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Accepted manuscript

# Interaction between genetics and inulin affects host metabolism in rainbow trout fed a sustainable all plant-based diet

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## Abstract

Inulin affects nutrition and metabolism in many animals. Although inulin is widely used in the diet of teleosts, its mechanism of action is unknown. Here, we investigated the effect of inulin (2%) on the intestinal microbiome and metabolism in rainbow trout (*Oncorhynchus mykiss*) selected for growth and survival when fed a 100% plant-based diet (*suave*) and a control line (*temoin*). Metabolic responses to the two factors (line and inulin) in liver, intestine, muscle, and adipose were tissue-specific, with line and interaction between the two factors influencing overall expression in liver. In the intestine, inulin and line and in muscle, line influenced the expression of metabolic genes. Microbiota between the mucus and digestive contents was significantly different, with genera from *Proteobacteria* being more abundant in the mucus, whereas genera from the *Firmicutes* and *Planctomycetes* being more abundant in contents. Effect of inulin and interaction between factors on the microbiome was evident in contents. The significant taxa of control and inulin fed groups differed greatly with *Streptococcus* and *Weissella* being significantly abundant in the inulin-fed group. There was a general trend showing higher levels of all SCFAs in *temoin* group with propionic acid levels being significantly higher. An OTU belonging to the *Ruminococcaceae* was significantly abundant in *suave*. The tissue-specific correlations between OTUs and gene expression may indicate the link between microbiome and metabolism. Together, these results suggest that line and inulin impact the gene expression in a tissue specific manner, possibly driven by specific OTUs enriched in inulin-fed groups and *suave*.


**Keywords:** Rainbow trout, Inulin, *Firmicutes*, Short chain fatty acid, microbiome, sustainable diet, metabolism

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## 1 Introduction

The rainbow trout is a cold-water carnivorous species with high commercial value. In a commercial setting, the diet of this species consists of a high proportion of protein and oil from marine fish (1). The supply of these traditional fish feed ingredients is economically and ecologically unsustainable due to high fishing pressure, which directly leads to the collapse of wild fish stocks (1). As an alternative, a feed consisting exclusively of plant-based ingredients has been developed and extensively tested to meet all the nutritional requirements of rainbow trout (2–4). This next-generation sustainable diet formula is a major step towards achieving the Sustainable Development Goals (SDG14) set by the United Nations. However, these total plant-based diets are known to cause severe metabolic abnormalities such as glucose intolerance and high visceral fat when fed to rainbow trout (5,6).

To overcome this limitation, a genetic line (hereafter "line") of fish that grow and survive better when fed a 100% plant-based diet was developed through selective breeding (4 generations) (3). This line of fish is able to digest and metabolise the total plant-based diet better than the naïve fish and maintain a similar growth profile to fish fed conventional ingredients (3). However, knowledge about metabolic adaptations, the intestinal microbiome and the interaction between diet, intestinal microbiome and host metabolism is unknown for the selected line (*suave*).

On the other hand, prebiotics such as inulin are potential modulators of various metabolic and immune processes in different animals, including fish (7). Inulin is known to affect energy metabolism, regulation of inflammation, and immune homeostasis in the intestine via its microbially derived metabolites (short-chain fatty acids: SCFA) (8,9). Systemically, these dietary fibre-derived metabolites are transported to the liver via the portal vein and are involved in the pathways of fatty acid synthesis, oxidation, and fat storage (10). In addition, SCFAs are known to affect glucose uptake in muscle and adipose tissue (11). The members of the intestinal microbiome involved in the breakdown and utilization of inulin in humans and livestock are well documented (12). The microbial species encoding inulin-degrading functions may vary, but the mechanism of inulin degradation and utilization by the microbiota, as well as receptor-mediated uptake and utilization of SCFAs by the host, are conserved in humans and other mammals (13,14).

The effect of inulin on growth, disease resistance, immune parameters, digestive enzymes, and metabolism has been demonstrated in teleosts with a wide range of dietary habits (15–18). In rainbow trout, there are several studies demonstrating the beneficial effects of inulin

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on growth and immune status (19–21). Recently, the involvement of inulin in metabolic processes in teleosts has also been demonstrated. Studies show that inulin can attenuate the negative metabolic syndrome caused by high-carbohydrate feeding in tilapia (22) and alter the expression of genes involved in various metabolic pathways in rainbow trout (4). Taken together, these results suggest that the mechanism of action of inulin in teleosts may be similar to that in mammals. Given the overwhelming interest in exploiting these beneficial aspects of the interaction between inulin and the gut microbiome for better health and nutritional management in teleosts, a more detailed investigation of this aspect is warranted.

Therefore, in this study, we investigate the effects of inulin and line on the metabolism (via microbially derived SCFA) and microbiome of rainbow trout fed a 100% plant-based basal diet. To this end, two lines (*temoin* and *suave*) of rainbow trout (mean weight: 128.6 g±8.4) were fed a 100% plant-based basal diet containing either 2% inulin or devoid of it for a period of 120 days. In addition to growth and plasma parameters, host metabolic responses were measured by examining gene expression in various organs, including liver, intestine, muscle, and adipose tissue. Since the established link between the inulin and the host is the microbially derived metabolites such as SCFA, we investigated the changes in the microbiome as well as the changes in the content of SCFAs in the intestine. Changes in microvillar length are also reported, as inulin is known to affect these epithelial structures.

## 2 Materials and methods

### 2.1 Ethical approval

PEIMA INRAE facilities (Sizun, France) are authorized for animal experimentation under French regulation (D29-277-02). The fish handling and sampling was conducted in accordance with the guidelines of French and European legislation on the use and care of laboratory animals (Decree No. 2013-118 of 1 February 2013 and Directive 2010/63/EU, respectively). Experiments were conducted under the animal experimentation authorization of Karine Brugirard Ricaud and Laurent Labbé.

### 2.2 Diet and experimental setup

The feeding trial was conducted at the PEIMA fish breeding and rearing facility (INRAE, Sizun, France). Two genetic lines of rainbow trout (hereafter referred to as line) were used for the feeding trial, namely temoin (the INRAE synthetic strain; a domesticated strain maintained at the PEIMA facility with a large number of spawners and without artificial selection in order to maintain the genetic variability) and suave (a selected line from the

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synthetic strain obtained after 4 generations of selective breeding based on the ability to survive and grow when fed a 100% plant-based diet). A two-factorial design was used, with line and inulin intake as the factors. A total plant-based diet (containing only plant ingredients and vegetable oils supplemented with free amino acids) with (2%) or without (0%) inulin was prepared at the feed manufacturing facility (INRAE Donzacq, France). The dosage of inulin was decided based on our previous study (4) The diets were isoproteic (~45% crude protein), isolipidic (~22% crude fat) and isoenergetic (~24 KJ/Kg dry matter) and were prepared to meet the nutritional requirements of rainbow trout (23). The composition of the ingredients and the proximate composition of the diet are given in Table 1 and Supplementary Table 1, respectively.

Fifty juvenile rainbow trout with a stocking density of ~3.5 kg/$m^3$ were introduced into each of the 1800 L fibreglass circular tanks. The average initial weight of the fish in each group is given in table 2. During subsequent growth, number of fish was reduced once by random elimination to keep a density below 15 kg/m3 in each tank. At the same time, early maturing males (at one year) were discarded. There was a total of 4 groups consisting of TVO: *temoin*-0% inulin; TVI: *temoin*-2% inulin; SVO: *suave*-0% inulin; SVI: *suave*-2% inulin. Each group was allocated 3 tanks. Fish were reared under standard conditions during the experimental period, i.e., water oxygen level of 9 mg/L, temperature between 6.0 and 18 °C and pH of 6.5, water flow rate of 0.7 L/s and natural photoperiod. The fish were fed by automatic feeder five times a day for 16 weeks. Total weight of fish in each tank was measured every three weeks to assess growth parameters. The amount of feed given was recorded daily to calculate the consumption index. The total amount of feed consumption increased steadily with growth and the feeding rate was adjusted accordingly.

### 2.3 Sampling

At the end of the feeding experiment, we randomly sampled 12 fish per group (4/tank). The fish were first anaesthetised with tricaine methane sulfonate; (MS-222) (50 mg/L) and then euthanised with a higher dose of MS-222 (100 mg-L). The fish were weighed and then blood samples were collected using heparinised syringes and tubes and centrifuged at 3000 g for 10 minutes to isolate the plasma. Plasma samples were stored at -20°C until analysis of plasma parameters. The liver was dissected and weighed. Muscle samples were also taken. Then the viscera were dissected out and the adipose tissue was removed. The mid intestine was cut open and the digestive contents were separated from the mucus. The mucus samples were obtained by scraping with a glass slide. The contents and mucus were kept in separate tubes for microbiome analysis. Part of the mid

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intestinal tissue was also collected for gene expression analysis. From a separate group of fish, 2/tank (6/group) intestinal content samples were collected for short chain fatty acid (SCFA) analysis. All samples (except for electron microscopy) were frozen with liquid nitrogen and then stored at -80°C. Intestinal tissue (mid intestine) was stored in 4% formaldehyde for electron microscopy.

