

# ARE FOODS ENRICHED WITH QUORUM SENSING AUTOINDUCERS CAPABLE OF AFFECTING THE NEONATAL ASSEMBLY OF THE GUT MICROBIOTA IN RATS?

C MICHEL<sup>1</sup>, R TAREB<sup>2</sup>, M CROYAL<sup>3</sup>, A MEYNIER<sup>4</sup>, N LCONTE<sup>5</sup>, H BILLARD<sup>1</sup>, L BRULE<sup>2</sup>, M VIAU<sup>4</sup>, JP SEGAIN<sup>1</sup>, G JAN<sup>5</sup>, MF PILET<sup>2</sup>



With the help of : B CASTELLANO, A DAVID-SOCHARD R, A GANDON, I GRIT, A. LEFEBVRE, S SUZANNE

1: Nantes Université, INRAE, UMR 1280-PHAN, F-44000 Nantes; 2: Oniris, INRAE, SECALIM, 44300 Nantes, France; 3: CRNH Ouest Mass Spectrometry Core Facility, F-44000 Nantes; 4: INRAE, BIA, 44300 F-Nantes; 5: INRAE, Institut Agro, STLO, F-35000 Rennes.



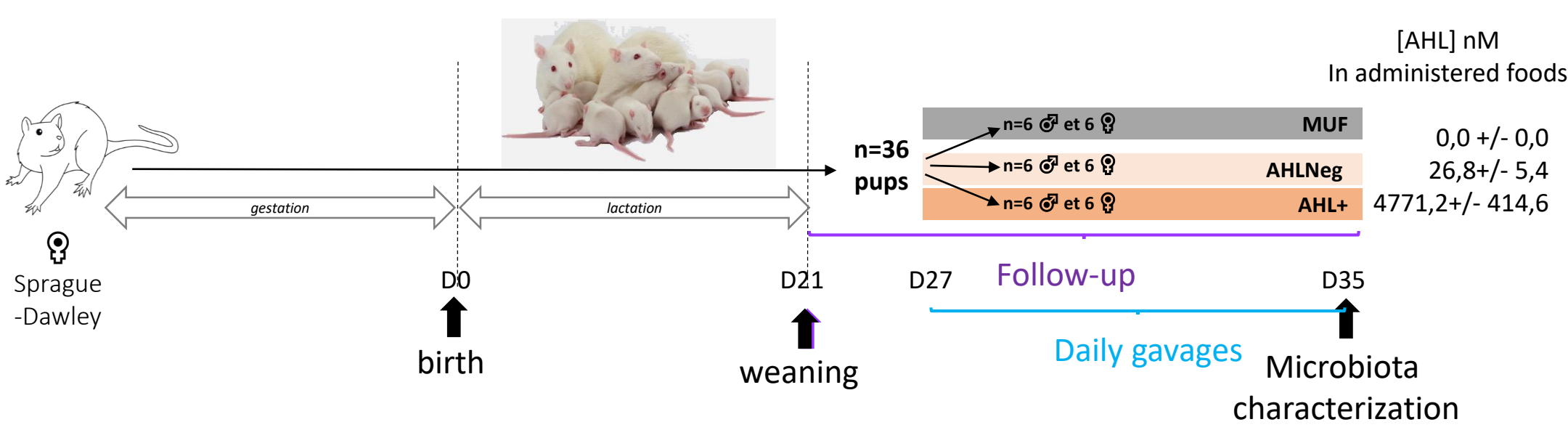
The traditional use of bacteria enriched -fermented or -uncooked foods [1], the growing interest in alternative food preservation using protective bacteria [2] and the current popularity of new fermented plant products [3] trivialise the consumption of food products containing high levels of live bacteria. This is perceived as beneficial to health, probably through modulation of the gut microbiota [4], although the underlying mechanisms are not fully understood.

The bacterial density in such bacteria-enriched food products is compatible with production of autoinducers (AIs) [5] that mediate the density-dependent communication system, termed quorum sensing (QS), which regulates cooperative behaviors (e.g. biofilm development, enzyme secretion, ...) [6]. These AIs include AHLs whose chemical structure should enable them to resist digestion and therefore, may modulate the gut microbiota of the consumers.

