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Oligosaccharides improve the flesh quality and nutrition value of Nile tilapia fed with high carbohydrate diet

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ABSTRACT

High level of carbohydrate in aquafeed could achieve cost-sparing effect, but it may cause adverse effects on flesh quality of aquatic products. An eight-week trial was conducted to investigate whether oligosaccharides-supplementation, including Galacto-oligosaccharides (GOS) and xylo-oligosaccharide (XOS), could systematically improve the growth performance, texture characteristics and nutrition composition of Nile tilapia fed with high-carbohydrate diet. The results indicated that GOS-supplementation improved the amino acid composition, while XOS-supplementation showed beneficial effects on growth performance. High-carbohydrate diet had adverse effects on fillet texture, while oligosaccharide-supplementation regulated the expression of muscle development-related genes to help restoring muscle texture properties. Furthermore, either high-carbohydrate or addition of oligosaccharides could change the intestinal microbiota composition and their metabolites. Further correlation analysis suggested that intestinal microbiota may account for the improvement in fish growth condition and texture characteristics. Application of oligosaccharides may be an innovative strategy for flesh quality modulation in aquaculture.

1. Introduction

Globally, aquatic products are the third-largest source of dietary protein for human consumption, accounting for 6.5% of the total protein supply or 16.4% of the total animal protein supply (Albert & Marc, 2018). Aquaculture becomes a vital contributor to the food supply, however, some issues including the increasing price of fishmeal caused by world supply fluctuations are restricting the rapid development of aquaculture (Shi et al., 2017). Carbohydrates as important non-protein energy sources for fish were incorporated in feed formulation to substitute expensive feeding ingredients. High-level of carbohydrates supplementation is regarded as an efficient way to promote the cost-sparing effect in aquafeed, but excessive supplementation of carbohydrates may induce metabolic disturbance, thus cause adverse effects on fish health. Juvenile mirror carp (*Cyprinus carpio*) fed with high-carbohydrate diet showed lower feed utilization and protein efficiency ratio, with a higher food conversion ratio (Li, Xu, Wang, Wang, Zhao, & Luo, 2016). Furthermore, high dietary carbohydrate could induce abnormally high blood glucose levels and excessive lipid accumulation in Nile tilapia (Xu et al., 2021). Considering the close relationship between the growth condition and flesh quality, excessive carbohydrates in the diet could also affect the texture and flesh quality. In the blunt snout bream, higher dietary carbohydrate significantly increased the content of monounsaturated fatty acid (MUFA) in muscle, while decreased the polyunsaturated fatty acid (PUFA) content (Wang et al., 2017). In addition, increased dietary carbohydrate significantly affected the hardness and gumminess of dorsal muscle in black sea bream (*Acanthopagrus schlegelii*) (Li, Tang, Yan, Lu, & Shao, 2014).

The quality of flesh consists of sensory properties (fillet color, texture, flavor), nutritional value (amino acids, fatty acids, protein) and freshness. These characteristics strongly depend on many factors including the inherent properties of fish (species, age, sex), environmental factors (temperature, salinity, pH), feeding history (nutrition composition, feeding ratio) (Grigorakis, 2007), and nutritional status (Lie, 2001). Previous studies suggested that supplementation of functional additives is a feasible way to reduce the side effects caused by high-carbohydrate diet (Li, Hu, Qiao, Du, & Zhang, 2020). However, it is still unclear whether the functional additives could restore the flesh quality caused by high carbohydrate diet.

Galacto-oligosaccharides (GOS) and xylo-oligosaccharides (XOS) are

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commonly used in aquaculture to promote fish immunity and growth performance (Guerreiro, Oliva-Teles, & Enes, 2018). GOS consists of β-linked galactose moieties with galactose or glucose at the reducing end. XOS is carbohydrate oligomers consisting of two to six xylose residues linked by β-(1,4)-xylosidic bonds. Positive effects of GOS and XOS were found in enhancing the systemic immune response in fish (Azeredo et al., 2017; Hoseinifar, Zoheiri, Dadar, Rufchaei, & Ringø, 2016). In addition, GOS could ameliorate diarrhea in suckling rats and XOS could improve the production performance of laying hens through the modulation of gut microbiota and intestinal healthy (Azagra-Boronat et al., 2018; Zhou et al., 2021), suggesting these two oligosaccharides could improve host physiological characteristics by affecting the intestinal health. It has been reported that addition of β-glucan, an immunomodulator, to the diet could improve the texture of fish fillets compared with the control group in Pengze crucian carp (*Carassius auratus* var. Pengze) (Cao et al., 2019). However, it is unclear whether GOS and XOS, often used as immunomodulators, could alleviate high-carbohydrate diet-induced systemic impacts, including the growth condition and the flesh quality in aquatic animals.

Nile tilapia is the second most farmed fish species worldwide. Although Nile tilapia have a higher capacity for carbohydrate utilization, high concentration of carbohydrate in the diet also caused metabolic syndromes in Nile tilapia (Boonanuntanasarn et al., 2018). An eight-week nutritional trial was conducted to determine whether the growth and flesh quality of Nile tilapia could be improved fed with high-carbohydrate diet supplemented with XOS and GOS. Growth performance, nutritional composition, texture characteristics and intestinal microbiota were investigated.

2. Materials and methods

2.1. Ethics statement

All experiments were performed under the guidance for the care and use of laboratory animals in China. This research was approved by the Committee on the Ethics of Animal Experiments of East China Normal University (ECNU) (No. F20201002).

2.2. Experimental design

Oligosaccharides, including xylo-oligosaccharide (XOS) and galacto-oligosaccharide (GOS), were purchased from Qingdao FTZ United International Co., Ltd., (Shandong, China). Juvenile Nile tilapia were obtained from shanghai Ocean University (Shanghai, China), and acclimatized in the tanks for 2 weeks before the beginning of the experiment. Male Nile tilapia were divided into four diet treatments, including control (CK, 30% carbohydrate diet), high-carbohydrate (HC, 42% carbohydrate diet), high-carbohydrate supplemented with GOS (HCG, 10 g·Kg^{-1} GOS) and high-carbohydrate supplemented with XOS (HCX, 10 g·Kg^{-1} XOS). The diet composition of four experimental diets was indicated in Supplementary Table 1. Dietary ingredients were ground and powdered and then mixed with oil and distilled water to form a paste. The paste was extruded through a screw extruder, and then was dried to<10% moisture at 50 °C to produce 2-mm-diameter pellets using a feed grinder. Juvenile Nile tilapia (an average body weight as group 5.51 ± 0.16 g) were randomly allocated to tanks (256L), with three tanks per treatment and each tank contained 30 individuals. The experimental cultivation system was an indoor static water model. The water used for fish culture was tap water aerated for one day, and half of the water in the tank was renewed daily. All fish were fed at 5% (Qian et al., 2021) of their average body weight per day for 8 weeks. Feeding consumption was adjusted every two weeks according to the weight. Fish were fed three times daily at 8:00, 12:00 and 16:00. During the whole trial, fish were supplied with compressed air via air-stones from air pumps, and the content of dissolved oxygen was 5.0–6.0 mg·L^{-1}. Water temperature was measured daily and kept at 27–30 °C. The content of nitrite in the water is not more than 0.1 mg·L^{-1} and the content of ammonia nitrogen is not more than 0.02 mg·L^{-1}, pH was between 7.3 and 7.6, and the natural photoperiodicity (12 h light-12 h dark) was used for light.

