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► To cite this version:

Christel Marie-Etancelin, Beatrice Gabinaud, Didier Marcon, Flavie Tortereau, Dominique Francois, et al.. Genetic links between feeding behavior and microbiota of Romane lambs. 11. World Congress on Genetics Applied to Livestock Production (WCGALP), Feb 2018, Auckland, New Zealand. Massey University, 1130 p., 2018, Proceedings of the 11th World Congress on Genetics Applied to Livestock Production (WCGALP). hal-02736414

HAL Id: hal-02736414

<https://hal.inrae.fr/hal-02736414>

Submitted on 2 Jun 2020

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Genetic links between feeding behavior and microbiota of Romane lambs

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Summary

The aim of this study was to explore the ruminal microbiota of lambs genetically extreme for their feeding speed when eating concentrates. From a database of 1001 lambs phenotyped for their feeding behavior, we estimated breeding values for their feeding speed at the visit level, and selected amongst 77 lambs phenotyped, 20 extreme lambs: 10 “high feeding speed” lambs with an average value of 57.8 g/min and 10 “low feeding speed” lambs with an average value of 26.8 g/min. The ruminal microbiota of these animals was profiled –after a first period of concentrates diets then a second period of forage-based diets - using 16S rRNA gene sequencing were analysed with FROGS software leading to 228 OTUs with taxonomic affiliation and characterized by their relative abundances. Microbiota differences was mainly due to the type of feed ingested -with higher *bacteroidetes* abundances when lambs ingested concentrates- since 211 of the 228 OTUs have abundance differences with respect of diet. Only 6 OTUs were significantly different according to feeding speed of animal and concerned only minor bacterial taxa such as *Bacteroidales* S24_7 group family and the genus *Syntrophococcus*, *Lachnospiraceae* NC2004 group, *Ruminiclostridium* and *Eubacterium cellulosolvens* group.

Keywords: microbiota, feeding behavior, lambs.

Introduction

Ley *et al.* (2006) have shown an association between the composition of the intestinal microbiota and the metabolic phenotype of the host. Thus, obese mice possess half the *bacteroidetes* and a proportional increase in firmicutes than normal mice. In addition, the obese subjects when losing weight restore an intestinal microbiota similar to that of lean individuals. Although the relationship between feeding and intestinal microbiota composition is demonstrated in several species, the microbiota influence on host behavior appears only more recently. Thus, in terms of feeding behavior, Breton *et al.* (2016) demonstrated that the relative importance of *E. coli* in the murine intestinal flora can lead to anorexia of host or bulimia. Indeed, the multiplication of *E. coli* of the intestinal microbiota induces a production in greater quantity of the protein ClpB. This protein, a melanotropin-hormone-satiated look-alike, will pass into the blood and decrease the appetite of the host. In dairy cattle, evidence were published (Khafipour *et al.*, 2011) that rumen *E. coli* population was three log higher in a SARA-diet (SubAcute Ruminal Acidosis) than in a control one. Moreover, in young bulls fed with a highly concentrated diet, Mialon *et al.* (2008) showed that animals adapt their feeding behavior to concentrated diets to reduce SARA by spreading the intake all over the day and increasing the straw intake. Desnoyers *et al.* (2011) studied the feeding behavior of

dairy goats in terms of several chewing activities (ruminating, eating and jaw resting) and defined different feeding patterns associated with different rumen pH patterns. According to these authors, feeding behavior provides information of predisposition of the animal to ruminal acidosis.

Therefore, we explored the ruminal microbiota of lambs genetically extremes for their feeding behavior when ingesting concentrates to verify if the feeding speed was linked to the microbiota composition, both when the animals ingest exclusively concentrates or a 2/3 forage diet.

Material and methods

One thousand and one Romane lambs breeding on a straw pen at INRA experimental farm of “La Sapinière” were involved in this experiment. From 2009 to 2016, 125 lambs per year on average have their feeding behavior monitored with an Automatic Concentrated Feeders (ACF) during 8 weeks at 4 months of age. The last year (2016), 60 of the 77 lambs phenotyped with ACF were additionally monitored for their feeding behavior when eating a forage based diet (2/3 hay + 1/3 concentrates), with an Automatic Forage Feeders (AFF) during 6 weeks at 7 months of age (François *et al.*, 2016). A sample of the ruminal fluid was collected for each animal at the end of the ACF period and at the end of the AFF period. Twenty divergent animals on feeding speed index when eating concentrates were selected and their microbiota sequenced.