### 2.4 Diet and whole-body proximate composition

The same protocol was used to analyse the nutrient composition of the diet and the whole body. The nutrient composition of the feed was performed with fresh samples, while the nutrient composition of the whole body was derived from freeze-dried samples. The moisture content of the samples was measured by drying the samples at 105°C for 24 hours. The weight of the post-dried samples was subtracted from the weight of the pre-dried samples. The ash content of the samples was measured by burning the samples at 550°C for 16 hours and subtracting the weight of the post-combusted samples from that of the pre-combusted samples. The energy content of the samples was measured using the adiabatic bomb calorimeter (IKA, Heitersheim Gribheimer, Germany). Total lipids were measured by petroleum ether extraction using the Soxtherm system (Gerhardt analytical systems, Koenigswinter, Germany). Crude protein content was measured by the Kjeldahl method using the Kjeltek™ 8400 system (FOSS, Nanterre, France) after acid extraction.

### 2.5 Measurement of the plasma biochemical parameters

Plasma parameters were measured using commercial kits in combination with a microplate reader. Various biochemical plasma parameters such as glucose (Glucose RTU, bioMérieux, Marcy l'Etoile, France) (24), triglycerides (PAP 150, bioMérieux) (25), cholesterol (Cholesterol RTU, bioMérieux) (26) and free fatty acids (NEFA C Kit, Wako Chemicals, Neuss, Germany) (27) were measured. Total free amino acid was quantified according to the method of Moore (28), with glycine as standard.

### 2.6 Microbiome analysis

#### 2.6.1 DNA extraction

DNA from the intestinal contents and mucus samples was extracted using the QIAamp fast DNA stool kit (Qiagen, France) according to the manufacturer's instructions. Some modifications were made to the protocol to achieve a better yield from the difficult-to-lyse bacterial cells (29). The purity and integrity of the extracted DNA was assessed using the NanoDrop 2000c (Thermo, Vantaa, Finland) and an agarose gel, respectively.

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### 2.6.2 Preparation of the 16s rRNA (V3-V4) sequencing libraries

The sequencing libraries were prepared according to the standard protocol recommended by Illumina® (Illumina, France) and as described elsewhere (4,30). Briefly, the V3 and V4 regions of the bacterial 16s rRNA gene were amplified using the recommended set of primers (31) linked to the Illumina® adaptor overhangs. The final primer pairs were as follows. Forward 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and Reverse 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'. The preparation of the library involved 2 stages of PCR. In the first stage, the PCR mix (25 µL) contained 12.5 µL of KAPA HiFi Master Mix (Roche, France) together with 5 µL each of forward and reverse primers (1 µM each) and 2.5 µL of DNA (~100 ng). Reactions were performed in duplicate. Thermocycling conditions included pre-incubation for 3 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 30 sec. A final extension was performed at 72 °C for 5 minutes. After PCR, duplicate reactions belonging to one sample were pooled and an aliquot was run on the agarose gel to confirm a positive reaction (~550bp). The positive control with a mock bacterial DNA (ZymoBIOMICS Microbial Community DNA Standard, Zymo Research Irvine, USA) and a negative control with water were also included. The negative PCR control showed no visible band on the agarose gel after PCR. The PCR products were transported to La Plateforme Génome Transcriptome de Bordeaux (PGTB, Bordeaux, France) to perform the second stage PCR. In the second stage, index PCR was performed to add sample-specific barcodes to the PCR products using the Nextera XT index kit according to the manufacturer's protocol (Illumina, France). The PCR setup was the same as in stage 1 except that only 8 cycles were used. PCR products were purified using AMPure XP beads (Beckman Coulter, Paris, France) and quantified using the KAPA library quantification kit for Illumina platforms (Roche, France) according to the manufacturer's instructions. The sequencing libraries were pooled equimolarly (4nM) and sequenced using a 250 bp Paired End Sequencing Kit v2 (Illumina, France).

### 2.6.3 Sequence data analysis

The paired-end sequencing data were analysed using the UPARSE pipeline (32) as described elsewhere (33). The paired-end sequences were merged and the primer binding sites were removed. Sequences were then quality filtered using the maximum error rate strategy

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(threshold=1) (34). The sequences from different samples were merged after being uniquely labelled and the dataset was dereplicated and the singletons were removed. Operational Taxonomic Unit (OTU) clustering was performed at 97% similarity. The raw reads were mapped to the OTUs to create the OTU abundance table. Taxonomies were assigned using the SINTAX algorithm (35). Assignments with < 0.8 confidence value were filtered out. A phylogenetic tree in Newick format was created. The OTU table, taxonomy table and phylogenetic tree were exported to the phyloseq package for downstream analysis (36).

### 2.7 Short-chain fatty acid measurement

The SCFA measurements are performed as previously described (4). Briefly, frozen intestinal content samples (1g) were placed in glass bottles filled with clean air zero supplied by an F-DGS air zero generator (Evry, France). These bottles were connected to heated inlet line (100 °C) of the SIFT-MS instrument via the sample inlet. To compensate for the dispersion in the bottle during SIFT-MS sampling, a Tedlar bag (Zefon International Inc., Florida, USA), filled with dry and clean zero air was connected to the bottle inlet. The closed bottle was incubated at 60 °C ± 2 for 2 hours before SIFT-MS analysis.

Full-scan mass spectra were recorded for each positive precursor ion ($H_3O^+$, $O_2^{\bullet+}$, $NO^+$) in a *m/z* range from 15 to 250 with an integration time of 60 s. Quantification was performed using the $NO^+$ precursor ion as described before (37,38).

### 2.8 Electron microscopy

Electron microscopic examinations were carried out at the Bordeaux Imaging Centre - University of Bordeaux, a core facility of the French network "France Bio Imaging". The processing and sectioning of the samples has been described in detail previously (4). Mid-intestinal tissue samples were fixed with 2.5% (v/v) glutaraldehyde in 0.1M phosphate buffer (pH=7.4) for 2 hours before being stored at 4 °C. Samples were then washed in phosphate buffer and fixed in 1% (v/v) osmium tetroxide in 0.1M phosphate buffer for 2h in the dark (RT) and then washed. Then the samples were dehydrated and embedded in epoxy resin using the automated microwave tissue processor for electron microscopy (Leica EM AMW; Leica Microsystems, Vienna, Austria). After polymerisation, the samples were cut with a diamond knife (Diatome, Biel-Bienne, Switzerland) on an ultramicrotome (EM UCT, Leica Microsystems, Vienna, Austria). After localisation of the regions of interest, ultrathin sections (70 nm) were picked up on copper grids and subsequently stained with uranyless and

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lead citrate. The grids were examined with a transmission electron microscope (H7650, Hitachi, Tokyo, Japan) at 80kV.

### 2.9 Gene expression analysis

RNA from liver, intestine and muscle was extracted using the TRIzol reagent method (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. RNA from the adipose tissue was extracted using the RNeasy lipid tissue mini kit according to the manufacturer's instructions (Qiagen, France). The quality and quantity of the RNA was checked on a 1% agarose gel and a nanodrop (Thermo scientific, Bordeaux, France), respectively. Two µg of RNA was converted to cDNA using the enzyme superscript III reverse transcriptase and random hexamers (Invitrogen, France). After reverse transcription, the cDNA was diluted 50-fold before being used in RT -qPCR.

RT-qPCR was performed on a LightCycler® 384 system (Roche Diagnostics, Neuilly-sur-Seine, France) according to the protocol described previously (including primer details) (4). The data normalization was performed using geNorm (39). The reference genes *eef1a* and *rna18s* were used to calculate the normalisation factor in liver and intestine, while a combination of *actb* and *gapdh* was used in muscle and *actb* and *eef1a* in adipose tissue.

### 2.10 Statistical analysis

Feeding efficiency (FE), bulk fish weight and specific growth rate (SGR) were measured per tank (n=3 per group). For SCFA and electron microscopy, 2 fish were sampled per tank (n=6 per group). All other parameters were measured on 4 individual fish per tank (n=12 per group).

All statistical analyses were performed using R software (version 3.6.3) (40) in combination with *phyloseq* (36). The heatmap was created with *heatmapper* (41). All data subjected to either one-way ANOVA or two-way ANOVA were tested for normal distribution and homogeneity of variances. If the data did not meet any of these assumptions, a non-parametric Kruskal-Wallis test or a non-parametric equivalent of the factorial ANOVA (aligned rank transform ANOVA) was used.

Alpha diversity (observed OTUs and Shannon index), beta diversity (Bray-Curtis dissimilarity index) and OTU compositions were calculated using the phyloseq package. The effects of 2 factors (inulin and line) on alpha diversity were calculated separately for the 2 sample types (i.e., contents and mucus) using two-way ANOVA. For the beta diversity measures, the homogeneity of sample dispersions between groups was checked using the

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*betadisper* function of the *vegan* package (42). Permutative multivariate analysis of variance (PERMANOVA) was used to test the significance of Bray-Curtis distance between samples belonging to different sample types (content and mucus) and to analyse effect of the factors (inulin or line) on beta diversity in contents and mucus.

The effect of 2 factors (inulin and line) on the expression levels of each gene was analysed using two-way ANOVA. A similar approach was used to test the effect of experimental factors on microvilli length and SCFA levels. Based on the relative expression of the genes, a Bray-Curtis distance matrix was calculated and a group-wise comparison was performed using two-way PERMANOVA after checking the homogeneity of dispersions.

LEfSe (Linear discriminant analysis Effect Size) was performed to identify the significant features (OTUs) belonging to specific groups (43). The p-values and LDA score thresholds were set at 0.05 and 4, respectively. Regularised canonical correlation analysis (rCCA) was performed using the mixOmics package to understand the correlations between relative gene expression in different tissues and OTU abundances in content and mucus samples (44). Zero inflated OTU data were transformed using centered log transformation (clr) before analysis. Correlations with a value above 0.45 are shown.