Table 1
Growth performance and body composition (%) of Nile tilapia (*Oreochromis niloticus*) fed with different diets for 8 weeks.

Parameters	Groups				
	CK	HC	HCG	HCX	P-value
WGR (%)	531.13 ± 12.64^{b}	562.41 ± 20.52^{ab}	564.42 ± 0.89^{ab}	598.86 ± 38.16^{a}	0.039
Body length (cm)	9.83 ± 0.65^{b}	10.08 ± 0.30^{ab}	10.17 ± 0.65^{ab}	10.45 ± 0.59^{a}	0.150
CF (%)	3.13 ± 0.19^{a}	3.47 ± 0.17^{b}	3.49 ± 0.25^{b}	3.48 ± 0.17^{b}	0.002
BMI	3.07 ± 0.12^{a}	3.49 ± 0.19^{b}	3.54 ± 0.29^{b}	3.64 ± 0.34^{b}	0.000
FCR	1.18 ± 0.03	1.16 ± 0.07	1.16 ± 0.01	1.18 ± 0.03	0.870
MFI (%)	1.14 ± 0.27^{bc}	1.53 ± 0.39^{a}	1.44 ± 0.22^{ab}	0.97 ± 0.20^{c}	0.012
HSI (%)	1.87 ± 0.13^{b}	2.21 ± 0.35^{a}	2.33 ± 0.24^{a}	2.11 ± 0.21^{ab}	0.028
Muscle moisture (%)	77.68 ± 0.42	77.46 ± 0.31	77.26 ± 0.53	77.61 ± 0.46	0.307
Muscle protein (%)	85.37 ± 2.54	85.16 ± 1.88	86.61 ± 0.66	86.53 ± 2.49	0.554
Muscle lipid (%)	7.98 ± 0.65	7.85 ± 0.84	8.68 ± 0.94	8.13 ± 1.23	0.399
Total moisture (%)	72.92 ± 0.86^{a}	71.91 ± 0.78^{ab}	71.11 ± 1.13^{b}	71.86 ± 0.80^{ab}	0.021
Total protein (%)	15.53 ± 0.22^{b}	16.05 ± 0.43^{ab}	15.62 ± 0.69^{b}	16.26 ± 0.32^{a}	0.009
Total lipid (%)	5.20 ± 0.48^{b}	5.82 ± 0.32^{a}	5.84 ± 0.47^{a}	4.79 ± 0.36^{b}	0.004

WGR, weight gain rate (n = 3 tanks); Body length (cm) (n = 9 individuals); CF, condition factor (n = 9 individuals); BMI, Body Mass Index (n = 9 individuals); FCR, Feed conversation ration (n = 3 tanks); MFI, Mesenteric fat index (n = 9 individuals); HSI, Hepatosomatic index (n = 9 individuals); Muscle moisture (%) (n = 6 individuals); Muscle protein (%) (n = 6 individuals); Muscle lipid (%) (n = 6 individuals); Total protein (%) (n = 6 individuals); Total lipid (%) (n = 6 individuals); Total moisture (%) (n = 6 individuals). Data was presented as means ± SEM. Statistical significance was determined by using one-way analysis of variance (ANOVA). P-vaule < 0.05 indicates a statistically significant difference among groups. Values in the same row with different letter superscripts were significantly different (P < 0.05).

2.3. Sample collection and preparation

At the end of the feeding trial, the body length of fish was measured to calculate the condition factor. Fish were euthanized with diluted tricaine methanesulfonate (20 mg·L^{-1} MS-222; Sigma, Saint Louis, USA) before sampling and dissected to obtain tissues for biochemical and molecular biological analyses. Gut content was collected and promptly frozen at −80 °C for SCFAs and intestinal microbial composition analysis. Six fish were used to analyze the body composition. All the procedures were carried out on the ice.

2.4. Biochemical analysis

Proximate composition of the diet and the whole fish body was determined by measuring moisture, crude protein and crude lipid contents according to the methods (AOAC, 2005; Folch, Less, & Sloane Stanley, 1957). In brief, moisture content was detected by drying the samples to a constant weight at 105 °C. Total protein content of samples was determined by a semi-automatic Kjeldahl System (FOSS, Hilleroed, Denmark) after the acid digestion. The total lipid of samples was

extracted using chloroform/methanol (2:1, v/v; Sinopharm, Shanghai, China). The differences in the weight of samples before and after experimental processing were used to calculate the moisture and crude lipid contents.

2.5. Texture profile analysis of muscles

Considering different cooking procedures may have different influences, the Texture Profile Analysis (TPA) was performed on raw meat using the QTS-25 surface analyzer (CNS Farnell, London, Britain) with the program of Texture Pro adaptation 2. Prior to the test, muscle samples were equilibrated to room temperature and cut into 0.5 cm thick sections. The samples were compressed twice at a crosshead speed of 0.50 mm·s^{-1} using a 6-mm diameter cylindrical probe, and the target esteem of compression was 0.5 mm. From the resulting force–time curves, the following parameters were determined: hardness (maximum force required to compress the sample); cohesiveness (the attractive forces within the same material which keep it together); springiness (ability of sample to recover its original form after the deforming force was removed); chewiness (the energy required to chew a solid food to the point adequate for swallowing); adhesiveness (the strength of the physical attraction between different materials) (Peleg, 2019).

2.6. Histological observation

Muscle samples were immediately fixed in 4% paraformaldehyde (Servicebio, Wuhan, China) after sampling and then embedded in paraffin. Sections with 5 μm thickness were stained with the hematoxylin-eosin (Servicebio, Wuhan, China) and then washed with 70% alcohol (Sinopharm, Shanghai, China). The stained samples were examined under a light microscope (Nikon, Tokyo Metropolis, Japan). The number of muscle fibers in each photo and the diameter (long diameter) of all muscle fibers were measured by using imaging software (Nis-Elements F package version 4.60). The percentage of muscle fiber diameter in each sample was counted within three ranges, including 0–35 μm, 35–55 μm and 55–130 μm. Six samples were used to quantify the diameter of muscles in each treatment.

