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Fermentation of Arabinoxylan-Oligosaccharides, Oligofructose and their Monomeric Sugars by Hindgut Bacteria from Siberian Sturgeon and African Catfish in Batch Culture *in vitro*

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Abstract

The *in vitro* fermentation of two Non-Digestible Oligosaccharide (NDO) preparations, Arabinoxylan-Oligosaccharides (AXOS) and Oligofructose (OF), and their respective monomeric sugars, xylose and fructose, were investigated by hindgut microbiota of two major aquaculture fish species, Siberian sturgeon (*Acipenser baerii*) and African catfish (*Clarias gariepinus*). Inocula from the hindgut of both fish species were incubated for 48 h in bottles containing 1.0% of one of four substrates, i.e. AXOS, OF, xylose or fructose. Amounts and profiles of produced Short-Chain Fatty Acids (SCFAs) differed between the two fish species and substrates. The hindgut microbiota of Siberian sturgeon has a higher fermentation capacity than the microbiota from African catfish. Xylose was much easier fermented than AXOS by microbiota from Siberian sturgeon whereas OF was quicker fermented than fructose with African catfish inoculum. The SCFAs were dominated by acetic acid for both fish species and for all substrates. Fermentation of OF and fructose by hindgut microbiota of Siberian sturgeon also yielded high amounts of butyric and branched-chain fatty acids after 48 h incubation. Results of this study suggest that AXOS, OF, and their monomeric sugars have an impact on microbial fermentation activity of hindgut microbiota from Siberian sturgeon and African catfish in a substrate and species dependent manner.

Keywords: Non-digestible oligosaccharide; Siberian sturgeon; African catfish; SCFA; *in vitro*

Introduction

There is currently increasing interest to understand better the composition of gut bacterial community, and their role in animal nutrition, physiology and immunology. It is well known that most terrestrial vertebrate herbivores contain populations of symbiotic organisms that play a key role in digestion by breaking down plant cell walls (cellulose and hemicellulose) to simple compounds such as Short-Chain Fatty Acids (SCFAs). Short-chain fatty acids, the main acidic products of bacterial fermentation, contribute towards a low colonic pH, have a direct inhibitory activity towards important gastrointestinal pathogens [1], and provide a major source of useful energy and nutrients for the host and for the colonocytes [2]. The presence of SCFAs in the hindgut of herbivorous, omnivorous and carnivorous fish species suggests the occurrence of microbial fermentation in fish, inducing some physiological effects [3-10].

In recent years, many research efforts have focused on the modulation of the colonic microbiota and their fermentation processes, using Non-digestible Oligosaccharides (NDOs) as prebiotic agents, with the aim of improving host health [11-13]. Non-digestible oligosaccharides are low molecular weight carbohydrates intermediate in nature between simple sugars and polysaccharides. The NDOs possess important physicochemical and physiological properties and are claimed to behave as dietary fibers and prebiotics. It has been well proven that NDOs that target the colon, affect the internal environment and bacterial community composition, and enhance the concentration of SCFA such as acetate, propionate and butyrate. Therefore, their industrial applications have rapidly increased in the last few years, both in prebiotic formulations and in symbiotic products (containing probiotic organisms and prebiotic oligosaccharides). According to Roberfroid [14] and Van Loo [15], only a few dietary NDOs, namely fructan inulin and oligofructose (OF, enzymatic hydrolysate of inulin) and trans-galactooligosaccharide, classify as prebiotics for humans, livestock and companion animals.

Reported beneficial health effects of prebiotics in humans as well as in livestock or companion animals have recently attracted scientific attention to their possible utilization for economically important aquaculture organisms [16-19]. Intestinal microbiota fermentation of carbohydrates has been studied in red seabream (*Pagrus major*) fed diets containing lactosucrose [20], in tilapia (*Oreochromis niloticus*) fed α-starch, sodium alginate, kaolin, cellulose or chitin diets [21], in common carp (*Cyprinus carpio* L.) fed soybean–oligosaccharide, raffinose, gentobiose, isomalto-oligosaccharides, lactosucrose, xylo-oligosaccharide, lactosucrose, kestose, lactulose, or 6-&4-galactosyllactose in diets [22]. Positive effects of prebiotics have been shown on growth performance, innate immunity, haematological and serum biochemical parameters, microbial fermentation and autochthonous intestinal microbiota of sturgeon species [23-26].

