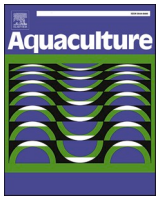
Aquaculture 578 (2024) 740002

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Effects of dietary supplementation of lignocellulose-derived cello-oligosaccharides on growth performance, antioxidant capacity, immune response, and intestinal microbiota in rainbow trout (*Oncorhynchus mykiss*)

Aprajita Singh [a,*], Aleksandar Vidakovic [a], Bernt Hjertner [b], Eleni Krikigianni [c], Anthi Karnaouri [c], Paul Christakopoulos [c], Ulrika Rova [c], Johan Dicksved [a], Kartik Baruah [a], Torbjörn Lundh [a]

[a] *Department of Animal Nutrition and Management, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden*

[b] *Department of Biomedical Science and Veterinary Public Health, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden*

[c] *Department of Biochemical Process Engineering, Luleå University of Technology, 97187, Luleå, Sweden*

ARTICLE INFO

Keywords:
Cello-oligosaccharides
Rainbow trout
Microbiota
Forest waste
Gut immunity
Oxidative stress

ABSTRACT

This study evaluated the prebiotic potential of cello-oligosaccharides (COS) produced from birch (*Betula pendula*), an under-utilised lignocellulosic source from the forestry industry, on growth performance, mucosal immunity, gut microbiota composition, and antioxidant capacity of juvenile rainbow trout (*Oncorhynchus mykiss*). In a 45-day trial, the fish were fed with diets containing 0%, 0.1%, 0.5% and 1.5% COS, while a diet containing fructo-oligosaccharides (0.5% FOS) was used as a positive control. Fish fed with the 0.5% and 1.5% COS diets showed significantly ($P < 0.05$) higher abundance of Ruminococcaceae, Bacillaceae and Lactobacillaceae, in the faecal microbiota. The COS diets also induced higher antioxidant capacity in the gut and serum, but there were no treatment effects ($P > 0.05$) on growth of rainbow trout. Gene expression analysis of the intestine showed significant elevation ($P < 0.05$) in expression of complement (*c3* and *c-type lectin*) and receptor (*tlr2*) genes of the innate immune system in COS-fed fish. However, for cytokine and adaptive immune genes, no significant differences ($P > 0.05$) in gene transcripts were observed between the COS/FOS diets with the control diet. These results suggest that dietary cello-oligosaccharides can be a useful feed supplement for rainbow trout, which can modulate intestinal microbial communities, innate immune response and antioxidant capacity of the host.

1. Introduction

With stagnation in capture fisheries over recent decades, aquaculture is key to fulfilling the United Nation's sustainable development goals. Global aquaculture, which is projected to produce 109 million tonnes of fish by 2030 (FAO, 2022), has increasingly shifted to intensive and super-intensive culture systems. However, intensification of cultivation is often accompanied by a vicious cycle of frequent disease outbreaks that disrupt production chains and lead to significant economic losses. The common response of producers to disease outbreaks is indiscriminate use of unsustainable antimicrobial drugs or antibiotics, which in turn cause detrimental environmental consequences. It also poses a direct or indirect threat to human health through the development of drug-resistant pathogens (Schar et al., 2021).

To overcome these issues, sustainable prophylactic strategies based on the use of probiotics (beneficial bacteria), prebiotics (dietary supplements that nurtures beneficial bacteria) and/or synbiotics (synergistic mixture of dietary supplements and beneficial bacteria) are being investigated, and are reported to have beneficial effects on growth, metabolism and health of cultured fish (Song et al., 2014; Montalban-Arques et al., 2015; Hoseinifar et al., 2015; Ringø and Song, 2016; Huynh et al., 2017). Prebiotics consisting of short-chain functional

* Corresponding author.
E-mail addresses: aprajita.singh68@gmail.com, aprajita.singh@slu.se (A. Singh).

https://doi.org/10.1016/j.aquaculture.2023.740002
Received 9 April 2023; Received in revised form 26 July 2023; Accepted 17 August 2023
Available online 19 August 2023

oligosaccharides show promise as dietary supplements that can nurture beneficial microorganisms in the host intestine and confer health benefits (Zhou et al., 2007; Li et al., 2009; Wang et al., 2010; Zhang et al., 2012; Sang et al., 2014; Li et al., 2018; Hoseinifar et al., 2020). Reported health benefits of supplementing fish diets with functional oligosaccharides include disease resistance via modulated gut microbiota and improved gut physiology (Mussatto and Mancilha, 2007; Qiang et al., 2009; Sinha et al., 2011; Akrami et al., 2013); enhancement of growth and metabolic activity (Ortiz et al., 2013; Zhang et al., 2014); and orchestration of protective immune responses (Nayak, 2010; Guerreiro et al., 2016; Wang et al., 2018).

Short-chain oligosaccharide-based prebiotics can withstand hydrolysis by the host's digestive enzymes and act as a substrate or nutrient source for fermentation by selective microorganisms in the fish gut, thereby favouring colonisation by specific intestinal bacteria. These prebiotic-augmented bacteria are believed to have a direct or indirect effect on the immune system and on overall health of the fish (Grisdale-Helland et al., 2008; Nawaz et al., 2018). Among the oligosaccharides, fructo-oligosaccharides (FOS) are the most studied prebiotic used as a nutritional supplement for fish. They show high potential for improvement of growth performance, modulation of gut microbiota composition and enhancement of non-specific immunity to pathogenic bacterial infection in several non-salmonid fish species (Soleimani et al., 2012; Akrami et al., 2013; Zhang et al., 2014; Wang and Li, 2020). However in salmonid fish species, such as Atlantic salmon (Grisdale-Helland et al., 2008) and rainbow trout (Ortiz et al., 2013), evaluation of FOS efficiency has been confined mainly to their beneficial effect on growth performance and feed utilisation. Comprehensive investigation of the impact of FOS supplementation on other health aspects, such as immune responses, antioxidant capacity and modulation of gut microbiota would provide a deeper understanding of the effects of this prebiotic on salmonids.

Apart from FOS, oligosaccharides that originate from cellulose, namely cello–oligosaccharides (COS), have also been shown to have great potential as a prebiotic in higher vertebrates, such as cattle, pigs and humans, with beneficial effects on host digestion and intestinal ecology (Satouchi et al., 1996; Otsuka et al., 2004; Song et al., 2013; Uyeno et al., 2013; Zhong et al., 2020). However, the impact of COS as a prebiotic has not yet been evaluated in fish.

Structurally, cello-oligosaccharides are linear, non-digestible oligosaccharides composed of 3–10 short-chain β-(1, 4) glucopyranose units produced by controlled enzymatic hydrolysis of cellulose (Karnaouri et al., 2018, 2019a; Barbosa et al., 2020). In addition to oligosaccharides, the cellulose-derived disaccharide cellobiose is also reported to have high prebiotic potential (Karnaouri et al., 2018; Pokusaeva et al., 2011). Cellobiose and cello-oligosaccharides are produced from lignocellulosic biomass, including currently under-utilised agricultural by-products and forest residues. This abundant resource can be used for sustainable preparation of high value-added prebiotic, thus contributing to the circular economy. Given their potential health benefits, as demonstrated in higher animals, and their ability to promote growth of lactic acid bacteria (LAB) *in vitro* (Kontula et al., 1998; Karnaouri et al., 2018), use of cellobiose and cello-oligosaccharides as a feed additive could therefore be a viable and novel option in prebiotic-based strategies for aquaculture.

Considering the need for alternative strategies in fish health management, together with the great potential effect and high availability of cellobiose and cello-oligosaccharides, the aim of this study was to test their use as a supplement in fish diets. A mixture of cello-oligosaccharides and cellobiose (referred as COS) obtained from enzymatic hydrolysis of birch (*Betula pendula*) was added as a potential prebiotic supplement to the feed of rainbow trout (*Oncorhynchus mykiss*), and thereby its effects on fish growth performance, intestinal microbiota, antioxidant capacity and immune activity were investigated. In addition, a feed containing FOS was used as a positive control for comparative assessment of FOS- and COS-mediated prebiotic effects on various health aspects in rainbow trout.

2. Materials and methods

2.1. Ethics statement

The fish experiments were carried out in the Aquatic Facility at the Centre for Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, (Uppsala, Sweden). Full compliance with laws and regulations on procedures and experiments on live animals in Sweden, which are overseen by the Swedish Board of Agriculture (Registration number: 5.8.18–16,347/2017) was ensured.

2.2. Fish husbandry

A total of 225 juvenile rainbow trout (30.5 ± 10.2 g) purchased from Vilstena Fiskodling AB, Fjärdhundra, Sweden, were randomly distributed between 15 experimental tanks (water capacity 200 L) with $n = 15$ fish/tank. Each tank was equipped with belt collectors (Hølland teknologi, Sandnes, Norway) for waste feed and faeces, and with a partial water recirculation system, where tank water was replaced by fresh municipal water at a rate of 3 L min^{-1}. Rearing conditions followed a 12:12 h-light cycle (08:00–20:00 h), with temperature 11 ± 1 °C and dissolved oxygen level 8 ± 2 mg/L (measured by HQ40D Portable Multi Meter, Hach, Loveland, CO, USA) during the whole experiment. Before the start of the experiment, the fish were acclimatised to the experimental conditions for two weeks, during which they were fed a commercial diet (EFICO Enviro 920 Advance, Biomar, Denmark), twice per day at 2% of body weight.

2.3. Diet preparation

Feed was prepared by cold pelleting (Singh et al., 2021) at the Feed Technology Laboratory, Swedish University of Agricultural Sciences. Lignocellulose-derived COS was prepared by enzymatic hydrolysis of organosolv-pretreated birchwood using the commercially available enzyme mixture Celluclast® (Sigma-Aldrich, USA), according to a previously described protocol (Karnaouri et al., 2019b). The resulting powder contained 13.5% cellobiose on a dry weight (dw) basis and a negligible level of glucose (<0.1%). The FOS (Sigma-Aldrich, USA) used in the experiment was procured commercially. Using the COS and FOS prebiotics, five experimental diets were prepared: A control diet without inclusion of any prebiotic compounds; a positive control diet with 0.5% (*w*/w) inclusion of FOS; and three diets with different inclusion rates (w/w) of COS (COS 0.1%, COS 0.5%, COS 1.5%).

2.4. Proximate composition analysis

Following preparation, samples of the five experimental diets were freeze-dried, milled and stored at −20 °C for proximate composition analysis. To determine dry matter content of both feed and feed waste, samples were dried in a hot-air oven for 16 h at 103 °C and then cooled in a desiccator before weighing. All experimental feeds were analysed for crude protein content (nitrogen, N × 6.25) by the Kjeldahl method (Nordic Committee on Food, 1976), using a 2020 Digester (with Cu as catalyst) and 2400 Kjeltec Analyser unit (FOSS Analytical A/S, Hilleröd, Denmark). Crude lipid content was analysed according to the Official Journal of the European Communities (1984), using a Soxhlet extraction unit (1047 Hydrolysing Unit, Soxtec System HT 1043, FOSS Analytical A/S). Neutral detergent fibre (NDF) was measured based on the method described by Chai and Udén (1998), using a 100% neutral detergent solution, with amylase and sulphite used for reduction of starch and protein. Gross energy (GE) content was determined in an isoperibol bomb calorimeter (Parr 6300, Parr Instrument Company, Moline, IL, USA). Dry matter content and ash content were determined according to standard methods (AOAC, 1995). Feed ingredient composition and

Table 1
Feed ingredient composition and proximate composition of the control diet and experimental diets containing fructo-oligosaccharides (FOS 0.5%) and cello-oligosaccharides (COS 0.1%, COS 0.5%, COS 1.5%). Bold figure represents the variation of ingredients in different experimental diets.