2.7. Identification and quantification of amino acids and fatty acids

Amino acid profiles of muscle samples were detected by using an L-8900 High-speed Amino Acid Analyzer (Hitachi, Tokyo, Japan) (Junhao, Hu, Li, & Jiang, 2018). Briefly, samples of approximately 20 mg freeze-dried muscle were put into a thread screw neck vial with a screw cap. 5 mL HCl (6 N; Sinopharm, Shanghai, China) and 20 μL phenol (Sinopharm, Shanghai, China) were added. The tube was sealed under N_2 and put into the oven at 110 °C for 22 h for digestion. After cooling, the digested samples were centrifuged at 11000 × g for 20 min under 4 °C. The supernatant was dried in the nitrogen-blowing instrument (Quandao, Shanghai, China), then 1 mL HCl (0.02 N) was added and the mixture was centrifuged at 11000 × g for 20 min under 4 °C. Finally, 700 μL of the supernatant was used for amino acid determination. The packed column was Hitachi ion-exchange resin 2622 (4.6 mm × 60 mm, particle size 5 μm; Hitachi, Tokyo, Japan) and ninhydrin coloring solution was used as the reactive reagent for amino acids detection. Results were represented as mg·g^{-1} matter with all determinations performed in triplicate.

Fatty acid methyl ester (FAME) analysis was conducted by GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) with a GC Columns (60 m × 0.25 mm ID × 0.25 μm df; Restek, PA, USA). Two milligrams of lipid and 0.1 mg internal standard solution (1 mg·mL^{-1} C19:0; Dr. Ehrenstorfer GmbH, Augsburg, Germany) were put into a brown bottle. After drying with a vacuum dryer (Jinghong, Shanghai, China), 1 mL of 0.5 mol·L^{-1} KOH-CH_3OH (Sinopharm, Shanghai, China) (65 °C, 30 min), 1 mL of 14% BF_3-CH_3OH (Aladdin, Shanghai, China) (75 °C, 30 min), 1 mL of ddH_2O and 1 mL of Hexane (for HPLC; Sigma, Saint Louis, USA) were added. The bottles stand still for 2 min and centrifuged at 400 × g (centrifuge diameter 16.2 cm) for 5 min under 4 °C. The supernatant was then filtered through an organic phase filter and dried using a vacuum dryer. Finally, 200 μL hexane was added to dissolve the FAME for the subsequent test. High purity nitrogen was used as the carrier gas with a flow rate of 7.8 mL·min^{-1}. The temperature increased at a speed of 4 °C·min^{-1}, and the temperature was maintained at 240 °C for 30 min. The injector and detector were set at 250 and 260 °C, respectively. By comparing with the peak area of Nonadecylic acid (C19:0, 0.1 mg/2 mg lipid), the FAME content of each sample was quantified (mg·g^{-1}).

2.8. Gene expression quantification

Total RNA of sample was isolated by Tri Pure Reagent (Aidlab, Beijing, China). The quality and quantity of RNA were tested by NANODROP 2000 Spectrophotometer (Thermo, Waltham, USA). The 260/280 nm absorbance ratios of all RNA samples were 1.9–2.0. cDNA was synthesized by FastQuant RT Kit with gDNase (TIANGEN, Beijing, China) by S1000™ Thermal Cycler (Bio-Rad, California, USA) following the instructions. The primers for Quantitative PCR (qRT-PCR) were designed in National Center for Biotechnology Information Search database (NCBI) (https://www.ncbi.nlm.nih.gov/) (Supplementary Table 2). The qRT-PCR reaction was conducted at 95 °C for 10 min, 40 cycles of 95 °C for 5 s and 60 °C for 15 s. Elongation factor 1 α (*ef1α*) and *β-actin* were used as the reference genes. Melting curves of amplified products were generated to ensure the specificity of assays at the end of each run. Each qRT-PCR run was performed in triplicate and the negative control (no cDNA) was included. The relative mRNA expressions were calculated by using $2^{-\Delta\Delta CT}$ method.

Table 2
The absolute content of hydrolyzed amino acids (mg·g^{-1} tissue) and fatty acids composition (mg·g^{-1} total fatty acids) in the muscle of Nile tilapia (*Oreochromis niloticus*) fed with different diets for 8 weeks.

	Groups				
	CK	HC	HCG	HCX	P-value
[a]EAA	87.13 ± 0.75[a]	87.77 ± 1.25[a]	90.67 ± 0.55[b]	87.87 ± 0.43[a]	0.059
[b]NEAA	81.17 ± 1.01	81.75 ± 1.03	83.76 ± 0.19	81.54 ± 0.71	0.183
[c]DAA	80.51 ± 1[a]	81.04 ± 0.81[ab]	82.98 ± 0.17[b]	80.87 ± 0.49[ab]	0.129
[d]TAA	168.30 ± 2.98[a]	169.52 ± 3.94[ab]	174.43 ± 1.27[b]	169.41 ± 1.96[ab]	0.095
C18:3n-3	0.23 ± 0.02	0.21 ± 0.01	0.25 ± 0.00	0.25 ± 0.03	0.278
C20:5n-3	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.889
C22:6n-3	0.47 ± 0.02[b]	0.4 ± 0.01[a]	0.36 ± 0.01[a]	0.39 ± 0.03[a]	0.009
[e]SFA	9.09 ± 0.08	8.42 ± 0.15	8.84 ± 0.12	8.66 ± 0.33	0.181
[f]MUFA	0.79 ± 0.09	0.83 ± 0.03	0.93 ± 0.01	0.81 ± 0.02	0.268
[g]PUFA	5.52 ± 0.09[bc]	5.84 ± 0.22[c]	5.2 ± 0.06[b]	4.1 ± 0.09[a]	0.000
[h]n-3/n-6	8.36 ± 1.28	7.49 ± 0.36	6.54 ± 0.33	8.75 ± 0.76	0.269
[a]EAA	87.13 ± 0.75[a]	87.77 ± 1.25[a]	90.67 ± 0.55[b]	87.87 ± 0.43[a]	0.059

[a] EAA, essential amino acids; [b]NEAA, nonessential amino acids; [c]DAA, delicious amino acids; [d]TAA, total amino acids. Data was presented as means ± SEM (n = 3). [e]SFA, saturated fatty acids; [f]MUFA, mono-unsaturated fatty acids; [g]PUFA, poly-unsaturated fatty acids. [h]n-3 PUFA/n-6 PUFA: the ratio of n-3 poly-unsaturated fatty acids to n-6 poly-unsaturated fatty acids. Data was presented as means ± SEM (n = 6). Statistical significance was determined by using one-way analysis of variance (ANOVA). P-vaule < 0.05 indicates a statistically significant difference among groups. Values in the same row with different letter superscripts were significantly different (P < 0.05).

2.9. Determination of the intestinal microbial diversity

Total bacterial community DNA of the intestinal contents was extracted following the instruction of E.Z.N.A™ Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The V3V4 region of the bacterial 16S rRNA gene was amplified by using primers 338F (5′- ACTCCTACGGGAGG-CAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). After the purification, the amplified products were sequenced by Illumina MiSeq PE300 platform by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were quality-filtered by Trimmomatic and merged by Fast Length Adjustment of SHort reads (FLASH) according to certain criteria. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%. The principal co-ordinates analysis (PCoA) based on OTU compositions were determined to distinguish microbial compositions among different groups. All data were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com). The raw reads of all samples are available in the GenBank database (accession no. PRJNA613504).