To assess these hypotheses, we generated modelled foods enriched or not with AHL from cultures of *Hafnia alvei* (a usual cheese-ripening starter known as an AHLs-producer [7]) on milk ultrafiltrate, quantified the survival of AHL during an *in vitro* digestibility test, then investigated the impacts of these model foods on the intestinal microbiota of recently weaned rats.

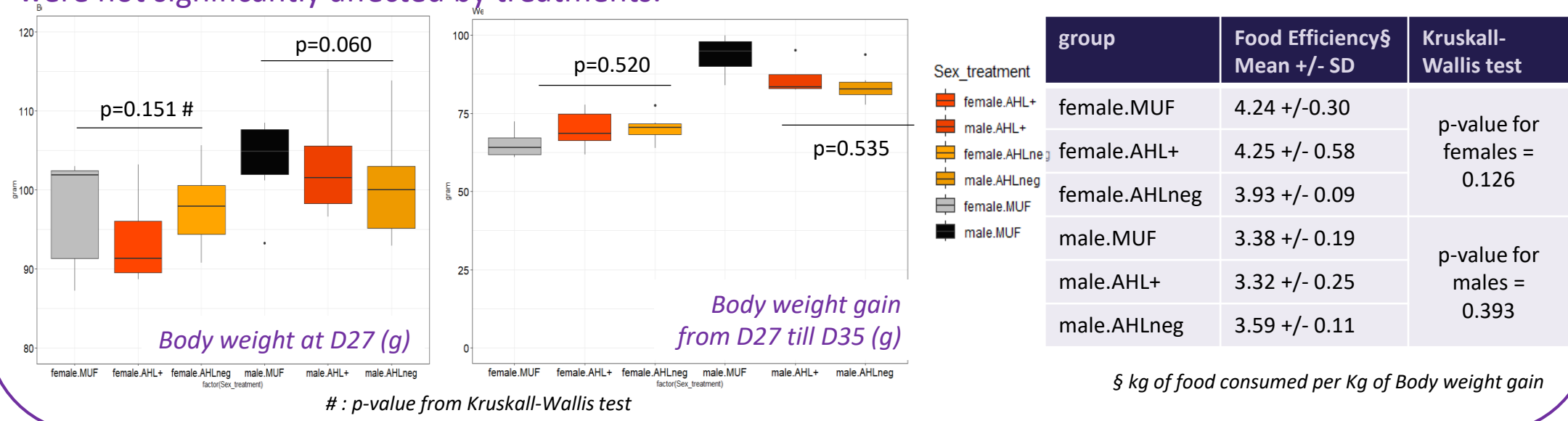
#### 4) Overall design of the animal study

Recently weaned pups originating from 3 different litters were supplemented from D27 till D35 with either uninoculated milk ultrafiltrate (MUF) or filter-sterilized supernatants from *H alvei* culture on MUF that had been submitted to alkalisation (AHLneg) or not (AHL+). All supplements have been concentrated by freeze-drying then resolubilized before used. Animals gavages and follow-up as well as intestinal microbiota characterization were carried out as described in [10]



#### 5) AHL enriched food had no significant impact on pups growth nor food consumption

Studied pups exhibited comparable body weights at the beginning of the supplementation and their growth -as summarized by bodyweight gain- no their food consumption -as reflected by food efficiency- were not significantly affected by treatments.



