 70 days, and milk performance and BCS were recorded once a week over a period of 4 months during their 2nd (in 2018) and 3rd lactation (in 2019).

A first calculation of feed efficiency was made from the individual measured data (reference value). In order to mimic the SMARTER protocol, data simplifications were performed by taking a single common value for all ewes for the following variables: BW, BCS, forage intake and standardized milk production. For each simplified variable, the single common value set for all ewes was first the average value of the group, then a gradient below and above (e.g. the average live weight of the group is 78.5 kg in 2019, the values set are 70, 75, 78.5, 80 and 85 kg for all ewes). The variables are simplified one by one, and then the simplifications are aggregated over several variables at once. The reclassification of the ewes after simplification of the variables had allow to define the importance of each one in the calculation of feed efficiency.

11.6 Appendix 6 – description of feed efficiency (through NEICMR) in French dairy sheep breeds

Lactation stage 1 corresponds to the month of lamb suckling, which explains the low number of animals in this category. The lactation stages ranged from month 2 to 8. The range of feed efficiency values is from 0.5 to 1.75 for all breeds.

Estimated feed efficiency results (mean, standard deviation, minimum, maximum and coefficient of variation) by ewe's milk production area, parity and milk recording number

Milk production Area	Parity	Lactation stage (month)	N	Mean	Sd	Minimum	Maximum	CV
Roquefort area	primiparous	1	22	0.87288	0.23262	0.55209	1.45904	27
Roquefort area	primiparous	2	388	0.86321	0.17223	0.50160	1.34726	20
Roquefort area	primiparous	3	470	0.87959	0.23446	0.50010	1.69423	27
Roquefort area	primiparous	4	548	0.89464	0.23324	0.50536	1.69304	26
Roquefort area	primiparous	5	632	0.94445	0.2543	0.50028	1.74556	27
Roquefort area	primiparous	6	468	0.83005	0.24357	0.50160	1.67324	29
Roquefort area	primiparous	7	401	0.79336	0.18943	0.50033	1.73400	24
Roquefort area	primiparous	8	178	0.76354	0.23624	0.50222	1.50810	31
Roquefort area	multiparous	1	19	0.99048	0.32268	0.53380	1.70809	33
Roquefort area	multiparous	2	949	0.96944	0.22346	0.51398	1.72555	23
Roquefort area	multiparous	3	1422	0.95913	0.22826	0.50032	1.74386	24

Roquefort area	multiparous	4	1529	0.94413	0.23235	0.50294	1.70529	25
Roquefort area	multiparous	5	1531	0.98717	0.28738	0.50048	1.74635	29
Roquefort area	multiparous	6	1464	0.86318	0.22042	0.50004	1.72674	26
Roquefort area	multiparous	7	922	0.77983	0.20272	0.50053	1.70222	26
Roquefort area	multiparous	8	627	0.74945	0.19179	0.50103	1.53188	26
Western Pyrenean area	primiparous	1	45	0.66523	0.12746	0.50783	1.06462	19
Western Pyrenean area	primiparous	2	170	0.75691	0.19341	0.50826	1.53593	26
Western Pyrenean area	primiparous	3	230	0.80652	0.22623	0.50104	1.72183	28
Western Pyrenean area	primiparous	4	292	0.78035	0.1906	0.50416	1.71918	24
Western Pyrenean area	primiparous	5	170	0.81603	0.23231	0.50365	1.57239	28
Western Pyrenean area	primiparous	6	149	0.89847	0.29713	0.50120	1.74245	33
Western Pyrenean area	primiparous	7	194	0.84088	0.25411	0.50171	1.69808	30
Western Pyrenean area	primiparous	8	74	0.89582	0.2682	0.51394	1.69685	30
Western Pyrenean area	multiparous	1	21	0.78562	0.25666	0.51713	1.36742	33
Western Pyrenean area	multiparous	2	168	0.83414	0.2049	0.50432	1.54072	25
Western Pyrenean area	multiparous	3	156	0.80343	0.20277	0.50259	1.60854	25
Western Pyrenean area	multiparous	4	159	0.73515	0.19821	0.50140	1.60109	27
Western Pyrenean area	multiparous	5	110	0.83881	0.27162	0.50443	1.67493	32
Western Pyrenean area	multiparous	6	122	0.87875	0.27911	0.50269	1.58738	32
Western Pyrenean area	multiparous	7	131	0.81849	0.26316	0.50014	1.67960	32
Western Pyrenean area	multiparous	8	75	0.78909	0.23886	0.50027	1.73049	30