2.10. Analysis of short-chain fatty acids

Intestinal content (200 mg) of three fish per treatment was suspended with 200 μL distilled water and then centrifuged at 500 × g for 10 min under 4 °C. 0.5 V mL 50 % sulphuric acid (Sinopharm, Shanghai, China) was added to the supernatant (V mL) for acidifying. Then, SCFAs were extracted with 2 V mL diethyl ether (Sinopharm, Shanghai, China). The samples were centrifuged at 12 000 g for 10 min under 4 °C, and the supernatants were collected for detection. The analysis was conducted with GC by using GC-2030 (Shimadzu, Kyoto, Japan). The temperature of the injector port and detector was 180 °C. The oven temperature increased from 80 to 155 °C at the rate of 5 °C·min^{-1} and hold on 80 °C for 2 min. The content of SCFAs was measured according to the external standard curve.

2.11. Statistical analysis

All the results were presented as means ± SEM (standard error of the mean). Normal distribution was tested using Shapiro-Wilk test in SPSS statistics 22.0 software (IBM, New York, USA). Statistical analyses were performed using one way analysis of variance (ANOVA) followed by Duncan's multiple range tests. The correlations between the parameters and the selected bacteria were conducted by using multiple regression analysis in R software (ver. 4.0.2; R Studio, Boston, USA). Result with p-value < 0.05 were considered to be statistically significant. Bar graphs were made by GraphPad Prism 6.0 software (Prism, California, USA).

3. Results

3.1. The influence of oligosaccharide-supplementation on the growth performance of Nile tilapia fed with high-carbohydrate diet

The influence of different diets on the growth performance was detected (Table 1). High-carbohydrate diet (HC) showed no significant difference on the weight gain compared with CK groups, while combination of XOS and high-carbohydrate diet (HCX) significantly increased the weight gain in Nile Tilapia compared with CK diet (Table 1). Consistent with the weight gain, HC group had no significant difference in the body length compared with CK group, while HCX group significantly increased the body length compared with CK group (Table 1). In addition, we also found HC diet increased the condition factor relative to CK diet, and the supplementation of oligosaccharides (HCG and HCX) sustained this effect (Table 1). BMI, an indicator of obesity (Oka et al., 2010), was also measured. Similarly, HC, HCG and HCX diets increased the BMI relative to CK diet. No significant difference was found in the feed conversion ratio among four groups (Table 1).

Mesenteric fat index (MFI) and hepatosomatic index (HSI) were significantly higher in the HC group than in CK group, but MFI was significantly lower in the HCX group than in HC group (Table 1). HC diet did not influence the content of total protein and moisture, while GOS-supplementation diet significantly decreased the moisture and XOS-supplementation diet significantly increased total protein compared with CK diet (Table 1). HC diet significantly increased the total lipid compared with CK diet, while HCX diet significantly decreased it (Table 1). Further analysis showed that HCX diet could alleviate lipid accumulation by down-regulating genes related to lipogenesis and up-regulating genes related to β-oxidation (Supplementary Fig. 1). The moisture, protein and lipid content of muscle were also detected and no significant difference was found among four dietary treatments (Table 1).

3.2. The influence of oligosaccharide-supplementation on muscle textural properties and muscle growth of Nile tilapia fed with high-carbohydrate diet

Texture is one of the important indicators to reflect the flesh quality. Textural properties of muscle were detected and the results showed that HC diet significantly decreased the hardness of muscle compared with CK diet, while addition of GOS or XOS in high-carbohydrate diet (HCG or HCX diet) reversed this trend (Fig. 1a). HC diet significantly decreased the adhesiveness of muscle, while addition of GOS significantly increased it. Addition of XOS had an increase trend in adhesiveness, but no significant difference was detected (Fig. 1b). The springiness of muscle was higher in HC group, and addition of GOS decreased the springiness caused by high-carbohydrate diet (Fig. 1c). The chewiness of muscle was significantly lower in HC group than in CK group, and addition of XOS increased it significantly (Fig. 1d). HC diet did not influence the cohesiveness of muscle, while GOS-supplementation diet significantly decreased it (Fig. 1e). These data indicated that addition of oligosaccharides restored muscle textural properties caused by a high-carbohydrate diet and the influences of different oligosaccharides varied.

Considering that muscle textural properties were closely related to muscle fiber characteristics, the diameter and density of muscle fibers were detected (Fig. 1f-i). Statistics suggested that the average fiber diameter of HC diet group was significantly lower than CK group. Oligosaccharide-supplementation (HCG and HCX) increased fiber diameter compared with HC, appearing a similar diameter to CK group (Fig. 1g). Accordingly, HC group had the highest density of muscle fiber among four groups (Fig. 1h). The percentage of muscle fiber diameter within a certain range was also counted (Fig. 1i) and the results showed that HC group had a significantly higher percentage in the range of 0–35 μm and a lower percentage in the range of 55–130 μm compared with other groups (Fig. 1j). These data indicated that high carbohydrate diet increased the proportion of thin fibers, but decreased the proportion of thick fibers. Addition of oligosaccharides could restore the muscle fiber composition, which may account for the restoration of muscle textural properties.

The mRNA expression levels of genes related to muscle development were also analyzed (Fig. 1k). The expression level of *myhc*, which is a major functional protein for muscle contraction, showed no significant differences among four groups (Fig. 1k). The expression level of *myogenic determining factor* (*myod*), which is related to the determination and differentiation of vertebrate skeletal muscle, was not influenced by the high-carbohydrate diet but increased by addition of GOS or XOS. Concurrently, the expression levels of *calpain 1* (*capn1*) and *forkhead box o1* (*foxo1*), negative regulators of muscle development, were also detected (Fig. 1k). The results showed that the expression levels of *capn1*