#### 6) AHL enriched food did no significant modify colonic microbiota richness nor evenness

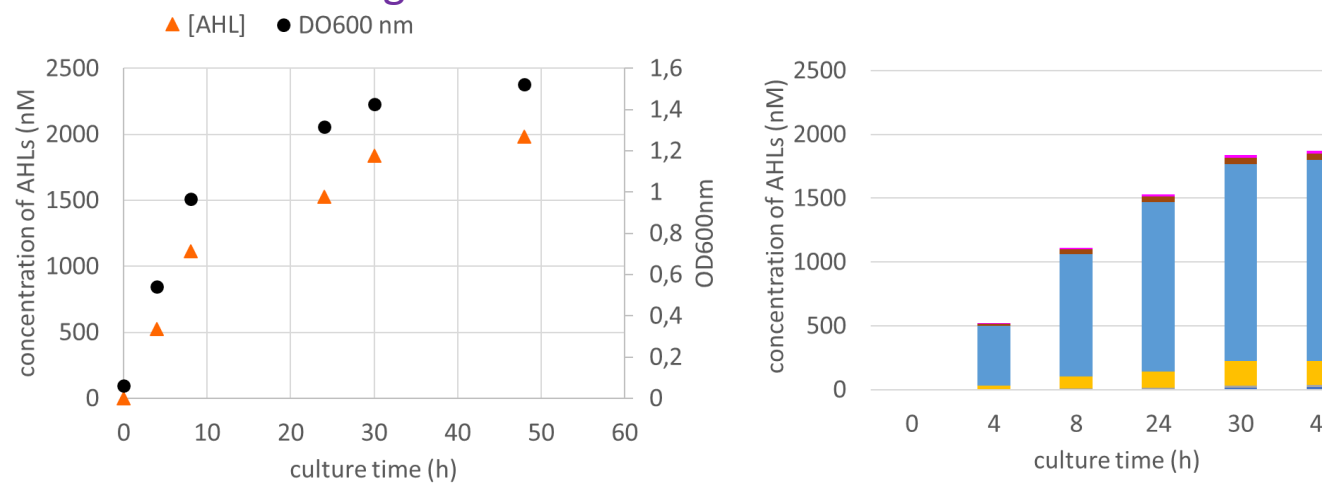
None of the indices reflecting the alpha-diversity of colonic microbiota collected from pups at the end of supplementation was significantly affected by the treatments.

	S.obs	S.chao1	Simpson	Shannon.H
female.MUF	421.33 +/- 77.78	466.99 +/- 65.84	0.949 +/- 0.040	3.961 +/- 0.783
female.AHL+	470.17 +/- 32.59	499.71 +/- 32.74	0.969 +/- 0.010	4.390 +/- 0.251
female.AHLneg	437.33 +/- 113.35	464.81 +/- 113.59	0.972 +/- 0.008	4.333 +/- 0.374
P-value from KW test	0.566	0.834	0.778	0.670
male.MUF	451.00 +/- 60.38	502.15 +/- 38.11	0.963 +/- 0.015	4.182 +/- 0.320
male.AHL+	435.00 +/- 93.11	486.62 +/- 107.12	0.953 +/- 0.039	4.163 +/- 0.566
male.AHLneg	447.83 +/- 67.11	492.60 +/- 67.07	0.960 +/- 0.029	4.247 +/- 0.499
P-value from KW test	0.927	0.927	0.994	0.476

Our study confirmed that food matrices can give rise to the production of AHLs and demonstrated that these signal molecules can resist - at least partially - digestion. These molecules therefore have the potential to interact with the intestinal microbiota. Despite this, under our operating conditions, the AHL-enriched food did not modify the colonic microbiota. Our first data about the ileal impact are however more encouraging and deserve further investigation.

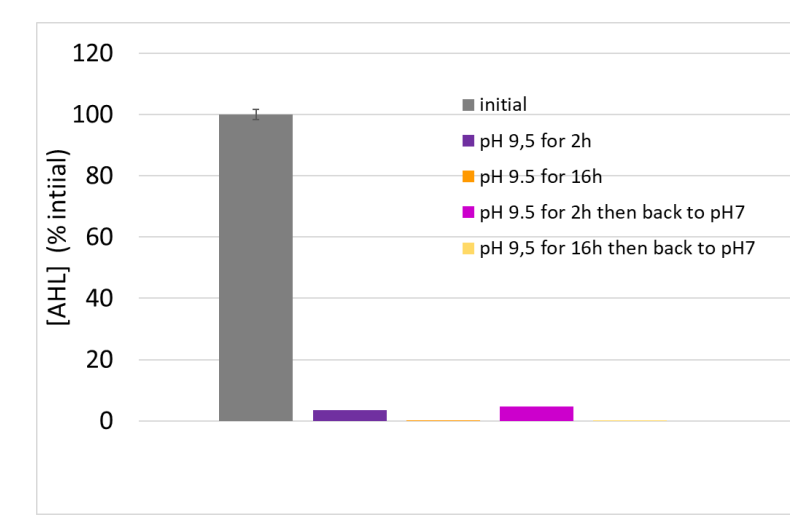
#### 1) *H alvei* produces AHLs on milk ultrafiltrate