Sd : standard deviation ; CV : coefficient of variation

11.7 Appendix 7 : beta-hydroxybutyrate phenotyping with portable blood sugar/ketone meter

Protocole de dosage du BHB sanguin à l'aide du lecteur Freestyle Abbot

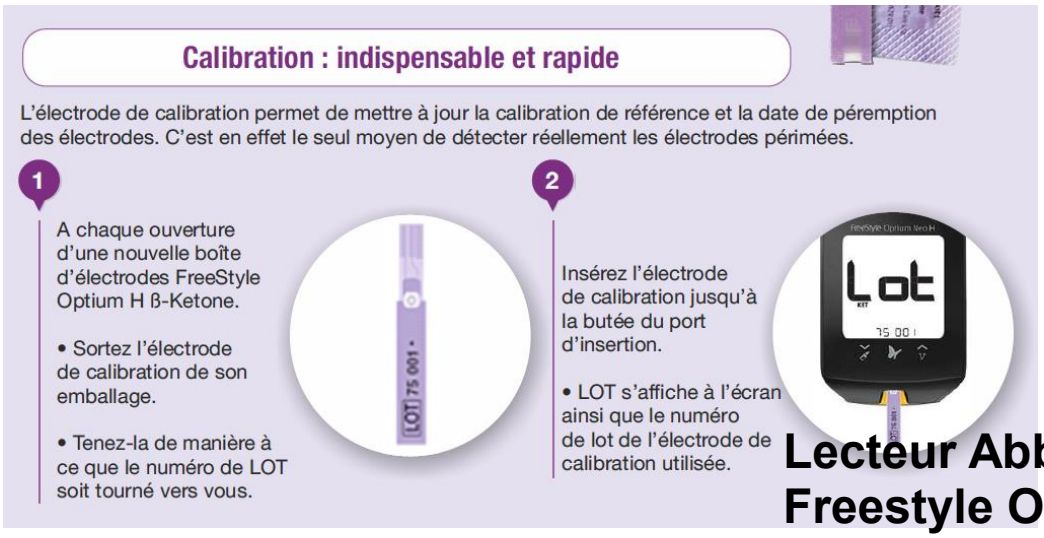
Le dosage de β-hydroxybutyrate (BHB) se place dans le cadre général d'approches innovantes pour l'élevage durable des petits ruminants, permettant de concilier l'efficience d'utilisation des ressources alimentaires et la résilience des individus en termes de santé, de bien-être et de longévité productive (projet européen SMARTER ; https://www.smarterproject.eu/).

Le BHB est un corps cétonique, dont la mesure permet un suivi individuel ou de troupeau des changements de l'équilibre énergétique de l'animal (Sadjadian et al., Comp Clin Path, 2012).

Il s'agira d'évaluer l'intérêt de ce marqueur (BHB) pour diagnostiquer des troubles métaboliques et/ou prédire d'autre troubles de santé en relation avec le bien-être des animaux. Nous chercherons également à caractériser la variabilité inter individuelle de la cétonémie (BHB) et l'influence de certains facteurs d'élevage (système alimentaire) dans les phases cataboliques (mobilisation des réserves) et anaboliques (reconstitution des réserves). L'étude permettra également d'étudier l'association entre ce marqueur du métabolisme énergétique et des caractères de production (poids, production laitière) et de reproduction (réussite à l'insémination animale).

Le mode opératoire proposé repose sur un dispositif de mesure direct sur du sang total réalisable à la ferme à l'aide du **lecteur Freestyle Optium Neo H de Abbot** et de languettes dédiées au dosage du BHB (**électrodes Freestyle Optium H β-ketone**).

Préambule : la calibration de l'appareil est à réaliser en amont des mesures, à chaque nouvelle ouverture d'une boite d'électrodes.



Lecteur Abbott Freestyle Optium NeoH

1		**1 - Collecter du sang total par prise de sang** - Sur tube sec, si la mesure est réalisée dans les minutes qui suivent la prise de sang - Sur tube EDTA, si la mesure est réalisée jusqu'à 2 heures après la prise de sang Le dosage ne requiert que 1-2 ml de sang (maximum) Le tube de sang EDTA peut-être réutilisé pour l'extraction d'ADN le cas échéant
2	  	**2 - Dosage** 2.1 - Sortir une électrode (Freestyle Optium H β-ketone) de son emballage individuel violet 2.2 - Insérer l'électrode dans le lecteur (Freestyle Optium Neo H), coté stries noires, jusqu'à la butée. 2.3 - Le lecteur s'allume automatiquement et le symbole  clignote 2.4 - Ouvrir le tube de sang, après l'avoir retourné 2-3 fois pour homogénéiser le contenu, et tremper la zone de dépôt blanche de l'électrode dans le tube. Une alternative est de verser une goutte de sang sur la zone de dépôt de la languette 2.5 - Trois traits s'affichent et disparaissent lorsque suffisamment de sang est déposé 2.5 - Un temps d'attente de 10 secondes s'affiche avant de donner le résultat. Celui-ci est généralement compris entre 0.2 et 1.2 mmol/L 2.6 - Enlever l'électrode et recommencer à l'étape 2.1 pour l'échantillon de sang suivant

Avertissement : le lecteur n'est pas opérant s'il fait trop froid.
Si un code erreur s'affiche à l'écran :

i) Enlever et réinsérer l'électrode si le sang n'est pas déjà déposé
ii) changer l'électrode si le sang est déjà déposé.