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ORIGINAL RESEARCH

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Assessment of nutritional contents and microbial community of three populations of cultured Beluga sturgeon (*Huso huso*) broodfish

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Abstract

Beluga sturgeon (*Huso huso*) is a valuable fish all over the world to produce caviar and meat. It is a good candidate for aquaculture due to its acceptance of formulated diets and high resistance to stressors from increased density during farming. This study evaluated three groups of sexually unknown farm-raised Beluga sturgeon populations that differed by age to understand differences in growth rate, nutrient utilization and microbial community by gut sections. Weight, length, and liver samples were collected to determine growth parameters. Proximate compositional and histopathological analyses were conducted. Luminal samples from the stomach and midgut and hindgut sections were collected for 16S rRNA gene characterization. The results showed that fish weight differed by age group ($p < 0.05$). The condition factor ranged from 0.61 to 0.78 for all the age groups. Fillet fatty acid compositions showed that erucic acid (monounsaturated) was significantly different

($p < 0.005$) between the three age groups, but not for other fatty acids and amino acids. The gastrointestinal section was a stronger factor than age modulating the microbial compositions in beluga ($p < 0.05$), and their compositions and diversity varied between stomach, midgut, and hindgut sections. The abundance of *Lactococcus* and *Lactobacillus* genera increased in the hindgut section of the gastrointestinal tract and differed by age group. Proteobacteria correlated positively and significantly with the essential amino acids ($p < 0.05$). The genus *Haloimpatients* from phylum Firmicutes showed a positive correlation with phenylalanine and threonine in the Beluga stomach ($p < 0.05$). A similar trend was found between *Staphylococcus* and histidine in the hindgut. More studies should be directed to address the functionality of the microbiota highlighted in the gut sections in this study and their involvement in the metabolism of essential amino acids to improve the conditions and optimal nutrient requirements of farm-raised Beluga sturgeon.

KEYWORDS
amino acids, bacterial community, fatty acids, gastrointestinal tract, nutrition, sturgeon

1 | INTRODUCTION

Sturgeons are valuable fishes worldwide (Hung, 2017). However, many species, particularly Beluga, are listed as critically endangered by the International Union for Conservation of Nature, making research to improve production critical for sustainability. Optimum dietary requirements for commercial diet development are essential when culturing Beluga for fish protein or broodstock to enhance its metabolic capacities, but it is currently unavailable in the industry. Farmers either use commercially available diets intended for other fishes or use diets formulated based on the dietary requirements of other freshwater fishes as a model for Beluga.

One of the nutritional challenges farmers encounters is the ability of Beluga broodstock to accumulate excess fat in the tissue during growth (van Eenennaam et al., 2004), which limits the performance of the fish. There is a need to develop diets that specifically meet the requirements of the fish at various stages of development to enhance its growth and reproductive performances, which may help surmount the challenge of Beluga taking 8–10 years of feeding to reach maturity because of nutritional-related physiological issues. Diets targeted to meet different developmental stages of Beluga become essential to meet production goals. Further, the need to prioritize changes in amino acid and fatty acid profiles in the diets to improve survival, growth, and egg quality and quantity in cultured Beluga broodstock is essential to meeting the 2030 Blue-transformation agenda of sustainable aquaculture production (FAO, 2023).

Historically, commercial fish feeds are formulated using ocean-harvested fishmeal to meet the requirement of the cultured fishes for quality protein. Because of the continuous expansion of the aquaculture industry and the finite state of marine-harvested products, research has been intensified to source alternative ingredients to replace

ocean-derived products in finfish nutrition (Caimi et al., 2020; Liu et al., 2009). However, the goal in animal nutrition is not just finding a replacement for fishmeal but identifying a continuous source of high-quality alternative protein that is sustainable and cost-effective for the aquaculture industry.