## 3. Results

### 3.1 Diet and whole-body composition, growth and plasma parameters

In the present study, the composition of the various macronutrients did not differ between the control and experimental diets ($P>0.05$; Supplementary Table 1). We also did not detect any effect of either factor (line and inulin) or the interaction between them on the proximate composition of the whole- body ($P>0.05$; Supplementary Table 2). The final bulk body weight and weight gain were significantly higher (10.77%) in *suave* ($P<0.05$, Table 2). Similarly, there was a significant effect of line on plasma glucose ($P=0.0012$) and triglycerides ($P=0.0026$; Table 3).

### 3.2 Expression of different genes involved in metabolism

We investigated the expression of several genes involved in amino acid metabolism, energy metabolism, fatty acid oxidation, fatty acid conversion, gluconeogenesis, glucose transport, glycolysis, lipogenesis and SCFA uptake in different tissues.

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### 3.2.1 Liver

The hepatic gene expression profile is shown in Figure 1A. The overall (group-wise) expression was significantly affected by line (PERMANOVA, $P$=0.016) and the interaction between line and inulin (PERMANOVA, $P$=0.030). Effect of factors and interaction ($P$<0.05) on expression of genes in different pathways is given below.

*Line*: Expression of genes of amino acid metabolism (*asat2* and *asat3*), gluconeogenesis (*fbp1b1*, *g6pca*, *g6pcb1b*, and *g6pcb2a*), and glycolysis (*pfkla*) was downregulated in *suave*.
*Inulin*: Inulin fed groups were showing an upregulation of amino acid metabolism (*alat2* and *gdh3*), energy metabolism (*atp5a* and *cox4*) and fatty acid oxidation (*cpt1c* and *hoad*).
*Interaction*: The expression of genes in amino acid metabolism (*asat1*), energy metabolism (*cox4* and *cs*), gluconeogenesis (*g6pcb2a*) and lipogenesis (*acly* and *g6pdh*) were upregulated in inulin fed groups only in the *suave*. On the contrary, the expression of a fatty acid oxidation gene (*cpt1b*) was down-regulated in the *suave* when fed with inulin.

### 3.2.2 Intestine

The intestinal gene expression profile is shown in Figure 1B. The group-wise expression was significantly affected by both factors, line (PERMANOVA, $P$=0.001) and inulin (PERMANOVA, $P$=0.001). Effect of factors and interaction ($P$<0.05) on expression of genes in different pathways is given below.

*Line*: The expression of genes in amino acid metabolism (*asat1*, *asat2*, *asat3* and *gdh2*), energy metabolism (*cox2*, *cox4*, *cs*, *qcr2* and *sdhb*) and glycolysis (*pfkla* and *pfklb*) were significantly downregulated in *suave*.
*Inulin*: The expression of genes involved in amino acid metabolism (*asat1*, *asat2*, *asat3*, *gdh2* and *gdh3*), energy metabolism (*atp5a*, *cox2*, *cox4*, *cs*, *qcr2* and *sdhb*) and glucose transport (*glut1ba* and *glut1bb*) were significantly downregulated in the inulin fed group.
*Interaction*: No significant interaction effect between the inulin and the line was found.

### 3.2.3 Muscle

The gene expression profile of muscle is shown in Figure 2A. The overall (group-wise) expression pattern was significantly affected by line (PERMANOVA, $P$=0.004). Effect of factors and interaction ($P$<0.05) on expression of genes in different pathways is given below.

*Line*: The expression levels of genes in amino acid metabolism (*alat2*, *asat3*, *gdh2* and *gdh3*), energy metabolism (*atp5a*, *cox2*, *cs* and *qcr2*), fatty acid oxidation (*cpt1a* and *cpt1d*), glucose

transport (*glut1bb* and *glut4a*) and lipogenesis (*fas*) were significantly downregulated in *suave*.

*Inulin*: The expression of *alat2* (amino acid metabolism), *cpt1a* (Fatty acid oxidation) and *g6pdh* (lipogenesis) was upregulated in inulin fed groups. On the other hand, the expression of a gene in glycolytic pathway (*hk1*) was down-regulated in inulin fed group.

*Interaction*: The levels of *cpt1a* and *cpt1b* (fatty acid oxidation) was higher in inulin fed group only in the *temoin*. On the other hand, *fas* (lipogenesis) was upregulated in the inulin fed *suave*.

### 3.2.4 Adipose

The gene expression profile of adipose tissue is shown in Figure 2B. Group-wise expression profile was not affected by any of the factors. Effect of factors and interaction ($P<0.05$) on expression of genes in different pathways is given below.

*Line*: The expression of *gdh2* (amino acid metabolism) was lower in *suave*, and the expression of *cox2* (energy metabolism) and *fbp1b1* (gluconeogenesis) was upregulated in *suave*.

*Inulin*: The expression of *g6pcb1a* (gluconeogenesis) was down-regulated when fed inulin.

*Interaction*: The expression of genes of energy metabolism (*atp5*, *cox4* and *qcr2*), fatty acid oxidation and conversion (*hoad* and *fad*), gluconeogenesis (*fbp1b1*) and lipogenesis (*acly* and *g6pdh*) was upregulated only in inulin fed *suave*.

## 3.3 Intestinal microbial diversity and composition

### 3.3.1 Effect of sample type on the microbial diversity and composition

We analysed the microbial diversity and composition in the two sample types, namely mucus and digestive contents (contents), separately. There was a significant effect of sample type on microbial diversity and composition. The alpha diversity of the contents was significantly higher than that of the mucus ($P=0.0002$). We found no significant effect of line or the inulin on alpha diversity measures (observed OTUs and Shannon index) in the mucus or digestive contents ($P>0.05$) (Figure 3A).

PERMANOVA of Bray-Curtis distances between samples shows a significant effect of sample type ($P=0.0001$), with the mucus and contents samples forming separate clusters. The individual samples are plotted in a two-dimensional space using NMDS (Figure 3B).

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The top twenty OTUs in terms of total abundance (after removal of the genera *Mycoplasma* and *Streptophyta*) are shown in Figure 3C. These OTUs include *Bacillus*, *Janthinobacterium*, *Lactobacillus*, *Moraxella*, *Pseudomonas*, *Ralstonia*, *Singulisphaera*, *Sphingomonas*, *Streptococcus*, *Weissella* and others. The different abundances of OTUs between the mucus and the contents were analysed using LEfSe (Figure 3D). The relative abundance of *Firmicutes* was significantly higher in the contents than in the mucus. Among this phylum, the families *Lactobacillaceae* (genus: *Lactobacillus*), *Leuconostocaceae* (genus: *Weissella*) and *Streptococcaceae* were the most important representatives. Phylum *Planctomycetes* was also a significant feature in the content, which comprised of one significant OTU in the genus *Singulisphaera*. On the other hand, *Proteobacteria* was the most abundant phylum in the mucus samples. Among this phylum, *Alphaproteobacteria* (genus: *Sphingomonas*), *Betaproteobacteria* and *Gammaproteobacteria* (genus: *Pseudomonas*) were found in significantly higher amounts compared to the content. In the *Betaproteobacteria* class, there were 2 significantly abundant families, including *Burkholderiaceae* (two OTUs belonging to the genus *Ralstonia*) and *Oxalobacteriaceae* (genus: *Janthinobacterium*).

### 3.3.2 Effect of inulin and line on the microbial beta diversity and composition

Two-way PERMANOVA revealed that there was a significant effect of inulin and the interaction between inulin and the line on the beta diversity of the samples (*P*: I=0.026; *P*: I*L=0.025) (Figure 4A). In contrast, mucus samples showed no such responses (*P*>0.05) (Figure 4B).

LEfSe analysis to identify the differentially abundant groups between 2 dietary conditions revealed 13 features, 9 of which belonged to the control group and 4 to the inulin fed group. Two phyla were significantly abundant in the control group, including *Proteobacteria* and *Actinobacteria*. Among the *Proteobacteria*, 2 families, namely *Enterobacteriaceae* and *Pseudomonadaceae* (genus: *Pseudomonas*), were significantly abundant (Figure 4C). significant OTUs in the inulin fed group included *Weissella* and *Streptococcus* (Figure 4C). Comparison between the two lines revealed 4 features, 3 of which belonged to the *temoin* (genus: *Pseudomonas* and *Brevundimonas*) and one to the *suave* (family: *Ruminococcaceae*) (Figure 4D).

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### 3.4 Correlation between the OTU abundance and gene expression

*Liver vs content OTUs*: The OTUs belonging to *Lactobacillus*, *Weissella* and *Ruminococcaceae* showed a strong negative correlation with the genes involved in glycolysis, gluconeogenesis and fatty acid oxidation. *Moraxella* and *Bacillus* were showing a negative correlation with genes involved in lipogenesis, amino acid metabolism and energy metabolism (Figure 5A). In contrast to the OTUs belonging to *Lactobacillus*, *Bacillus* showed a positive correlation with some genes involved in glycolysis and fatty acid oxidation (Figure 5A).

*Intestine vs content OTUs*: The OTU belonging to *Pseudomonas* was positively correlated with genes involved in many pathways, notably glycolysis, amino acid metabolism and energy metabolism. On the other hand, *Streptococcus* was negatively correlated with several pathways, most notable being the glucose transport, lipogenesis and amino acid metabolism (Figure 5B). *Planctomycetaceae*, was negatively correlated with all genes, especially those involved in glycolysis (*pfklb*), amino acid metabolism (*asat1* and *asat2*) and energy metabolism (*cox4*, *cs*, *atp5a* and *sdhb*) (Figure 5B).