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Fermentation of various carbohydrates using inocula of fish hindgut microbes has also been reported in common carp (*Cyprinus carpio*) [22], tilapia (*Oreochromis niloticus*) and European seabass (*Dicentrarchus labrax*) [27].

In this study, we aimed to investigate the degrading capacity of Arabinoxylan-oligosaccharides (AXOS) and xylose, compared with one of the most known prebiotics, Oligofructose and its constituent monomer fructose, by the microbiota from hindgut of two major aquaculture fish species, African catfish (*Clarias gariepinus*) and Siberian sturgeon (*Acipenser baerii*), either preadapted or not to dietary AXOS or OF.

Arabinoxylan-oligosaccharides, a new class of candidate prebiotics, are fragmentation products of arabinoxylans (AX), which occur in the cell wall of many cereal grains, consisting of a main chain of beta-1,4-linked D-xylopyranosyl units to which O-2 and/or O-3-L-arabinofuranosyl units are linked [28,29]. Based on the fragmentation processes, different preparations of AXOS with varying Degrees of Polymerization (DP) and arabinose-to-xylose ratio (DS) are produced. The structural properties of AXOS determine to a large extent their physicochemical effects and fermentation processes [30-34] as well as their immunological modulation in different organisms [31,35].

Our earlier study on prebiotic potential of AXOS in juvenile Siberian sturgeon indicated that administration of AXOS in fish diet increased SCFAs production in Siberian sturgeon's hindgut [36]. Moreover, our results showed that AXOS fermentation in hindgut of Siberian sturgeon is structure dependent. Concentrations of acetate, butyrate and total SCFAs were higher in the hindgut of fish fed 2% AXOS with average degree of polymerization (avDP) of 32 and average degree of substitution (avDS) of 0.30 (AXOS-32-0.30) in comparison to 2% AXOS-3-0.30. *In vitro* analysis of AXOS (with different (DP) and (DS)) and its constituent monomeric sugar, xylose, may be important for future studies investigating the prebiotic potential of new AXOS sources in fish. A concern related to investigating *in vitro* fermentation is the extent to which microbial adaptation to a dietary fiber source affects the outcomes measured. The diet consumed by the animal may greatly influence the results of any *in vitro* analysis performed [37].

This encouraged us to study the fermentation rate and profile of these groups of candidate prebiotics in the hindgut of two different fish species, African catfish and Siberian sturgeon. In addition the capacity of hindgut bacteria from both species to adapt to dietary NDOs was also investigated.

African catfish and Siberian sturgeon differ considerably in feeding habit (African catfish being an omnivorous and Siberian sturgeon a carnivorous fish) and gastrointestinal anatomy. Previous workers have suggested that specialized intestinal morphology is required for efficient hindgut fermentation [38]. Therefore, these species are expected to possess different fermentation capabilities, due to different intestinal morphology and most likely also gut microbial composition.

Materials and Methods

Fish and diets

In two parallel feeding experiments, African catfish and Siberian sturgeon were reared in 60-l glass tanks connected to a flow through system and daily fed experimental diets at 3.0% of their body weight for 8 and 10 weeks, respectively. Additionally to commercial feeds, experimental diets contained 2.0% AXOS-32-0.30 (Laboratory of Food Chemistry and Biochemistry, KU Leuven, Belgium), 2.0% OF (avDP=4; Orafti, Tienen, Belgium) or 2.0% cellulose (control diet).

Collection of hindgut contents

At the end of the experimental feeding period and 10 h after the last meal, fish were killed and disinfected with a benzalkolium chloride solution (0.1%). From each species, the hindgut was aseptically removed from six fish fed control diets as well as from six fish per experimental diet (AXOS or OF). Individual hindgut contents were quickly collected in pre-weighed plastic tubes.