	Experimental diet				
	Control	FOS 0.5%	COS 0.1%	COS 0.5%	COS 1.5%
Ingredient (g kg^{-1})					
Fish meal	330.0	330.0	330.0	330.0	330.0
Soy protein concentrate	130.0	130.0	130.0	130.0	130.0
Wheat gluten	160.0	160.0	160.0	160.0	160.0
Wheat meal	**135.0**	**130.0**	**134.0**	**130.0**	**120.0**
Fish oil	110.0	110.0	110.0	110.0	110.0
Rapeseed oil	40.0	40.0	40.0	40.0	40.0
Vitamin mineral premix	10.5	10.5	10.5	10.5	10.5
Monocalcium phosphate	10.0	10.0	10.0	10.0	10.0
Methyl cellulose	10.0	10.0	10.0	10.0	10.0
Gelatin	60.0	60.0	60.0	60.0	60.0
0.5% FOS	–	**5.0**	–	–	–
0.1% COS	–	–	**1.0**	–	–
0.5% COS	–	–	–	**5.0**	–
1.5% COS	–	–	–	–	**15.0**
Proximate composition (g kg^{-1} dry weight)					
Dry matter (DM)	94.1	93.8	93.8	93.6	93.6
Ash	8.2	8.5	8.2	8.1	8.7
Crude lipids	19.4	19.3	19.0	19.1	19.0
Crude protein	52.1	50.9	52.0	51.9	52.1
Crude fibre	0.79	0.78	0.79	0.76	0.77
Neutral detergent fibre	2.0	2.2	2.0	2.0	2.6
Gross energy (MJ/kg)	21.7	21.5	21.5	21.6	21.5

Table 2
Growth parameters of rainbow trout fed the control diet and experimental diets containing fructo-oligosaccharides (FOS 0.5%), and cello-oligosaccharides (COS 0.1%, COS 0.5%, COS 1.5%) for 45 days.

Experimental group	Growth parameter			
	WG (%)	SGR	FCR	Survival (%)
Control	131.8 ± 10.9	1.77 ± 0.09	0.65 ± 0.01	100
FOS 0.5%	137.9 ± 9.5	1.85 ± 0.08	0.68 ± 0.04	100
COS 0.1%	144.3 ± 11.6	1.89 ± 0.09	0.65 ± 0.02	100
COS 0.5%	123.9 ± 8.8	1.72 ± 0.08	0.75 ± 0.09	100
COS 1.5%	142.7 ± 11.8	1.87 ± 0.09	0.66 ± 0.04	100
	P value: 0.648	*P* value: 0.639	*P* value: 0.607	–

Values shown are mean ± SE.
WG (%) = Weight gain percentage; SGR = Specific growth rate; FCR = Feed conversion ratio.
One-way ANOVA was performed for each parameter and *P*-values are included in the respective column.

proximate composition of the five diets are shown in Table 1.

2.5. Experimental design and feeding

The five experimental diets (Control, FOS 0.5%, COS 0.1%, COS 0.5%, COS 1.5%) were randomly assigned to three tanks each, with n = 15 fish per tank and a total of 45 fish per treatment. Fish were fed twice a day for 45 days with the respective experimental diet, at a rate of 1.5% of total tank biomass. The feed was distributed by an automatic belt feeder (Hølland teknologi, Sandnes, Norway).

Table 3
Alpha diversity index (Shannon Simpson, Chao-1) of the gut microbiota of rainbow trout fed the control diet and experimental diets containing fructo-oligosaccharides (FOS 0.5%) and cello-oligosaccharides (COS 0.1%, COS 0.5%, COS 1.5%).

Experimental group	Alpha diversity index		
	Shannon	Simpson	Chao-1
Control	2.68 ± 0.13	0.78 ± 0.01	429.9 ± 46.6
FOS 0.5%	2.63 ± 0.20	0.80 ± 0.02	387.8 ± 61.8
COS 0.1%	3.06 ± 0.21	0.83 ± 0.02	474.2 ± 62.8
COS 0.5%	2.87 ± 0.18	0.81 ± 0.03	435.4 ± 41.2
COS 1.5%	2.99 ± 0.15	0.83 ± 0.03	523.0 ± 45.4
	P value = 0.445	*P* value = 0.652	*P* value = 0.295

Values shown are mean ± SE.
One-way ANOVA was performed for each index and *P*-values are included in the respective column.

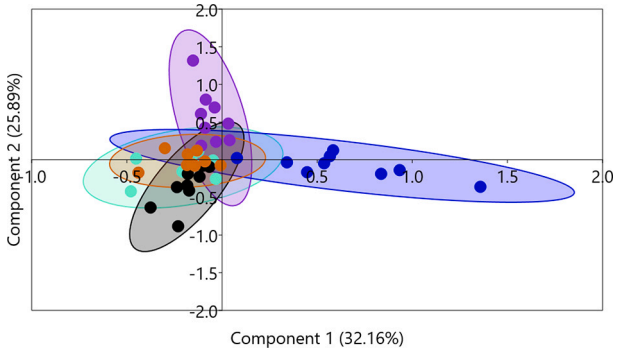


Fig. 1. Principal component analysis (PCA) plot of gut microbial communities in rainbow trout fed different experimental diets: Control (●), FOS 0.5% (●), COS 0.1% (●), COS 0.5% (●) and COS 1.5% (●) (FOS = fructo-oligosaccharides, COS = cello-oligosaccharides).

2.6. Sampling

Initial and final body weight of fish from each treatment was measured at the start and end of the feeding trial, respectively, for determination of growth parameters. Sampling for analysis of fish serum, intestinal tissues and digesta was carried out at the end of the 45-day experimental period. Prior to sampling, nine fish per treatment (3 fish/tank) were randomly selected and anesthetised using tricaine methane sulphonate (MS-222; 300 mg/L, Western Chemical Inc., Ferdale, WA, USA). Blood was collected from the caudal vein of three fish from each treatment (1 fish/tank/treatment) using a non-heparinised syringe, held at 22 °C for 30 min for clotting, followed by centrifugation at 2000 ×g at 4 °C for 15 min. The serum was collected and stored at −80 °C until use for antioxidant enzyme activity assay. Following blood collection, all nine euthanised fish from each treatment were swabbed with ethanol under a fume hood and aseptically dissected out from the ventral side. The hindgut of each fish was dissected from the ileocaecal valve to 0.5 cm above the anus and faeces were collected in cryotubes, snap-frozen in liquid nitrogen and then stored at −80 °C until DNA extraction for microbiota analysis. After collection of faeces, the distal segment of the six intestinal samples per treatment (2 fish/tank) were collected and stored in RNAprotect Tissue Reagent (Qiagen, Germany) for 24 h at 4 °C and then stored at −20 °C until RNA extraction for gene expression analysis.

2.7. Growth parameter analysis

From the initial and final body weight data, the growth parameters weight gain (WG %), feed conversion rate (FCR), specific growth rate

Table 4
Results of similarity analysis of gut microbiota of rainbow trout fed the control diet and experimental diets containing fructo-oligosaccharides (FOS 0.5%) and cello-oligosaccharides (COS 0.1%, COS 0.5%, COS 1.5%).

Group comparison	R-value	*P*-value	Dissimilarity (%)	ASVs
Control – FOS 0.5%	0.053	0.193	56.66	Ruminococcaceae, *Mycoplasma penetrans*, *Brevinema*
Control – COS 0.1%	−0.020	0.537	51.79	*Photobacterium*, *Mycoplasma penetrans*, *Brevinema*
Control – COS 0.5%	0.115	0.048	59.16	Ruminococcaceae, *Photobacterium*, *Mycoplasma penetrans*
Control – COS 1.5%	0.06	0.171	59.03	Ruminococcaceae, *Photobacterium*, *Mesomycoplasma moatsii*
FOS 0.5% – COS 0.1%	0.135	0.073	53.06	*Photobacterium*, *Mycoplasma penetrans*, *Brevinema*
FOS 0.5% – COS 0.5%	0.296	0.003	59.16	Ruminococcaceae, *Brevinema*, *Mycoplasma penetrans*
FOS 0.5% – COS 1.5%	0.222	0.022	59.51	Ruminococcaceae, *Mycoplasma penetrans*, *Photobacterium*
COS 0.1% – COS 0.5%	0.205	0.015	54.41	Ruminococcaceae, *Photobacterium*, *Mycoplasma penetrans*
COS 0.1% – COS 1.5%	0.162	0.034	56.39	Ruminococcaceae, *Photobacterium*, *Mycoplasma penetrans*
COS 0.5% – COS 1.5%	−0.035	0.673	53.29	Ruminococcaceae, *Photobacterium*, *Mesomycoplasma moatsii*

Analysis of similarity (ANOSIM) based on Bray Curtis index followed by Bonferroni correction was performed to calculate R and *P* value. Overall average dissimilarity (%) and amplicon sequence variants (ASVs) contributing to dissimilarity were estimated by similarity of percent analysis (SIMPER) using Bray-Curtis dissimilarity index.

(SGR) and survival percentage (Survival %) were calculated, using the following equations:

$$\text{WG}\ (\%) = [\text{Final weight}\ (\text{g}) – \text{Initial weight}\ (\text{g})\,]/\text{Initial weight}\ (\text{g}) \times 100$$

$$\text{FCR} = \text{Feed intake}\ (\text{g})/\text{Weight gain}\ (\text{g})$$

$$\text{SGR} = [\mathit{ln}(\text{Final weight}) – \mathit{ln}(\text{Initial weight})\,]/\text{Time}\ (\text{days}) \times 100$$

$$\text{Survival}\ (\%) = [\text{Number of fish at end}/\text{Number of fish at start}] \times 100$$

2.8. Gut microbiota analysis

2.8.1. Extraction of DNA

Faeces samples (20–100 mg) were transferred to sterile cryotubes containing 1 mL of InhibitEX buffer (Qiagen, Germany) and 0.5 g silica beads (0.1 mm diameter). The samples were homogenised in a Precellys Evolution homogeniser (Bertin Technologies, France), in two bouts at 6000 rpm for 1 min, with a 5-min interval on ice (to avoid heating). DNA was then isolated from the homogenised samples using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol.

2.8.2. Sequencing and DNA library preparation

PCR (polymerase chain reaction) amplicons from the V4 region of 16S rRNA genes were generated using specific target primers (515F and 806R). An additional PCR step was then applied to attach sample specific barcodes. All PCR reactions were carried out using Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, USA). Successful PCR amplification was confirmed by agarose (2%) gel electrophoresis. The bar-coded PCR products from the different samples were then mixed into equimolar concentrations. The samples were detected by 2% agarose gel electrophoresis and the target bands were recovered using QIAquick Gel Extraction Kit (Qiagen, Germany). The DNA library was constructed using a NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs, USA), and the constructed library was quantified using a Qubit fluorometer and qPCR. After quality analysis, the library was sequenced using NovaSeq6000 at Novogene, United Kingdom.

