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## **SMARTER – Which novel traits to improve feed efficiency?**

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### **Abstract**

The SMARTER (SMAll RuminanT breeding for Efficiency and Resilience) H2020 project aims to develop and implement innovative strategies to improve resilience and efficiency (R&E) related traits in sheep and goats. Regarding feed efficiency, the objective is to identify novel traits that would be relevant, easy to measure and cheap enough to be collected in many animals to identify the most efficient individuals. In practice, feed efficiency can be assessed by different criteria such as residual feed intake and feed conversion ratio. Both criteria require feed intake to be measured for each individual which remains an expensive trait to get and hardly feasible under farm conditions. Thus, our objective is to identify novel traits related to feed efficiency and use them as proxies for feed intake and/or feed efficiency.

First, novel phenotypes are identified and studied in experimental farms where feed intakes of concentrate and forage are recorded for each animal. Different novel phenotypes are being considered to study feed efficiency in sheep and goats, including (i) biomarkers (from blood or milk metabolomics), (ii) differentially expressed genes in targeted tissues, (iii) genomic polymorphisms, (iv) ruminal microbiota, (v) faecal NIR spectra, (vi) greenhouse gas emissions and body composition traits. These novel traits will be recorded either under classical feeding or under nutritional restriction to quantify, for example, the impact of a shortage of concentrate inputs.

Then, the most promising novel traits will be measured in commercial populations. From these larger datasets, we will estimate heritabilities of the novel traits and genetic correlations to other traits in the breeding goal.

Finally, for some case studies, we will quantify GxE interactions, particularly by considering the same breed under different breeding systems or regions.

*Keywords: small ruminants, sheep, goat, resource use efficiency, novel phenotypes*

### **Introduction**

Feed efficiency is the ability of livestock to transform feed into food edible by humans. Breeding feed-efficient livestock is of high interest both at environmental and economic levels. Among livestock, ruminants are the only ones able to transform fibers into proteins. There is

growing interest in breeding ruminants mainly fed with increasing amounts of forage and decreasing quantities of concentrates. Breeding programs are willing to include feed efficiency in their breeding objectives. Residual feed intake (RFI) is currently one of the most common criteria used to improve feed efficiency. RFI has been shown to be variable and moderately heritable in meat sheep (Cammack et al., 2005; Johnson et al., 2018; Paganoni et al., 2017; Snowden and Van Vleck, 2003; Tortereau et al., 2020) and dairy goats (Desire, S. et al., 2017). Selecting on RFI is thus possible, but it requires feed intake to be recorded, which is the major limit to the deployment of this recording on a broader scale, particularly in small ruminants. Therefore, there is an increasing interest in identifying proxies for feed intake and/or feed efficiency directly. In order to be widely recorded, proxies must be easy to collect and low in cost. In the SMARTER project, we do not only focus on such new phenotypes, but also on others that are more difficult to collect and/or more expensive to acquire. The overall objective is to understand the biological pathways underlying feed efficiency in small ruminants, which will help in identifying proxies.

The objective of this work is to gather data from different experimental and commercial farms of small ruminants to dissect feed efficiency and to propose proxies that could be widely collected to estimate their genetic parameters and their ability to predict feed intake or feed efficiency.

## **Material and methods**

### **Animals**

Two categories of animals are considered: animals from experimental farms and animal from commercial farms. In dairy sheep, experimental protocols rely on 4 different breeds : Chios (n=8), Lacaune (n=62), Frizarta (n=8) and Assaf (n=47), and in commercial farms, ewes from 5 breeds are being phenotyped : Chios (n=250, in 2 farms), Frizarta (n=500, in 2 farms), Lacaune (n=3,972 in 8 farms), Pyrenean breeds (n=876, including ewes from Manech Tête Rousse and Basco-Béarnaises breeds, in 7 farms). An experiment rely on a nutritional challenge in 40 Assaf ewe replacement lambs: half of the animals are subjected, during their prepuberal stage, to a diet contained 42% less crude protein than controls. In both control and restricted group, animals have been chosen from each of the extremes of the distribution of genetic values (paternal average) for milk production. An experiment in Lacaune ewes (n=55) relies on divergent lines selected on milk persistency.