Inoculum preparation, Substrates and Incubation

Individual fish inocula were homogenized by vortex for 3 min and were diluted 5 times in phosphate buffer (0.1M, pH=7.4) containing Na-thioglycolate (1g l^{-1}) to ensure anaerobic conditions. Inocula (10 ml) from control fish and from fish fed AXOS diets were added to 50 ml tryptic soy broth containing 0.6 g AXOS or xylose as substrate. The same amount of inoculum (10 ml) from control fish and from fish fed an OF diet was added to 50 ml tryptic soy broth containing 0.6 g OF or Fructose as substrate. The final concentration of saccharide substrates in the cultures was 1.0% (w/v). Each substrate condition was prepared in triplicate with inocula from three fish fed identical diets. The first sampling was taken 30 min after inoculation (time 0), the content of each bottle was mixed, incubated anaerobically (flushing of the headspace with nitrogen (N2) for 30 min to remove oxygen) in darkness at 25°C for 48 h under permanent soft agitation. Samples were taken at subsequent time points (2, 4, 24 and 48 h), each time followed by flushing. The samples were stored at −20°C for later analysis of SCFA.

Analysis of Short Chain Fatty Acid (SCFA)

An aliquot (2.0 ml) of each sample was vortex, brought in a centrifuge tube and the following products added: 0.5 ml H_2SO_4 (9.0 M), 0.4 g NaCl, 0.4 ml of internal standard (2-methyl hexanoic acid, 6 µl ml^{-1}) and 2.0 ml diethyl ether. Samples were rotated for 2 min and centrifuged at 2,000×g for 3 min. The ether layer containing SCFA extracts was transferred to the vitatrontubes. The ether extract was injected in a CE Instruments GC 8000 gas chromatograph with an AS 800 auto sampler and a flame ionization detector. Nitrogen gas was used as carrier gas. Temperatures during the separation ranged from 120°C to 200°C with a linear increase of 10°C min^{-1}. The injector temperature was 220°C and the detector temperature 200°C. Concentrations of different SCFA were calculated based on standards with known concentrations of the different acids.

Statistical analysis

Values were expressed as means of three replicates. A three way repeated measures ANOVA was used to investigate the effect of *in vivo* diet (control or oligosaccharide), *in vitro* substrate (AXOS, xylose, OF, fructose), and incubation time (0, 2, 4, 24 or 48 h) on SCFA production, using Statistical Analysis System software 9.1 (SAS Institute, Cary, NC). Data are presented as mean ± standard error of mean (SEM) and test results evaluated at p=0.05 significance level.

Results

SCFA profiles and rate

Tables 1 and 2 summarize the profiles of the most important fermentation end-products (acetic, propionic, butyric and branched-chain fatty acids) of different saccharides by microbiota from the hindgut of Siberian sturgeon and African catfish, respectively, at the fermentation peak times (24 h and 48 h). For both fish species and

Incubation time	Diet *in vivo*	Substrate *in vitro*	TotSCFA[1] mg l^{-1}	Ac mg l^{-1}	Pr mg l^{-1}	Bu mg l^{-1}	BC mg l^{-1}	Ac (%)	Pr (%)	Bu (%)	BC (%)
24 h	Control	xylose	3296 ± 459^{a}	3073 ± 442^{ad}	123 ± 18^{ab}	9 ± 1	86 ± 59	93	4	0	2
		AXOS	1520 ± 229^{b}	1368 ± 179^{b}	117 ± 55^{ab}	10 ± 1	7 ± 1	91	7	1	1
	AXOS	xylose	3539 ± 439^{a}	3459 ± 411^{ac}	35 ± 3^{a}	37 ± 31	5 ± 1	98	1	1	0
		AXOS	1896 ± 158^{ab}	1686 ± 146^{bd}	178 ± 14^{b}	10 ± 2	6 ± 1	89	9	1	0
	Control	Fructose	2653 ± 397^{ab}	2580 ± 372^{abcd}	33 ± 14^{a}	13 ± 3	23 ± 18	97	1	0	1
		OF	2321 ± 392^{ab}	2096 ± 357^{abcd}	191 ± 25^{b}	8 ± 1	21 ± 16	90	8	0	1
	OF	Fructose	1929 ± 353^{ab}	1849 ± 320^{bd}	41 ± 14^{a}	13 ± 4	20 ± 14	96	2	1	1
		OF	2434 ± 298^{ab}	2178 ± 233^{abcd}	175 ± 11^{b}	8 ± 1	66 ± 54	90	7	0	2
48 h	Control	xylose	5073 ± 650	3832 ± 86a^{b}	112 ± 2^{ab}	338 ± 173	779 ± 404^{abc}	77	2	6	14
		AXOS	3653 ± 450	2808 ± 201^{b}	315 ± 32^{a}	40 ± 7	478 ± 247^{ac}	78	9	1	12
	AXOS	xylose	6072 ± 517	4443 ± 320^{a}	68 ± 10^{b}	868 ± 82	683 ± 295^{ac}	74	1	14	11
		AXOS	4447 ± 539	3538 ± 235^{ab}	253 ± 29^{ab}	63 ± 27	582 ± 278^{ac}	81	6	1	12
	Control	Fructose	4578 ± 1089	2979 ± 221^{b}	113 ± 61^{ab}	1119 ± 724	365 ± 104^{ac}	70	2	20	8
		OF	6730 ± 323	4400 ± 234^{a}	280 ± 47^{ab}	198 ± 38	1851 ± 144^{b}	65	4	3	28
	OF	Fructose	5144 ± 2117	1929 ± 400^{c}	102 ± 43^{ab}	2041 ± 1353	1021 ± 514^{abc}	55	2	27	15
		OF	6531 ± 42	3889 ± 227^{ab}	257 ± 84^{ab}	246 ± 137	2121 ± 31b	60	4	4	32