*Intestine vs mucus OTUs*: *Ralstonia*, *Janthinobacterium*, *Pseudomonas* and *Sphingomonas* were negatively correlated with genes involved in energy metabolism, amino acid metabolism, lipogenesis, glycolysis, fatty acid oxidation, fatty acid conversion and glucose transport (Figure 5C). There was a positive correlation between these OTUs and one of the fatty acid oxidation genes (*cpt1d*) (Figure 5C).

### 3.5 SCFA levels and microvilli length

The SCFAs acetic acid, butyric acid, caproic acid, propionic acid and valeric acid were measured in the intestinal contents (Figure 6A). There was a significant effect of line on the level of propionic acid ($P$=0.034). In general, the level of all SCFAs was higher in *temoin*. Within the *temoin* line, the levels of all SCFAs were generally higher in the inulin-fed group, except for butyric acid.

We observed a significant effect of both inulin and line on microvilli length (Figure 6B). Microvilli were significantly longer in *suave* ($P$=1.47e-15). On the other hand, they were significantly shorter in fish fed inulin ($P$=2.22e-16).

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## 4 Discussion

In the last decade, much research has been done on the diet-microbiome-host (metabolism) axis in mammals. One of the main focuses has been on the benefits of fibre-utilizing bacteria and their metabolites (SCFAs). In humans and livestock, a direct link has been established between dietary fibres and various metabolic processes in liver, skeletal muscle, intestine, and adipose tissue (45). Although there is great interest in harnessing the beneficial effects of prebiotic derived microbial metabolites to improve aquatic animal health and metabolism, the prebiotic-microbiome-host axis is not well understood. Prebiotics such as inulin have been used in the diets of teleosts for decades, although not much is known about whether inulin has the same effect on host metabolism (via SCFAs) in teleosts as it does in mammals. In addition, there is a lack of knowledge about the effects of genotype on inulin degradation and utilisation and the bacterial groups that respond to inulin in teleosts. To address this, in the present study we investigated the metabolic effects of feeding inulin (2%) for 16 weeks to two different lines (*temoin* and *suave*) of rainbow trout.

### 4.1 Growth and plasma parameters

Final body weight was significantly higher in fish selected for better utilization of the 100% plant-based diet (*suave*), as previously observed (3). The lower growth rate in naive lines of rainbow trout fed an all-plant diet has been attributed to a combination of factors, including lower feed intake and feed efficiency (3). However, in the present study, feed acceptance and feed efficiency did not differ significantly between the two lines. It should be noted that there was a significant weight difference between the 2 lines before the start of the experiment. And this difference remained throughout the experiment. It was important to keep the age of the 2 lines the same at the beginning of the experiment, so the weight difference had to be accepted (3,46). Moreover, the significant difference between the lines in weight gain also underscores the fact that these 2 lines naturally grow at different rates (46).

Inulin had no effect on growth parameters. This is interesting because inulin is known to positively affect the growth of many teleosts, including rainbow trout (21,47). The source of inulin, the genetic background of the fish used in the experiment, and the differences in basal diet could be the plausible reason for the discrepancies (48). In addition, the species-specific microbiota and the intra-species differences in the intestinal microbial communities (observed in trout) could also lead to such discrepancies (4,49).

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We found a significant difference in plasma glucose and triglyceride (TGL) levels between the two lines. It is likely that glucose uptake is not as efficient in the *suave* because of its adaptation to a plant-based diet, which is generally rich in dietary fibre. Glucose uptake from the high-fibre diet is relatively slow compared with the low-fibre diet (50). On the other hand, the higher triglycerides in *temoin* could be due to increased lipolysis, because carnitine palmitoyltransferase 1 was generally more highly expressed in liver and muscle of the *temoin* line.

### 4.2 Effect on the hepatic metabolism

In the liver, total group-wise gene expression was affected by line. Most of the tested genes in the different metabolic pathways showed lower expression in the *suave* compared to the *temoin*. Interestingly, selection of the fish on a plant-based diet resulted in decreased glycolysis. A higher plasma glucose level in the *suave* also suggests a lower availability of glucose for hepatic glycolysis. Amino acid catabolism is one of the major metabolic pathways in rainbow trout, providing substrates necessary for energy metabolism (51). In the present study, the expression of genes responsible for amino acid catabolism was also lower in the *suave*. In addition, genes involved in gluconeogenesis were also less expressed in *suave*. Taken together, these results could possibly indicate the use of prebiotic derived substrates (SCFAs) (instead of amino acids and glucose) for energy metabolism in *suave*. The involvement of SCFAs (acetate) instead of glucose in the production of acetyl-CoA, which is required for the TCA cycle, has been reported previously (52). Indeed, some genes (*cox4* and *cs*) involved in energy metabolism were affected by the interaction between line and inulin (higher expression only in inulin-fed *suave*), further supporting this assumption.

The overall gene expression was not significantly affected by inulin, although two genes of the fatty acid oxidation pathway showed high expression in the inulin groups. Inulin (via the action of various SCFA) has been shown to increase fatty acid oxidation in humans and other animals (53). It is known that these metabolic changes in the liver are due to the activity of acetate and propionate, because butyrate is generally preferentially taken up by intestinal cells (45,54,55). Although we did not measure the amount of the various SCFAs in plasma or liver, the amount of SCFAs in the intestine was generally higher in the inulin-fed groups, suggesting a possible relationship between inulin and fatty acid oxidation. Moreover, two genes of lipogenic metabolism were induced to a higher extent in the inulin-fed fish only in *suave*. This *suave* specific induction contradicts the anti-lipogenic effect of inulin in mammals and needs further investigation (45,55).

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### 4.3 Metabolic changes in the intestine

Regarding the collective expression of all tested genes in the intestine, we observed a significant effect of line and inulin. In particular, there was a strong decrease in the expression of several genes involved in amino acid catabolism and energy metabolism in *suave*. Rainbow trout is a carnivorous teleost. The efficient use of amino acids compared with glucose and fatty acids to meet energy requirements is already well established in this species (56). The lower expression of energy metabolism genes in the *suave* suggests that selection on a purely plant-based diet results in changes in the mechanisms of energy homeostasis due to reduced amino acid degradation and glycolysis. This observation contradicts what has been documented in mammals. SCFA are known to positively affect intestinal energy metabolism by introducing SCFA into the beta-oxidation pathway, leading to the production of acetyl-CoA, which is used in energy metabolism (45). Moreover, most of the metabolic effects in the intestine are mediated by butyrate, and intestinal butyrate levels were relatively low compared with other SCFAs, suggesting that the dynamics of SCFA production and utilization in the trout intestine may be different from those in mammals and need further investigation.

### 4.4 Metabolic changes in the muscle

The overall gene expression in muscle was significantly affected by line and expression was significantly lower in *suave*. The major groups of genes that were downregulated in *suave* include amino acid catabolism, energy metabolism, and glucose transporters. *Suave* is reported to gain 35.3% weight within one generation when fed a plant-based diet. Also, in the present study, weight gain was higher (10%) in *suave* than in *temoin*, and these advantages in weight gain may be due to the sensory, morphological, and metabolic changes that the selected line undergoes (46,57). The expression pattern in the present study may be indicative of the metabolic changes undergone by this line. In contrast, the control line (*temoin*) fed a plant-based diet appears to metabolize the diet poorly. It is likely that energy metabolism in this group is subject to regulatory mechanisms involving molecules from fatty acid oxidation and amino acid catabolism (58). In addition, the higher expression of genes for fatty acid oxidation and lower lipogenesis when fed inulin may again indicate the inability of the *temoin* group to efficiently utilize inulin.

Inulin had no significant effect on gene expression in muscle. An essential role of prebiotics and their derivatives (SCFA) in skeletal muscle metabolism has been demonstrated in

humans by increased uptake and oxidation of fatty acids and decreased lipogenesis (54). In addition, an increase in glucose uptake and retention of nitrogen (protein metabolism) has been suggested (54). In the present study, the effect of inulin on the expression of genes for fatty acid oxidation, lipogenesis, and glycolysis was not evident. This counterintuitive finding may be due to the fact that muscle is not the primary site of action for SCFAs in carnivorous rainbow trout. Moreover, in addition to the direct effects of SCFAs, metabolites released after hepatic assimilation of SCFAs are known to have effects on metabolic processes in muscle (54).

### 4.5 Metabolic changes in adipose tissue

There were no drastic group-wise changes in the expression of metabolic genes in adipose tissue. It should be noted that most of the genes that showed a change responded to the interaction effect of line and inulin. As observed in liver, lipogenic genes are upregulated only in the inulin-fed group of *suave*. The effect of SCFA on lipogenic pathways in the adipose tissue is uncertain, as there are some studies showing a lipogenic effect of SCFA, whereas others show the opposite (59–61). However, regardless of the effect on lipogenesis, higher levels of the fatty oxidation and energy metabolism are consistently observed (62,63). These observations are very similar to those observed in the present study, in which genes were upregulated in both the lipogenic pathway and fatty acid oxidation. It is likely that the higher fatty acid oxidation is in turn related to the higher energy metabolism observed in the same group of fish. This relationship between fatty acid oxidation and energy metabolism has been documented previously (63). As for adipose tissue, only the *suave* appears to have adapted to utilize inulin, as has been described in mammals, which may be indicative of the metabolic changes experienced by the *suave* as a result of selection.