2.8.3. Bioinformatic analysis of sequence data

Paired-end reads were assigned to each sample, based on their unique barcodes. The barcode sequence and primer sequence were truncated, and then FLASH (v1.2.11, http://ccb.jhu.edu/software/FLASH/) was used to merge the reads to get raw tags (Magoč and Salzberg, 2011). Fastp v0.20.1 software (Chen et al., 2018) was used for quality control of raw tags, from which high-quality clean tags were obtained. Finally, Vsearch v2.3.4 software (Rognes et al., 2016) was used to blast the clean tags against the database to detect and remove chimeras and obtain the final data, namely effective tags. For the effective tags, the DADA2 package in QIIME2 software was used to obtain Amplicon Sequence Variants (ASVs) and a featured table. The Classify-sklearn moduler in QIIME2 software was then used to compare the ASVs with the database, to obtain the species annotation of each ASV (Bokulich et al., 2018; Bolyen et al., 2019). Sequences classified as chloroplasts and mitochondria were filtered out from the dataset prior to calculation of relative abundance. Microbiota analyses were performed on relative abundance data from the ASV table. The sequences have been submitted to the Sequence Read Archive, under Bioproject accession number PRJNA868155.

2.9. Serum antioxidant activity

Total antioxidant activity (T-AOC) and the activity of the specific antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) content in the experimental serum samples were measured by colorimetric assay, using the respective Antioxidant Assay Kit (Elabscience Biotechnology Inc., Houston, TX, USA) according to the manufacturer's protocol (Catalogue No: *E*-BC-K136-M, E-BC-K019-M, E-BC-K031-M, E-BC-K096-M, E-BC-K025-M, respectively). All biochemical indices were determined in duplicate.

2.10. Quantification of stress-mediated and immune genes in intestinal tissue samples

2.10.1. RNA isolation and cDNA synthesis

Intestinal tissue samples (30 mg each) were added to RNase-free bead beating tubes containing 1–3 mm corundum, 3 mm steel beads and 600 μL of Buffer RLT Plus (Qiagen, Germany). The samples were homogenised twice at 6000 rpm for 30 s, using a Precellys Evolution homogeniser (Bertin Technologies, France). RNA extraction was carried out using the RNeasy Plus Mini kit (Qiagen, Germany) according to the manufacturer's protocol. The concentration and purity of the extracted RNA were measured using NanoDrop ND-1000 (NanoDrop Technologies Montchanin, USA). RNA quality (RIN) was determined using the Agilent Tapestation 4150 (Agilent Technologies, Germany). Genomic DNA contamination was removed by treating 1.2 μg of each RNA sample with RQ1 RNase-Free DNase (Promega, USA). Complementary DNA (cDNA) was synthesised using the GoScript™ Reverse Transcriptase (Promega, USA), following the manufacturer's protocol. The cDNA reaction was split so that 0.2 μg RNA was used for control without reverse transcriptase (−RT control). The cDNA samples were diluted at 1:5 ratio using nuclease-free water and stored at −20 °C until use.

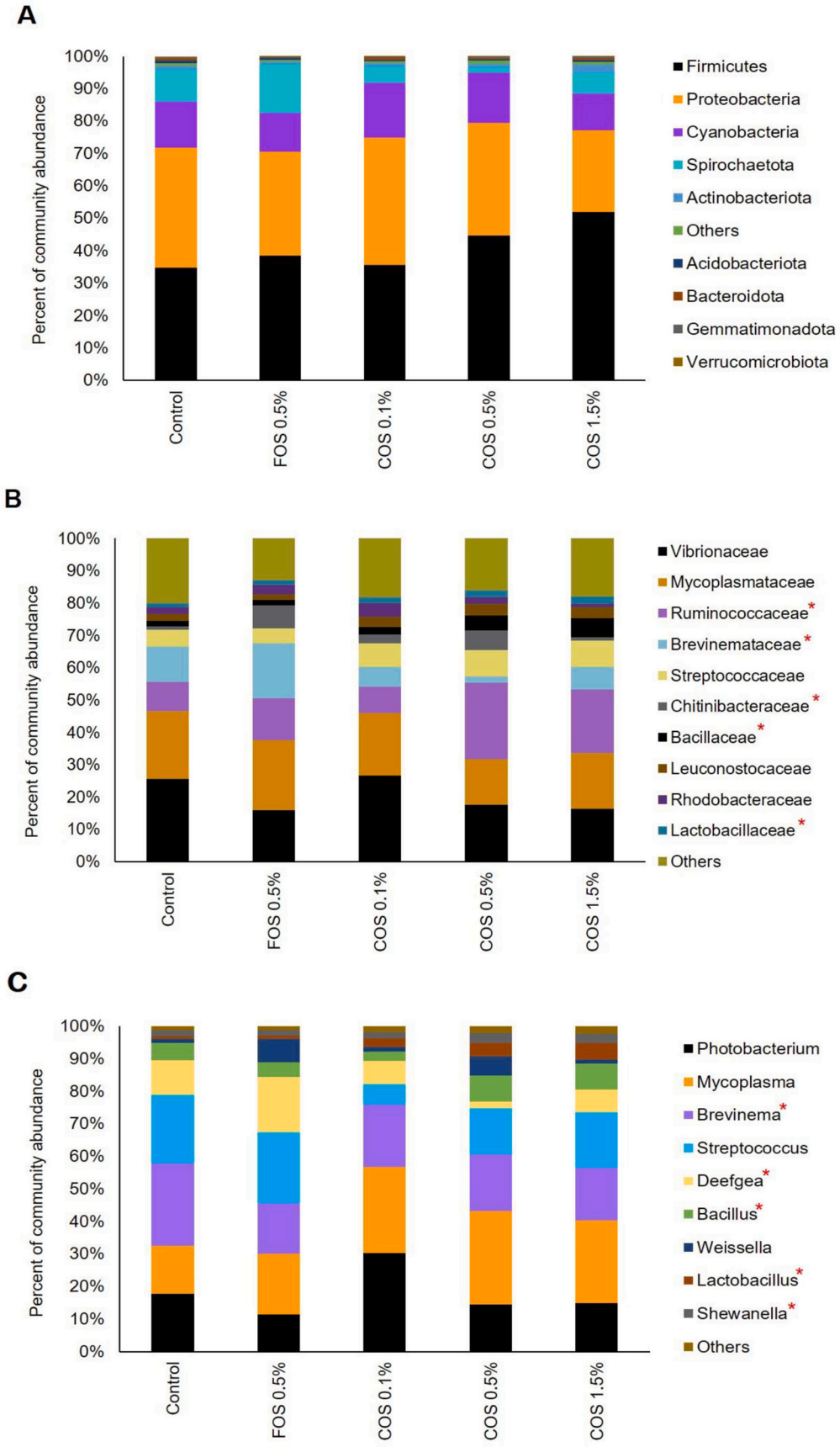


Fig. 2. Gut microbial composition in rainbow trout fed different experimental diets for 45 days: Control, FOS 0.5%, COS 0.1%, COS 0.5% and COS 1.5% (FOS = fructo-oligosaccharides, COS = cello-oligosaccharides). Microbial abundance at (A) phylum, (B) family and (C) genus level. Taxa showing significant differences ($P < 0.05$) are indicated by asterisks (*).

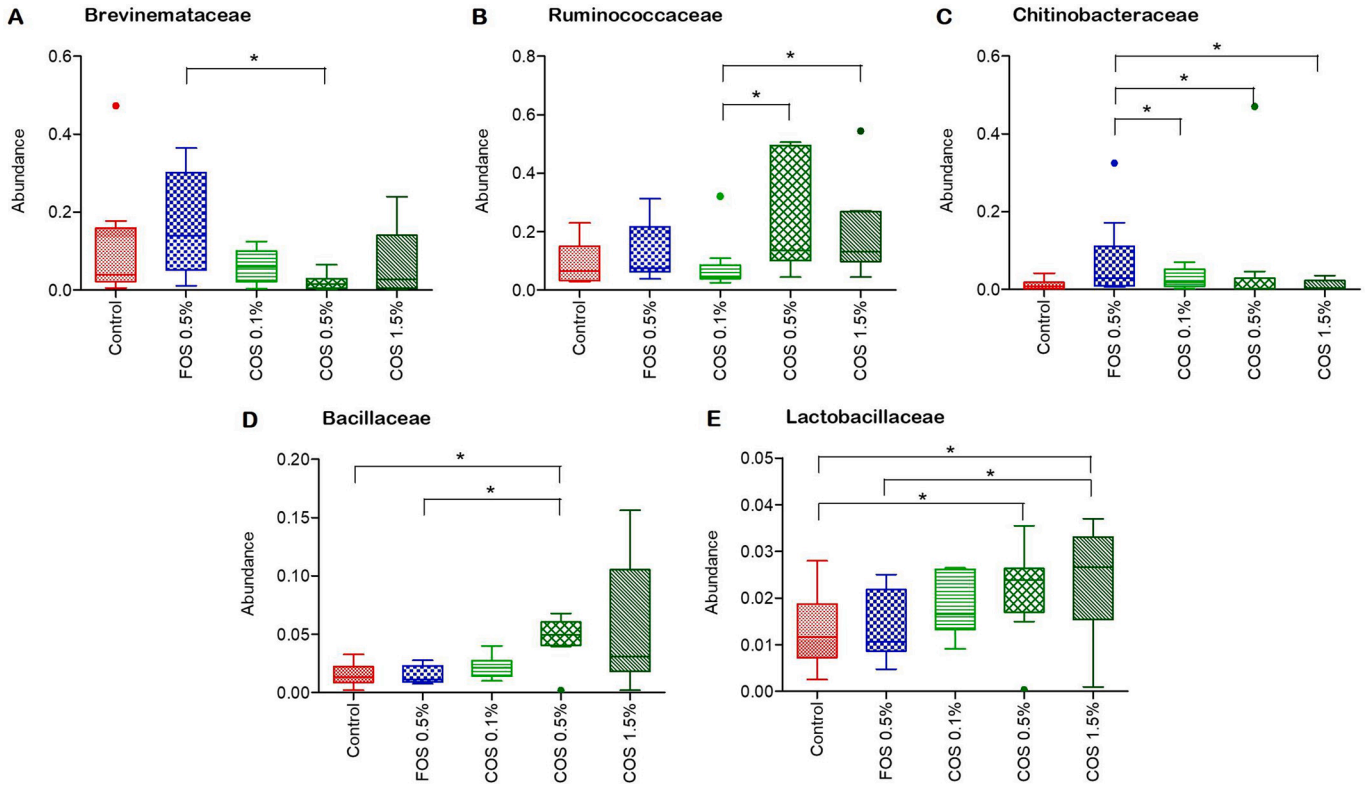


Fig. 3. Comparison of gut microbial community abundance at family level in rainbow trout fed different experimental diets for 45 days: Control, FOS 0.5%, COS 0.1%, COS 0.5% and COS 1.5% (FOS = fructo-oligosaccharides, COS = cello-oligosaccharides). Box and whisker plots of abundance of (A) Brevinemataceae, (B) Ruminococcaceae, (C) Chitinobacteraceae, (D) Bacillaceae and (E) Lactobacillaceae, where the line inside each box represents the median value. Significant differences ($P < 0.05$) between the experimental groups are indicated by asterisks (*). Outliers in the different groups are indicated by coloured dots.