In dairy goats, two experiments are conducted, the first in the Alpine breed (n=110) and the second in a mixed breed (n=3,421) based on Alpine, Saanen and Toggenburg breeds. A total of 11 commercial farms are involved in the collection of fine-phenotypes of goats: 6 farms with Saanen (n=1,678 goats) and 5 farms with Alpine (n=1,176 goats). The experiment in Alpine breed involves divergent lines on longevity. Goats are daughters of bucks selected according to their extremely high/low EBV on that trait.

In meat sheep, experiments are based on 7 different breeds: Romane (n=277), Merino (n=1002), Corriedale (n= 303), Dohne (n= 360), Texel and Texelx Lley (n= 2,340) and Scottish BlackFace (n~2,000). In commercial farms, data are being collected in 5 breeds: Mouton Vendéen (n=1,500 ewes in 5 farms), Rouge de l'Ouest (n= 800 ewes in 5 farms), Blanche du Massif Central (n=2,000 ewes in 5 farms), Norwegian White Sheep (n=1,600 ewes in 15 farms) and Texel (n~6,000 animals in 154 flocks). The experiment in the Romane breed relies on two divergent lines on Residual Feed Intake (RFI): male lambs that are phenotyped are selected for their extremely high/low EBV on this trait.

## Phenotypes

Different sets of phenotypes and biological samples are being collected, depending on the production type (meat vs. milk) and the farms (experimental vs. commercial). All the phenotypes directly collected in the animals or obtained after analysis of biological samples are described in table 1.

Other phenotypes are calculated from these elementary traits: Average daily gain (ADG), Feed Conversion Ratio (FCR), Residual Feed Intake (RFI). Differences in BCS between two successive physiological stages will also be computed. Milk fine compositions from MIRS were also estimated from previous equations. Microbiota abundances were get from 16S sequencing and metabolome abundances from NMR.

*Table 1: Phenotypes recorded on experimental and/or commercial dairy ewes, dairy goats and meat sheep.*

Group of traits	Trait	Production	Population	Comment
Intake	Total Feed intake (forage or concentrate)	Meat Milk	Experimental mainly	during given control periods, under different diets
Intake	Concentrate intake	Milk	Commercial Experimental	Concentrate supplied in milking parlour
Body composition	Live weight	Meat Milk	Commercial Experimental	collected at different ages per animal
Body composition	Chest width	Meat Milk	Commercial	at different production stages
Body composition	Chest depth	Meat Milk	Commercial	at different production stages
Body composition	Shoulder height	Meat	Commercial	at different production stages
Body composition	Muscle depth (ultrasound)	Meat Milk	Commercial Experimental	Only in experimental farms for dairy animals and at different production stages
Body composition	Back Fat Thickness (ultrasound)	Meat Milk	Commercial Experimental	Only in experimental farms for dairy animals and at different production stages
Body composition	Body condition score	Meat Milk	Commercial Experimental	at different production stages
Udder composition	Udder conformation	Milk	Commercial Experimental	
Production	Milk Production (MY, FY, PY, FC, PC)	Milk	Commercial Experimental	
Production	Carcass Traits	Meat	Commercial	
Gas emissions	GHG emissions (CH <sub>4</sub> , CO <sub>2</sub> )	Meat	Commercial Experimental	
milk samples	gene expression (RNA-seq)	Milk	Experimental	
milk samples	epigenetic marks	Milk	Experimental	

