

In situ production of prebiotics: making prebiotics in the animal itself

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Background

In animal husbandry, infectious disease is commonplace and antibiotics are routinely used in large amounts. The aim of this project was to develop a feed supplement as an alternative to antibiotics in the form of a prebiotic feed which could increase bacterial diversity and beneficial bacteria since these can combat pathogens in the GI-tract.

Because of the current cost-effectiveness of antibiotics, novel products should be effective as well as very low-cost, which current prebiotics are not. In this endeavour, the production of prebiotics was moved from the industrial setting to instead take place within the gastrointestinal tract of the animal, which is what we refer to as *in situ* production of prebiotics (**figure 1**). We used a waste product from the potato industry, namely potato pulp, along with pectinolytic enzymes to release galactose rich rhamnogalacturonan 1.

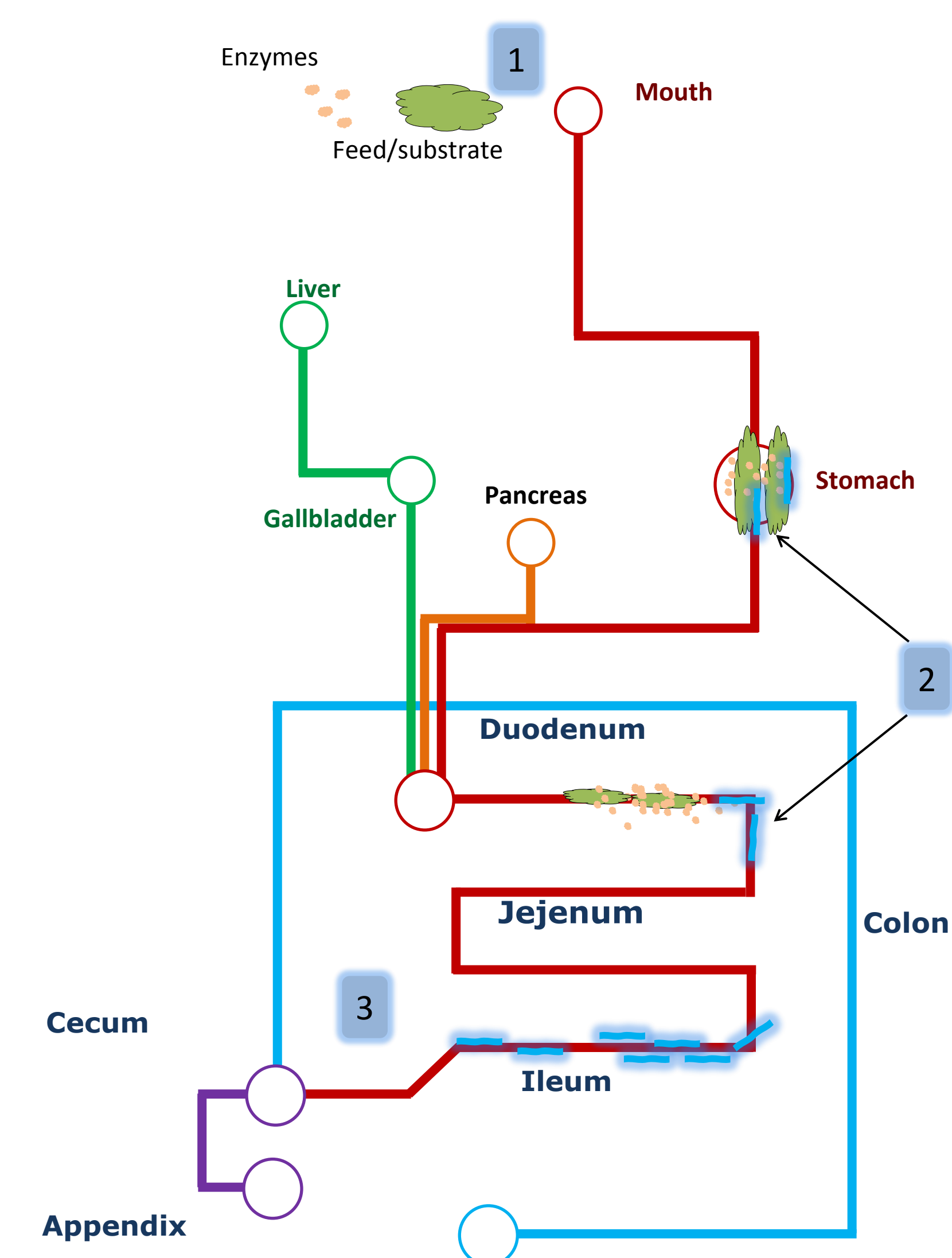


Figure 1: The *in situ* catalysis concept: 1) Enzymes and substrate is given along with feed. 2) concomitantly with regular digestion, the catalysis is initiated in the stomach and/or the small intestine. 3) The produced fiber travels to the ileum and colon where it acts as a prebiotic.

In vivo studies

When enzymes in combination with potato pulp were then fed to weaning piglets, we found up to 40% of the theoretically maximum amount of solubilized fiber in the gastrointestinal content. This was released within 20 minutes, suggesting that *in situ* production of fiber is feasible (**figure 2**).

