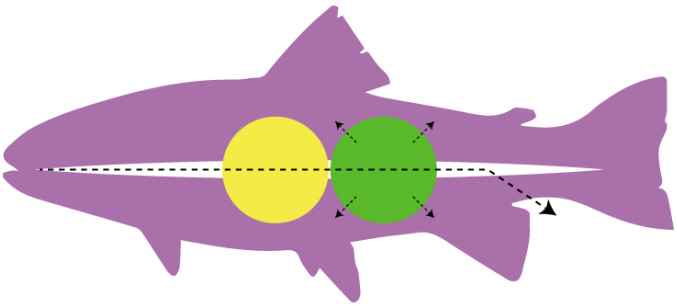
SALMOSIM ®



SalmoSim® – A salmon gut simulator to deliver cost-effective solutions for the development of aquaculture nutrition

Llewellyn M. S., Humble J. L., SalmoSim®, University of Glasgow, UK

SalmoSim® is an *in vitro* model of the Atlantic salmon (*Salmo salar*) gastrointestinal tract developed at the University of Glasgow in collaboration with research partners within the salmon aquaculture industry (MOWI, Alltech).

SalmoSim® was founded to address the growing need for sustainable and nutritious feed in salmonid aquaculture. SalmoSim® simulates the Atlantic Salmon marine-phase gastro-intestinal tract to predict the impact of feed additives on gut microbial communities and the intestinal environment. In parallel, SalmoSim-Digest® can predict the digestibility and absorbance profiles of novel feed ingredients.

SalmoSim® is designed to dramatically reduce the *in vivo* testing costs involved with bringing innovative feed ingredients to market by allowing rapid pre-screening of new products, or quality control of existing ones.

Digestibility and absorption

Analyse the enzymatic hydrolysis of aquafeed substrates and absorption profiles of products

Veterinary pharmaceuticals

Assess the activation and bioavailability of orally administered medicines

Pro-pre-syn biotics

Characterise the impact of prebiotic ingredients upon the diversity and activity of the bacteria community and nutrient absorption profiles.

Pathway to productivity

We provide assessments of the characteristics of different feed additives and ingredient in terms of their impact on salmon nutrition.

SalmoSim

Current and Previous Clients

Alltech® COPPENS

EKOREZERV

innova FEED

BIOKIND

CALYSTA®
MORE FROM LESS

GBS
gibios.ru/en

DSM

Testimonial

"Our experience with SalmoSim® was excellent. SalmoSim® provided us with a very valuable insight into our project, ranked against other generic ingredients on the market. Their team, led by Dr. Martin Llewellyn, was very open, engaging, and professional. I would recommend SalmoSim® to others and definitely use their services again in the future."

EKOREZERV

Case Study 1 - Validation of SalmoSim® concept

Case Study 2 – SalmoSim® to explore the impact of prebiotics on salmon gut health

Case Study 3 – Validation of SalmoSim-Digest® to assay protein digestibility and absorption

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Case Study 1

Validation of Concept

SalmoSim: the development of a three-compartment *in vitro* simulator of the Atlantic salmon GI tract and associated microbial communities

Kazlauskaite, R., Cheaib, B., Heys, C., Ijaz, U. Z., Connelly, S., Sloan, W., Russel, J., Rubio, L., Sweetman, J., Kitts, A., McGinnity, P., Lyons, P. & Llewellyn, M. S.

Microbiome: Volume 9

Article number: 179 (2021)

Commercial partner: **MOWI**

Study Question: Can the SalmoSim® simulate the salmon gut microbiome and predict the impact of novel feed ingredients?