Many plant protein ingredients have been studied as partial or complete replacement ingredients in aquaculture nutrition (Betiku et al., 2016; Betiku, Yeoman, Gaylord, Duff, et al., 2018; Karabulut et al., 2019). Soybean is the first-choice plant protein source due to its balanced nutritional profile. Despite this, its use is limited in cultured fishes due to its high price and the possibility of intestinal enteritis when included above 30% in the diet (Baeverfjord & Krogdahl, 1996; Kumar et al., 2020). Intestinal enteritis has restricted the use of soybean meal as a total replacement for fishmeal in fishes, including rainbow trout (Merrifield et al., 2009; Seibel et al., 2022), Atlantic salmon (Knudsen et al., 2008; Li, 2020), Carp (Urán et al., 2008), grouper (Zhang et al., 2021), and turbot (Gu et al., 2016) chiefly when long-term feeding is being considered. In addition, other antinutritional factors are present in plant protein ingredients, which have intensified plant-based alternative research in numerous fishes. However, nutritional studies on Beluga are limited, making many dietary effects of alternative ingredients used in the aquafeed on the fish unknown. Therefore, feeding Beluga with diets formulated with unknown nutritional requirements or for other farmed species may not be ideal, which is the current situation in many farms (Hung, 2017).

Diet has been shown to cause interaction between the aquatic host and gut microbiota (Betiku et al., 2023; Betiku, Yeoman, Gaylord, Americus, et al., 2018). Dietary components and manipulations modulate the diversity of microbes in the fish gut ecosystem (Ringø et al., 2016). The microbiota in the fish gastrointestinal tract (GIT) functions in nutrient digestion and utilization, the immune system, and the general well-being of the fish (Nayak, 2010). Absorption of nutrients, growth, and health can be impacted by a reduction in the diversity of microbes or an imbalance of microbiota in fish GIT (Petersen & Round, 2014; Vargas-Albores et al., 2021), making a balanced gut ecosystem of fish critical to meeting production targets. The amplicon sequencing method is well established in different fishes, which has helped unravel the diversity of microorganisms in the gut ecosystem (Ortiz-Estrada et al., 2019; Ringø et al., 2016) and the possible impact on fish health. Modern technology applications in nutritional studies are lacking in sturgeon species, including Beluga. The available reports are mostly conventional evaluation procedures.

Because age is an important factor modulating the diversity of microbes in the fish host (Zhang et al., 2018), fish diets are formulated to meet the nutritional requirements at different physiological life stages (Jobling, 2012). However, this is different in Beluga as farmers rely on commercial feeds for other fishes to grow the fish. Because of the restriction on Beluga, nutritional studies to address nutrient requirements in the fish are limited, and only a few commercial farms have permission to raise Beluga for caviar production. Therefore, using commercially farmed Beluga is the only available option to address some of the challenges Beluga farmers face in the US.

The current study assessed the nutritional and metagenomic profiles of three farm-raised Beluga populations cultured under the same dietary and water conditions. The objectives were to evaluate the nutritional compositions of three age groups of Beluga and identify the differences in microbiota in different gut sections of the fish and their relationship with the tissue nutritional compositions. Because nutritional studies on Beluga are limited, the information from the current study may help suggest the nutritional needs of Beluga when grown for caviar or as future broodstock.

2 | MATERIALS AND METHODS

2.1 | Fish and sampling

The parental Beluga generation was maintained in a recirculating system at a commercial farm. Three F1 cohorts were spawned in 2012, 2013, and 2018 (ages at sampling were 8, 7, and 2 years, respectively) and maintained under the same flowthrough hatchery conditions. Juveniles were kept in customized large round polyethylene tanks, and water was maintained at 19°C and with a long lighting period. Fish were initially fed a commercial rainbow trout diet containing 45% crude protein, 15% lipids, and 3% fiber for 6 months before transitioning to another diet with 42%

FIGURE 1 Pictures showing a Beluga fish and its intestinal sections.

crude protein, 16% lipid, and 1.0% fiber. During this period, gonad developments were monitored with ultrasound equipment. Before collecting samples, all fish were fasted for 24 h, and six fish were randomly selected from each year group. Handling of fish followed the recommended guidelines for fish food. Fish were weighed, and lengths were measured, and fillet samples were collected. In addition, each fish was dissected aseptically, and sex was determined as either male, female, or unknown. In addition, GIT samples, separated into stomach, midgut, and hindgut (Figure 1), were obtained, following the procedure previously described by Betiku, Yeoman, Gaylord, Americus, et al. (2018). Furthermore, the distal section of the intestinal samples was obtained and preserved in Davidson solution (VWR) for histopathological analysis. Samples collected were transported to the laboratory for further analysis.