### 4.6 Microbial mediation of prebiotic digestion

The effect of prebiotics (or dietary fibre) is mainly mediated by the intestinal microbiome via the production of metabolites such as SCFAs. These microbial processes are carried out in mammals by different groups of microbes belonging to the phyla Bacteroidetes and Firmicutes (64). The intestinal microbiome of rainbow trout is dominated by *Mycoplasma* (4,65), and is abundant in the mucus/epithelial samples (66). Therefore, we separated the mucus and digestive content samples in the present study. As expected, *Mycoplasma* was a common group in the mucus samples, while the contents were abundant with *Streptophyta* (most likely of dietary origin), as the diet was entirely plant-based (Supplementary Figure 1).

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Beta diversity indicated significantly different clustering of the samples depending on the sample type (either mucus or contents). Interestingly, these clusters persisted despite the removal of *Mycoplasma* and *Streptophyta* OTUs, with high abundance of *Ralstonia*, *Pseudomonas*, *Janthinobacterium* and *Sphingomonas* (all belonging to Proteobacteria) in the mucus, while members belonging to Firmicutes (*Lactobacillus*, *Streptococcaceae*) and Planctomycetes (*Singulisphaera*) was a significant feature in the contents, indicating the adaptation of the microbes to the specific microenvironment (either mucus or contents). Together with these results, the significantly lower alpha diversity in the mucus compared to the contents suggests that the contents may offer a nutrient rich niche (high fibre) and harbour a higher diversity of bacterial populations compared to the mucus. In support of this idea, a prevalence of Firmicutes in high-fibre diets has already been described in many animals (64,67). Although Planctomycetes are not a known species in the intestinal microbiome of animals, this group is known to contain an enzyme repertoire required for the degradation of polysaccharides (68), suggesting their potential role in dietary fibre utilization.

We also analysed the effect of inulin and line on the beta diversity of the mucosal microbiome and content separately. There was no effect of line on the beta diversity of the microbiome, but an OTU belonging to the Ruminococcaceae was a significant feature in *suave*, indicating the adaptability of this OTU to the intestinal environment of *suave*. A relationship between genotype and microbial population has been described in many species, including teleosts (69–71). In addition, several species of *Ruminococcus* have been described as one of the most efficient fibre degrading groups in ruminants (67).

The significant effect of inulin was observed only in the contents further supporting the hypothesis that fibre-degrading bacteria are more abundant in the contents and that they respond more readily to dietary inulin compared with the mucosal microbiome. This type of differentiation between the mucosal and content microbiome in terms of response to dietary components has been observed previously in Atlantic salmon (72).

### 4.7 Correlation between the gene expression and microbial abundance

There were negative correlations between several genes (hepatic glycolysis and gluconeogenesis) and OTUs belonging to *Lactobacillus*. On the other hand, a positive correlation of these pathways with *Bacillus* OTUs was observed. A similar finding has been previously reported in rainbow trout (4). It needs to be investigated whether the abundance of *Lactobacillus* and *Bacillus* is related to the high plasma glucose levels observed in *suave*.

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Interestingly, the most abundant bacterial groups in the contents (OTUs among Firmicutes) were not correlated with the expression of genes in the intestine. The OTUs that were predominant in the mucosa were negatively correlated with the genes down-regulated in intestine (energy metabolism and amino acid catabolism) of *suave* fed with inulin. This counterintuitive downregulation of energy metabolism genes in the intestine could be due to the uptake of SCFAs by the mucosal microbiota, resulting in reduced availability of these metabolites to epithelial cells (73).

**4.8 SCFA in intestine**

It was evident that the levels of all the SCFA in general were higher in the intestinal content of *temoin* (especially in the inulin fed group). This difference was significant in the case of propionic acid. It has been previously shown that the SCFA are absorbed by the intestinal cells quite rapidly after their release from the intestinal microbiome (45). Presumably, the rate of absorption of the SCFAs in the intestine of *suave* is higher due to their adaptation to a plant-based diet through selection (which is generally rich in SCFA). Measuring SCFA levels in multiple organs such as intestine, liver and blood simultaneously in the future studies would be more insightful.

SCFA receptor expression levels were not drastically modulated in any of the tissues studied. This underscores the need for a detailed study of the mechanisms involved in SCFA production, uptake by host cells, and metabolism in the various organs of teleosts. Moreover, in the present study, no direct relationship can be established between the content of SCFAs and their biological effect, because the measurement of SCFAs in the intestine is complicated due to 1) complex molecular cross-feeding mechanisms, which in turn depend on the microbial composition (56) and 2) different SCFAs have different sensitivities to different SCFA receptors and are therefore preferentially utilized by different organs (74).

The length of the intestinal microvilli was higher in *suave* and apparently this is an adaptation of the selected lineage to better nutrient absorption. This adaptation has been noted previously in several animals (75). The relationship between the higher villus length and the higher weight gain in *suave* needs further investigation. However, we observed a decrease in microvillus length in the groups fed inulin. Although the reason for this decrease is not clear, a similar effect was observed in another carnivorous teleost, the gilthead seabream (76).

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## 5. Conclusions and future perspectives

In summary, feeding 2% inulin to different lines of rainbow trout has a strong effect on the expression of several metabolic genes depending on the tissue. In the liver, the expression of several metabolic pathways is influenced by the line and the interaction between line and inulin, while in the intestine, both inulin and line influence the expression of metabolic genes. The overall expression in muscle was also influenced by line. From the present study, microbial communities differed drastically in mucus and contents and line-specific and inulin-specific abundance profiles were observed only in contents. The high abundance of specific genera among Proteobacteria (in mucus) and Firmicutes (in contents), indicates their metabolic adaptation to the specific intestinal microenvironment. The high abundance of OTUs among Firmicutes in fish fed 2% inulin may indicate their ability to degrade inulin, and the genomes of these groups need to be further studied. The association of an OTU belonging to the Ruminococcaceae with the selected line (*suave*) is interesting and its involvement in the better utilisation of plant ingredients in *suave* needs further investigation. The correlation between several members of the Firmicutes and Proteobacteria and the expression of genes involved in different metabolic pathways in both the liver and the intestine (especially gluconeogenesis in the liver and amino acid metabolism and energy metabolism in the intestine) could be indicative of the diet-microbiome-host axis and should be a focus of future research.

## Availability of data

All sequence data are available at the NCBI sequence read archive under accession number PRJNA797224.
https://dataview.ncbi.nlm.nih.gov/object/PRJNA797224?reviewer=lnsgt3mfb34gian8d1vkbq4fmd

## Conflict of interest

The authors declare no conflict of interest.

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## Author's contributions

JL designed and performed the wet lab experiments and data analysis, and drafted the first version of the manuscript. MD and RD performed the RNA extraction and qPCR experiments. MLB and TP performed the SCFA quantification. MDN designed the selection and maintenance of the selected line *suave*. TK, LL and LG oversaw the feeding experiments and organised the sampling. FT formulated and manufactured the feed. KR and SP conceived and coordinated the study. All authors contributed to the review of the manuscript. All authors read and approved the final manuscript.

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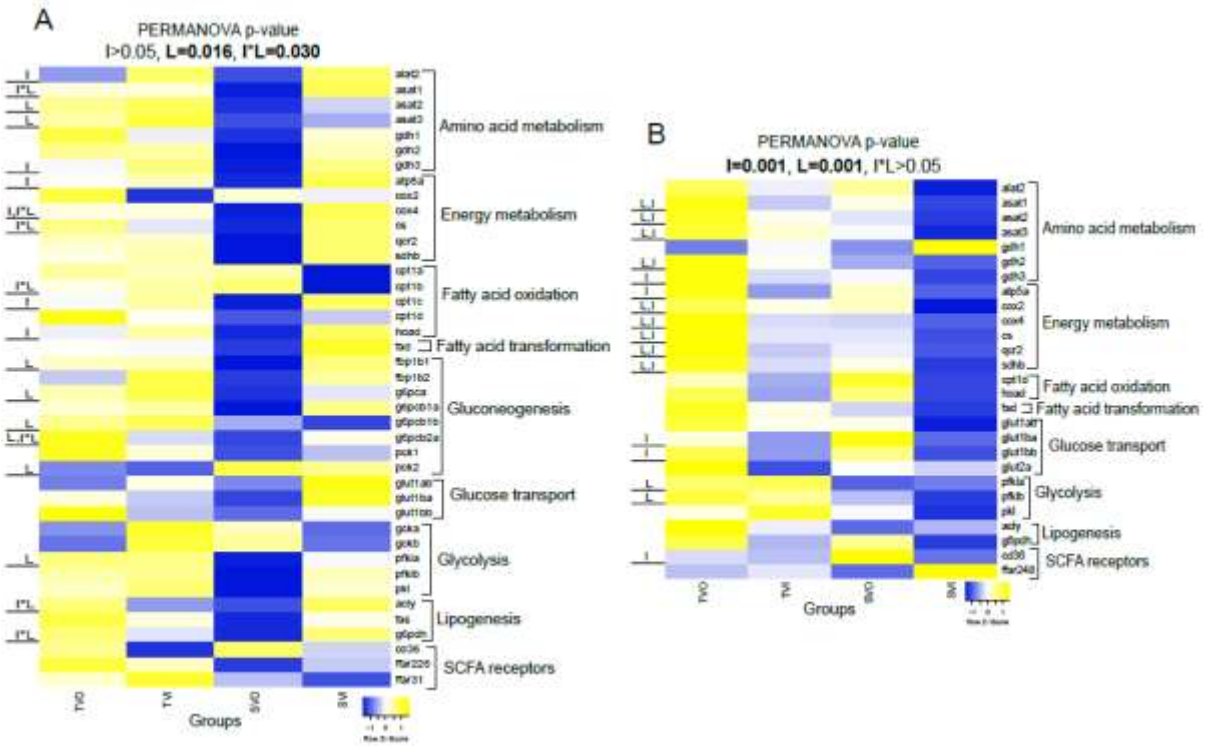



