Evaluate the impact of abiotic stressors (heat and suboptimal diet) on the gut microbiota of two chicken lines diverging in feed efficiency, using the 16S rRNA high throughput sequence technology

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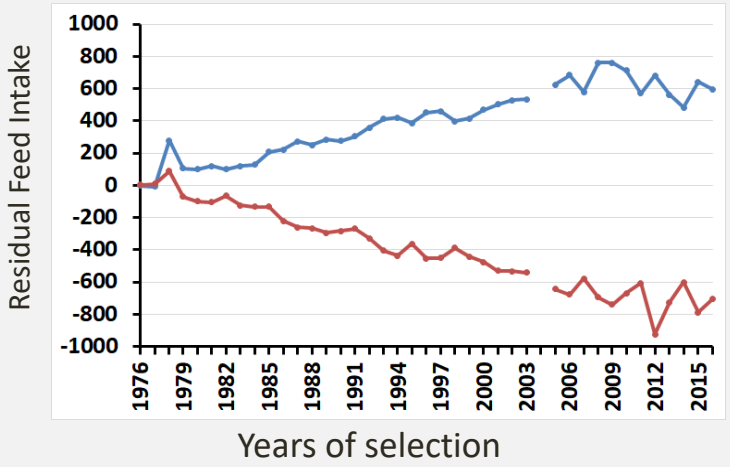
Animal model

Chicken lines divergently selected for feed efficiency

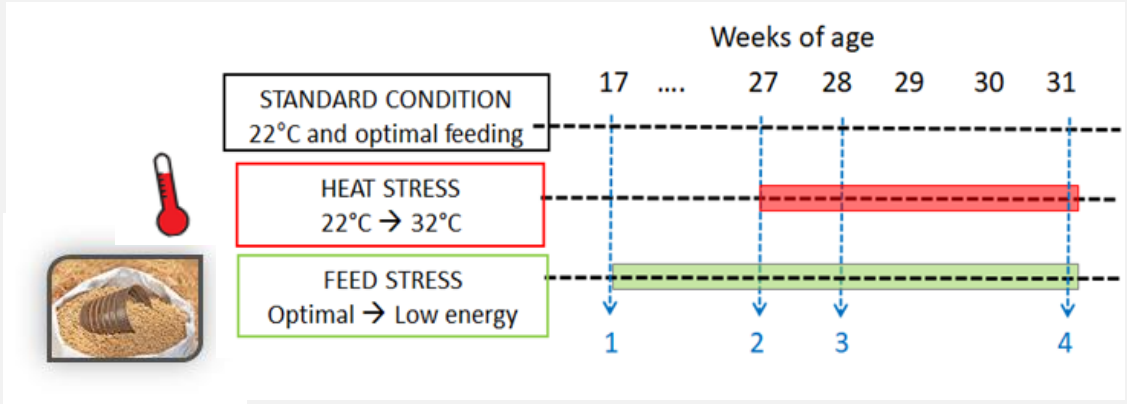


R+: low efficient line

R-: high efficient line

Experimental design

Three experimental conditions



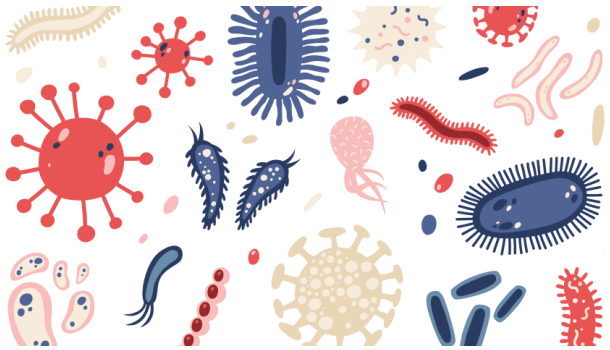
	Control	Food Stress	Heat Stress
R-	17	13	17
R+	18	11	19

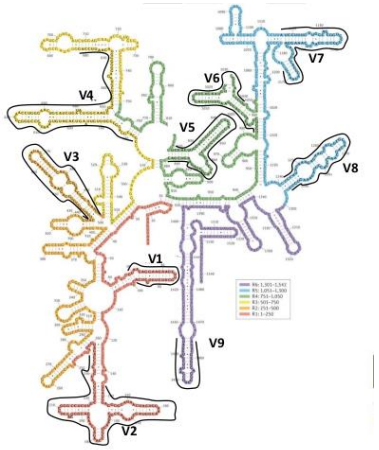
Objectives:

1. Is feed efficiency (genotypes) linked to gut microbiota composition?
2. Under abiotic stress (feed or heat), is there a difference in microbiota composition between lines?
3. Are these changes dependent on the genetic background of the chicken?