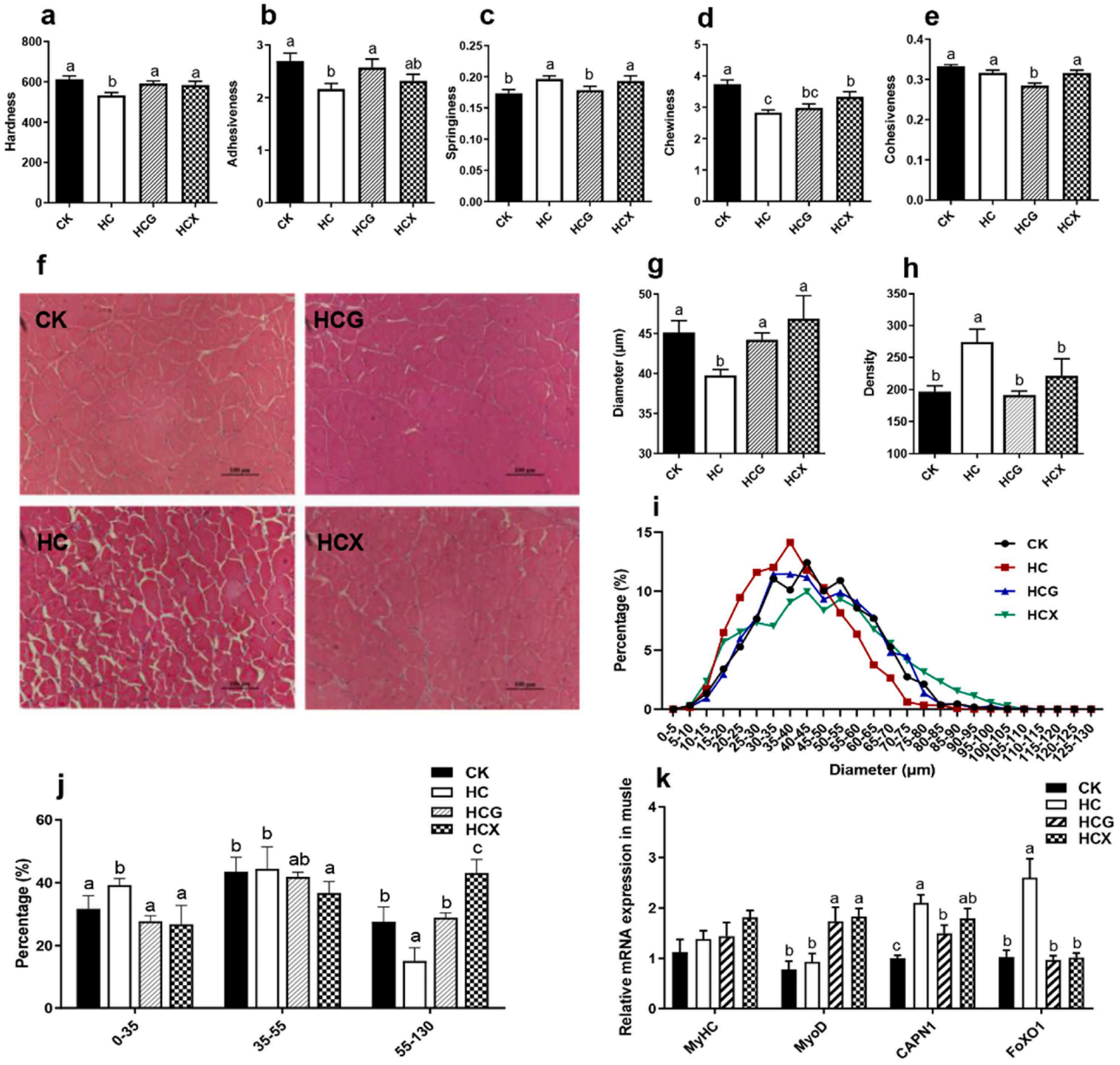

Fig. 1. The influence of four diet treatments on the muscle texture and muscle growth of Nile tilapia. (a) Hardness of muscle; (b) Adhesiveness of muscle; (c) Springiness of muscle; (d) Chewiness of muscle; (e) Cohesiveness of muscle; (f) Muscle histological sections of CK, HC, HCG and HCX groups under 200 × magnification (Scale bars, 100 μm); (g) Average diameter of muscle fiber (μm); (h) Muscle fibers density; (i, j) Statistics of muscle fiber diameter distribution (percentage of muscle fiber diameter within a certain range); (k) The expression level of regulators of muscle development. *myhc*: myosin heavy chain; *myod*: myogenic determining factor; *capn1*: calpain 1; *foxo1*: forkhead boxo1.Vertical bars represented the mean ± SEM. Statistical significance was determined by using one-way analysis of variance (ANOVA). Values marked with different letters indicated significant difference ($P < 0.05$).

and *foxo1* were significantly increased in HC group compared with CK group, but the expression levels of *capn1* significantly decreased in HCG group and the expression level of *foxo1* was significantly increased in HCG and HCX groups. These results revealed that HC diet inhibited muscle development by upregulating the expression of negative regulators of muscle development. Nevertheless, oligosaccharides induced the positive regulators and suppressed the negative regulators to promote muscle development.

3.3. The influence of oligosaccharide-supplementation on amino acid and fatty acid composition of Nile tilapia fed with high-carbohydrate diet

Considering that amino acid composition is one of the most important factors in evaluating nutrition and flavor value, the composition of protein-bound amino acids in Nile tilapia fillets was measured (Table 2; Supplementary Table 3). The result of the absolute content showed that HC diet had no significant influence on amino acid composition compared with CK diet, but HCG increased the content of total amino acids (TAA) (174.43 $mg \cdot g^{-1}$). Meanwhile, HCG group had a significantly higher content of essential amino acids (EAAs) (90.67 $mg \cdot g^{-1}$) and delicious amino acids (DAAs) (82.98 $mg \cdot g^{-1}$). In addition, HCX group did not significantly influence the amino acid composition.

Fatty acid profiles of total fat in muscle were also detected (Table 2; Supplementary Table 4). HC group had a significantly lower content of docosahexaenoic acid (DHA, C22:6n-3), while HCG and HCX did not reverse this change. No significant difference was observed in saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFA) among four treatments. In addition, HC group had a similar content of poly-

unsaturated fatty acids (PUFAs) compared with CK group, but the content of PUFAs significantly decreased in HCG (5.20 $mg \cdot g^{-1}$) and HCX (4.1 $mg \cdot g^{-1}$). Moreover, there was no significant difference on the ratio of n-3/n-6 PUFAs among four groups.

3.4. The influence of oligosaccharide-supplementation on intestinal microbiota composition and their metabolites

In order to evaluate whether the influence caused by oligosaccharides was correlated with the alteration of intestinal microbiota, the intestinal bacterial composition was detected by high-throughput sequencing (Fig. 2a). The relative community abundance at the phylum level showed that Actinobacteria, Proteobacteria, Firmicutes, Chlamydiae and Fusobacteria were dominant in the intestine of Nile tilapia (Supplementary Table 5). In the CK group, the relative abundance of these five phyla were 67.7%, 8.3%, 7.0%, 5.2%, and 4.5%, respectively. Although no significance was found, HC group tended to increase the abundance of Proteobacteria (13.3%), Firmicutes (19.7%), and Chlamydiae (7.7%) as well as decreasing the abundance of Actinobacteria (54.8%) and Fusobacteria (0.2%). GOS-supplementation group did not show obvious effect on the abundance of these dominant phyla compared with HC diet, while XOS-supplementation group significantly increased the abundance of Firmicutes (37.2%) and decreased the abundance of Chlamydiae (1.0%). The principal coordinates analysis (PCoA) (Fig. 2b) indicated that HC diet changed the intestinal microbiota compared with the CK group, and addition of oligosaccharides altered the microbiota composition compared with the HC group.