In vitro studies

In an *in vitro* digestion, 24.6% of the potato pulp could be water solubilized by enzymes and this solubilized galactose-rich fraction (rhamnogalacturonan 1) was then fermented by bacteria present in contents from piglet terminal ileum. The fermentations resulted in high levels of organic acids as determined by HPLC, lactate in particular, and an increase in the Genera *Lactobacillus* and *Veillonella* determined by deep sequencing of the 16S rRNA gene, suggesting some prebiotic potential.

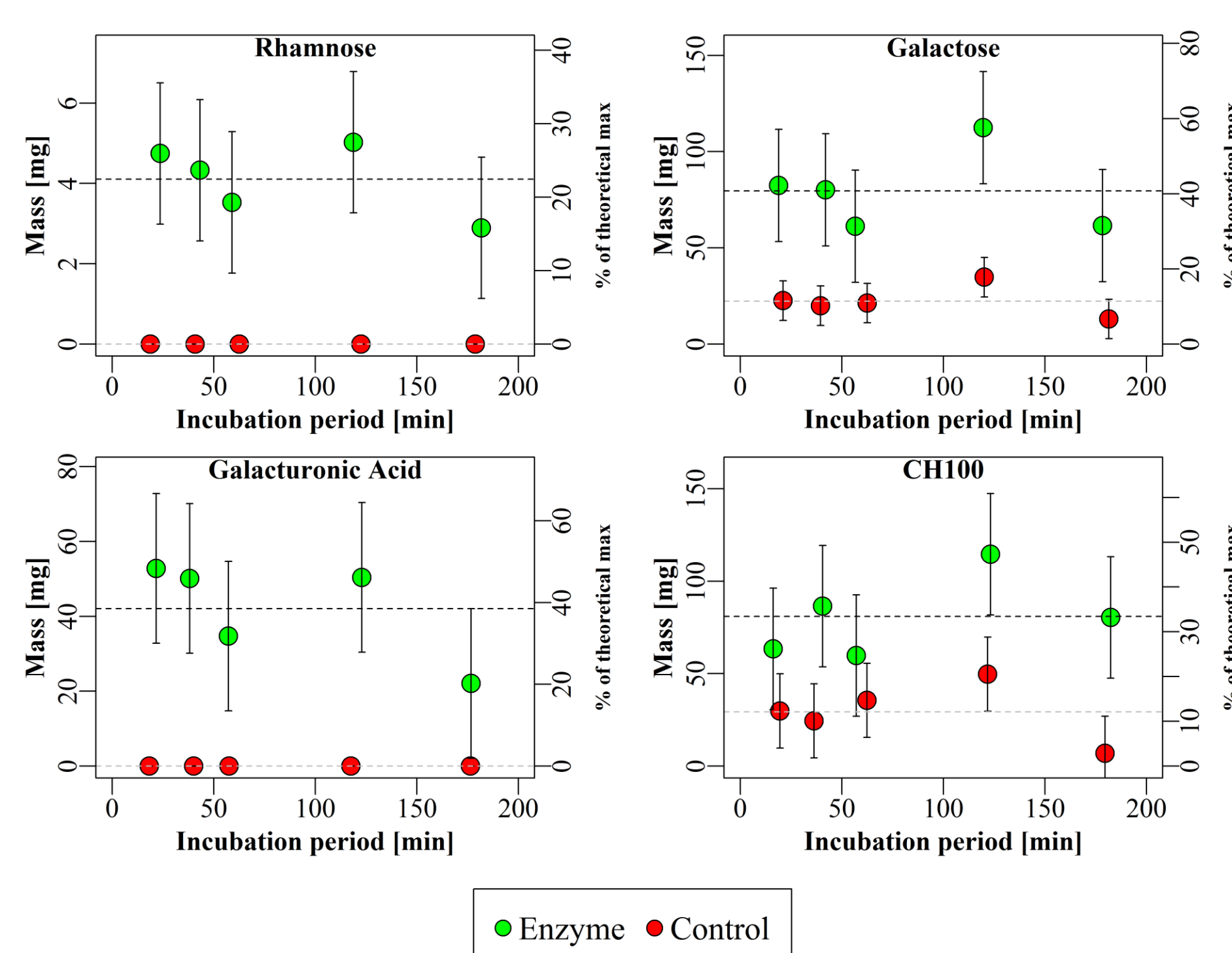


Figure 2: When enzymes along with potato pulp were administered *in vivo*, we observed release of rhamnogalacturonan 1 within 20 minutes, suggesting that *in situ* production is feasible in the weaning piglet. Values are means \pm SEM and are total amounts from the entire GI. CH100 is carbohydrate larger than 100kDa.