[1]Tot SCFA=total short-chain fatty acids (acetic+propionic+butyric+iso-butyric+valeric+iso-valeric+caproic+isocaproic acid; mg l^{-1}); Ac=acetic acid; Pr=propionic acid; Bu=butyric acid; BC=branched-chain SCFA; Ac (%), Pr (%), Bu (%), BC (%)=acetic, propionic, butyric and branched-chain acid as relative proportion to Tot SCFA.

Mean values with different superscripts within the same column and incubation time point differ significantly ($p<0.05$).

Table 1: Fermentation end-products after incubation of hindgut inocula of Siberian sturgeon with different substrates for 24 and 48 h.

Incubation time	Diet *in vivo*	Substrate *in vitro*	Tot SCFA[1] mg l^{-1}	Ac mg l^{-1}	Pr mg l^{-1}	Bu mg l^{-1}	BC mg l^{-1}	Ac (%)	Pr (%)	Bu (%)	BC (%)
24 h	Control	xylose	888 ± 72	850 ± 70	26 ± 2^{a}	4 ± 2^{a}	5 ± 2	96	3	0	1
		AXOS	943 ± 141	851 ± 112	29 ± 1^{abc}	9 ± 1^{b}	6 ± 1	91	3	1	1
	AXOS	xylose	1626 ± 162	1594 ± 158	21 ± 5^{ab}	4 ± 0^{ab}	5 ± 1	98	1	0	0
		AXOS	1145 ± 80	1063 ± 80	52 ± 13c	8 ± 0^{ab}	6 ± 1	93	5	1	1
	Control	Fructose	932 ± 35	907 ± 37	10 ± 2^{a}	7 ± 0^{ab}	4 ± 1	97	1	1	0
		OF	850 ± 76	820 ± 72	15 ± 6^{a}	7 ± 1^{ab}	3 ± 1	96	2	1	0
	OF	Fructose	1275 ± 427	1246 ± 425	11 ± 4^{a}	7 ± 1^{ab}	5 ± 1	97	1	1	0
		OF	1089 ± 155	1017 ± 151	58 ± 6^{b}	6 ± 0^{ab}	4 ± 1	93	5	1	0
48 h	Control	xylose	3472 ± 287^{a}	3419 ± 286^{a}	52 ± 2^{a}	2 ± 1	0 ± 0	98	2	0	0
		AXOS	949 ± 113^{b}	857 ± 111^{b}	86 ± 5^{b}	5 ± 0	1 ± 0	90	9	1	0
	AXOS	xylose	2891 ± 72^{a}	2878 ± 63^{a}	10 ± 10^{d}	2 ± 0	0 ± 0	100	0	0	0
		AXOS	999 ± 71^{b}	903 ± 73^{b}	78 ± 4^{b}	5 ± 0	3 ± 0	90	8	1	0
	Control	Fructose	485 ± 35^{b}	481 ± 35^{b}	1 ± 0^{bd}	2 ± 0	0 ± 0	99	0	0	0
		OF	1122 ± 88^{bc}	1018 ± 84^{bc}	99 ± 3^{b}	2 ± 1	0 ± 0	91	9	0	0
	OF	Fructose	646 ± 120^{b}	639 ± 119^{b}	2 ± 2^{bd}	3 ± 0	1 ± 0	99	0	0	0
		OF	1694 ± 93^{c}	1552 ± 87^{c}	136 ± 4^{c}	3 ± 1	1 ± 0	92	8	0	0

[1]Tot SCFA=total short-chain fatty acids (acetic+propionic+butyric+iso-butyric+valeric+iso-valeric+caproic+isocaproic acid; mg l^{-1}); Ac=acetic acid; Pr=propionic acid; Bu=butyric acid; BC=branched-chain SCFA; Ac (%), Pr (%), Bu (%), BC (%)=acetic, propionic, butyric and branched-chain acid as relative proportion to Tot SCFA.
Mean values with different superscripts within the same column and incubation time point differ significantly ($p<0.05$).