Group of traits	Trait	Production	Population	Comment
milk samples	MIRS	Milk	Commercial Experimental	
milk samples	metabolomic pattern	Milk	Experimental	
rumen samples	Fatty Acids (volatile and long)	Meat Milk	Experimental	under different diets
rumen samples	NMR	Meat Milk	Experimental	under different diets
rumen samples	16S – microbial abundances	Meat Milk	Experimental	under different diets
Blood samples	Targeted metabolites*	Meat Milk	Commercial Experimental	
Blood samples	NMR	Meat Milk	Experimental	under different diets
Blood samples	genotypes	Meat Milk	Commercial Experimental	
Faeces samples	NIRS	Meat Milk	Experimental	under different diets

MY: Milk Yield, FY: Fat Yield, PY: Protein Yield, FC: Fat Content, PC: Protein Content, MIRS: Mid Infra-Red Spectra, NMR: Nuclear Magnetic Resonance NIRS: Near Infra-Red Spectra

\* targeted metabolites : glucose, non-esterified fatty acids, beta-hydroxybutyrate, insulin

Feed intakes are obtained with automatic feeders that record each visit of any animal. Feeding behavior traits were calculated from these datasets.

Not all the phenotypes are available for all individuals. However, live weights and body composition traits obtained through ultrasounds are recorded for almost all meat sheep, and milk production traits are recorded for all dairy sheep and goats. Blood samples are obtained for almost all individuals with phenotypes, at least for genotyping purposes.

### Statistical analyses

First, each trait will be analysed independently within each experiment. Fixed effects and covariates will be selected according to each trial. For example, for divergent lines experiment, the line will be considered as a fixed effect. Each trait will be considered as a proxy for feed intake or feed efficiency. Combinations of elementary traits listed in table 1 will also be considered as proxies. Proxies will be identified in each protocol and will be tested in the other trials when possible. The quality of a proxy will be assessed through different methodologies, adapted to the different protocol dimension. Here, we analysed an experiment in 30 dairy ewes from 4 breeds (8 Chios, 7 Lacaune, 8 Frizarta and 7 Assaf), with sensitivity/sensibility approach. The receiver operating characteristic analysis was used to define thresholds for changes in body composition traits as predictors of negative energy balance. Moreover, mixed linear models were used to test the association of blood biomarkers with fat and muscle reserves and their mobilization.

Multivariate approaches will be applied for large datasets such as microbiota and metabolomics matrices. The MixOmics package implements such methods and will be used (Le Cao, et al., 2016).

In commercial populations, the higher numbers of phenotyped individuals will allow genetic parameters to be estimated in each population. Pedigrees are recorded in the frame of national genetic evaluation programs. These data are currently under collection, so no results are available yet.

## Results and Discussion

In dairy ewes from the Chios, Assaf, Lacaune and Frizarta breeds (total=30 ewes), NEFA were used to analyse energy balance: an increase in NEFA being synonymous with a negative energy balance. Association analyses highlighted that a NEFA status of more than 0.3 mmol / L could be predicted by a decrease in Back Fat Thickness (BFT) and in the sum of BFT and *Longissimus Dorsi* muscle Thickness (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 in their total sum of more than 0.15 mm, 0.065 mm and 0.350 mm, respectively. In the same 30 ewes, a significant positive association of serum albumins with LDT was found. 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 tissues.

In meat sheep, Romane from the 3<sup>rd</sup> generation of divergent selection on RFI exhibited significant differences (p-value<0.05) in feed intake, with more efficient lambs eating 195g less of concentrate than less efficient lambs, and RFI, with a difference of 1.9 genetic standard deviations between both groups. Live weight differences were observed, with inefficient individuals being heavier (+1.75 kg at 5 months old). Ruminal microbiota analyses highlighted differences in composition, and plasmatic amino acids were also different between both groups: more efficient lambs have lower plasmatic levels than less efficient lambs.

## Conclusion

The collection of phenotypes is still on-going. However, first analyses of experimental datasets highlight that some phenotypes are linked to feed intake, feed efficiency or energy status and can be proposed as promising candidates for proxies of those traits.

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