Compared to CK group, the HC diet increased the proportions of OTU1552 (*Bifidobacterium*), OTU1451 (*Brachybacterium*), OTU567 (*Brevibacterium*), OTU799 (*Ralstonia*), OTU1466 (*Enterococcus*), OTU1585 (*Burkholderia Caballeronia Paraburkholderia*), OTU1521/OTU1037 (*Hyphomicrobium*), OTU1443/OTU947/OTU1495 (*Lactobacillus*), OTU1116 (*Legionella*), OTU912 (*Microbacterium*), OTU165 (*Marmoricola*), OTU971 (*Nocardioides*), OTU962 (Parachlamydiaceae) and OTU1423 (*Rhodococcus*) (Fig. 2c). Some of these OTUs were decreased by the addition of GOS, including OTU567 (*Brevibacterium*), OTU1116 (*Legionella*), OTU912 (*Microbacterium*), OTU165 (*Marmoricola*), OTU971 (*Nocardioides*) and OTU962 (Parachlamydiaceae), and some were decreased by the addition of XOS, including OTU567 (*Brevibacterium*), OTU1037 (*Hyphomicrobium*), OTU1116 (*Legionella*) *and* OTU912 (*Microbacterium*). The HC diet decreased the abundances of OTU916/OTU1593 (*Actinomycetospora*), OTU371 (*Aeromicrobium*), OTU338 (*Blastococcus*), OTU273 (*Geodermatophilus*), OTU347 (*Gordonia*), OTU326 (*Janibacter*), OTU479 (*Kocuria*), OTU1569 (*Legionella*), OTU900 (Dependentiae), OTU1494 (*Pseudonocardia*) and OTU269 (Parachlamydiaceae) significantly, while addition of XOS induced the abundance of OTU912 (*Legionella*) (Fig. 2c).

The production of SCFAs was also detected. Compared with the control diet, high-carbohydrate diet did not affect production of acetic acid, propionic acid and butyric acid (Fig. 2d-f). But the supplement of GOS significantly increased the content of butyric acid in gut content (Fig. 2f), and the supplement of XOS significantly increased the content of propionic acid and butyric acid in gut content (Fig. 2e-f).

3.5. Correlation of intestinal microbiota and their metabolites with the growth condition and flesh quality

The correlations between the intestinal microbiota and the significantly changed parameters were calculated (Fig. 3). GOS-supplementation mainly promoted the muscle texture and amino acid composition, and the results showed that the expression level of *capn1* and *foxo1*, and fiber density were positively correlated with OTU962, OTU912, OTU567, OTU1449, OTU165, OTU971, OTU1116, OTU1656, OTU948 and OTU936 (Fig. 3a). The expression level of *myod* were positively correlated with OTU847 and OTU1242. OTU1242 also showed a positive association with EAA, while OTU994 were negatively correlated with EAA (Fig. 3a). In addition, butyric acid, which significantly increased in HCG, was positively related to OTU847 and OTU1242 but negatively related to OTU994 in GOS-supplementation group (Fig. 3a).

XOS-supplementation group mainly improved the growth performance, lipid metabolism, fatty acid composition and muscle texture. There was a significant positive correlation between OTU1650, OTU1552 and WGR, while OTU326, OTU692 and OTU353 were negatively associated with WGR (Fig. 3b). OTU567 and OTU912 all showed a negative correlation with C18:3n-3, n-3 PUFA and n-3/n-6 PUFA, and some bacteria were positively correlated with fatty acid composition such as OTU866, OTU1451, OTU1471 and OTU1242 (Fig. 3b). OTU1037 showed a positive association with fiber density and the expression level of *capn1* and *foxo1*. Similar to GOS treatment, OTU1116, OTU567 and OTU912 in XOS treatment also showed a positive association with *capn1* and *foxo1* (Fig. 3b). In addition, the content of propionic acid and butyric acid positively correlated with OTU 1569, OTU1242, OTU1650, OTU1471 and OTU1552, but they were negatively correlated with OTU1033, OTU994, OTU326, OTU692 and OTU353 (Fig. 3b). The above results suggested that systemic impacts caused by oligosaccharides may be related to the intestinal microbiota composition.

4. Discussion

Carbohydrate was regarded as an effective energy substitute in aquafeed to reduce the culturing cost. However, long-term feeding of a high-carbohydrate diet not only caused metabolic disorders but also affected the flesh quality. Our results indicated that two oligosaccharides improved the adverse effects induced by high carbohydrate diet, and the beneficial effects on flesh quality of different oligosaccharides varied (Fig. 4).

Muscle texture properties are important sensory attributes of fish to influence the consumer's acceptability and the following mechanical processing. Oligosaccharide-supplementation, including GOS and XOS, alleviated the texture parameters altered by high-carbohydrate diet. Zhao *et al.* found that grass carp fillet with higher hardness and shear force had thicker muscle fibers in histologic slices (Zhao et al., 2018). Oligosaccharide-supplementation significantly increased the fiber diameter by increasing the thick muscle fibers and decreasing the thin muscle fibers, suggesting that oligosaccharides could restore the texture properties by changing the muscle fiber composition. The effect of diet on muscle growth-related genes have been reported in many fish species, including *D. rerio* (Ulloa et al., 2013) and *Solea senegalensis* (Valente, Cabral, Sousa, Cunha, & Fernandes, 2016). However, how oligosaccharides regulated the expression of genes related to muscle development remains unknown. In order to explain the underlying mechanism, the expression level of genes related to the muscle development was detected. HC diet upregulated the negative regulators such us *capn1* and *foxo1*, while oligosaccharides upregulated the myogenic regulatory factors and suppressed the negative regulators. In recent years, "gut-muscle axis" has been received a lot of attention in mammals (Lustgarten, 2019). Compared to specific pathogen-free mice, germ-free mice displayed more obvious signs of muscle atrophy and lower muscle strength with higher expression level of *foxo*, *atrogin-1*, *murf-1*, and *myod* (Lahiri et al., 2019). Probiotic-supplementation also improved muscle mass and increased the numbers of oxidative muscle associated with increased muscle endurance in mice (Chen et al., 2016). In the present study, OTU1116 (Legionella), OTU567 (Brevibacterium), OTU912 (Microbacterium) and OTU936 (*Candidatus Protochlamydia*) showed similar correlations with muscle phenotypes in both GOS and XOS supplementation groups.

Addition of XOS to a high-carbohydrate diet promoted the growth performance in Nile tilapia compared with control diet. Oligosaccharides have been proved to promote host health by modifying the