Table 2: Fermentation end-products after incubation of hindgut inocula of African catfish with different substrates for 24 and 48 h.

for all substrates, acetic acid showed the highest SCFA concentration. The peak amount of acetic acid was produced by inocula of Siberian sturgeon previously fed a control diet and incubated by oligofructose for 48 h. Moreover, at time point 24 h, the amount of acetic acid was significantly higher for fish inocula incubated by xylose than those incubated by AXOS, irrespective of the previous *in vivo* feeding. For African catfish, the highest amount of acetic acid production was observed by the inocula of fish previously fed control diet and incubated by xylose for 48 h. Unrelated to *in vivo* feeding, at the point of 48h, incubation of African catfish inocula by xylose resulted in higher concentration of acetic acid than incubation by other substrates. In both fish species, incubation of fish inocula by OF resulted in higher acetic acid production, regardless to their *in vivo* feeding.

Very low concentrations of iso-butyric, valeric, iso-valeric, caproic and iso-caproic acids were also detected (results not shown). The proportion of propionic, butyric and branched-chain fatty acids in both fish species was very low (max. 7 mg l^{-1}) for all substrates during early incubation times (0, 2 and 4 h), but had increased after 24 and 48 h (Tables 1 and 2). This increase was more important with inocula from Siberian sturgeon than from African catfish. Unrelated to *in vivo* feeding, the highest production of propionic acid was observed in batches of Siberian sturgeon inoculated by OF for 24 ($p<0.05$) or 48 h ($p>0.05$). The proportion of butyric and branched-chain fatty acids measured after 48 h of incubation reached 28% and more in batches inoculated with Siberian sturgeon hindgut contents in the presence of fructose and OF (Table 1), but remained very low (≤1%) in the case of African catfish inocula (Table 2). Overall results demonstrated that batch cultures inoculated with Siberian sturgeon hindgut contents produced on average 350% more SCFA than those inoculated with African catfish hindgut contents (Figures 1 and 2). At least at 24 and 48

h of incubation, the SCFA profiles were different between these two fish species (Tables 1 and 2), acetic acid being more dominant in African catfish than in Siberian sturgeon.

SCFA production in fish inocula incubated by AXOS and xylose

Figure 1A and B show the total SCFA production during 48 h of *in vitro* fermentation of AXOS or their respective monomeric sugar (xylose), by hindgut microbiota from the Siberian sturgeon and African catfish. *In vitro* incubation of Siberian sturgeon hindgut contents with AXOS produced more SCFA than incubation with xylose during the earlier incubation stages (0, 2 and 4 h) ($p<0.001$), but not any longer at later incubation times (24 and 48 h) where xylose incubations produced the highest SCFA levels (Figure 1A). This difference was significant ($p<0.01$) at 24 h, but no longer at 48 h ($p=0.07$). Similar, but less pronounced patterns in SCFA production were observed in batch cultures inoculated with African catfish hindgut contents. Batch cultures incubated for 48 h with xylose resulted in strongly significant ($p<0.001$) higher production of SCFA than with AXOS (Figure 1B).

For both fish species, SCFA production remained very low during the first 4 h *in vitro* incubation, whereas it increased significantly for all conditions after 24 and 48 h. A significant different impact on SCFA production was noticed at different incubation times in both fish species for *in vitro* addition of AXOS compared with xylose (Figures 1A and 1B). For Siberian sturgeon this was most pronounced after 24 h and for African catfish after 48 h.

SCFA production in fish inocula incubated by Oligofructose and Fructose

Incubation of Siberian sturgeon hindgut contents with either OF or fructose did not affect differently the SCFAs production at any of the incubation times (Figure 2A). In African catfish, an increase in SCFA production ($p<0.001$) was only observed at 48 h of incubation when OF was used as substrate (Figure 2B). This increase was even higher after *in vivo* pretreatment with OF.