SalmoSim

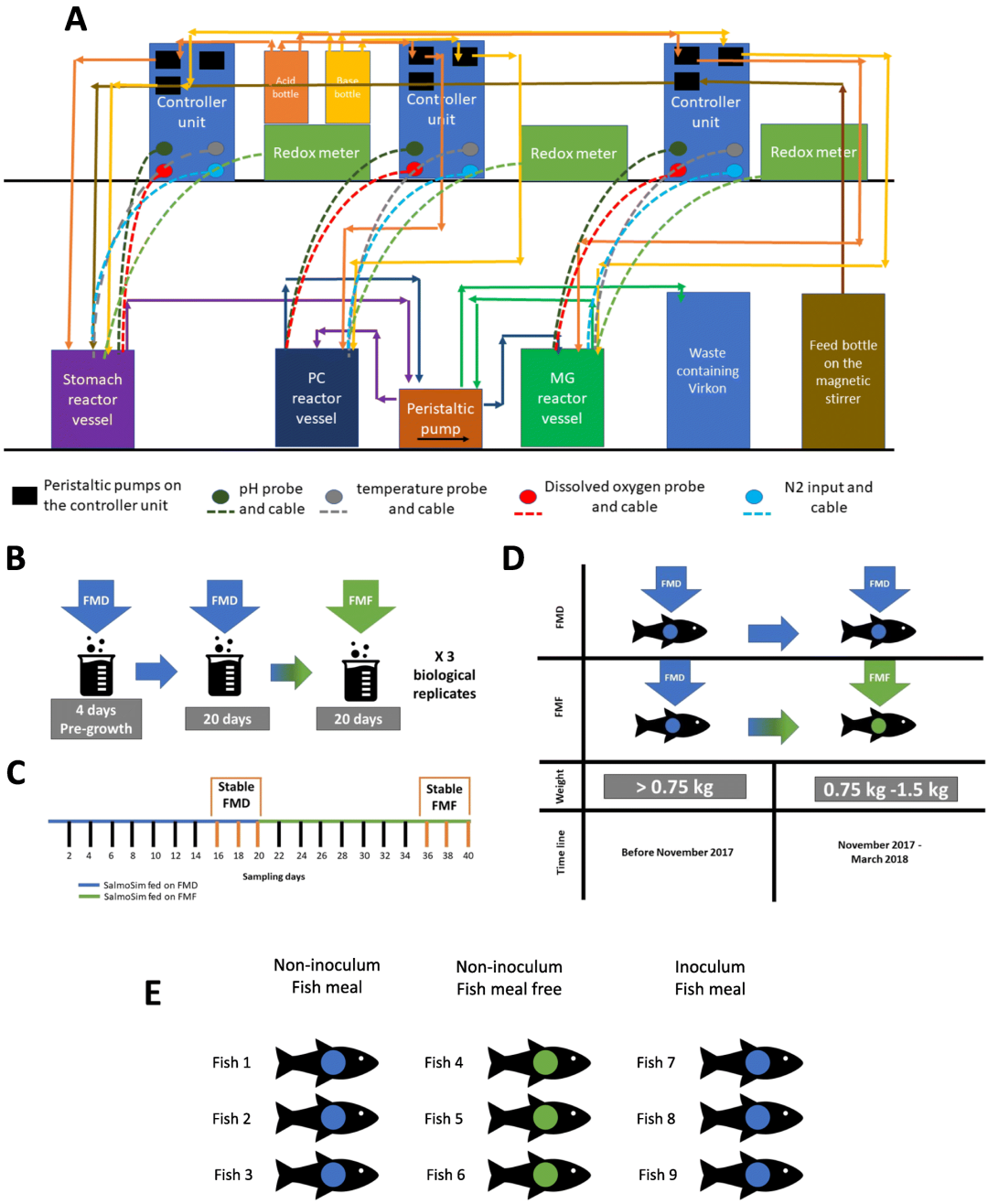

Figure 1: Salmon gut in vitro simulator. *(A) Schematic representation of SalmoSim® system bioreactor. (B) SalmoSim® feed trial design. (C) SalmoSim® sampling time points. (D) in vivo feed trial design. (E) Real salmon sacrificed for non-inoculum and inoculum samples*

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Outcome:

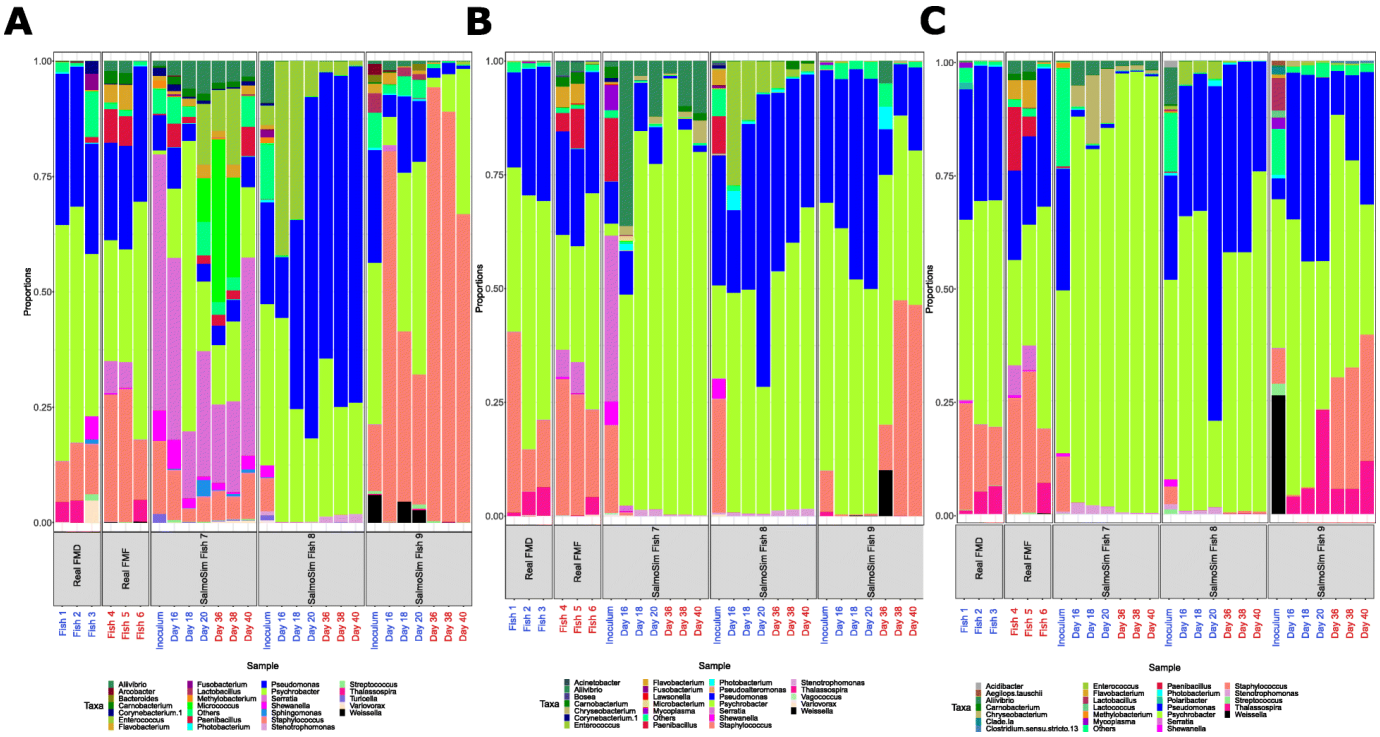

Figure 2: Microbial profiles from stomach (A), Pyloric caecum (B) and hindgut (C) from SalmoSim and real salmon across the course of the experiment.

Take-home messages:

- SalmoSim microbiomes were indistinguishable (p = 0.230) from their founding inocula at 20 days and the most abundant genera proliferated within SalmoSim.
- Real salmon and SalmoSim responded similarly to the introduction of novel feed, with the majority of taxa (96% Salmon, 97% SalmoSim) unaffected, while a subset of taxa was differentially affected across both systems.
- Consistent with a low impact of the novel feed on microbial fermentative activity, volatile fatty acid profiles were not significantly different in SalmoSim pre- and post-feed switch

SalmoSim

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Case Study 2

Prebiotics

Deploying an *In vitro* Gut Model to Assay the Impact of the Mannan-Oligosaccharide Prebiotic Bio-Mos on the Atlantic Salmon (*Salmo salar*) Gut Microbiome

Kazlauskaite, R., Cheaib, B., Humble, J., Heys, C., Ijaz, U. Z., Connelly, S., Sloan, W. T., Russell, J., Martinez-Rubio, L., Sweetman, J., Kitts, A., McGinnity, P., Lyons, P. & Llewellyn, M. S.

ASM Journals: Microbiology Spectrum

Article Number: e0195321 (2022)

Commercial Partner: **Alltech-Coppens**

Study Question: What is the predicted impact of Bio-MOS® pre-biotic on salmon gut microbial communities?