**Figure 1: Heatmap showing the relative expression of different genes involved in metabolism in (A) liver and (B) intestine of two lines (*temoin* and *suave*) of rainbow trout fed 0% and 2% inulin**. The different feeding groups are symbolized as follows: TVO: *temoin*-0% inulin; TVI: *temoin*-2% inulin; SVO: *suave*-0% inulin; SVI: *suave*-2% inulin. The effect of line and inulin on the expression of each gene was evaluated using two-way ANOVA. Significant effects ($P<0.05$) of either factor (line or inulin) or interaction are indicated on the left panel (I: effect of inulin; L: effect of line; I*L: interaction effect). The gene names and the pathways to which they belong are shown on the right panel. Group-wise differences in expression levels of all genes were statistically tested using PERMANOVA (Liver *P* value: I>0.05, L=0.016, I*L>0.030; Intestine *P* value: I=0.001, L=0.001, I*L>0.05) based on the Bray-Curtis dissimilarity matrix derived using the relative expression levels and shown in the top panel.

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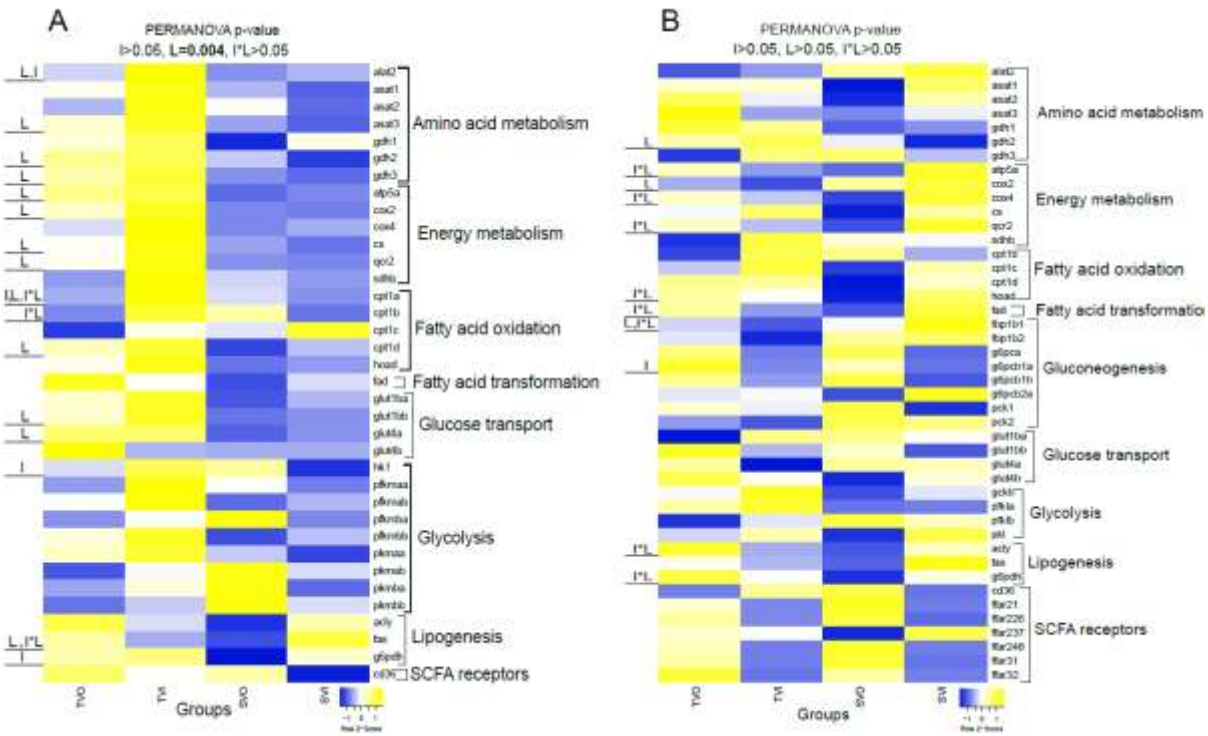



**Figure 2: Heatmap showing the relative expression of different genes involved in metabolism in the muscle (A) and adipose (B) of two lines (*temoin* and *suave*) of rainbow trout fed 0% and 2% inulin**. The different feeding groups are symbolized as follows: TVO: *temoin*-0% inulin; TVI: *temoin*-2% inulin; SVO: *suave*-0% inulin; SVI: *suave*-2% inulin. The effect of line and inulin on the expression of each gene was evaluated using two-way ANOVA. Significant effects ($P$<0.05) of either factor (line or inulin) or interaction are indicated on the left panel (I: effect of inulin; L: effect of line; I*L: interaction effect). The gene names and the pathways to which they belong are shown on the right panel. Group-wise differences in expression levels of all genes were statistically tested using PERMANOVA ($P$ value: I>0.05, L=0.004, I*L>0.05) based on the Bray-Curtis dissimilarity matrix derived using the relative expression levels and shown in the top panel.

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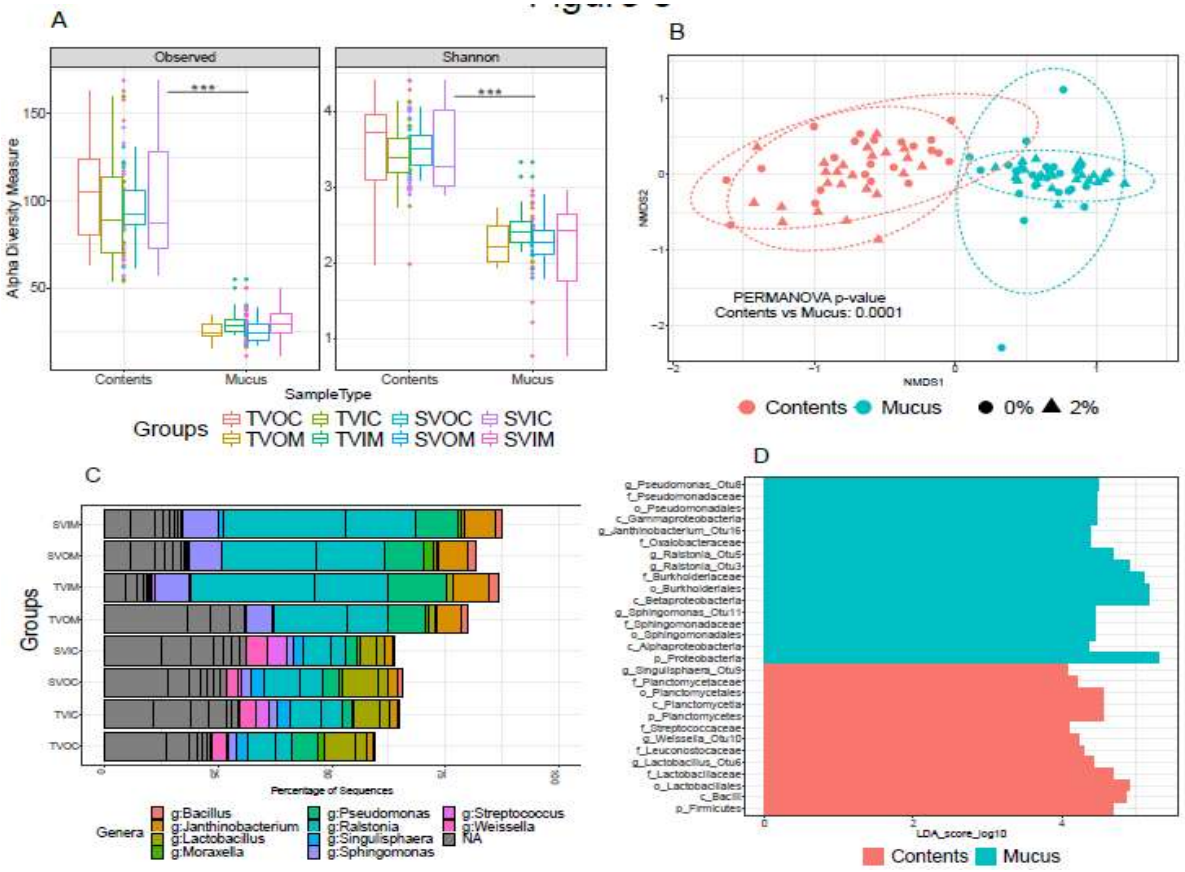



**Figure 3: Plot of alpha and beta diversity, OTU composition and the discriminant features in the intestine of two lines (*temoin* and *suave*) of rainbow trout fed 0% and 2% inulin (*Mycoplasma* and *Streptophyta* OTUs are filtered out)**. Feeding groups are symbolized as TVO: *temoin*-0% inulin; TVI: *temoin*-2% inulin; SVO: *suave*-0% inulin; SVI: *suave*-2% inulin. (A) Alpha diversity measures (Observed OTUs and Shannon index) in different feeding groups. Measurements were performed separately for the content (C) and mucus (M) samples. There was no statistically significant effect of inulin or line on the alpha diversity measurement (Two-way ANOVA; $P>0.05$). The differences between the mucus and content samples were significant for both the observed and Shannon indexes (One-way ANOVA; $P=0.0001$). (B) Beta diversity calculated using the Bray-Curtis dissimilarity matrix is shown in an NMDS plot. Sample types (content and mucus) are colour coded. Feeding groups are represented by different shapes (0% inulin: sphere; 2% inulin: triangle). There was a significant effect of sample type (mucus and contents) on sample dispersion (PERMANOVA: $P=0.0002$). (C) Composition of the 20 most abundant OTUs in the content (C) and mucus (M) samples. Taxonomies assigned to OTUs are color-coded (at genus level). OTUs with grey colour indicate non-assignment of the taxonomy at the genus level. (D) LEfSe analysis showing the discriminant genera between the content and mucus samples (LDA score >4).