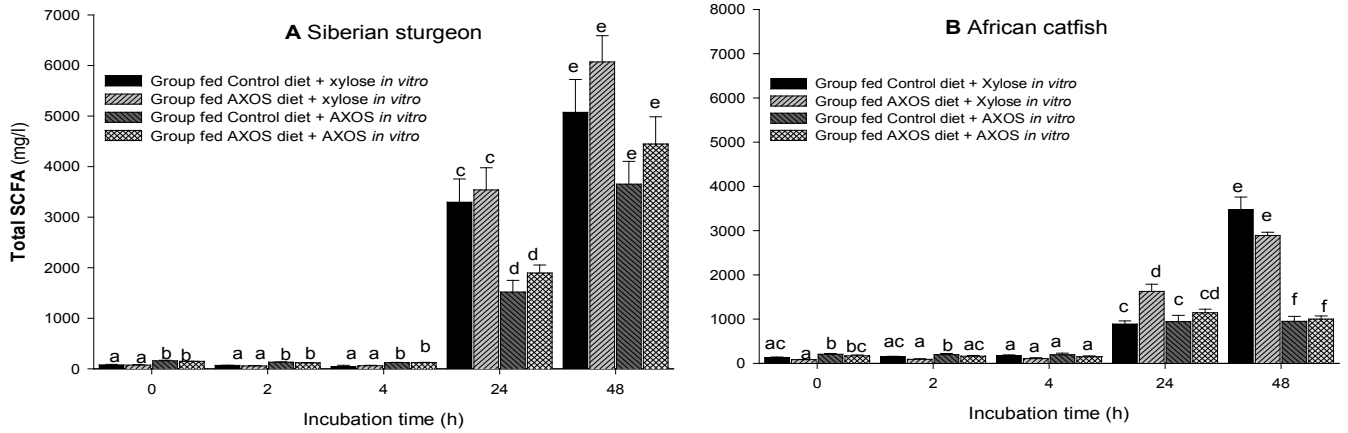

Figure 1: Short-chain fatty acids (SCFAs) production *in vitro* in cultures of hindgut contentsfrom Siberian sturgeon **(A)** and African catfish **(B)** fed either control diets or diets containing 2%AXOS-32-0.30 and incubated *in vitro* for different times with 1% AXOS or xylose. Data wasexpressed as mean ± SEM (n=3). For each fish species and time point, bars with differentsuperscript differ significantly ($p<0.05$).