SalmoSim

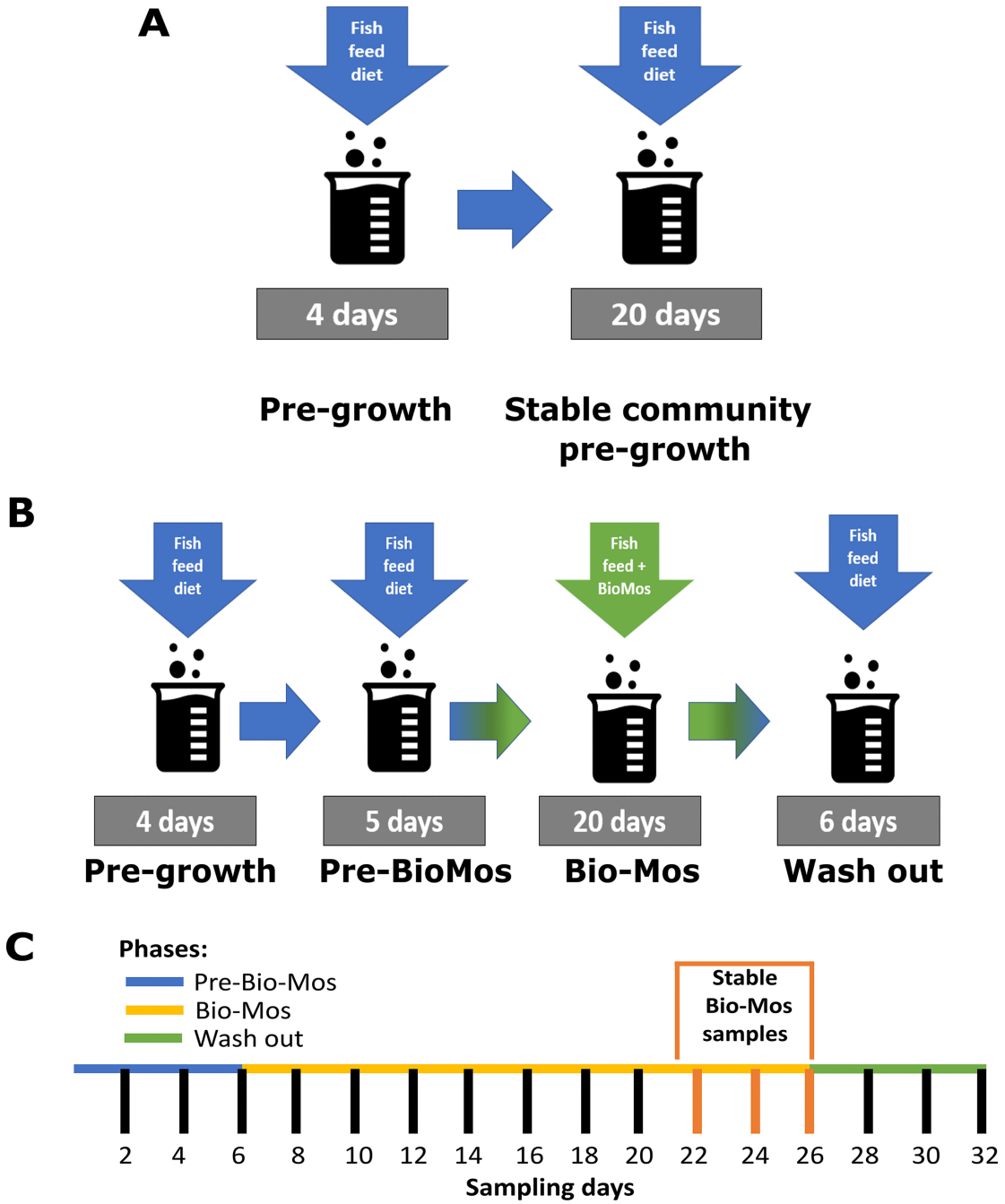

Figure 2: *In vitro setup.* ***(A)*** *Stable community pre-growth run within the SalmoSim system.* ***(B)*** *Experimental run involving four stages (i) Pre-growth. (ii) Feeding system pre-Bio-Mos. (iii) Feeding system with Bio-Mos. (iv) Wash out of the system without the addition of pre-biotic.* ***(C)*** *SalmoSim sampling time points.*

Outcome(s):