As observed on other broths [7], N-(3-Oxohexanoyl)-L-homoserine lactone (3oxoC6-AHL) and N-hexanoyl-L-Homoserine lactone (C6-AHL) accumulated in the media during culture of *H alvei* CIRM-BIA 1832 on milk ultrafiltrate



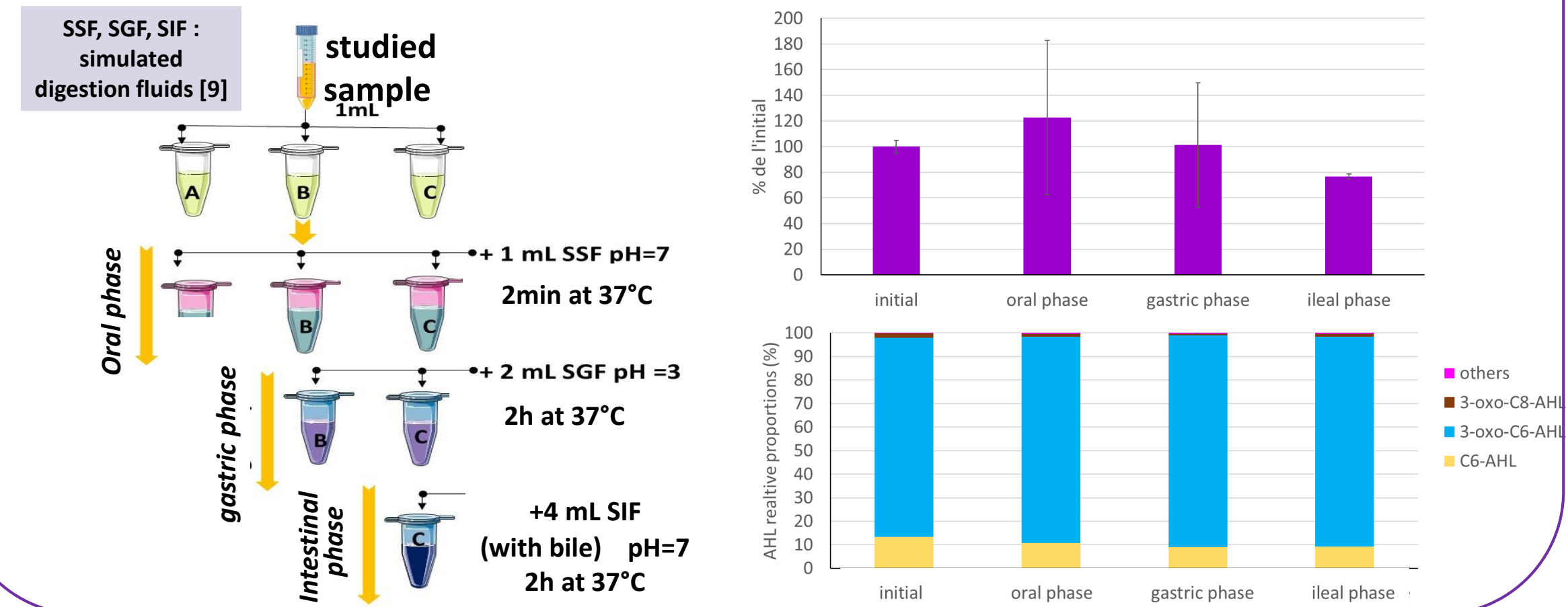
#### 2) AHL were degraded by alkalization