Technical approach and bioinformatics analysis

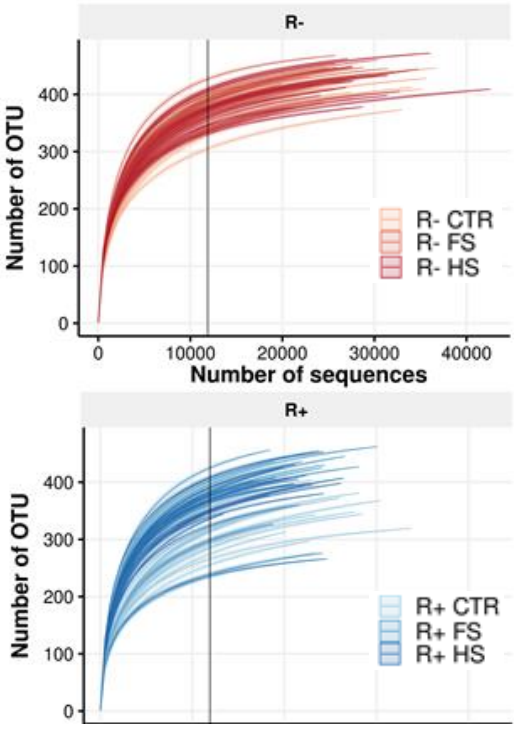
FROGS
F.Escudié et al, FROGS: Find, Rapidly, OTUs With Galaxy Solution, *Bioinformatics* 2018, doi: 10.1093/bioinformatics/btx791





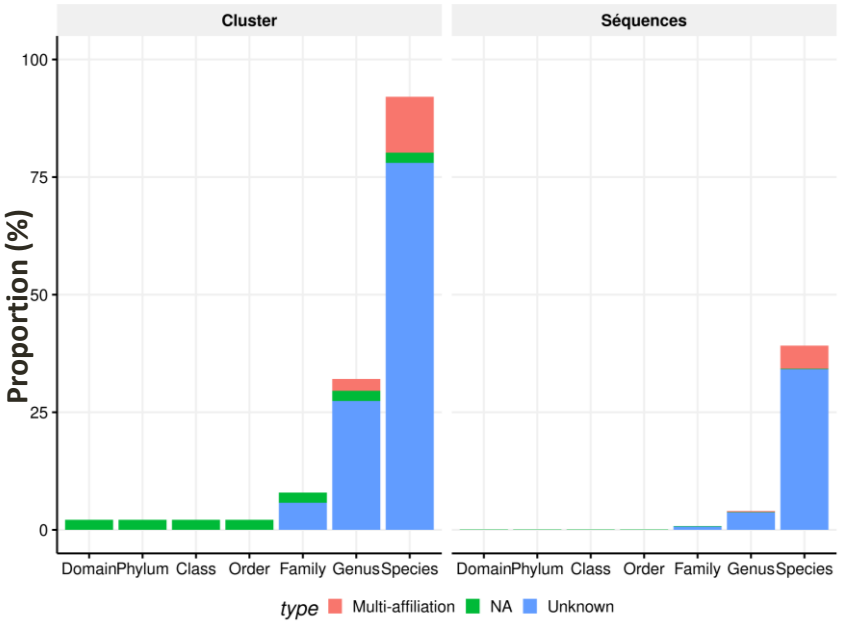
FROGS

silva
high quality ribosomal RNA databases



DNA from the caecum was extracted and the V3V4 region of the 16S rRNA gene was amplified and sequenced in paired end 2x250bp on an Illumina Miseq sequencer. OTU were constructed with the FROGS pipeline, with a filtering step on low abundance ($<5.10^{-5}$) and on a presence prevalence of at least 50% in at least one group. Taxonomical affiliations were obtained using NCBI Blast+ against the SILVA 132 database (filtered on pintail > 50).