2.10.2. Gene expression by qPCR

All specific primers for qPCR of the targeted and reference genes were designed using Primer3 software or selected from previously published studies (Tsujita et al., 2004; Løvoll et al., 2011; Ballesteros et al., 2012, 2014; Tacchi et al., 2015; Johansson et al., 2016; Dupuy et al., 2019; Huyben et al., 2019; Mehrabi et al., 2019; Rawling et al., 2021). For details, see Table S1 in Supplementary File 1. In all experimental samples, qPCR amplification of two reference genes [beta actin (*β-actin*) and ribosomal protein S20 (*rsp20*)], three oxidative stress-mediated genes (*sod*, *cat* and *gpx*) and 11 mucosal immune-related genes [interleukin-1beta (*il-1β*), tumour necrosis factor-alpha (*tnf-α*), interleukin-10 (*il-10*), transforming growth factor-beta (*tgf-β*), mucin-2 (*muc-2*), immunoglobulin tau heavy chain (*igt*), cluster of differentiation 4 (*cd4*), complement factor 3 (*c3*), *c-type lectin*, toll-like receptor 2 (*tlr2*) and toll-like receptor 5 (*tlr5*)] was carried out in a CFX96 Touch PCR machine (Bio-Rad, California, USA) using Quantitect SYBR Green (Qiagen, Germany). Each reaction was prepared in duplicate to a total volume of 25 μL per reaction with the reaction mixture consisting of 12.5 μL Quantitect SYBR Green (2×), 1.25 μL forward primer, 1.25 μL reverse primer, 8 μL nuclease-free water and 2 μL cDNA samples as a template. The thermal profile used for qPCR amplification consisted of an initial cycle of denaturation at 95 °C for 15 min, followed by 39 cycles of 95 °C for 15 s, annealing at gene-specific annealing temperature (T_a) for 30 s and extension at 72 °C for 30 s. The thermal cycle ended with melt curve analysis to verify the PCR product. The relative expression of each stress-mediated and immune-responsive gene was normalised with the two selected reference genes and calibrated with respect to the control samples. The efficiency of *β-actin* (M value: 0.28, Stability: 1.26) and *rsp20* (M value: 0.17, Stability: 1.77) was calculated using the CFX software. The ΔΔCt value of each sample was determined by subtracting the average ΔCt value of the control from the ΔCt of the test sample. Relative quantification or the fold change in expression for each gene compared with the control was thus expressed as $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001).

2.11. Statistical analysis

To identify significant differences between the microbiota data (ASVs) and diets, Kruskal Wallis analysis was conducted on ASVs with mean abundance >1% in the dataset, followed by Dunn test for post hoc analysis using GraphPad PRISM 9.3.1. Principal component analysis (PCA) was used to investigate potential differences between the diets, followed by one-way analysis of similarity (ANOSIM) based on Bray-Curtis dissimilarity index and then Bonferroni correction to statistically confirm clustering using Paleontological Statistics Software version 3.25 (PAST). Similarity percentage analysis (SIMPER) based on Bray-Curtis dissimilarity index was performed to identify the dominant taxa using PAST. The data obtained on growth parameters, antioxidant enzyme activity and gene expression were subjected to one-way ANOVA, followed by Tukey's comparison test using GraphPad PRISM 9.3.1 for pair-wise comparisons of the different diets. The significance level was set at $P < 0.05$. In addition, for the gene expression ANOVA values, which showed a distinct trend ($P \leq 0.1$), an unpaired *t*-test was conducted for pair-wise comparison between prebiotic (FOS and COS) and control diets.

3. Results and discussion

3.1. Fish growth performance

The growth performance of rainbow trout fed diets supplemented with FOS (0.5%) and COS (0.1%, 0.5% and 1.5%) and those fed the control diet are presented in Table 2. No significant ($P > 0.05$) differences were observed for any of the three growth parameters studied (WG

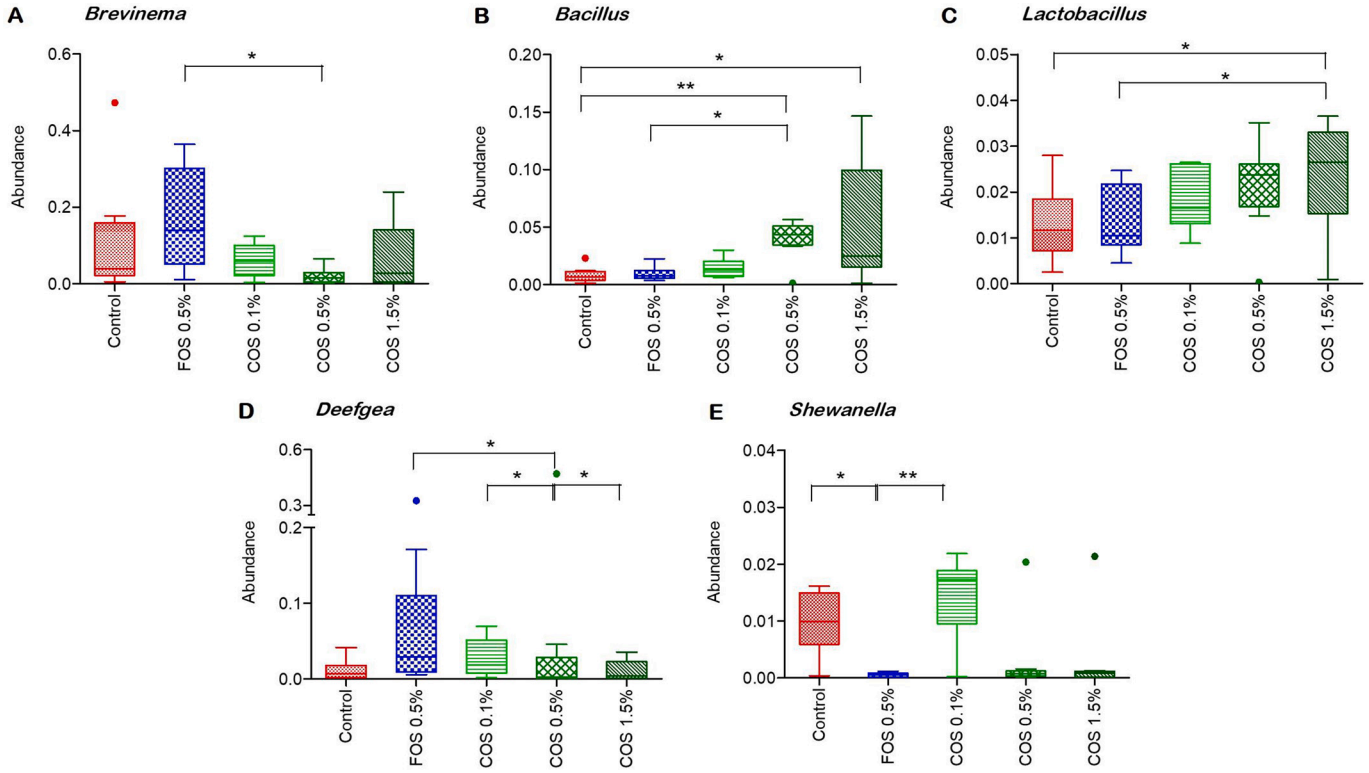


Fig. 4. Comparison of gut microbial community abundance at genus level in rainbow trout fed with different experimental diets for 45 days: Control diet, FOS 0.5%, COS 0.1%, COS 0.5% and COS 1.5% (FOS = fructo-oligosaccharides, COS = cello-oligosaccharides). Box and whisker plot of abundance of (A) *Brevinema*, (B) *Bacillus*, (C) *Lactobacillus*, (D) *Deefgea* and (E) *Shewanella*, where the line inside each box represents the median value. Significant differences between the experimental groups are indicated by asterisks (*$P < 0.05$, **$P < 0.01$). Outliers in different groups are indicated by coloured dots.

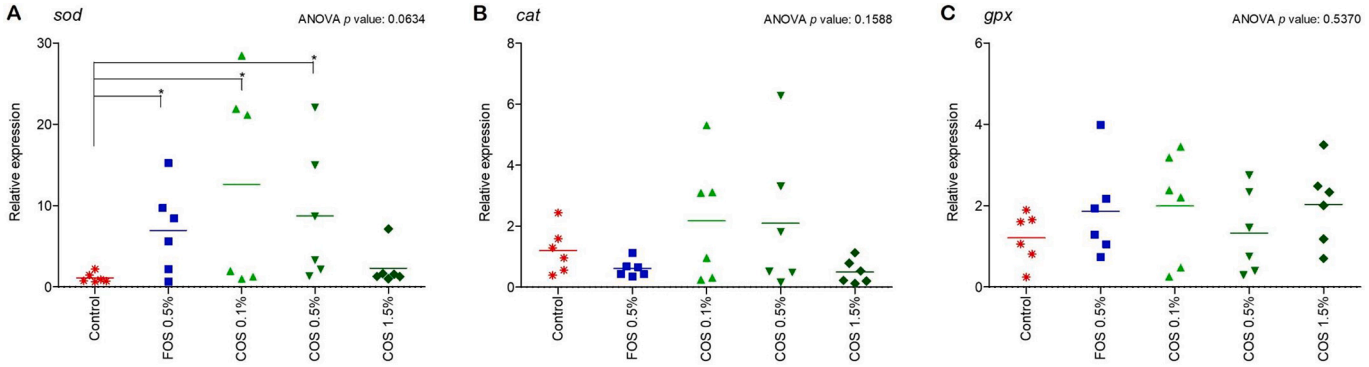


Fig. 5. Relative expression of oxidative stress-related genes in intestine tissue of rainbow trout fed different experimental diets for 45 days: Control, FOS 0.5%, COS 0.1%, COS 0.5% and COS 1.5% (FOS = fructo-oligosaccharides, COS = cello-oligosaccharides). Expression levels of each gene in the experimental groups were compared with those in the control. Relative expression levels of (A) *sod*, (B) *cat*, and (C) *gpx*, plotted as individual and mean ($n = 6$) fold change in gene transcript level. Significant differences among the diets (one-way ANOVA) are indicated by *P* value ($P < 0.05$) and pairwise comparison between COS- and FOS- diets with the control (unpaired *t*-test) are indicated by asterisks (*$P < 0.05$).

%, SGR, FCR), for the prebiotic-supplemented diets compared with the control. This lack of significant difference in growth performance in fish fed the COS and FOS diets supports previous findings on oligosaccharide-supplemented diets in Gulf sturgeon (Pryor et al., 2003), turbot (Mahious et al., 2006), hybrid tilapia (Genc et al., 2007), Atlantic salmon (Grisdale-Helland et al., 2008), common carp (Hoseinifar et al., 2016) and sea bream (Guerreiro et al., 2016). All those studies observed no effect of oligosaccharide-based prebiotics on growth of the respective host fish. In the present study, there were no adverse health effects of inclusion of COS during the 45-day feeding trial, as indicated by the 100% survival rate in the fish fed the COS diets.