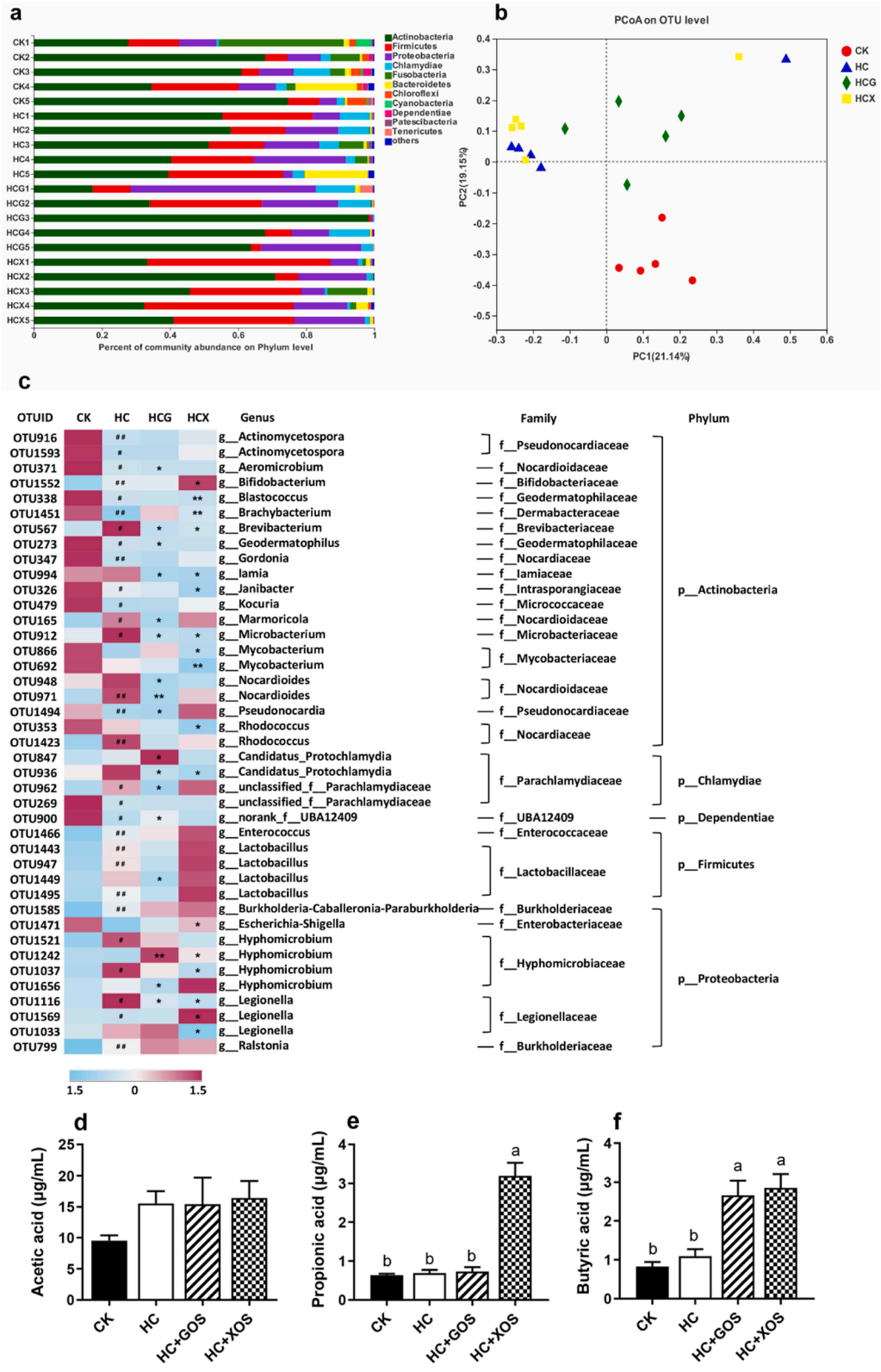

Fig. 2. The influence of four diet treatments on the intestinal microbiota composition (n = 5) and concentration of SCFAs (n = 3) in Nile tilapia. (a) Bacterial composition in four groups at the phylum level. The phyla with the abundance<1% were showed. (b) The principal co-ordinates analysis (PCoA) based on OTUs level. (c) Heatmap analysis of OTUs. The color bar of each OTU in each treatment is shown. The taxonomy of the OTUs (genus, family, and phylum) is depicted on the right. Differences were detected using Kruskal-Wallis in R package. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, HC groups versus CK groups; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, HC groups versus HCG or HCX groups. The concentration of (d) Acetic acid; (e) propionic acid;(f) butyric acid. Vertical bars represented the mean ± SEM (n = 3). Statistical significance was determined by using one-way analysis of variance (ANOVA). Values marked with different letters indicated significant difference ($P < 0.05$).

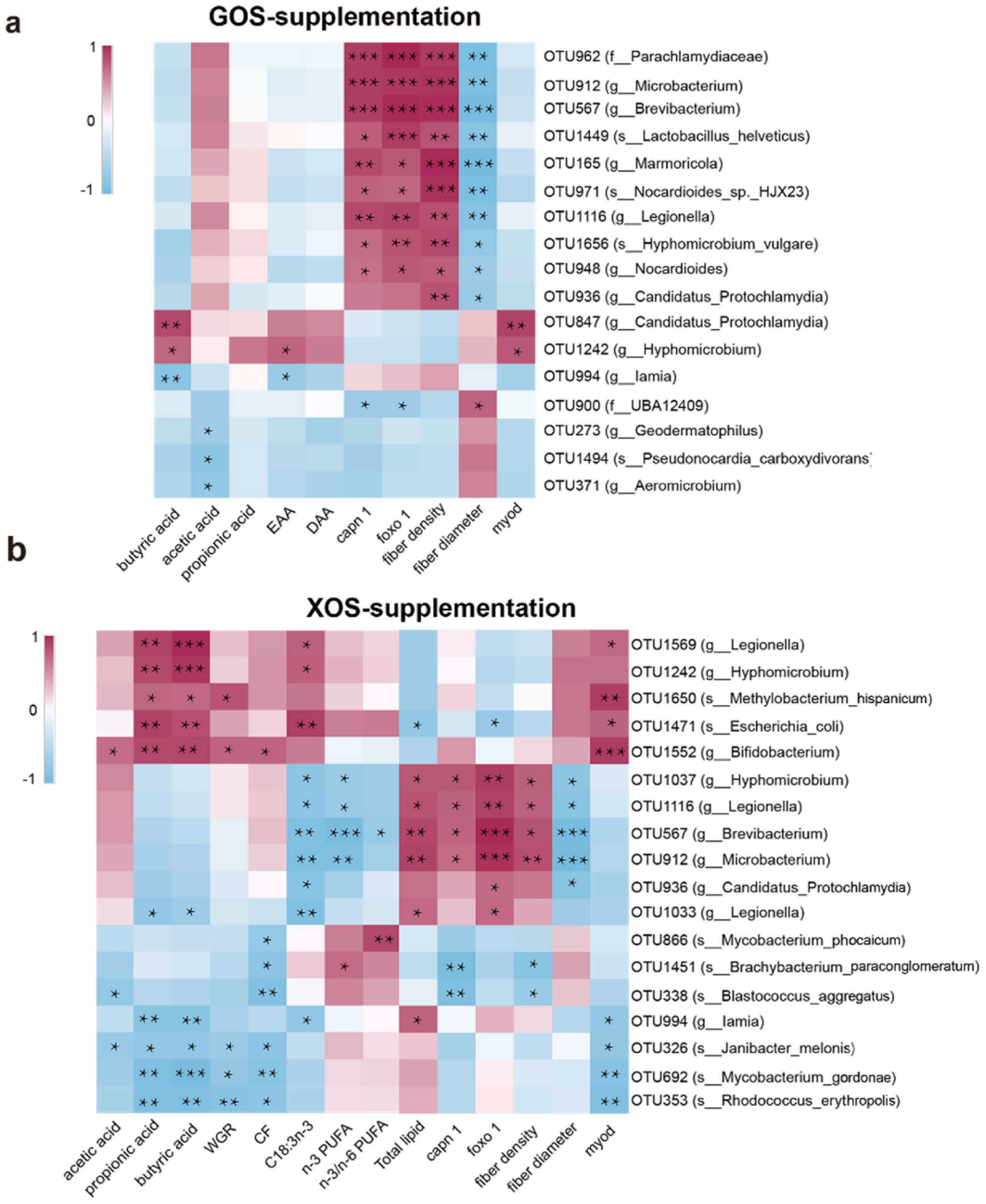

Fig. 3. Correlation analysis of the intestinal microbiota and systematic parameters. (a) The correlation analysis of the microbiota and systematic parameters by comparing CK, HC and HCG groups. (b) The correlation analysis of the microbiota and systematic parameters by comparing CK, HC and HCX groups. Positive correlations were indicated by red cubes and negative correlations by blue cubes. Spearman test, *P < 0.05, **P < 0.01, ***P < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