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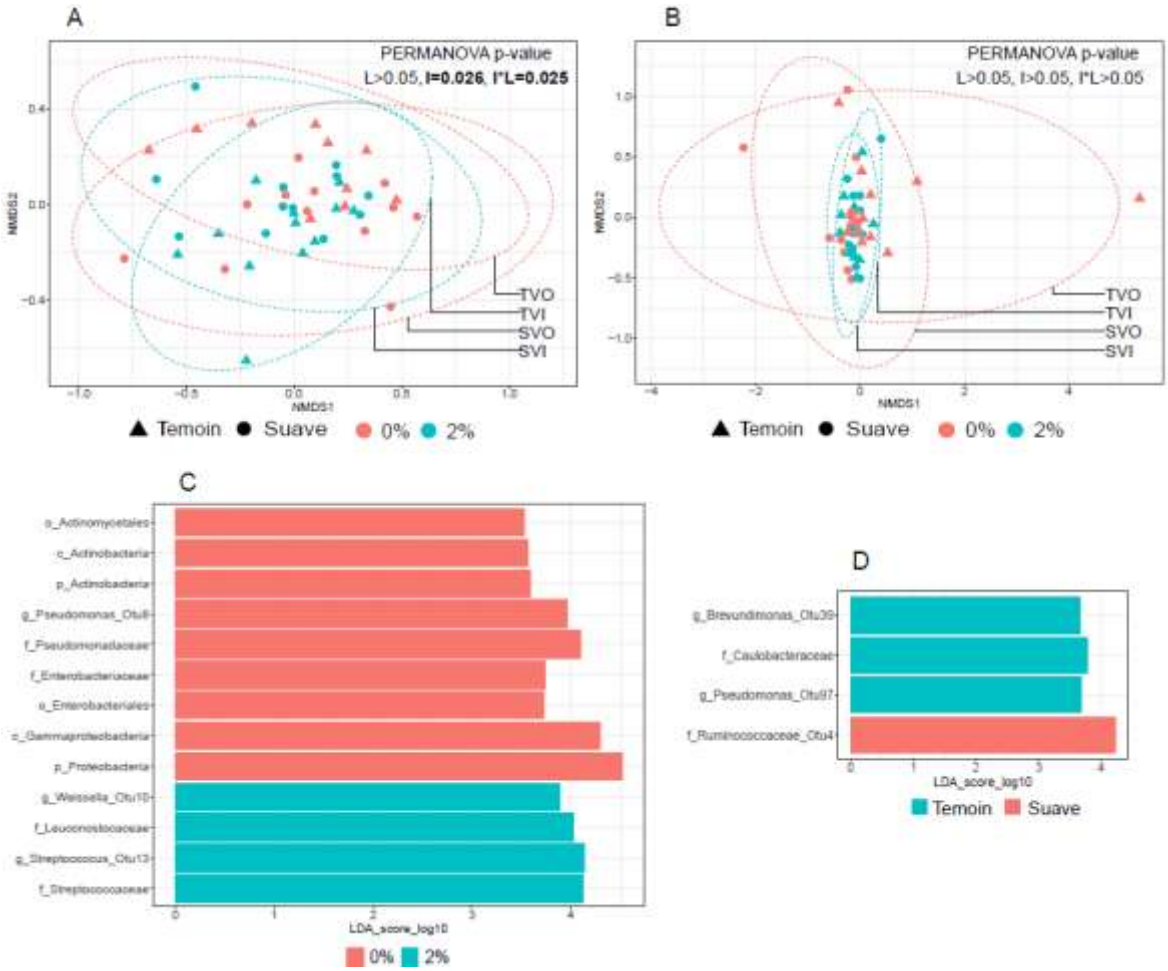



**Figure 4: NMDS and bar plot showing the dispersion of the samples in a multivariate space and the discriminating features belonging to different groups in the contents**. Beta diversity of the (A) content samples and (B) mucus samples calculated based on the Bray-Curtis dissimilarity index are shown using NMDS plot. Feeding groups are symbolized as TVO: *temoin*-0% inulin; TVI: *temoin*-2% inulin; SVO: *suave*-0% inulin; SVI: *suave*-2% inulin. Groups fed with different levels of inulin are colour coded and the lines are represented by different shapes (*temoin*: triangle; *suave*: sphere). Statistical significance of group dispersion was analysed using two -way PERMANOVA (Contents *P* value: I=0.026, L>0.05, I*L=0.025; Mucus *P* value: I>0.05, L>0.05, I*L>0.05). LEfSe analysis showing the discriminant genera (C) between the fish fed with either control diet (0% inulin) or diet containing 2% inulin and (D) between the 2 lines of fish (LDA score >4).

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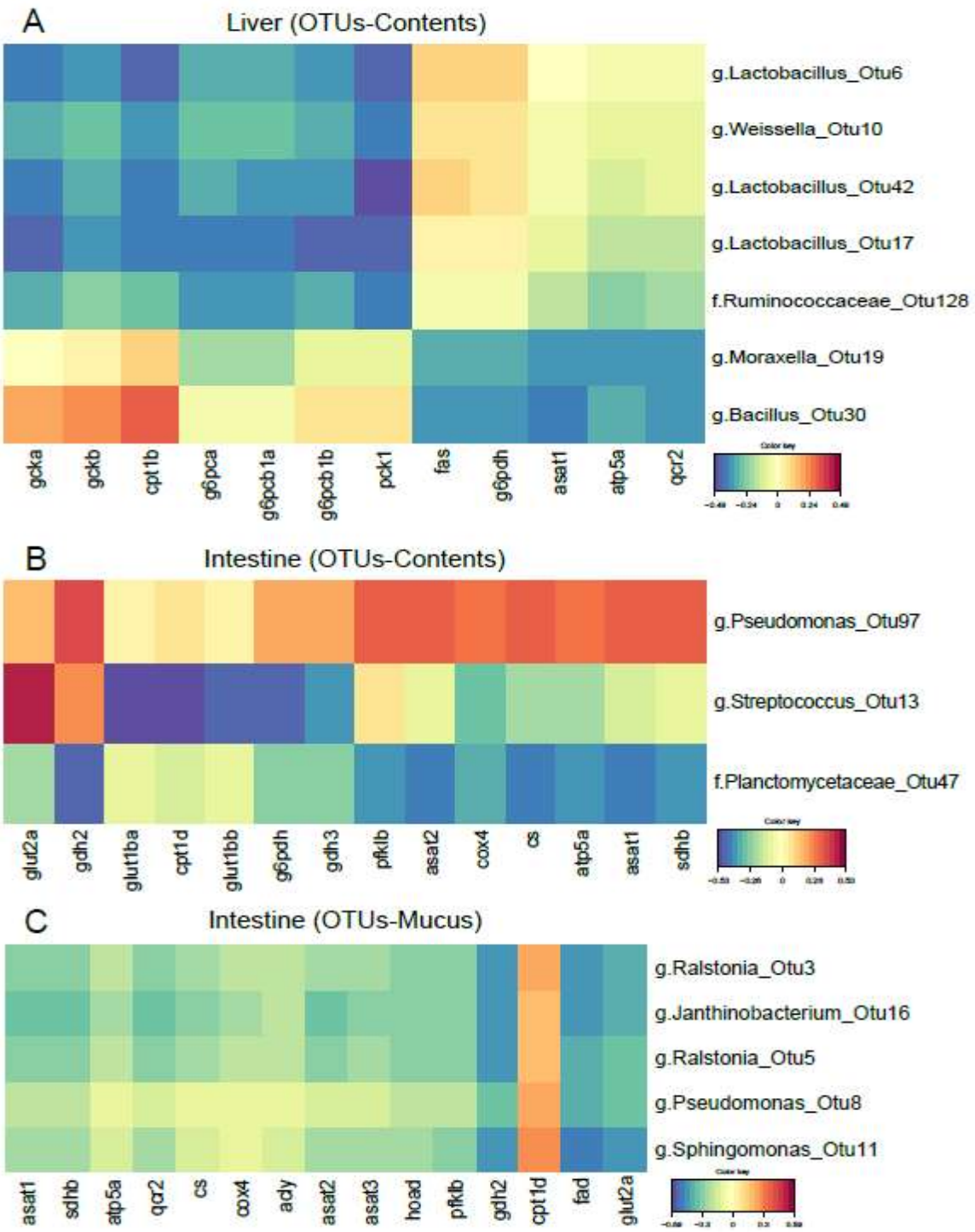



**Figure 5:** Correlation between relative abundance of OTUs found in intestinal content samples (Y-axis) and expression levels of different genes in the (A) liver, (B) intestine. (C) Correlation between relative abundance of OTUs found in intestinal mucus samples (Y-axis) and expression levels of different genes in the intestine. The correlations were computed with rCCA (Regularised Canonical Correlation Analysis). Colour key shows the strength of the correlation.