3.2. Microbial alpha and beta diversity

After removal of the ASVs for mitochondria, chloroplasts and unassigned taxa, a total of 2.4 million effective reads, with an average of 54,011 reads per intestinal sample, were obtained from the sample set. These sequences were divided into 3799 effective ASVs. Analysis of the alpha diversity of gut bacteria in fish fed different experimental diets showed that the COS and FOS diets had no significant influence ($P > 0.05$) on Shannon, Simpson and Chao-1 index of gut microbiota (Table 3). Previous studies on dietary supplementation with non-starch polysaccharides in rainbow trout (Zhou et al., 2022), short-chain FOS in sea bream (Guerreiro et al., 2016), and pectin and xylan in yellow catfish

Table 5
Antioxidant activity in serum of rainbow trout fed the control diet and experimental diets containing fructo-oligosaccharides (FOS 0.5%) and cello-oligosaccharides (COS 0.1%, COS 0.5%, COS 1.5%).

	Antioxidant activity				
Experimental diet	T-AOC (U/mL)	SOD (U/mL)	CAT (U/mL)	GPx (U/mL)	MDA (μmol/L)
Control	5.4 ± 1.3	20.2 ± 5.2[a]	47.1 ± 2.1	274.4 ± 54.4	5.7 ± 1.6
FOS 0.5%	4.4 ± 0.8	49.1 ± 4.5[b]	38.9 ± 3.9	288.1 ± 65.3	14.3 ± 4.7
COS 0.1%	6.9 ± 1.4	44.5 ± 7.4[b]	57.8 ± 2.9	328.5 ± 73.9	7.9 ± 3.3
COS 0.5%	6.3 ± 1.4	52.9 ± 5.7[b]	39.3 ± 9.2	364.3 ± 84.7	10.9 ± 4.8
COS 1.5%	6.8 ± 1.1	37.7 ± 3.5[b]	39.4 ± 1.2	132.7 ± 36.9	19.5 ± 6.1
	P value: 0.581	*P* value: 0.012	*P* value: 0.319	*P* value: 0.198	*P* value: 0.262

Values shown are mean ± SE.
One-way ANOVA was performed for each parameter and *P*-values are included in the respective column. Values within lines with different superscript letters are significantly different ($P < 0.05$).

(Zhang, 2014) also found limited or non-significant effects on microbial alpha diversity in the respective host species. Thus, it can be suggested that alpha diversity, which is commonly used as a tool in microbiota studies, is more closely related to fish species and less to prebiotic supplements in the diet. The PCA plot showed a clear distinction in overall gut microbial composition in COS 1.5% treated fish compared with fish in the control and FOS 0.5% treatments (Fig. 1). Moreover, the ANOSIM cluster analysis showed significant differences in control vs. COS 0.5% ($P = 0.048$), FOS 0.5% vs. COS 0.5% ($P = 0.003$), FOS 0.5% vs. COS 1.5% ($P = 0.022$), COS 0.1% vs. COS 0.5% ($P = 0.015$) and COS 0.1% vs COS 0.5% ($P = 0.034$) (Table 4). The SIMPER results showed that Ruminococcaceae, *Photobacterium, Mycoplasma penetrans*, and *Brevinema* were the top four ASVs contributing to the significant differences in microbial beta diversity observed for the experimental diets.

3.3. Gut microbiota and effect of COS- and FOS- supplemented diets on specific taxa

Gut microbiota composition in the samples was dominated by three phyla, Firmicutes (35–52%), Proteobacteria (25–39%), and Cyanobacteria (11–17%) (Fig. 2A). This is consistent with observations in previous studies (Singh et al., 2021; Foysal and Gupta, 2022; Zhou et al., 2022), confirming the high relative abundance of these phyla in fish gut microbiota. At the family level, Vibrionaceae (16–26%), Mycoplasmataceae (14–22%), and Ruminococcaceae (9–24%) were the three most common ASVs (Fig. 2B). At the genus level, *Photobacterium* (14–22%) was found to be the most abundant ASV, followed by *Mycoplasma* (16–26%), *Brevinema* (7–17%), and *Streptococcus* (5–8%) (Fig. 2C). The dominance of *Photobacterium* (belonging to the Vibrionaceae) in the gut microbiota reflects the role of this genus in host fish's digestion (Ray et al., 2012; Wang et al., 2018).

Effects of the COS- and FOS-supplemented diets on abundance in microbial community were also seen at family and genus level, with the bacterial families Brevinemataceae ($P = 0.020$), Ruminococcaceae ($P = 0.018$), Chitinobacteraceae ($P = 0.039$), Bacillaceae ($P = 0.001$), and Lactobacillaceae ($P = 0.044$) differing significantly between the diets (Fig. 3). The relative abundance of Brevinemataceae (Fig. 3A) and Chitinobacteraceae (Fig. 3C) was highest for the FOS 0.5% diet and lowest for the COS 0.5% diet. Likewise, the genus *Brevinema* ($P = 0.02$) (Fig. 4A) and *Deefgea* ($P = 0.04$) (Fig. 4D), both belonging to the Brevinemataceae, showed significantly higher relative abundance in the gut of fish fed the FOS 0.5% diet than fish fed all three COS-supplemented diets. This dominance could be explained by the fact that members of the Brevinemataceae (Lyons et al., 2017) and Chitinobacteraceae (Molinari et al., 2007; Sichert et al., 2020) are capable of degrading complex carbohydrates such as FOS, which comprise of heterogeneous mixture of oligosaccharides containing mainly β-(2–1) glycosidic bond, whereas the COS product used in the present study, consisted mainly of cello-oligosaccharides with β-(1–4) glycosidic bonds and might not be preferred by these two bacterial families. In contrast, Ruminococcaceae which are associated with production of short-chain fatty acids by fermentation of ingestible carbohydrates such as resistant starch or dietary fibre (Walker et al., 2011; Ze et al., 2013; Rimoldi et al., 2020), was found to be the most abundant family in the gut of fish fed the COS 0.5% diet (Fig. 3B). Moreover, the relative abundances of Bacillaceae ($P = 0.001$) (Fig. 3D) and the underlying genera *Bacillus* (Fig. 4B) was highest in COS 0.5%, followed by COS 1.5%, COS 0.1%, FOS 0.5% and lowest in the control. While the relative abundance of Lactobacillaceae ($P = 0.001$) (Fig. 3E) and *Lactobacillus* (Fig. 4C), was highest for COS 1.5%, followed by COS 0.5%. Higher abundance of Bacillaceae and Lactobacillaceae in the diets with COS compared with FOS is in agreement with findings in a study by Ortiz et al. (2013). They observed that FOS fermentation in the gut of rainbow trout did not affect short-chain fatty acid and/or lactic acid production, resulting in lower abundance of lactic acid bacteria (LAB). A study by Karnaouri et al. (2019a), demonstrated that birch- and spruce-derived cellobiose-rich hydrolysates supported the growth of two *Lactobacillus* probiotic strains in vitro. Thus, the present study indicates COS as a preferential prebiotic for LAB as compared to FOS. Further, the γ-proteobacterial strain *Shewanella*, which is reported to be a natural resident with beneficial characteristics in the salmonid gut (Navarrete et al., 2010; García de la Banda et al., 2012), showed high abundance in the gut of fish fed the COS 0.1% and control diets, but the abundance was lower in fish fed with the COS 0.5%, COS 1.5% and FOS 0.5% diets (Fig. 4E).

3.4. Effects of COS and FOS incorporated diets on antioxidant capacity

Besides microbiota analysis, in the present study the effects of COS and FOS diets on the antioxidant capacity of rainbow trout were evaluated using comparative mRNA expression profiling of three important oxidative stress-related genes (*sod*, *cat* and *gpx*) in the distal intestine tissue (Fig. 5). Their downstream impact on enzyme activity in the blood serum was also analysed (Table 5). Overall, the expression profile of the *cat* (Fig. 5B) and *gpx* (Fig. 5C) genes showed non-significant differences at mRNA transcript level in the diets with prebiotic compared with the control. However, dietary inclusion of COS and FOS significantly ($P < 0.05$) improved the expression level of the *sod* gene compared with the control (Fig. 5A). It is important to note that the expression patterns of individual fish within each treatment showed wide variation for all three genes, with 2–3 fish displaying higher transcript values than the respective mean level. The apparent differences in antioxidant activity in individual fish are unclear and merit further study.

In agreement with the gene expression data, the enzymatic assays also demonstrated significant enhancement ($P < 0.05$) of SOD enzyme activity in the diets with prebiotic compared with the control, and no such significant differences were observed for CAT enzymes. However, GPx enzyme activity showed significant differences in the pairwise comparison between the diets Likewise, experimental diet did not have any significant impact on T-AOC or MDA level in the serum. Previous studies have demonstrated that the antioxidant capacity in serum and intestine of fish species depends on the type and dose of the dietary supplement used. One study found that inclusion of 8% insoluble cellulose (iNSP) had no effect on antioxidant activity, but at 16% soluble cellulose (sNSP) and 28% iNSP, antioxidant capacity (SOD and CAT levels) was lowered and MDA levels were elevated (Deng et al., 2021). Another study examining sNSP and mucosal health observed that the higher level of sNSP tested (>9%) lowered the antioxidant activity of SOD and CAT in the intestine of juvenile largemouth bass (Liu et al., 2022). In contrast, a study on blunt snout bream found enhanced