composition of intestine microbiota (Sanders, Merenstein, Reid, Gibson, & Rastall, 2019), and the abundance of Bifidobacterium and Lactobacillus was found to increase in HCX group. Correlation analysis showed that Bifidobacterium was positively correlated with WGR and CF, which was consistent with the previous studies (Luo et al., 2020; Sahandi, Jafaryan, Soltani, & Ebrahimi, 2019).

Excessive carbohydrate in the diet could induce lipid accumulation in fish (Kamalam, Medale, & Panserat, 2017). In the present study, the supplement of XOS alleviated the lipid accumulation by down-regulating genes related to lipogenesis and up-regulating genes related to β-oxidation (Fig S1). Consistent with our results, the study in mammals also showed that XOS-supplementation could influence the intestinal microbiota and metabolites to regulate lipid accumulation by decreasing lipogenesis and increasing energy expenditure (Lensu et al., 2020; Li et al., 2020). In the present study, propionic acid, one of the short-chain fatty acids, increased significantly only after XOS addition. The addition of prebiotic showed a positive correlation between changes in short-chain fatty acids and certain intestinal microbiota in mice (Han, Ma, Liu, Zhao, & Li, 2021), which is consistent with our results. Furthermore, the addition of propionic acid could prevent or alleviate diet-induced obesity in mice (Tengeler et al., 2020). Similarly, prebiotic-supplementation to a high-carbohydrate diet could reduce lipid accumulation, which was accompanied by an increase in the intestinal propionic acid of Nile tilapia (Wang et al., 2020). All these studies suggested that although fish and mammals harbor different intestinal microbiota composition, increased propionic acid may be related to the decreased

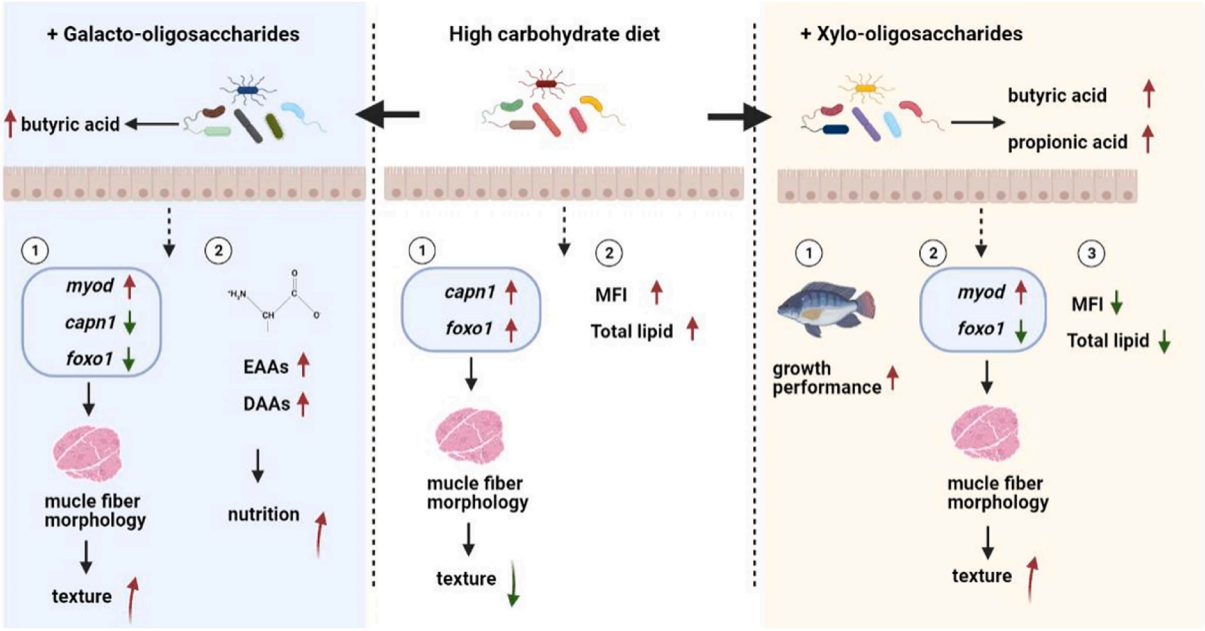

Fig. 4. The influence of two oligosaccharides on the growth performance and texture characteristics of Nile tilapia fed with high carbohydrate diet.

lipid accumulation in XOS supplemented group. In the present study, significant changes in lipid metabolism were not showed in muscle, which may be related to the growth stage of the fish. It has been shown that different carbohydrate utilization capacity of fish at different developmental stages makes the metabolic status of fish at late developmental stages be different from those at early stages, thus leading to the changes in flesh quality (Liu, Mai, Xu, Zhang, Zhou, & Ai, 2015). It should be noted that fish at adult stages need to be involved in the subsequent study.

Differed from XOS, GOS-supplementation improved the amino acid composition in the fillet. High-carbohydrate diet had no obvious influence on the content of amino acids, while addition of GOS in high-carbohydrate diet significantly increased the absolute content of EAAs and DAAs, suggesting that addition of GOS in high-carbohydrate diet promoted the amino acid nutrition level of fillets. A previous report found that changes in metabolic pathways including amino acid in zebrafish were associated with the intestinal microbiota (Wang, Zhao, & Jin, 2020). Several studies indicated that the gut microbiota can regulate a variety of metabolic pathways and homeostasis (Neis, Dejong, & Rensen, 2015), but the underlying mechanism remains to be explored. More informative methods including metagenomics or metabolomics should be used in the future to reveal the exact function of the intestinal microbiota.

5. Conclusions

In summary, the present study found that high-carbohydrate diet affected growth condition, lipid accumulation and flesh quality in Nile tilapia. Addition of oligosaccharides could alleviate the adverse effects and the influence of different oligosaccharides varied. Compared with high-carbohydrate group, GOS-supplementation in high-carbohydrate diet increased the essential amino acids (EAAs) and delicious amino acids (DAAs) contents, while XOS-supplementation showed beneficial effects on the growth performance and lipid metabolism. In addition, both XOS-supplementation and GOS-supplementation could restore the texture and the intestinal bacterial composition and their metabolites may be involved in these processes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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