as previously suggested [8] but even when it was only for 2h and transitory



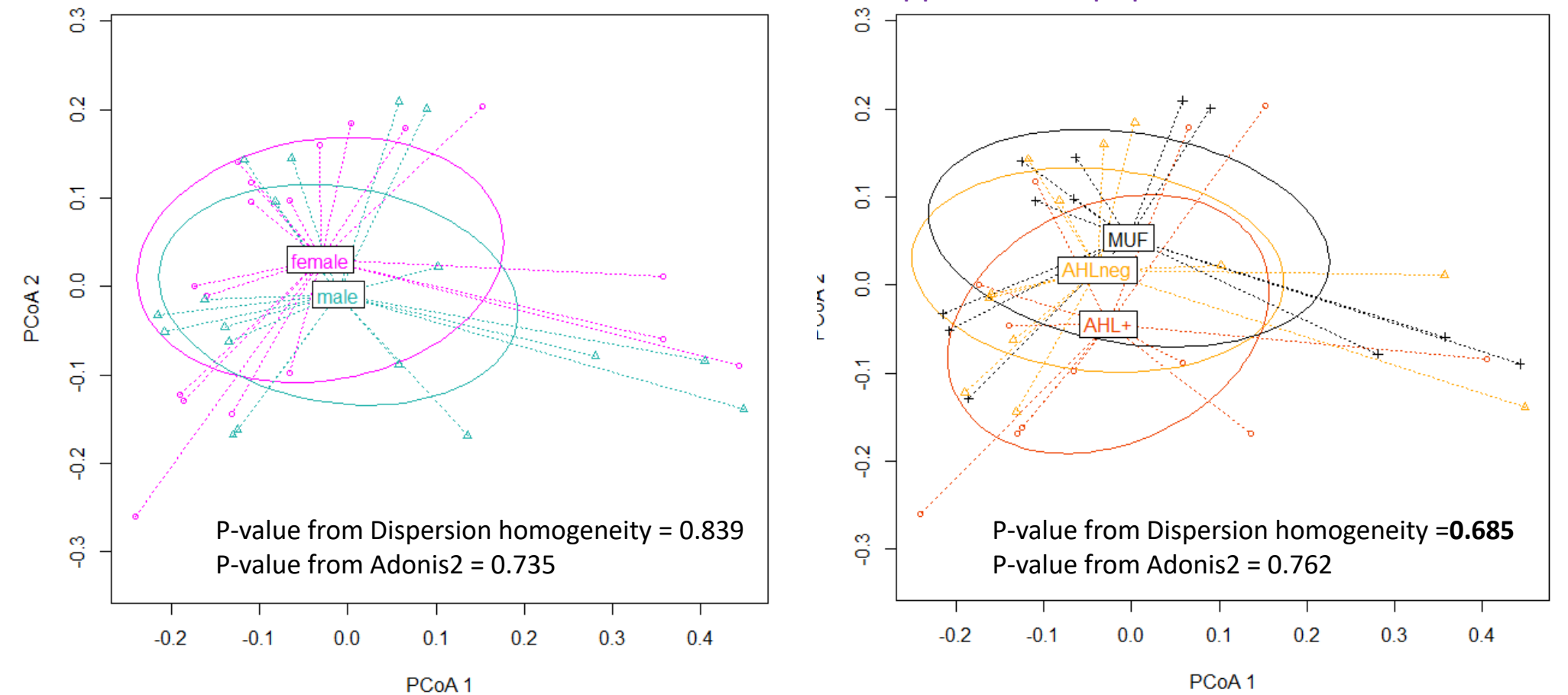
#### 3) most of AHL survived to conditions mimicking digestion

Indeed, 77 +/- 2% of the initial AHL load was recovered at the end of the *in vitro* digestion test, without any marked change in relative proportions. Partial degradation occurred mainly during the most alkaline phase (ie ileal).