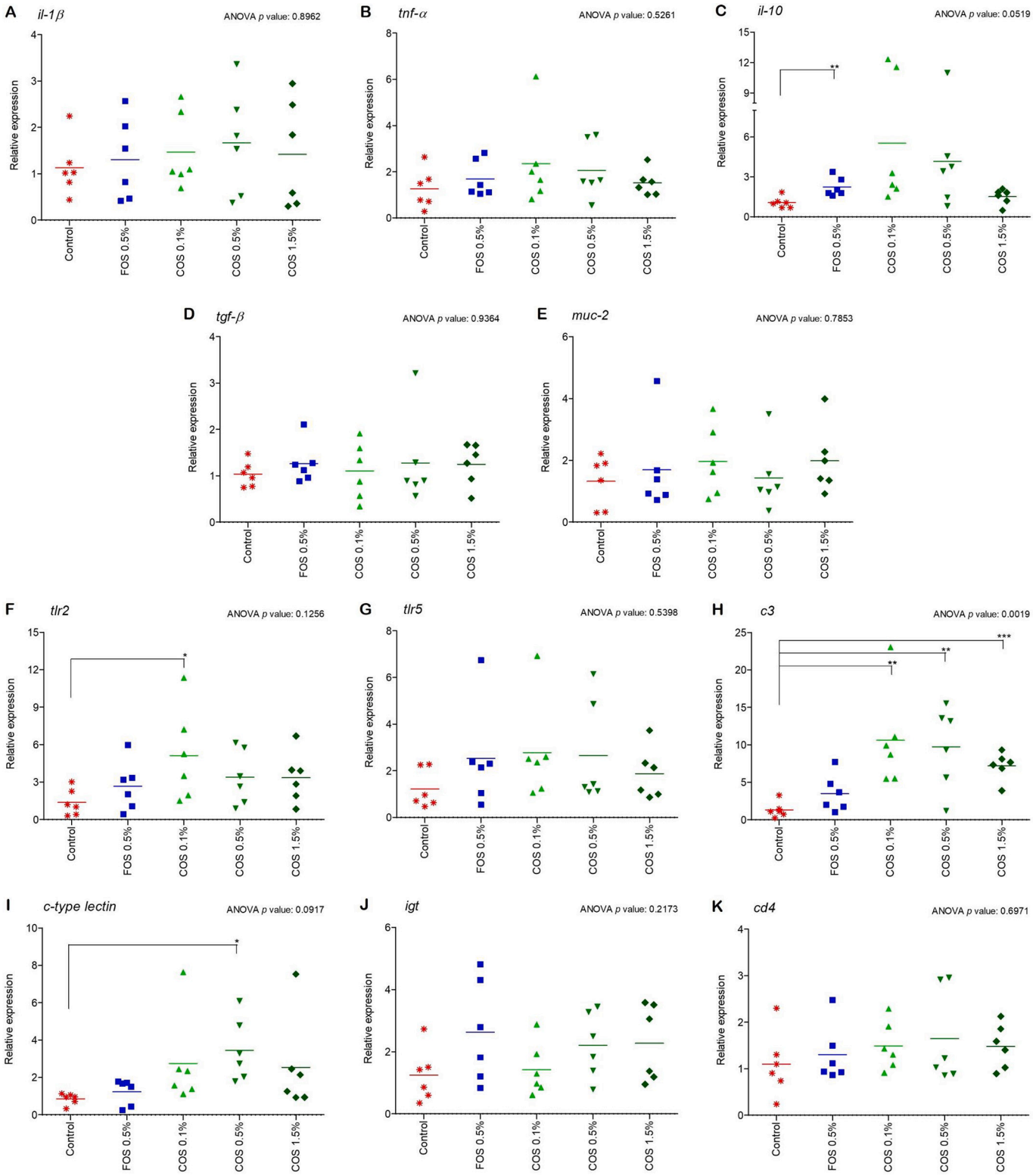


Fig. 6. Relative expression of immune-related genes in intestine tissue of rainbow trout fed different experimental diets for 45 days: Control, FOS 0.5%, COS 0.1%, COS 0.5% and COS 1.5% (FOS = fructo-oligosaccharides, COS = cello-oligosaccharides). Expression levels of each gene in the experimental groups were compared with those in the control. Relative expression levels of (A) *il-1β*, (B) *tnf-α*, (C) *tgf-β*, (D) *il-10*, (E) *muc-2*, (F) *tlr2*, (G) *tlr5*, (H) *c3*, (I) *c-type lectin*, (J) *igt* and (K) *cd4*, plotted as individual and mean (n = 6) fold change in gene transcript level. Significant differences among the diets (one-way ANOVA) are indicated by *P* value ($P < 0.05$) and pairwise comparison between COS- and FOS- diets with the control (unpaired *t*-test) are indicated by asterisks (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$).

activity of SOD, CAT and GPx, and low levels of MDA, when the fish were fed a diet supplemented with a lower level (0.8%) of FOS (Zhang et al., 2014). Similarly, Ren et al. (2020) observed that dietary inclusion of mannan oligosaccharides (MOS), even at low levels (0.3–2%), successfully enhanced T-AOC and SOD levels in the serum of juvenile hybrid grouper fish; whereas, Hoseinifar et al. (2017) demonstrated significant increase in the SOD level in galactooligosaccharide (GOS), FOS and inulin treated *Cyprinus carpio*. Therefore, it can be concluded that certain prebiotics influence the antioxidant capacity of the host fish, but the correlation depends on a range of other factors that need to be further investigated. Nevertheless, the results in the present study indicated that COS is a suitable prebiotic candidate for rainbow trout, since it was able to reduce the level of oxidative stress and enhance the antioxidant capacity of the host fish, even at relatively low concentrations.

3.5. Effects of COS- and FOS-supplemented diets on immune gene expression in the intestine

The effects of the COS and FOS diets on relative expression of various classes of immune-related genes in the distal intestinal tissue of rainbow trout were also investigated (Fig. 6). The intestine is the main region where digestion and absorption of feed nutrients take place, while it also acts as an important immune organ for fish (Xu et al., 2021). Thus, determining the expression profile of different genes in the intestinal mucosa can help in determining the health of the intestine, and of the fish as a whole. The distal intestine was selected for analysis in the study because enterocytes in this region are more capable of antigen sensing and uptake, due to the presence of irregular microvilli and high pinocytotic activity (Rombout et al., 2011).

Dietary inclusion of FOS and COS had no effect ($P > 0.05$) on expression of pro-inflammatory cytokines *il-1β* (Fig. 6A) or *tnf-α* (Fig. 6B) or the anti-inflammatory *tgf-β* gene (Fig. 6C). The anti-inflammatory cytokine *il-10* gene transcript showed numerically, but not significantly, higher expression (~1.5- to 5.5-fold upregulation) in fish fed with COS and FOS diets compared with control fish (Fig. 6D). This might be attributable to the low inclusion level of COS and FOS not affecting the physical layer of the gut mucosa or causing fluctuation in gut microbiota. This is similar to previous observations that reported regulation of pro- and anti-inflammatory cytokines in the intestine is directly related to the inclusion level of dietary NSP or cellulose (Liu et al., 2022; Zhang et al., 2019). Further, our results showed no significant difference ($P > 0.05$) in expression of the *muc-2* gene (precursor for mucin secretion) in COS- and FOS-fed fish compared with the control (Fig. 6E), indicating normal intestinal mucosa and absence of inflammatory reaction in fish with prebiotic-supplemented diets.

Beside cytokines, the expression profile of other genes involved in the innate and adaptive immune systems in the different treatment groups was also investigated. In fish, innate immune receptors such as toll-like receptors (*tlrs*) play an important function in recognising bacterial pathogen-associated molecular patterns (PAMPs), which in turn leads to antigen processing and presentation by the adaptive immune system (Rebl et al., 2010). In this study, *tlr2* (which recognises bacterial lipopolysaccharides) and *tlr5* (which recognises bacterial flagellin) showed numerically higher expression (~2- to 5-fold upregulation) in the prebiotic-treated fish compared with the control fish. Although, this difference in gene expression was not statistically significant ($P > 0.05$), likely due to wide variation in the expression pattern seen for individual fish within each diet (Fig. 6F-G), but a statistical trend ($P \leq 0.1$) was observed for *tlr2* gene. Apart from receptors, the complement cascade constitutes an integral part of the teleost innate immune defence (Boshra et al., 2006). There was significant ($P < 0.05$) upregulation of *c3* (Fig. 6H) gene transcript in COS-fed fish compared with the control, indicating greater efficiency of COS as an innate immune stimulator in rainbow trout. Meanwhile, the non-significant transcript level of *c3* gene in FOS fish compared with the control contradicts findings for FOS-treated blunt snout bream, where the level showed distinct enhancement (Zhang et al., 2014). The *c-type lectin* (Fig. 6I) gene showed distinct elevation in the COS diets; however, only a statistical trend ($P \leq 0.1$) could be observed from the transcript expression.

Moreover, the *igt* (Fig. 6J) and *cd4* (Fig. 6K) gene transcripts did not differ significantly ($P > 0.05$) in expression between the prebiotic-fed and the control fish. Similarly, in a previous study dietary NSP had no effect on *igt* gene expression in the intestine of largemouth bass (Liu et al., 2022). This difference in expression pattern between innate and adaptive genes can be traced back directly to the immunological tolerance and pathogenicity of the prebiotic-mediated gut microbial population, where beneficial bacteria help in stimulating the non-specific innate immune system. However, due to lack of detection of ASVs for pathogenic bacteria, the host cell does not recognise the pathogenic antigen and might have curtails the $CD4^{+}$-T-cell-mediated antigen processing and B-cell proliferation for immunoglobulin (*igt*) production.

4. Conclusions

This study provided important new baseline data on COS as a potential functional feed in rainbow trout. The results demonstrated higher abundance of bacterial families related to production of short-chain fatty acids (Ruminococcaceae, Bacillaceae, Lactobacillaceae), which should be quantified in future studies to confirm its prebiotic potential. The results also demonstrated improved antioxidant capacity and higher immunomodulatory effect owing to upregulation in complement (*c3* and *c-type lectin*) and receptor (*tlr2*) genes of the innate immune system. This indicates that dietary COS can be of biological significance for rainbow trout and can modulate the overall health status of the fish. However, due to the low levels of dietary COS tested in the present study, the immunomodulatory and prebiotic properties may have been underestimated. Thus, tests on higher inclusion levels of COS are needed to identify its maximum functionality.

Author contributions

AS, TL and KB designed the study. AS carried out planning and execution of the experiments, laboratory work, data analysis and manuscript writing. AS, TL and AV carried out the sampling. AS and AV performed the feed optimisation and feed production. AS was responsible for DNA analysis and performed the data analysis with the support of JD. AS carried out the gene expression and data analysis with the support of BH. EK and AK were involved in production of prebiotic COS and manuscript review and editing. PK and UR were involved in study conceptualisation, funding acquisition and supervising. All authors contributed to the article and approved the final version.

Declaration of Competing Interest

The authors declare that they have no potential conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

The authors acknowledge the strategic innovation program Bio-Innovation, a joint venture by Vinnova (Verket för innovations system; diarie number: 2017-02691), Formas and Swedish Energy Agency, for the financial support to carry out this study, and the Indian Council of Agricultural Research for providing a Ph.D. fellowship for the first author (AS); Francis Pius Mmanda for helping in feed preparation and wet laboratory during the experiment; and Sumalata Sonavane and Astrid Gumucio for carrying out sampling and/or laboratory help.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740002.

References

Akrami, R., Iri, Y., Rostami, H.K., Mansour, M.R., 2013. Effect of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival, lactobacillus bacterial population and hemato-immunological parameters of stellate sturgeon (Acipenser stellatus) juvenile. Fish Shellfish Immunol. 35, 1235–1239. https://doi.org/10.1016/j.fsi.2013.07.039.

AOAC, A, 1995. Official methods of analysis. In: Association of Official Analytical Chemists, 16th ed. Sci. Educ, Washington DC, USA.

Ballesteros, N.A., Saint-Jean, S.S.R., Encinas, P.A., Perez-Prieto, S.I., Coll, J.M., 2012. Oral immunization of rainbow trout to infectious pancreatic necrosis virus (Ipnv) induces different immune gene expression profiles in head kidney and pyloric ceca. Fish Shellfish Immunol. 33, 174–185. https://doi.org/10.1016/j.fsi.2012.03.016.

Ballesteros, N.A., Saint-Jean, S.R., Perez-Prieto, S.I., 2014. Food pellets as an effective delivery method for a DNA vaccine against infectious pancreatic necrosis virus in rainbow trout (Oncorhynchus mykiss, Walbaum). Fish Shellfish Immunol. 37, 220–228. https://doi.org/10.1016/j.fsi.2014.02.003.

Barbosa, F.C., Kendrick, E., Brenelli, L.B., Arruda, H.S., Pastore, G.M., Rabelo, S.C., Damásio, A., Franco, T.T., Leak, D., Goldbeck, R., 2020. Optimization of cello-oligosaccharides production by enzymatic hydrolysis of hydrothermally pretreated sugarcane straw using cellulolytic and oxidative enzymes. Biomass Bioenergy 141, 105697. https://doi.org/10.1016/j.biombioe.2020.105697.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 1–17. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Boshra, H., Li, J., Sunyer, J.O., 2006. Recent advances on the complement system of teleost fish. Fish Shellfish Immunol. 20, 239–262. https://doi.org/10.1016/j.fsi.2005.04.004.