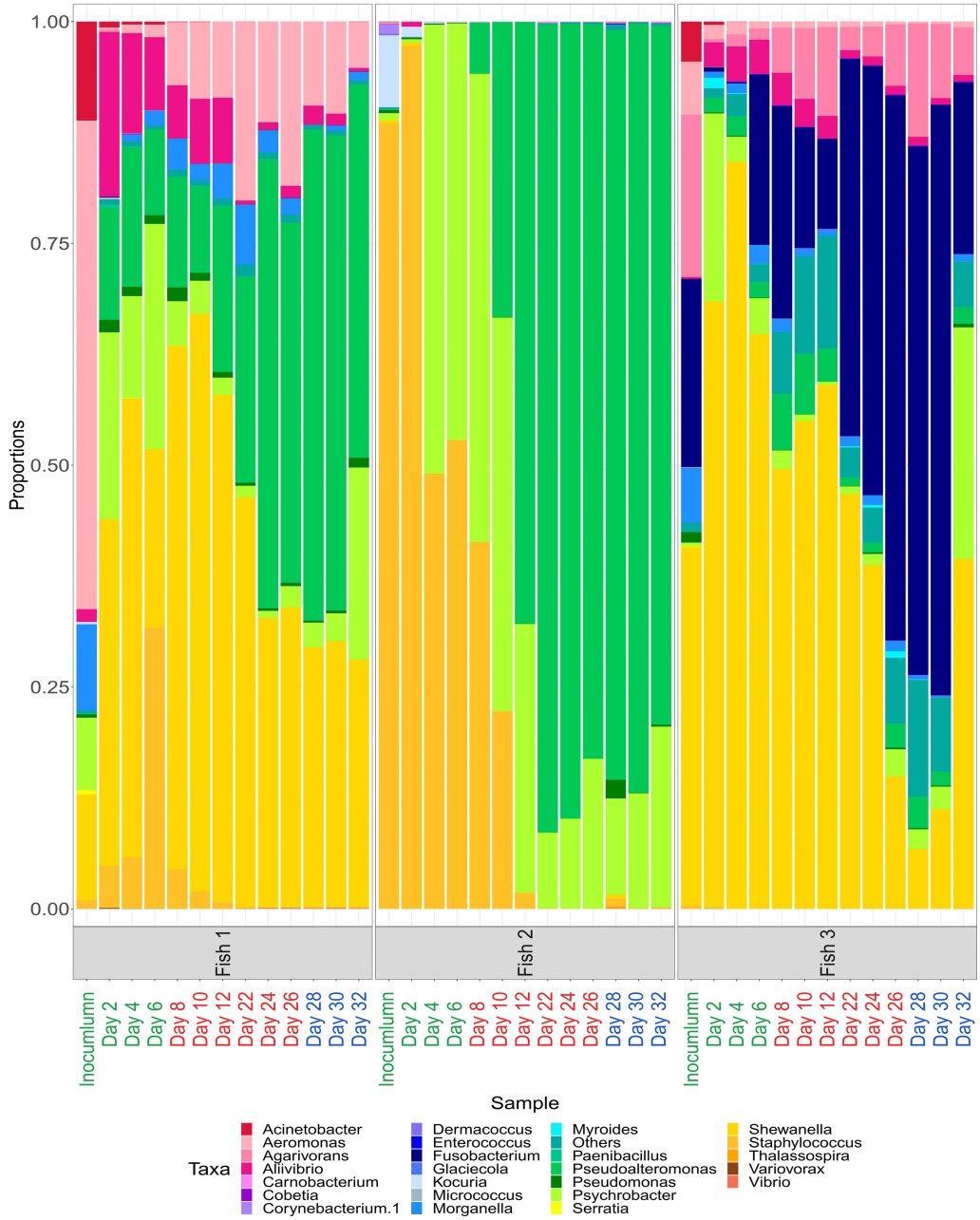

Figure 4: Microbial composition (25 most common genus + others) among different biological replicates and experimental phases Labels on x axis in green represent samples from Pre-Bio-Mos phases, in red samples fed on Bio-Mos phase and in blue samples from Wash out period. Only subset of time points is visualized for each phase: time points 2–6 for Pre-Bio-Mos, 8–12 and 22–24 for Bio-Mos, and 28–32 for Wash out.

Outcome(s):