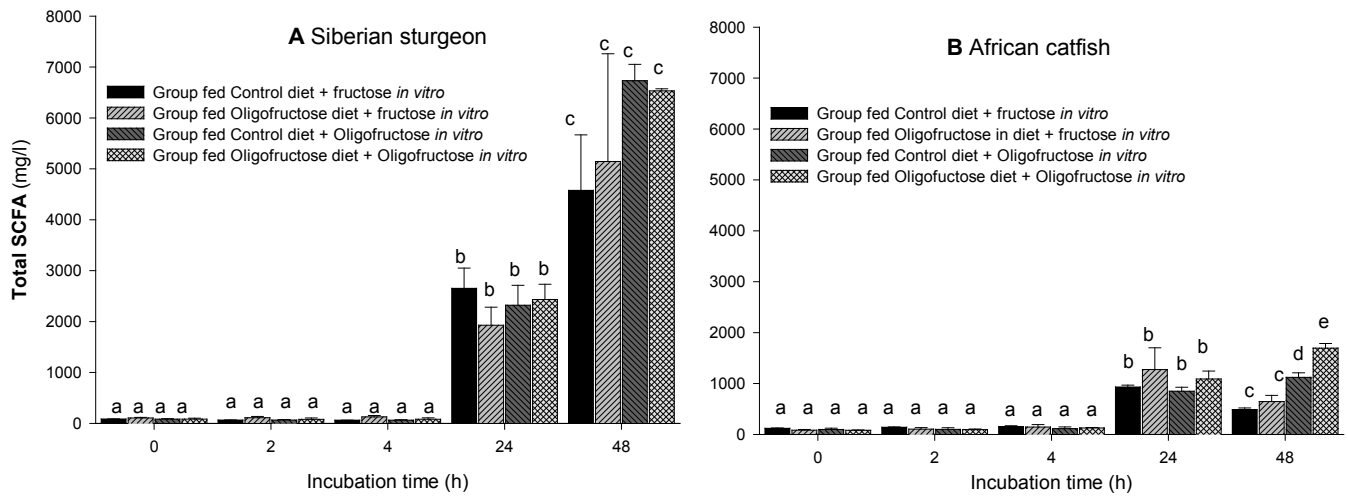

Figure 2: Short-chain fatty acids (SCFAs) production *in vitro* in cultures of hindgut contents fromSiberian sturgeon **(A)** and African catfish **(B)** fed either control diets or diets containing 2% OFand incubated *in vitro* for different times with 1% fructose or OF. Data are expressed as mean ± SEM (n=3). For each fish species and time point, bars with a different superscript differsignificantly ($p<0.05$).

Comparison of the impact of OF versus fructose as a substrate for *in vitro* incubation did not show a difference in the SCFA production in Siberian sturgeon and gave only limited differences for African catfish after 48 h incubation (Figures 2A and 2B). At that time the total *in vitro* SCFA production for both species was higher with OF substrate than with fructose.

Impact of feeding Siberian sturgeon/African catfish (*in vivo*) with dietary NDOs on *in vitro* fermentation capacity of hindgut microbiota

Production rates of SCFA were not affected by the dietary treatment of the fish, although adaptation by AXOS tended to increase *in vitro* SCFAs production in inocula incubated for 24 or 48 h by xylose or AXOS (Figure 2A). *In vivo* feeding of African catfish with an AXOS or OF diet positively affected the *in vitro* SCFAs production after 24 h or 48 h incubation by xylose or OF, respectively (Figures 1B and 2B).

Discussion

The focus was to examine the fermentation patterns of AXOS, OF and their monomeric building blocks by fish fecal microbiota. This study helps to better understand the *in vivo* consumption and effects of mono and oligosaccharides in the hindgut of fish, which is the result of a cooperative cross-feeding process between different bacteria.

Hindgut microbiota of both Siberian sturgeon and African catfish ferment NDOs and their monomeric sugars, as evidenced by substantial SCFA production (Figures 1 and 2). This can have important consequences for fish health in several ways. SCFA may be beneficial for the fish in terms of energy supply as already indicated for other fish species [39,40].

The same pattern of fermentation was observed for inocula of both fish species as all substrates were fermented slowly at the onset of incubation (0, 2 and 4 h). This might be linked to a lag phase of bacterial growth, the delay before the start of exponential growth. Significant amounts of SCFAs were produced after 24 and 48 h of incubation. These time points correspond to the stage at which most fish gut bacteria reach a maximum biomass *in vitro* [41].

For most fish species so far studied, acetic acid is in generally the most dominant SCFA produced during intestinal microbial fermentation of carbohydrates. The predominant production of acetic acid by hindgut microbiota from Siberian sturgeon and African catfish in this study is in agreement with results for other fish and terrestrial animals, both *in vitro* and *in vivo* [4,9,36,42]. In carp, however, certain carbohydrate substrates resulted in high amounts of propionic acid during an incubation period of 6 h [22]. Butyric acid production was relatively high using inocula from tilapia and seabass during incubation periods of up to 168 h [27]. In this study, the proportion of butyric and branched-chain fatty acids reached 28% in batches inoculated with Siberian sturgeon hindgut contents in the presence of fructose and OF at the end of the fermentation period (48 h) (Table 1). When compared to African catfish, hindgut microbiota from Siberian sturgeon produced relatively higher amounts of butyric acid in the presence of a fructose substrate and more branched-chain fatty acids in the presence of OF as substrate, at least after 48 h of incubation (Table 2).