Chai, W., Udén, P., 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. Anim. Feed Sci. Technol. 74, 281–288. https://doi.org/10.1016/S0377-8401(98)00187-4.

Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884–i890. https://doi.org/10.1093/bioinformatics/bty 560.

Deng, J., Zhang, X., Sun, Y., Mi, H., Zhang, L., 2021. Effects of different types of non-starch polysaccharides on growth, digestive enzyme activity, intestinal barrier function and antioxidant activity of rainbow trout (Oncorhynchus mykiss). Aquac. Reports 21, 100864. https://doi.org/10.1016/j.aqrep.2021.100864.

Dupuy, C., Cabon, J., Louboutin, L., Le Floch, S., Morin, T., Danion, M., 2019. Cellular, humoral and molecular responses in rainbow trout (Oncorhynchus mykiss) exposed to a herbicide and subsequently infected with infectious hematopoietic necrosis virus. Aquat. Toxicol. 215, 105282 https://doi.org/10.1016/j.aquatox.2019.105282.

FAO, 2022. In brief to the state of world fisheries and aquaculture 2022. The State of World Fisheries and Aquaculture 2022. https://doi.org/10.4060/ca9229en.

Foysal, M.J., Gupta, S.K., 2022. A systematic meta-analysis reveals enrichment of Actinobacteria and Firmicutes in the fish gut in response to black soldier fly (Hermetica illucens) meal-based diets. Aquaculture 549, 737760. https://doi.org/10.1016/j. aquaculture.2021.737760.

García de la Banda, I., Lobo, C., Chabrillón, M., León-Rubio, J.M., Arijo, S., Pazos, G., María Lucas, L., Moriñigo, M.Á., 2012. Influence of dietary administration of a probiotic strain Shewanella putrefaciens on Senegalese sole (Solea senegalensis, Kaup 1858) growth, body composition and resistance to Photobacterium damselae subsp piscicida. Aquac. Res. 43, 662–669. https://doi.org/10.1111/j.1365-2109.2011. 02871.x.

Genc, M.A., Yilmaz, E., Genc, E., Aktas, M., 2007. Effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (Oreochromis niloticus x 0. Aureus). Isr. J. Aquac. 59. https://search.iczhiku.com/paper/r7EoKEv0dJoDVsmL.pdf.

Grisdale-Helland, B., Helland, S.J., Gatlin III, D.M., 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar). Aquaculture 283, 163–167. https://doi.org/10.1016/j.aquaculture.2008.07.012.

Guerreiro, I., Serra, C.R., Enes, P., Couto, A., Salvador, A., Costas, B., Oliva-Teles, A., 2016. Effect of short chain fructooligosaccharides (scFOS) on immunological status and gut microbiota of gilthead sea bream (Sparus aurata) reared at two temperatures. Fish Shellfish Immunol. 49, 122–131. https://doi.org/10.1016/j.fsi.2015.12.032.

Hoseinifar, S.H., Esteban, M.Á., Cuesta, A., Sun, Y.Z., 2015. Prebiotics and fish immune response: a review of current knowledge and future perspectives. Rev. Fish. Sci. Aquac. 23 (4), 315–328. https://doi.org/10.1080/23308249.2015.1052365.

Hoseinifar, S.H., Eshaghzadeh, H., Vahabzadeh, H., Peykaran Mana, N., 2016. Modulation of growth performances, survival, digestive enzyme activities and intestinal microbiota in common carp (Cyprinus carpio) larvae using short chain fructooligosaccharide. Aquac. Res. 47, 3246–3253. https://doi.org/10.1111/are.12777.

Hoseinifar, S.H., Ahmadi, A., Raeisi, M., Hoseini, S.M., Khalili, M., Behnampour, N., 2017. Comparative study on immunomodulatory and growth enhancing effects of three prebiotics (galactooligosaccharide, fructooligosaccharide and inulin) in common carp (*Cyprinus carpio*). Aquac. Res. 48 (7), 3298–3307. https://doi.org/10.1111/are.13156.

Hoseinifar, S.H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., Carnevali, O., 2020. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev. Fish. Sci. Aquac. 29 (2), 198–217. https://doi.org/10.1080/23308249.2020.1795616.

Huyben, D., Vidaković, A., Hallgren, S.W., Langeland, M., 2019. High-throughput sequencing of gut microbiota in rainbow trout (Oncorhynchus mykiss) fed larval and pre-pupae stages of black soldier fly (Hermetia illucens). Aquaculture 500, 485–491. https://doi.org/10.1016/j.aquaculture.2018.10.034.

Huynh, T.-G., Shiu, Y.-L., Nguyen, T.-P., Truong, Q.-P., Chen, J.-C., Liu, C.-H., 2017. Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review. Fish Shellfish Immunol. 64, 367–382. https://doi.org/10.1016/j.fsi.2017.03.035.

Johansson, P., Wang, T., Collet, B., Corripio-Miyar, Y., Monte, M.M., Secombes, C.J., Zou, J., 2016. Identification and expression modulation of a C-type lectin domain family 4 homologue that is highly expressed in monocytes/macrophages in rainbow trout (Oncorhynchus mykiss). Dev. Comp. Immunol. 54, 55–65. https://doi.org/10.1016/j.dci.2015.08.005.

Karnaouri, A., Topakas, E., Matsakas, L., Rova, U., Christakopoulos, P., 2018. Fine-tuned enzymatic hydrolysis of organosolv pretreated forest materials for the efficient production of cellobiose. Front. Chem. 6, 128. https://doi.org/10.3389/fchem.2018.00128.

Karnaouri, A., Matsakas, L., Krikigianni, E., Rova, U., Christakopoulos, P., 2019a. Valorization of waste forest biomass toward the production of cello-oligosaccharides with potential prebiotic activity by utilizing customized enzyme cocktails. Biotechnol. Biofuels 12, 1–19. https://doi.org/10.1186/s13068-019-1628-z.

Karnaouri, A., Matsakas, L., Bühler, S., Muraleedharan, M.N., Christakopoulos, P., Rova, U., 2019b. Tailoring celluclast® cocktail's performance towards the production of prebiotic cello-oligosaccharides from waste forest biomass. Catalysts 9, 897. https://doi.org/10.3390/catal9110897.

Kontula, P., von Wright, A., Mattila-Sandholm, T., 1998. Oat bran β-gluco-and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. Int. J. Food Microbiol. 45, 163–169. https://doi.org/10.1016/S0168-1605(98)00156-1.

Li, J., Tan, B., Mai, K., 2009. Dietary probiotic Bacillus OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (Litopenaeus vannamei). Aquaculture 291, 35–40. https://doi.org/10.1016/j.aquaculture.2009.03.005.

Li, Y., Liu, H., Dai, X., Li, J., Ding, F., 2018. Effects of dietary inulin and mannan oligosaccharide on immune related genes expression and disease resistance of Pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 76, 78–92. https://doi.org/10.1016/j.fsi.2018.02.034.

Liu, Y., Huang, H., Fan, J., Zhou, H., Zhang, Y., Cao, Y., Jiang, W., Zhang, W., Deng, J., Tan, B., 2022. Effects of dietary non-starch polysaccharides level on the growth, intestinal flora and intestinal health of juvenile largemouth bass Micropterus salmoides. Aquaculture 557, 738343. https://doi.org/10.1016/j.aquaculture.2022.738343.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Løvoll, M., Austbø, L., Jørgensen, J.B., Rimstad, E., Frost, P., 2011. Transcription of reference genes used for quantitative RT-PCR in Atlantic salmon is affected by viral infection. Vet. Res. 42, 1–5. https://doi.org/10.1186/1297-9716-42-8.

Lyons, P.P., Turnbull, J.F., Dawson, K.A., Crumlish, M., 2017. Phylogenetic and functional characterization of the distal intestinal microbiome of rainbow trout Oncorhynchus mykiss from both farm and aquarium settings. J. Appl. Microbiol. 122, 347–363. https://doi.org/10.1111/jam.13347.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

Mahious, A.S., Gatesoupe, F.J., Hervi, M., Metailler, R., Ollevier, F., 2006. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, Psetta maxima (Linnaeus, C. 1758). Aquac. Int. 14, 219–229. https://doi.org/10.1007/s10499-005-9003-4.

Mehrabi, Z., Firouzbakhsh, F., Rahimi-Mianji, G., Paknejad, H., 2019. Immunostimulatory effect of Aloe vera (Aloe barbadensis) on non-specific immune response, immune gene expression, and experimental challenge with Saprolegnia parasitica in rainbow trout (Oncorhynchus mykiss). Aquaculture 503, 330–338. https://doi.org/10.1016/j. aquaculture.2019.01.025.

Molinari, L.M., Pedroso, R.B., de Oliveira Scoaris, D., Ueda-Nakamura, T., Nakamura, C. V., Dias Filho, B.P., 2007. Identification and partial characterisation of a chitinase from Nile tilapia, Oreochromis niloticus. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 146, 81–87. https://doi.org/10.1016/j.cbpb.2006.09.004.

Montalban-Arques, A., De Schryver, P., Bossier, P., Gorkiewicz, G., Mulero, V., Gatlin III, D.M., Galindo-Villegas, J., 2015. Selective manipulation of the gut microbiota improves immune status in vertebrates. Front. Immunol. 512 https://doi.org/10.3389/fimmu.2015.00512.

Mussatto, S.I., Mancilha, I.M., 2007. Non-digestible oligosaccharides: a review. Carbohydr. Polym. 68, 587–597. https://doi.org/10.1016/j.carbpol.2006.12.011.

Navarrete, P., Toledo, I., Mardones, P., Opazo, R., Espejo, R., Romero, J., 2010. Effect of Thymus vulgaris essential oil on intestinal bacterial microbiota of rainbow trout, Oncorhynchus mykiss (Walbaum) and bacterial isolates. Aquac. Res. 41, e667–e678. https://doi.org/10.1111/j.1365-2109.2010.02590.x.

Nawaz, A., Irshad, S., Hoseinifar, S.H., Xiong, H., 2018. The functionality of prebiotics as immunostimulant: evidences from trials on terrestrial and aquatic animals. Fish Shellfish Immunol. 76, 272–278. https://doi.org/10.1016/j.fsi.2018.03.004.

Nayak, S.K., 2010. Role of gastrointestinal microbiota in fish. Aquac. Res. 41, 1553–1573. https://doi.org/10.1111/j.1365-2109.2010.02546.x.

Nordic Committee on Food, 1976. Nitrogen. Determination in foods and feeds according to Kjeldahl.

Official Journal of the European Communities, 1984. Determination of Crude Oils and Fat. Method B.

Ortiz, L.T., Rebolé, A., Velasco, S., Rodríguez, M.L., Treviño, J., Tejedor, J.L., Alzueta, C., 2013. Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (O ncorhynchus mykiss). Aquac. Nutr. 19, 475–482. https://doi.org/10.1111/j.1365-2095.2012.00981. x.