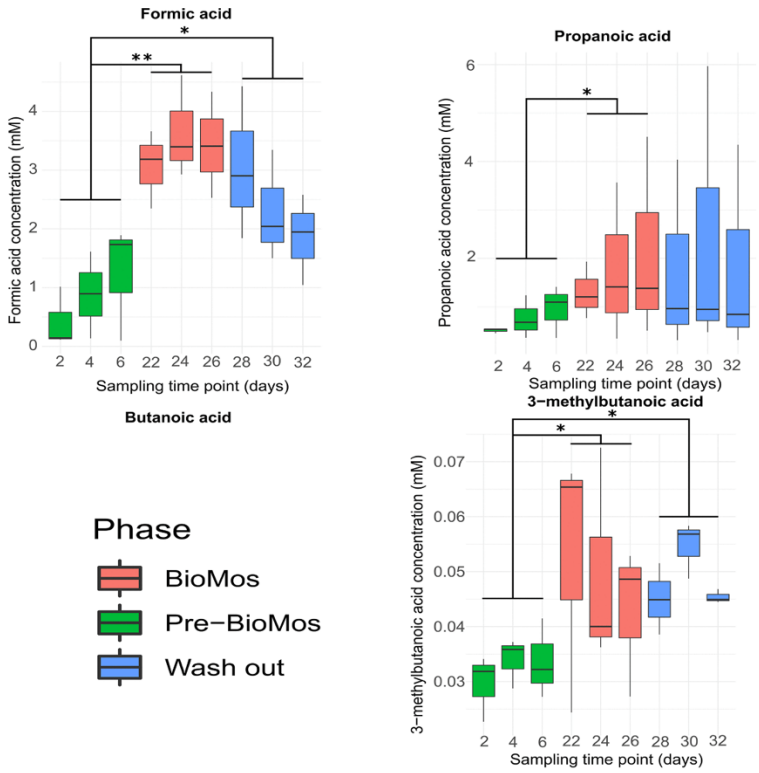

Figure 3: VFA responses in SalmoSim pyloric cecum compartment after Bio-Mos introduction and subsequent wash out period. The figure above visually represents volatile fatty acid production in three different experimental phases: (i) SalmoSim fed on Fish meal alone without prebiotic addition (Pre-Bio-Mos: green), (ii) SalmoSim fed on Fish meal with addition of Bio-Mos (Bio-Mos: red), (iii) wash out period during which SalmoSim was fed on Fish meal without Bio-Mos (Wash out: blue). x axis represents the concentration of specific volatile fatty acid (mM) while the y axis represents different sampling time points (days). The lines above bar plots represent statistically significant differences between different experimental phases. The asterisks show significance: *, $0.01 \leq P < 0.05$; **, $0.05 \leq P < 0.001$; ***, $P \leq 0.001$.

Take-home messages:

- Our data suggest that Bio-Mos may be of value in salmonid production as it enhances volatile fatty acid production by the microbiota from salmon pyloric ceca.
- Bio-MOS correlates with a significant shift in microbial community composition with an observed increase in lactic acid-producing *Carnobacterium.*

Case Study 3

Digestibility and absorption

Adapting SalmoSim® to assay protein digestibility and absorption – SalmoSim-Digest® - Comparison with *in vivo* digestibility data.

Humble, J., Sweetman, J., Lyons, P. & Llewellyn, M. S.

Manuscript in preparation

Commercial Partners: Innova Feed, Calysta, EKO-Reserv, Biokind, Biofeyn and others

Study Question: How well does SalmoSim-Digest® predict matching in vivo digestibility trial data?