Finally, in total 2,381,417 sequences were kept and distributed into **555 OTU** that belonged to 6 phyla, 11 classes, 12 orders, 30 families (26 known), 98 genus (80 known), 141 species (32 known).

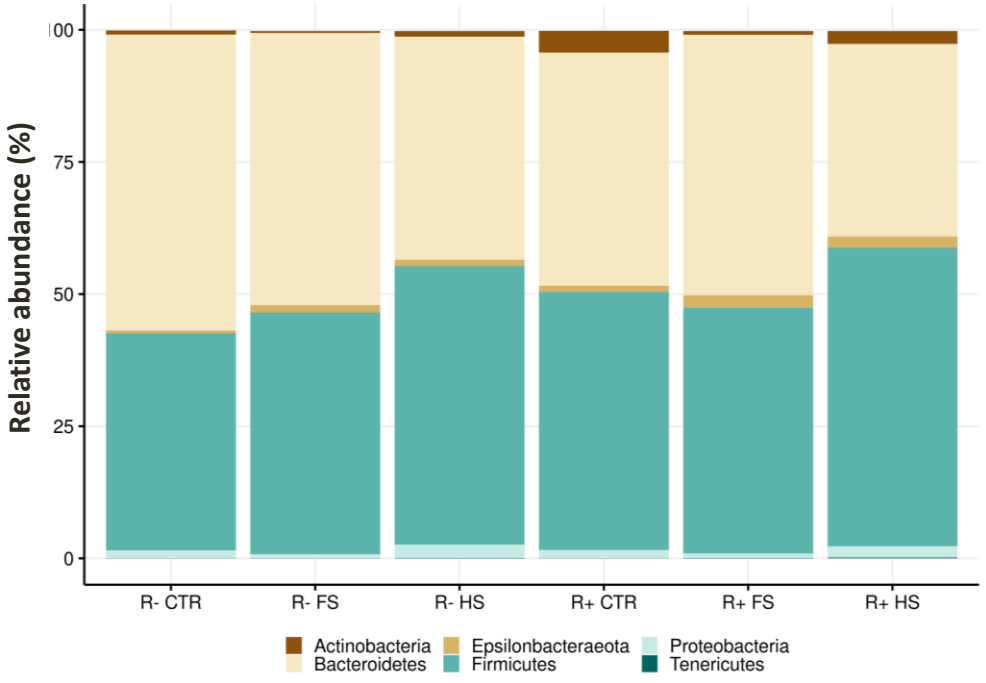


Microbiota composition analysis

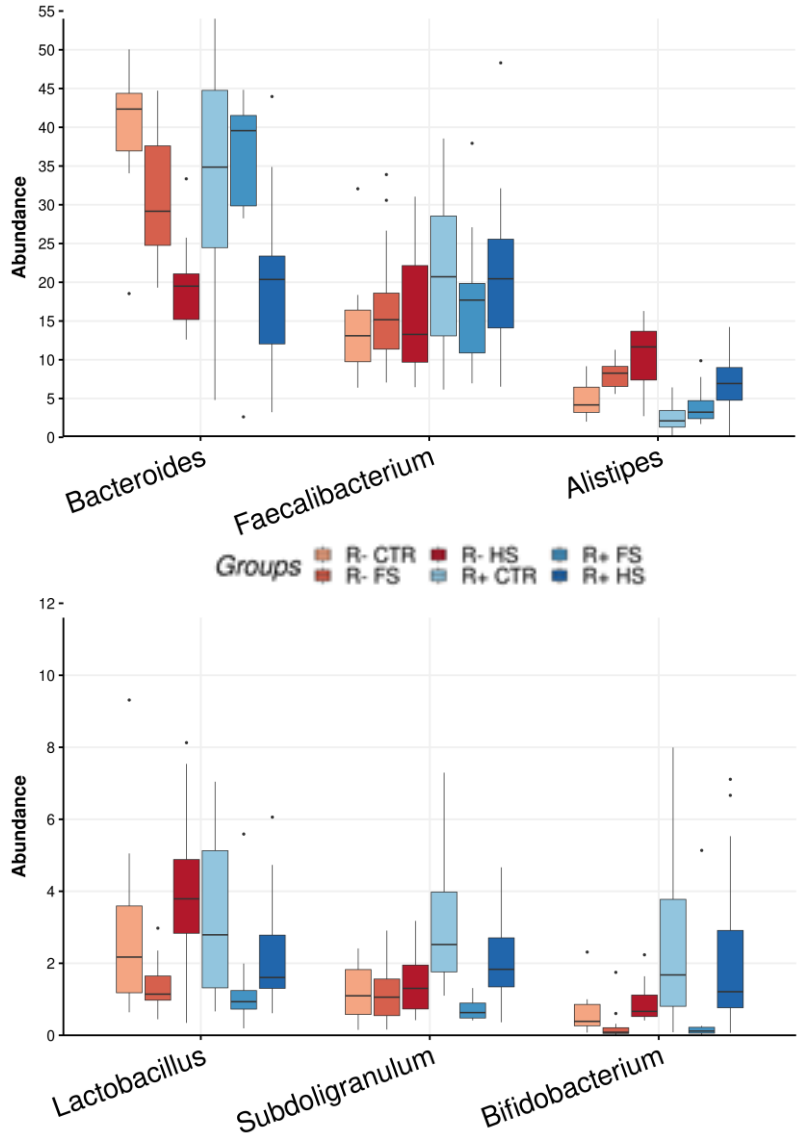
As previously described in litterature, the *Firmicutes* and the *Bacteroidetes* are the main phyla in the gut microbiota. Despite the high intra group variability (SD until 17.6%), we observed significant line and condition effects. Indeed, *Bacteroidetes* are more abundant in the R- line than in the R+ line, and this is the opposite for the *Firmicutes* or the *Actinobacteria*. The *Proteobacteria* abundance increase in the heat stress condition. Line and condition effects are also visible at the genus level.