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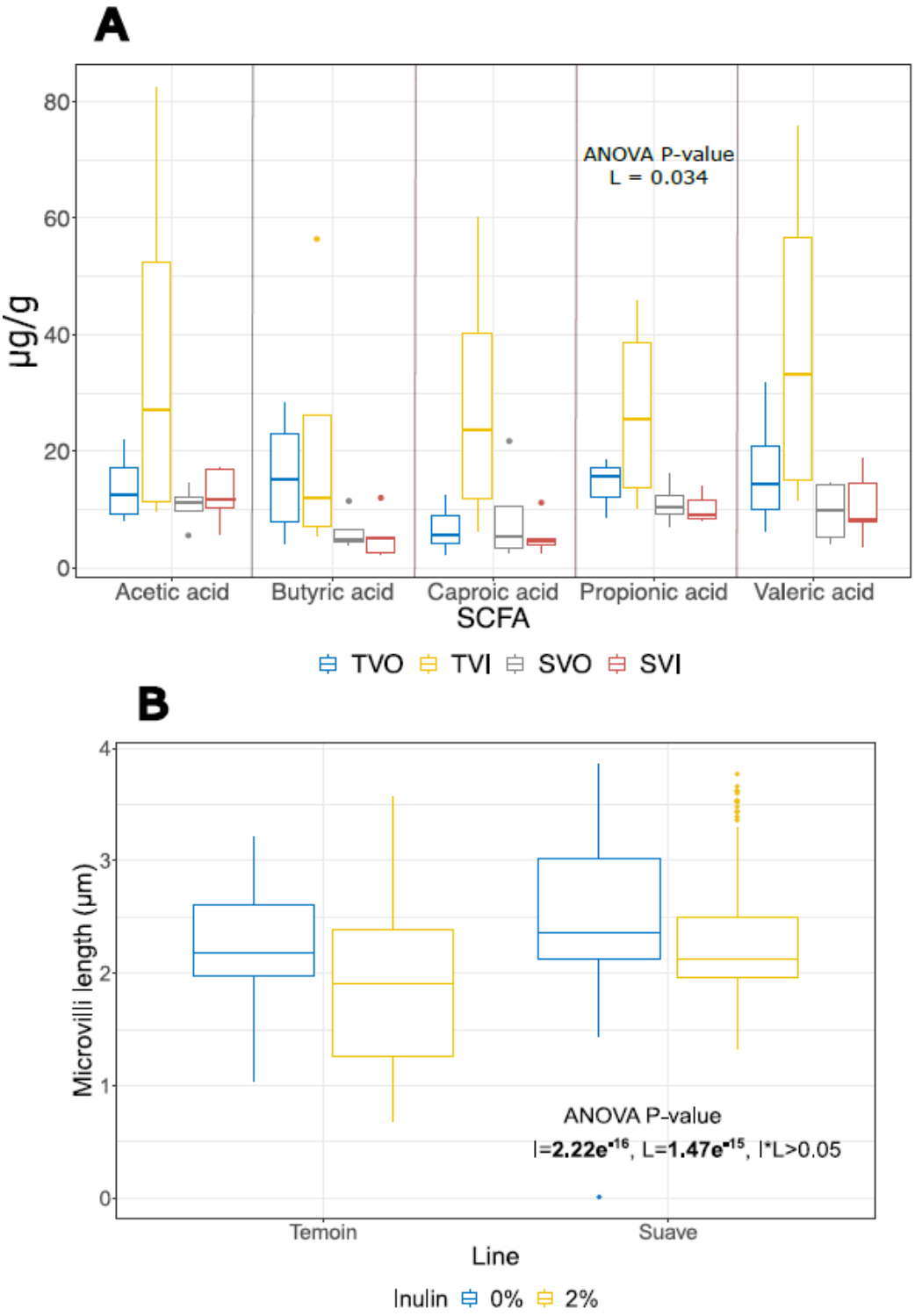



**Figure 6:** (A) Levels of different SCFAs including acetic acid, butyric acid, caproic acid, propionic acid and valeric acid in the mid-intestinal contents of two lines (*temoin* and *suave*) of rainbow trout fed 0% and 2% inulin. (B) Length of intestinal epithelial microvilli (µm) in two lines (*suave* and *temoin*) of rainbow trout fed 0% and 2% inulin.

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**Table 1**: Formulation of the diets used in the present study

| Ingredient (%) | Inulin 0% (SVO, TVO) | Inulin 2% (SVI, TVI) |
|---|---|---|
| Whole wheat | 5.70 | 5.70 |
| Lupine flour | 10.00 | 10.00 |
| Corn gluten | 8.49 | 8.49 |
| Wheat gluten | 15.00 | 15.00 |
| Concentrated protein extract of peas (LYSAMINE ®) | 12.50 | 12.50 |
| Concentrated faba bean protein extract | 8.00 | 8.00 |
| Traced soybean meal | 12.00 | 12.00 |
| L-Lysine | 0.50 | 0.50 |
| L-Methionine | 0.50 | 0.50 |
| Palm oil | 2.00 | 2.00 |
| Linseed Oil | 6.00 | 6.00 |
| Rapeseed Oil | 9.78 | 9.78 |
| Soy lecithin powder | 2.50 | 2.50 |
| Dicalcium phosphate | 2.00 | 2.00 |
| Vitamin premix | 1.50 | 1.50 |
| Mineral premix | 1.50 | 1.50 |
| Astaxanthin | 0.03 | 0.03 |
| Inulin | 0.00 | 2.00 |
| Cellulose | 2.00 | 0.00 |

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**Table 2**: Growth parameters of rainbow trout (*temoin* and *suave*) fed with the control diet (O) and the diet supplemented with 2% inulin (I)

| Growth parameters | TVO | TVI | SVO | SVI | *P* value | | |
|---|---|---|---|---|---|---|---|
| | | | | | Line | Inulin | Line*Inulin |
| Feed efficiency[1] | 0.91 ± 0.04 | 0.91 ± 0.04 | 0.91 ± 0.02 | 0.90 ± 0.05 | 0.9110 | 0.9110 | 0.8790 |
| Initial body weight (g) | 117.10 ± 3.99 | 122.12 ± 4.26 | 136.94 ± 2.29 | 136.77 ± 4.06 | **4.45e-05** | 0.2940 | 0.2650 |
| Final body weight (bulk) (g) | 487.83 ± 20.68 | 505.17 ± 3.51 | 555.33 ± 11.85 | 550 ± 18.48 | **0.0002** | 0.5010 | 0.2380 |
| Hepatosomatic index (%)[2] | 1.33 ± 0.20 | 1.32 ± 0.21 | 1.23 ± 0.17 | 1.29 ± 0.11 | 0.2420 | 0.3765 | 0.0985 |
| Specific growth rate (%/day)[3] | 1.10 ± 0.03 | 1.09 ± 0.03 | 1.08 ± 0.01 | 1.07 ± 0.04 | 0.2610 | 0.7610 | 0.9760 |
| Weight gain (g)[4] | 369.96 ± 18.99 | 383.04 ± 7.52 | 418.39 ± 10.63 | 413.56 ± 20.04 | **0.0020** | 0.6521 | 0.3390 |
| Survival rate (%) | 100 | 100 | 100 | 100 | 0.3470 | 0.3470 | 0.3470 |

The data is presented as the mean ± sd, n=3 tanks except for the final body weight (n=12) and hepatosomatic index (n=12). Means between the groups were compared using a two-way ANOVA. A *P* value < 0.05 was considered as significant and are presented in bold.

[1] Feed efficiency=wet weight gain (g)/feed intake (g).

[2] Hepatosomatic index=100 x (liver weight/total body weight)

[3] Specific growth rate=100 x [Ln (final body weight, g)-Ln (initial body weight, g)]/days

[4] Weight gain=Final weight-initial weight (g)

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**Table 3**: Plasma parameters in rainbow trout (*temoin* and *suave*) fed with the control diet (O) and the diet supplemented with 2% inulin (I)

| Plasma parameters | TVO | TVI | SVO | SVI | *P* value | | |
|---|---|---|---|---|---|---|---|
| | | | | | Line | Inulin | Line*Inulin |
| Cholesterol (g/L) | 2.05 ± 0.40 | 2.34 ± 0.64 | 1.76 ± 0.61 | 2.03 ± 0.46 | 0.0547 | 0.0761 | 0.9376 |
| Free fatty acids (mmol/L) | 0.46 ± 0.14 | 0.50 ± 0.10 | 0.40 ± 0.08 | 0.45 ± 0.10 | 0.0846 | 0.1344 | 0.7853 |
| Glucose (g/L) | 0.66 ± 0.08 | 0.72 ± 0.10 | 0.75 ± 0.08 | 0.77 ± 0.08 | **0.0012** | 0.2459 | 0.1919 |
| Total amino acids (µmol/mL) | 4.54 ± 0.76 | 4.49 ± 0.59 | 5.26 ± 2.69 | 4.31 ± 1.63 | 0.9680 | 0.5563 | 0.5563 |
| Triglycerides (g/L) | 3.86 ± 1.30 | 4.03 ± 1.78 | 2.57 ± 1.04 | 2.86 ± 1.10 | **0.0026** | 0.5519 | 0.8684 |

The data is presented as the mean ± sd, n=12. Means between the groups were compared using a two-way ANOVA. A *P* value < 0.05 was considered as significant and are presented in bold.

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