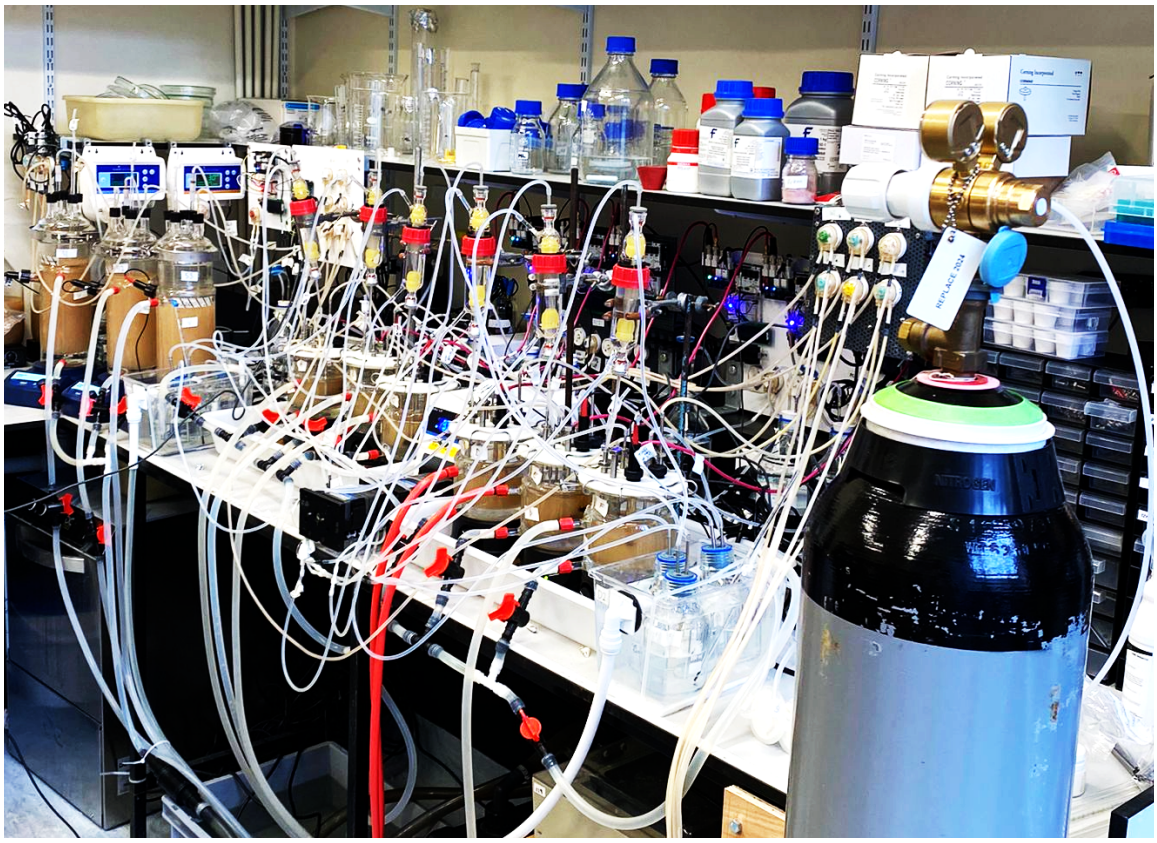

Figure 6: SalmoSim-Digest® in operation. Triplicate reactor vessels simulating the pyloric ceacum are run in parallel. Absorption of material across a semipermeable membrane is assessed and apparent digestibility calculated. Constituents of absorbed and retained fractions can analysed and rates of absorption calculated.

Outcome(s):

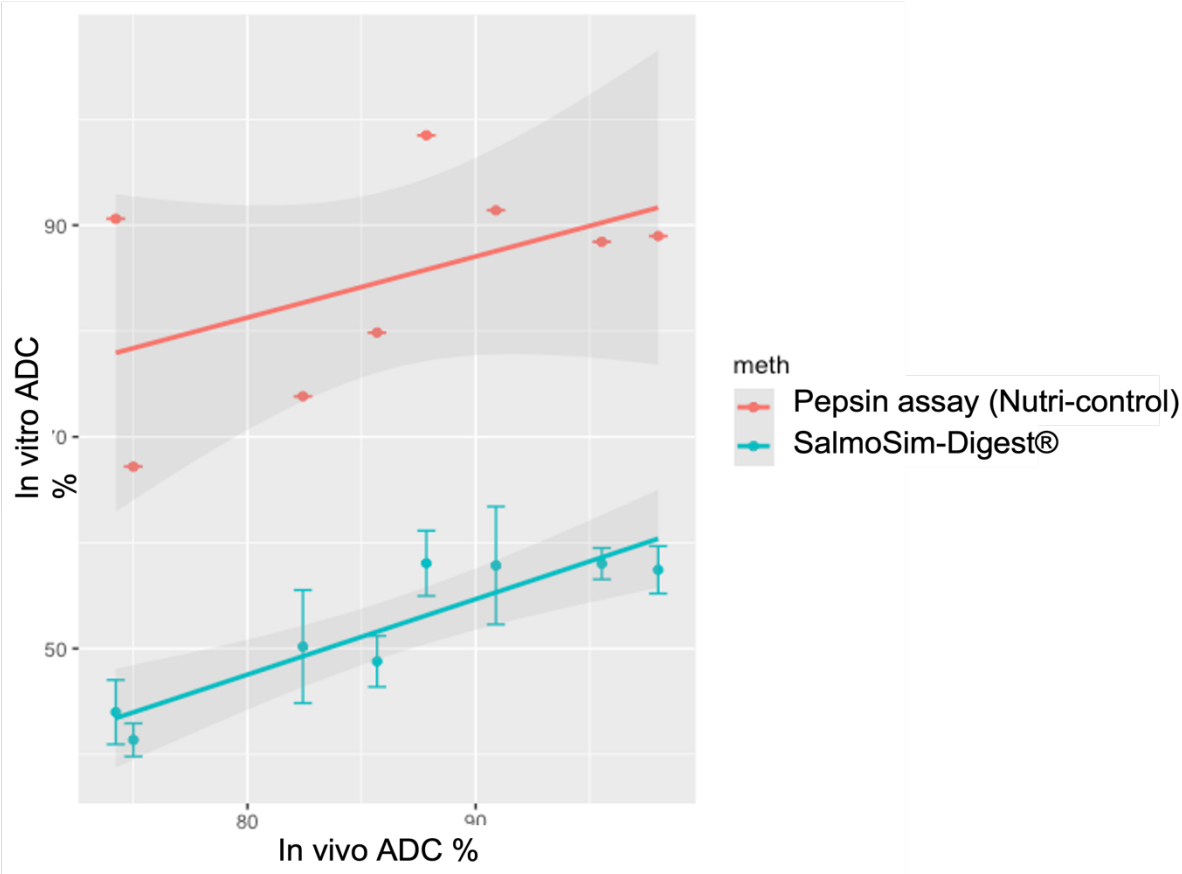

Figure 7: SalmoSim-Digest® comparison with *in vitro* trial data provided by Alltech-Coppens ltd for *Oncorhynchus mykiss* (Rainbow trout) (Green) (R^2 = 0.83). Nutricontrol digestible protein assay (R^2=0.23) (RED). Feed ingredients include; Poultry meal, Barley Protein, Poultry meal and bone, Fish meal, and four different single cell protein ingredients.