Bacteroides a well-known propionate producer often linked to high fiber diets, strongly decreases in both stressed conditions in the R- line, and only in the heat stress group for the R+ line. Short-chain fatty acid producers, like *Subdoligranulum, or Faecalibacterium*, present dynamic changes between conditions, and these changes are more pronounced in the R+ line.

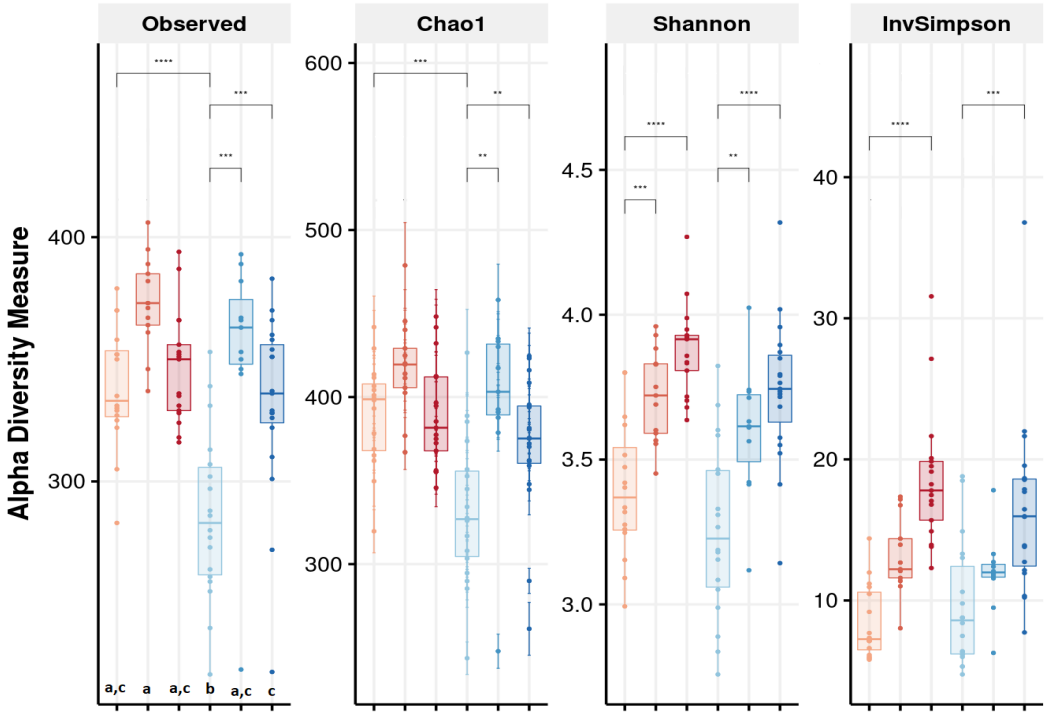
Phylum average relative abundance by groups