Our results indicate clear substrate preferences among fish inocula for fermentation. The chemical structures of saccharides differently affect the fermenting capacity of fish species-specific bacteria in Siberian sturgeon and African catfish. Incubation of fish inocula with xylose or OF induced a higher SCFA production, particularly acetic acid, than incubation with AXOS or fructose. This is valid for Siberian sturgeon and African catfish, regardless of previous *in vivo* diet. These results are in line with previous findings of Kihara and Sakata [22], who using various substrates differing in monomeric building blocks and in glycosidic linkages, concluded that structural differences affect the fermentability of oligosaccharides in common carp. Pollet et al. [43] reported that the extent of fermentation was structure-dependent and decreased with increasing structural complexity, represented by a higher arabinose to xylose ratio and avDP of AXOS. The substrate preferences under *in vivo* condition indicated that Siberian sturgeon hindgut is better adapted to dietary AXOS in comparison to OF, but African catfish hindgut microbiota presents a higher adaptability to OF than dietary AXOS (Figures 1 and 2). This may related with the difference in hindgut microbial composition between these two fish species. The resident microbiota in certain fish species, including Siberian sturgeon and African catfish, is a relatively less studied aspect that plays potentially a role in health conservation and nutrition optimization. Based on cultivable isolates, Al-Harbi and Uddin [44] reported that gram-negative bacteria dominate up to 89% of catfish intestine, including *Aeromonas hydrophila, Shewanella putrefaciens, Vibrio* spp. and *Staphylococcus* spp. Based on cultivable isolates, Mahious et al. [24] reported the predominance of the yeasts and the bacteria Bacillus subtilis and Plesiomonas shigelloides in the gut of the Siberian sturgeon. High-throughput sequencing technology (454 pyrosequencing) indicated that the dominant bacteria in the hindgut of Siberian sturgeon are *Cetobacterium somerae, Eubacterium budayi, Candidatus arthromitus* sp., *Rhodobacter* spp. and *Plesiomonas* spp. [36]. The present results indicated that fish hindgut bacteria adapt to dietary AXOS and OF, although the change was not that strong (Figures 1 and 2). This results are in agreement with our earlier studies where we demonstrated a higher production of SCFA in fish fed AXOS with an avDP of 32 versus short avDP of 3 [36]. We indicated that AXOS with a relatively longer DP have a stronger resistance to saccharolytic fermentation than AXOS with shorter DP. The higher SCFA production in the groups of fish fed an AXOS diet can be linked with some potentially beneficial microorganisms, including *Lactobacillus* spp., *Clostridium* spp., *Eubacterium* spp. and *Lactococcus lactis* [36]. *Bifidobacterium* spp, *Lactobacilli Us* spp., *Enterococcus* spp., *Bacteroides* spp., *Prevotella* spp., *Clostridium* spp., *Ruminococcus* spp., *Eubacterium* spp. and *L. lactis* are able to break down the AX molecule by different enzymes, including endo-1, 4-ß-xylanases, α-L-arabinofuranosidases, ß-xylosidases, α-glucuronidases and ferulic acid esterases [45]. Microbial fermentation of oligofructose by Siberian sturgeon hindgut microbiota has already been reported by Mahious et al. [24].

The higher microbial fermentation of NDOs in batches inoculated with Siberian sturgeon hindgut contents in terms of SCFA production, compared to batches inoculated with African catfish gut content, could be explained by differences in initial microbial density and gut microbiota composition between these two species. Although we used the same amount of inoculum for each fish species in all batches, this does not necessarily mean that the initial microbial concentrations are identical. Rao [46] indicated that initial concentration of Bifidobacteria in the feces, independent of the dose of the fructo-oligosaccharides, influences the fermentation. The bacterial composition may also be related to fish age or specific individuals, and be influenced by the nutritional status, environmental conditions, and the complexity of the fish digestive system [44,47-49]. However, it has already been shown in the simulator of human intestinal microbial ecosystem, that AXOS supplementation can affect fermentation activity without significantly impacting the total microbial community [50].

Conclusion

In conclusion, the results of this study suggest that AXOS, OF, and their monomeric sugars change microbial fermentation activity of hindgut microbiota from Siberian sturgeon and African catfish, in a substrate and species dependent manner. The bacteria from the hindgut of Siberian sturgeon and African catfish can ferment AXOS and OF as well as their monomeric building blocks. Although xylose or fructose is broken down preferentially, it might be tested in an *in vivo* experiment whether these monomers are able to reach to hindgut. Large differences in fermentability and composition of fermentation end-products were also observed between the two fish species. The hindgut microbiota of Siberian sturgeon has a higher fermentation capacity than the microbiota from African catfish. Increased levels of fermentation of AXOS and OF might therefore influence gut health characteristics in Siberian sturgeon more easily than in African catfish, through the local supply of energy by produced SCFA and by affecting microbial composition through altered pH as well as SCFA levels and patterns. Additional research is needed to investigate the possible changes in bacterial population upon incubation with AXOS preparations.

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