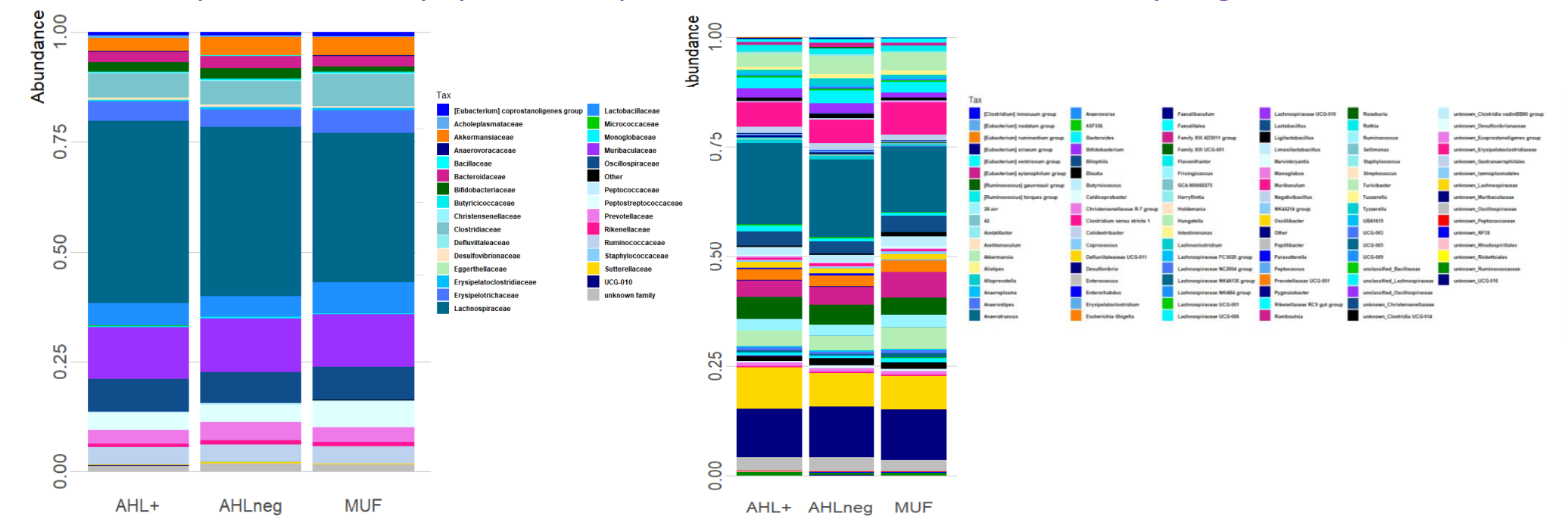


#### 7) AHL enriched food did not significantly change the colonic microbiota communities

No clear-cut differences related to sex or to treatment were evidenced from both Principal coordinate analysis (PCoA) and Permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray-Curtis dissimilarity matrix of bacterial communities from the colonic contents of supplemented pups