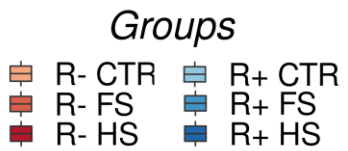


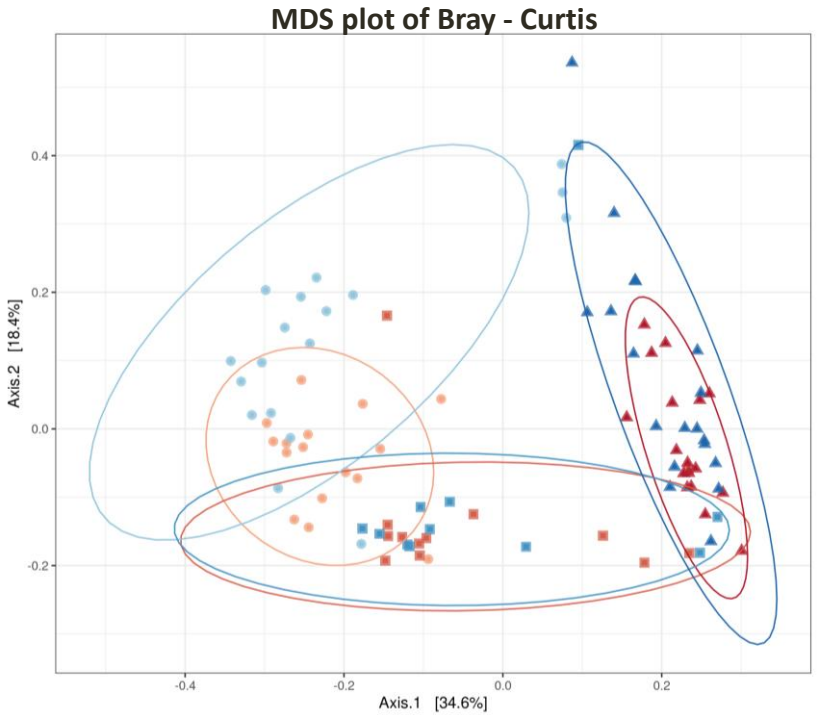
6 genera abundance distribution showing different evolution among groups



Microbiota composition analysis







Alpha diversities indices show:

- Under optimal conditions**, a strong richness difference exist between lines**, with R+ line presenting reduced flora diversity. This difference is not observed under stressful conditions. This is mainly due to **the huge increase of richness in R+ in under feed and heat conditions.**
- The abiotic stresses impact the eveness of OTU abundance as Shannon and InvSimpson increase. This means there is more abundant OTU than in optimal conditions.

Bray-Curtis beta diversities, as well as Jaccard and Unifrac, revealed a **strong condition effect**. Moreover, this analysis confirmed that **microbiota composition differs between lines but these differences are reduced** (in HS) **or even erased** (in FS) **in stressed conditions**.

Conclusions and Perspectives

	Optimal conditions			Feed stress		Heat stress	
	Feed intake	Feed efficiency	OTU richness	Feed intake	OTU richness	Feed intake	OTU richness
R+	4,1 kg/ 28 days	-	low	+ 10%	high	- 28%	high
R-	2,6 kg / 28 days	+	high	+ 10%	high	- 25%	high

Under optimal reared conditions, increased gut diversity is associated with increased feed efficiency. This could imply that the R- line, to reach its high feed efficiency, needs a rich flora to guarantee the maximum of efficiency from a relatively reduced feed intake. On the contrary, the R+ line that has a feed intake larger than expected, probably does not require a very efficient microbiota because it exceeds in nutrients.

Under stressful conditions, major changes are observed in the R+ line reaching the level observed in the R- line. This increasing richness probably reflects the need to guarantee microbiota functionality in response to host requirements under constraints.

Perspectives

There is still work to do to identify precise OTU involved in microbiota dynamics under these abiotic stresses and how it relates to feed efficiency parameters.

Our experiment lack of sensitivity to determine the taxonomy of each OTU. A finer taxonomy will allow us to estimate microbiota functions for example using PICRUSt2.

Acknowledgment

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