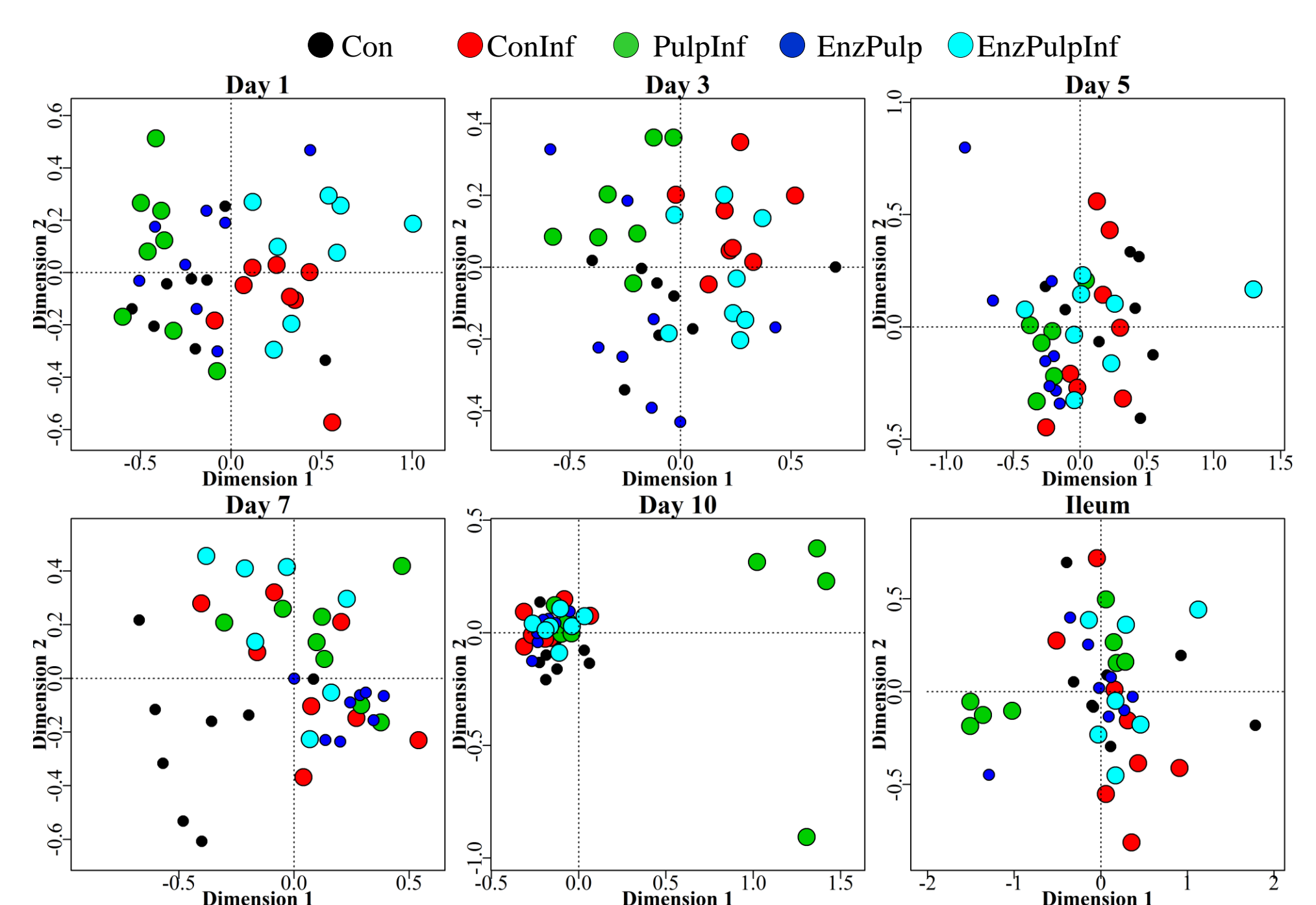


Figure 3: nMDS multivariate plot of the fecal and ileal microbiome across an experimental infection as assayed by 16S Illumina sequencing. Large points are infected animals.

In vivo infection

A pilot study testing an experimental infection was performed by feeding the pulp and enzyme supplement to weaning piglets challenged with *E. coli* F4+. Enzyme-released fibers as well as microbial changes (**figure 3**) were observed in the intestines of the animals but the experimental challenge unfortunately did not result in a clinical infection.

In conclusion

Overall, *in situ* production of fibers is possible in the weaning piglet, although it remains to be confirmed *in vivo* if these fibers are indeed prebiotic and/or inhibitory against PWD.

Acknowledgement

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References

- Strube ML, Meyer AS, Boye M. 2013. Basic physiology and factors influencing exogenous enzymes activity in the porcine gastrointestinal tract. *Animal Nutrition and Feed Technology* **13**: 441-459
 Strube ML, Ravn HC, Ingerslev HC, Meyer AS, Boye M. 2015. In situ prebiotics for weaning piglets: *in vitro* production and fermentation of potato galacto-rhamnogalacturonan. *Appl Environ Microbiol.* **81**(5):1668-78.
 Strube ML, Jensen TK, Meyer AS, Boye M. 2015. *In situ* prebiotics: Release of galacto-rhamnogalacturonan from potato pulp *in vivo* in the porcine gastrointestinal tract. *AMB Express* **5**:66.
 Strube ML, Rasmussen S, Jensen TK, Meyer AS, Boye M. The effect of *in situ* produced galacto-rhamnogalacturonan on an experimental *E. coli* infection in piglets. Manuscript in preparation.