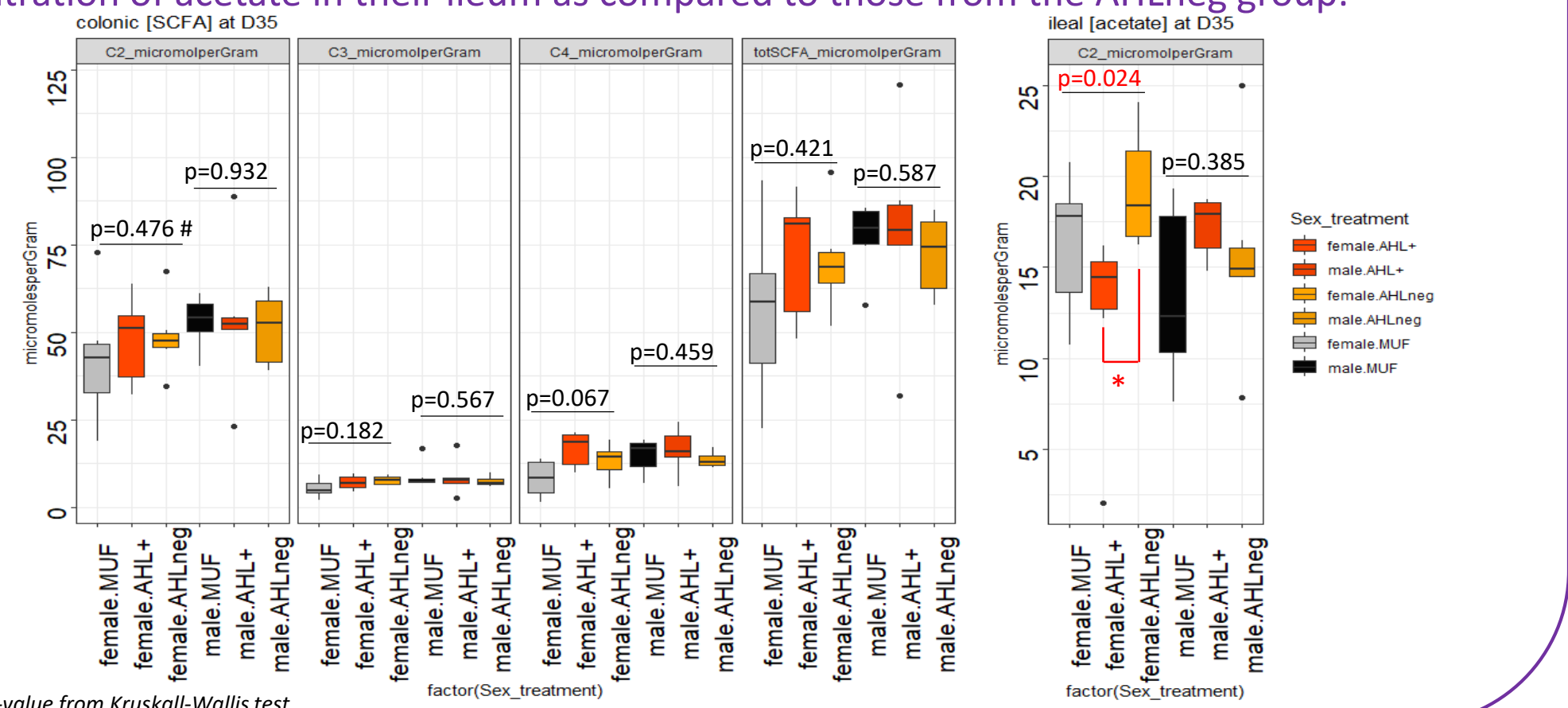


Similarly no significant differences were observed between pups supplemented with AHL enriched food as compared to other pups with respect to bacterial abundance at family or genus levels



#### 8) AHL enriched food may modulate microbiota activity in the ileum ...

In the colon, none of the short-chain fatty acids concentrations was significantly affected by treatments. Conversely, female pups from the AHL+ group exhibited significantly lower concentration of acetate in their ileum as compared to those from the AHLneg group.



[1] Tamang et al Diversity of Microorganisms in Global Fermented Foods and Beverages. Front Microbiol. 2016;7:377. doi: 10.3389/fmicb.2016.00377. [2] Wiernasz et al. Lactic Acid Bacteria Selection for Biopreservation as a Part of Hurdle Technology Approach Applied on Seafood. Front. Mar. Sci. (2017) 4:119. doi: 10.3389/fmars.2017.00119. [3] Torres et al An overview of plant-autochthonous microorganisms and fermented vegetable foods. Food Science and Human Wellness 9 (2020) 112 https://doi.org/10.1016/j.fshw.2020.02.006. [4] Wastyk et al. Gut-microbiota-targeted diets modulate human immune status. Cell. 2021;184(16):4137-4153.e14. doi: 10.1016/j.cell.2021.06.019. [5] Rezac et al Fermented Foods as a Dietary Source of Live Organisms. Front. Microbiol. (2018) 9:1785. doi: 10.3389/fmicb.2018.01785 [6] Stubbendieck & Straight (2020) Specialized metabolites for bacterial communication. In: Liu H-W, Begley TP (eds) Elsevier, Oxford, pp 66. [7] Li et al. Effect of the lux/R gene on AHL-signaling molecules and QS regulatory mechanism in *Hafnia alvei* H4. AMB Express. 2019;9(1):197. doi: 10.1186/s13568-019-0917-z. [8] Decho et al Autoinducers extracted from microbial mats reveal a surprising diversity of N-acylhomoserine lactones (AHLs) and abundance changes that may relate to diel pH. Environ Microbiol. 2009;11(2):409. doi: 10.1111/j.1462-2920.2008.01780.x. [9] Brodtkorb et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc. 2019;14(4):991-1014. doi: 10.1038/s41596-018-0119-1. [10] Le Dréan et al. Neonatal Consumption of Oligosaccharides Greatly Increases L-Cell Density without Significant Consequence for Adult Eating Behavior. Nutrients. 2019;11(9):1967. doi: 10.3390/nu11091967.