Outcome(s):

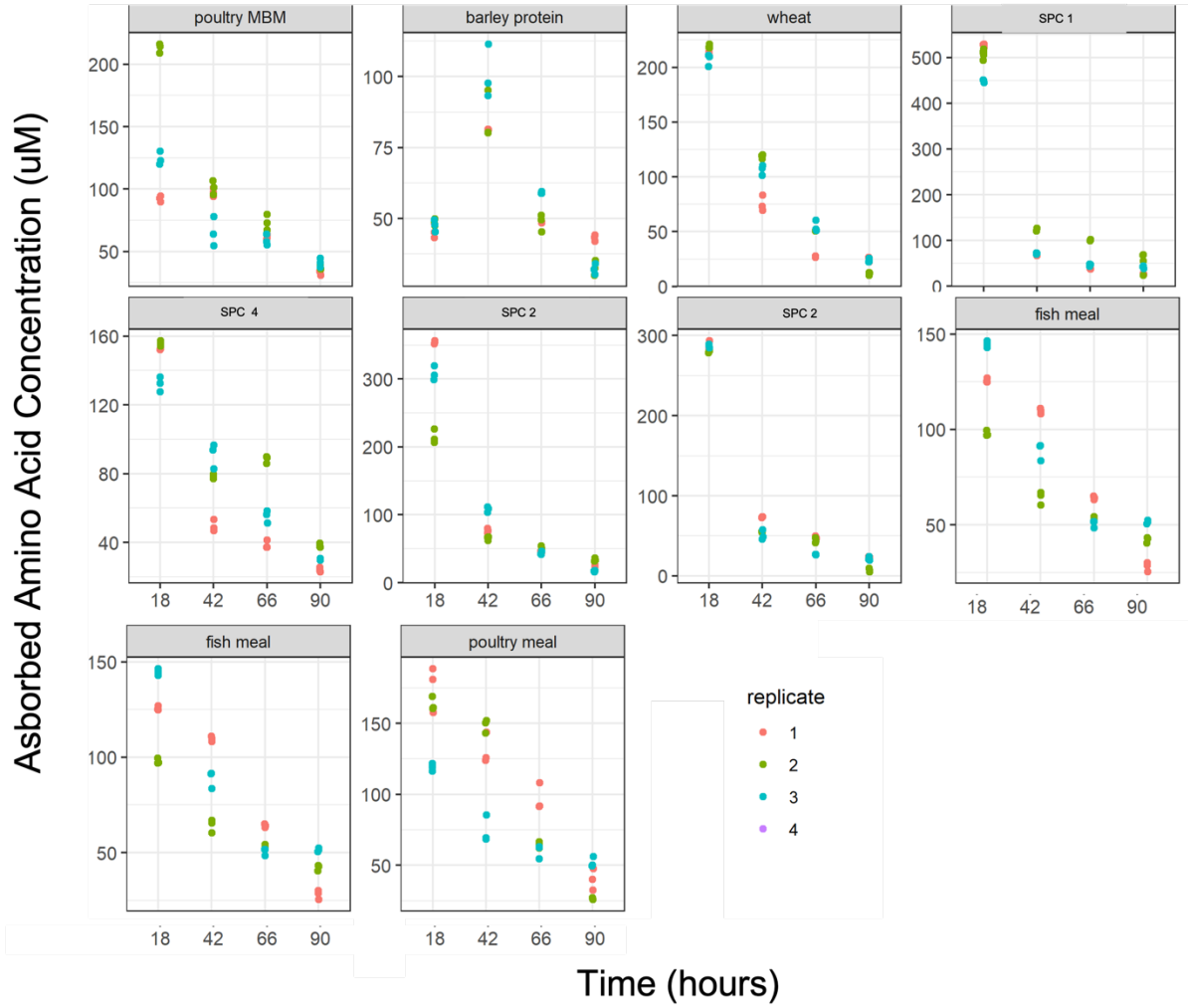

Figure 8: SalmoSim-Digest® amino acid absorption (uM) for different protein ingredients over 90 hours.

Take-home messages:

- SalmoSim-Digest® can accurately predict ($R^2 = 0.83$) *in vivo* digestibility trial data in salmonids for proteins and performs better than industry standard Pepsin digestibility assays ($R^2 = 0.23$)
- Trials are ongoing to in relation to lipid digestibility and absorption.