Otsuka, M., Ishida, A., Nakayama, Y., Saito, M., Yamazaki, M., Murakami, H., Nakamura, Y., Matsumoto, M., Mamoto, K., Takada, R., 2004. Dietary supplementation with cellooligosaccharide improves growth performance in weanling pigs. Anim. Sci. J. 75, 225–229. https://doi.org/10.1111/j.1740-0929.2004.00180.x.

Pokusaeva, K., O'Connell-Motherway, M., Zomer, A., MacSharry, J., Fitzgerald, G.F., van Sinderen, D., 2011. Cellodextrin utilization by Bifidobacterium breve UCC2003. Appl. Environ. Microbiol. 77, 1681–1690. https://doi.org/10.1128/AEM.01786-10.

Pryor, G.S., Royes, J.B., Chapman, F.A., Miles, R.D., 2003. Mannanoligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in Gulf of Mexico sturgeon. N. Am. J. Aquac. 65, 106–111. https://doi.org/10.1577/1548-8454(2003)65%3C106:MIFNEO%3E2.0.CO;2.

Qiang, X., YongLie, C., QianBing, W., 2009. Health benefit application of functional oligosaccharides. Carbohydr. Polym. 77, 435–441. https://doi.org/10.1016/j.carbpol. 2009.03.016.

Rawling, M., Leclercq, E., Foey, A., Castex, M., Merrifield, D., 2021. A novel dietary multi-strain yeast fraction modulates intestinal toll-like-receptor signalling and mucosal responses of rainbow trout (Oncorhynchus mykiss). PLoS One 16, e0245021. https://doi.org/10.1371/journal.pone.0245021.

Ray, A.K., Ghosh, K., Ringø, E., 2012. Enzyme-producing bacteria isolated from fish gut: a review. Aquac. Nutr. 18, 465–492. https://doi.org/10.1111/j.1365-2095.2012.00943.x.

Rebl, A., Goldammer, T., Seyfert, H.M., 2010. Toll-like receptor signaling in bony fish. Vet. Immunol. Immunopathol. 134 (3–4), 139–150. https://doi.org/10.1016/j.vetimm.2009. 09.021.

Ren, Z., Wang, Shifeng, Cai, Y., Wu, Y., Tian, L., Wang, Shaoqun, Jiang, L., Guo, W., Sun, Y., Zhou, Y., 2020. Effects of dietary mannan oligosaccharide supplementation on growth performance, antioxidant capacity, non-specific immunity and immune-related gene expression of juvenile hybrid grouper (Epinephelus lanceolatus♂× Epinephelus fuscoguttatus♀). Aquaculture 523, 735195. https://doi.org/10.1016/j.aquaculture.2020. 735195.

Rimoldi, S., Torrecillas, S., Montero, D., Gini, E., Makol, A., Valdenegro, V., Izquierdo, M., Terova, G., 2020. Assessment of dietary supplementation with galactomannan oligosaccharides and phytogenics on gut microbiota of European sea bass (Dicentrarchus Labrax) fed low fishmeal and fish oil based diet. PLoS One 15, e0231494. https://doi.org/10.1371/journal.pone.0231494.

Ringø, E., Song, S.K., 2016. Application of dietary supplements (synbiotics and probiotics in combination with plant products and β-glucans) in aquaculture. Aquac. Nutr. 22, 4–24. https://doi.org/10.1111/anu.12349.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.

Rombout, J.H.W.M., Abelli, L., Picchietti, S., Scapigliati, G., Kiron, V., 2011. Teleost intestinal immunology. Fish Shellfish Immunol. 31, 616–626. https://doi.org/10.1016/j.fsi.2010. 09.001.

Sang, H.M., Kien, N.T., Thanh Thuy, N.T., 2014. Effects of dietary mannan oligosaccharide on growth, survival, physiological, immunological and gut morphological conditions of black tiger prawn (Penaeus monodon Fabricius 1798). Aquac. Nutr. 20, 341–348. https://doi.org/10.1111/anu.12083.

Satouchi, M., Watanabe, T., Wakabayashi, S., Ohokuma, K., Koshijima, T., Kuwahara, M., 1996. Digestibility, absorptivity and physiological effects of cellooligosaccharides in human and rat. J. Japanese Soc. Nutr. Food Sci. (Japan) 49, 143–148. https://agris.fao.org/agris-search/search.do?recordID=JP1997001562.

Schar, D., Zhao, C., Wang, Y., Larsson, D.G.J., Gilbert, M., Van Boeckel, T.P., 2021. Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. Nat. Commun. 12, 5384. https://doi.org/10.1038/s41467-021-25655-8.

Sichert, A., Corzett, C.H., Schechter, M.S., Unfried, F., Markert, S., Becher, D., Fernandez-Guerra, A., Liebeke, M., Schweder, T., Polz, M.F., 2020. Verrucomicrobia use hundreds of enzymes to digest the algal polysaccharide fucoidan. Nat. Microbiol. 5, 1026–1039. https://doi.org/10.1038/s41564-020-0720-2.

Singh, A., Karimi, S., Vidakovic, A., Dicksved, J., Langeland, M., Ferreira, J.A., Taherzadeh, M.J., Kiessling, A., Lundh, T., 2021. Dietary filamentous Fungi and duration of feeding modulates gut microbial composition in rainbow trout (Oncorhynchus mykiss). Front. Mar. Sci. 8, 728569 https://doi.org/10.3389/fmars.2021.728569.

Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition–a review. Food Chem. 127, 1409–1426. https://doi.org/10.1016/j.foodchem.2011.02.042.

Soleimani, N., Hoseinifar, S.H., Merrifield, D.L., Barati, M., Abadi, Z.H., 2012. Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (Rutilus rutilus) fry. Fish Shellfish Immunol. 32, 316–321. https://doi.org/10.1016/j.fsi.2011.11.023.

Song, J., Jiao, L.F., Xiao, K., Luan, Z.S., Hu, C.H., Shi, B., Zhan, X.A., 2013. Cello-oligosaccharide ameliorates heat stress-induced impairment of intestinal microflora, morphology and barrier integrity in broilers. Anim. Feed Sci. Technol. 185, 175–181. https://doi.org/10.1016/j.anifeedsci.2013.08.001.

Song, S.K., Beck, B.R., Kim, D., Park, J., Kim, J., Kim, H.D., Ringø, E., 2014. Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol. 40, 40–48. https://doi.org/10.1016/j.fsi.2014.06.016.

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E.T., Salinas, I., 2015. Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (Oncorhynchus mykiss). Aquaculture 435, 120–127. https://doi.org/10.1016/j.aquaculture.2014.09.027.

Tsujita, T., Tsukada, H., Nakao, M., Oshiumi, H., Matsumoto, M., Seya, T., 2004. Sensing bacterial flagellin by membrane and soluble orthologs of toll-like receptor 5 in rainbow trout (Onchorhynchus mikiss). J. Biol. Chem. 279, 48588–48597. https://doi.org/10.1074/jbc. M407634200.

Uyeno, Y., Kawashima, K., Hasunuma, T., Wakimoto, W., Noda, M., Nagashima, S., Akiyama, K., Tabata, M., Kushibiki, S., 2013. Effects of cellooligosaccharide or a combination of cellooligosaccharide and live clostridium butyricum culture on performance and intestinal ecology in Holstein calves fed milk or milk replacer. Livest. Sci. 153, 88–93. https://doi.org/10.1016/j.livsci.2013.02.005.

Walker, A.W., Ince, J., Duncan, S.H., Webster, L.M., Holtrop, G., Ze, X., Brown, D., Stares, M.D., Scott, P., Bergerat, A., 2011. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J. 5, 220–230. https://doi.org/10.1038/ismej.2010.118.

Wang, C., Li, Z., 2020. Growth performance, digestive enzyme activity and immune response of Japanese sea bass, Lateolabrax japonicus fed with fructooligosaccharide. Aquac. Nutr. 26, 296–305. https://doi.org/10.1111/anu.12990.

Wang, G., Zhou, Y., Huang, W., Huang, Y., Liu, X., Dong, S., 2010. Effects of xylooligosaccharide on grow, body composition and non-specific immunity in Litopenaeus vannamei. Freshw. Fish 40, 55–58. http://en.cnki.com.cn/Article_en/CJFDTotal-DSYY201005011.htm.

Wang, A.R., Ran, C., Ringø, E., Zhou, Z.G., 2018. Progress in fish gastrointestinal microbiota research. Rev. Aquac. 10, 626–640. https://doi.org/10.1111/raq.12191.

Xu, X., Li, X., Xu, Z., Yao, W., Leng, X., 2021. Dietary Azomite, a natural trace mineral complex, improved the growth, immunity response, intestine health and resistance against bacterial infection in largemouth bass (Micropterus salmoides). Fish Shellfish Immunol. 108, 53–62. https://doi.org/10.1016/j.fsi.2020.11.016.

Ze, X., Le Mougen, F., Duncan, S.H., Louis, P., Flint, H.J., 2013. Some are more equal than others: the role of "keystone" species in the degradation of recalcitrant substrates. Gut Microbes 4, 236–240. https://doi.org/10.4161/gmic.23998.

Zhang, Z., 2014. The effects of pectin and Xylan on growth, digestion and intestinal Flora in Chinese mitten crab (Eriocheir sinensis) and yellow catfish (Pelteobagrus fulvidraco). Dr. Diss Soochow Univ 557, 738343.

Zhang, J., Liu, Y., Tian, L., Yang, H., Liang, G., Xu, D., 2012. Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 33, 1027–1032. https://doi.org/10.1016/j.fsi.2012.05.001.

Zhang, C.-N., Li, X.-F., Jiang, G.-Z., Zhang, D.-D., Tian, H.-Y., Li, J.-Y., Liu, W.-B., 2014. Effects of dietary fructooligosaccharide levels and feeding modes on growth, immune responses, antioxidant capability and disease resistance of blunt snout bream (Megalobrama amblycephala). Fish Shellfish Immunol. 41, 560–569. https://doi.org/10.1016/j.fsi.2014.10.005.

Zhang, Y., Chen, P., Liang, X.F., Han, J., Wu, X.F., Yang, Y.H., Xue, M., 2019. Metabolic disorder induces fatty liver in Japanese seabass, Lateolabrax japonicas fed a full plant protein diet and regulated by cAMP-JNK/NF-kB-caspase signal pathway. Fish Shellfish Immunol. 90, 223–234. https://doi.org/10.1016/j.fsi.2019.04.060.

Zhong, C., Ukowitz, C., Domig, K.J., Nidetzky, B., 2020. Short-chain cello-oligosaccharides: intensification and scale-up of their enzymatic production and selective growth promotion among probiotic bacteria. J. Agric. Food Chem. 68, 8557–8567. https://doi.org/10.1021/acs.jafc.0c02660.

Zhou, Z., Ding, Z., Huiyuan, L.V., 2007. Effects of dietary short-chain fructooligosaccharides on intestinal microflora, survival, and growth performance of juvenile white shrimp, *Litopenaeus vannamei*. J. World Aquac. Soc. 38, 296–301. https://doi.org/10.1111/j.1749-7345.2007.00099.x.

Zhou, H., Liu, Y., Fan, J., Huang, H., Deng, J., Tan, B., 2022. Feeding rainbow trout with different types of non-starch polysaccharides: impacts on serum metabolome and gut microbiota. Metabolites 12, 1167. https://doi.org/10.3390/metabo12121167.