Feeding behavior characterization and indexation

From a database of more than 843,000 elementary visits, three feeding behavior traits (feed intake, intake duration and feeding speed) were computed for each visit for the 1,001 lambs. For the animals phenotyped in 2016, the same three feeding behavior traits (feed intake, intake duration and feeding speed) at visit and day levels and the number of visits per day were computed for the ACF (n=77) and the AFF (n=60) periods.

Estimated breeding values for feeding speed (FS) at the visit level were computed on the whole database. Among the 77 animals recorded with the ACF in 2016, 10 “low FS” and 10 “high FS” lambs with extreme breeding values for the feeding speed were selected and their microbiota characterized. Fifteen of these 20 extreme lambs have also their microbiota characterized when feeding with forage.

Microbiota characterization

The profiling of the ruminal microbiota was performed using 16S rRNA gene sequencing at the INRA Genotoul Platform. The microbiota sequences obtained were analysed by FROGS a bioinformatics pipeline (Escudié *et al.*, 2016): clustering uses Swarm (Mahé *et al.*, 2014), chimera removal uses Vsearch (Rognes *et al.*, 2016) and taxonomic affiliation uses Silva 128 database (Quast *et al.*, 2013). At the end of the process, abundance tables of Operational Taxonomic Units (OTUs) and their taxonomic affiliation were produced. To normalize the data, a square root transformation was applied to each abundance taxa.

Statistical analyses

Estimated breeding values for feeding speed at the visit level were computed on the 1,001 lambs with PEST software (Groeneveld, 2006). Only two fixed factors of variation were considered: the year (8 levels) and the ACF devices (4 levels). Pedigrees were traced back up to 20 generations of ancestors and included 5,216 animals.

Abundance values of each ruminal taxa and feeding behavior traits were compared for the low versus high feeding speed lambs during ACF (10 FS- and 10 FS+) and AFF (8 FS- and 7 FS+) periods with a single factor analysis (the 2 groups of feeding speed) in a variance model, using the GLM procedure of SAS (SAS, 2002).

Results and discussion

At 4 months of age, the 1,001 Romane lambs have ingested an average of 1.97 kg of concentrate per day, during about 50 minutes (Table 1). This daily intake is split into 15 visits of 147 g each with a duration of 224 s, on average. The average of the feeding speed was very similar at the visit level (44.4 g/min) and at the day level (42.1 g/min).

Table 1. Feeding behavior traits at ACF of the Romane population (n=1001)

N =1 001	At the visit level		At the day level	
	Mean	Std	Mean	Std
Feed Intake	147 g	45 g	1 968 g	249 g
Feeding duration	224 s	77 s	50.5 min	12.2 min
Feeding speed	44.4 g/min	12.4 g /min	42.1 g/min	10.8 g/min
Nb visits per day			14.9	5.7

The 20 “extreme” lambs for their feeding speed were selected among the 77 lambs phenotyped in 2016: index difference between the 2 groups of FS was of 12.47 g/min, that is to say 1 phenotypic standard deviation. Regarding the feeding behavior traits (table 2), phenotypic differences between FS- and FS+ were mainly for feeding speed (on average 58 g/min for FS+ and 27 g/min for FS- either at the visit or the day levels). This discrepancy is expressive due to difference of feeding duration between the two groups (183 s for FS+ and 244 s for FS- at the visit level; 39.5 min for FS+ and 68.4 min for FS- at the day level), but not to the feed intake (on average 162 g and 2.02 kg at the visit and day levels respectively) or to the number of visit per day (13 on average).

Table 2. Feeding behavior traits comparisons between the 2 groups of extreme feeding speed index at ACF

	FS+ (n=10)		FS- (n=10)		P
	Mean	Std	Mean	Std	
<i>At the visit level</i>					
Feed Intake	171 g	49 g	153 g	26 g	NS
Feeding duration	183 s	58 s	244 s	66 s	***
Feeding speed	57.8 g/min	10.5 g/min	26.8 g/min	4.6 g /min	***
<i>At the day level</i>					
Feed Intake	2 204 g	273 g	1 829 g	269 g	NS
Feeding duration	39.5 min	6.8 min	68.4 min	12.2 min	***
Feeding speed	57.7 g/min	9.6 g/min	27.8 g/min	4.5 g/min	***
Nb visits per day	13.9	4.4	12.2	2.5	NS

The microbiota taxa abundances of these 20 lambs were analyzed after the concentrate feeding and after forage-based feeding. A total of 228 OTU with taxonomic affiliation were identified: 128 genus, 45 families, 28 orders, 17 classes and 10 phyla. The figure 1 showed the microbiota differences according to the type of feed ingested with clearly higher

bacteroidetes abundances when lambs ingested only concentrates compared to forage-based diet. More precisely 211 of the 228 OTUs had abundance differences with respect to diet. Ellison *et al.* (2015) already showed that 349 OTU were identified from rumen microbiota of 77 lambs, among which 52 abundance differences were observed according to the diet. Briefly, forage-based diet favored fibrolytic bacteria when concentrates favored amylolytic and lactolytic ones.

Only 6 bacterial abundances (among the 228) were significantly different according to the FS group (table 3): 4 abundances were different when lambs were fed with concentrates, and 2 abundances when they ate forage-based diet. It is noticeable that only minor bacterial taxa were concerned by the FS effect since abundances of these six taxonomic groups were each lower than 3% of the total bacterial abundances. It seems that the low feeding speed animals have more *Syntrophococcus* and *Lachnospiraceae* NC2004 group. Either with a concentrate diet or a forage one, animals with a higher intake rate have more *Ruminiclostridium*. *Ruminiclostridium* is cellulolytic bacteria, competing with major cellulolytic genus like *Ruminococcus* for their substrate utilization. As a minor one, they could probably access to more substrate when forage entrance rate in the rumen is high. *Syntrophococcus* use various sugars, in particular those from degradation of polysaccharides. A low feeding speed could be due to a low ruminal emptying rate, inducing a higher extent of polysaccharides degradation and more sugars available for their growth.

Table 3. Relative abundances (%) of taxa with significant differences between the 2 groups of extreme feeding speed index

	ACF period		AFF period		P
	FS+	FS-	FS+	FS-	
Families					
<i>Bacteroidales</i> S24_7 group	0.34	0.16			*
Genus					
<i>Syntrophococcus</i>	1.44	2.86			*
<i>Lachospiraceae</i> NC2004 group	0.02	0.10			*
<i>Ruminiclostridium</i> 9	0.01	0.00			*
<i>Ruminiclostridium</i>			0.13	0.06	*
<i>Eubacterium cellulosolvens</i> group			0.00	0.02	**

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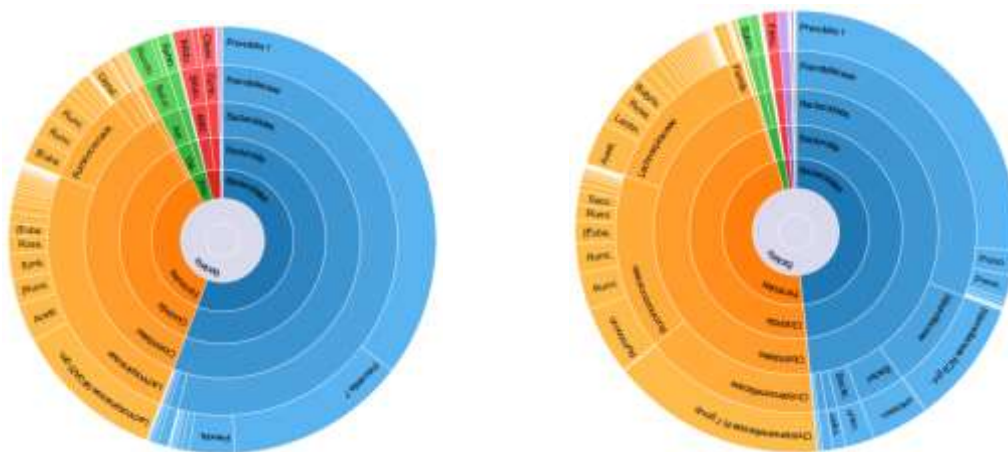


Figure 1. Taxa distribution (from phylum to genus) in rumen after concentrated feeding (left) and after forage-based diet (right).