2.2 | Body condition indexes and factor

Individual viscera weight, fillet weight, and liver weight obtained were used for the calculation of condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI), as shown in the following equations:

$$CF = \left(\text{Fish weight} / \text{Total lenght}^3\right) \times 100 \quad (1)$$

$$HSI = (\text{liver mass}/\text{body mass}) \times 100 \quad (2)$$

$$VSI = (\text{gut mass}/\text{body mass}) \times 100 \quad (3)$$

2.3 | Proximate composition and amino acid analyses

Frozen fillets were thawed and homogenized. Proximate composition analysis was carried out for individual samples ($n = 6$/year group) according to AOAC methods (AOAC, 2005). Moisture content was determined by drying the samples in an oven at 105°C until a constant weight was achieved (AOAC, 2005). Ash was determined in a furnace by incineration at 600°C for 3 h. Crude protein was determined with a LECO Nitrogen analyzer, and N values obtained were converted with a protein factor of 6.25. Crude fat content in samples was determined using the ether extract hydrolysis method (AOAC, 2005). Tissue samples were hydrolyzed and analyzed with an amino acid analyzer (Hitachi Amino Acid Analyzer L-8800). Amino acid levels of duplicate feeds and tissue samples were determined according to Dawczynski et al. (2007).

2.4 | Fatty acids analysis and lipid quality indices

Fillets from Beluga were thawed individually on ice to prevent muscle degradation. Following homogenization, extraction was performed according to methods by Folch et al. (1957) and Parrish (1999) with chloroform:methanol (2:1 v/v) solution containing 0.01% butylated hydroxytoluene as an antioxidant. Fatty acid methyl esters (FAMEs) were obtained by methylation methods, as described by Lepage and Roy (1984). The samples of FAMEs derived were analyzed with a Clarus 680/600 T GC–MS (Perkin-Elmer, Waltham, MA, USA) using a 30 m Thermo Fisher TR-5 general purpose column with a 250-μm diameter. With an 82-vial autosampler, 1.0 μL of each sample was injected into the injector column, heated to 250°C, and maintained for 10 min. Quantification of the fatty acid profile detected in each sample was determined by comparing the resulting fatty acids to a standard with known concentrations. The fatty acid composition was expressed as the percent of total fatty acids in the tissue. The atherogenic index (AI), thrombogenic index (TI), and hypocholesterolemia/hypercholesterolemia ratio (H/H), which are determinant of the lipid quality indices, were calculated using the percentages of saturated, monounsaturated, and polyunsaturated fatty acids as shown in the following equations (Santos-Silva et al., 2002; Ulbricht & Southgate, 1991):

$$\mathrm{IA} = [(\mathrm{C12:0} + (4 \times \mathrm{C14:0}) + \mathrm{C16:0})]/(\mathrm{MUFAs} + n-6\,\mathrm{PUFAs} + n-3\,\mathrm{PUFAs}) \tag{4}$$

$$\mathrm{IT} = [(\mathrm{C14:0} + \mathrm{C16:0} + \mathrm{C18:0})]/(0.5\,\mathrm{X\,MUFAs}) + (0.5\,\mathrm{X}\,n-6\,\mathrm{PUFAs}) + (3 \times n-3\,\mathrm{PUFAs}) + [(n-3\,\mathrm{PUFAs})/n-6\,\mathrm{PUFAs}] \tag{5}$$

$$\mathrm{H/H} = (\mathrm{C18:1}n-9 + \Sigma\,\mathrm{PUFAs})/(\mathrm{C12:0} + \mathrm{C14:0} + \mathrm{C16:0}) \tag{6}$$

2.5 | Histopathological analysis

A transverse section from each end of the intestinal segments and the center of the segment were taken to assess all regions for general anatomic conformation, inflammation, infection, and neoplasia using hematoxylin and eosin (H&E)-stained slides. The three sections from each fish were placed into an individual cassette labeled with the fish number and birth year. Tissues were processed using routine histological techniques, embedded in paraffin wax, sectioned at 3–5 μm, mounted on glass slides, and stained with hematoxylin and eosin for review. Slides were reviewed on a Nikon Eclipse 80i (Tokyo, Japan), and photomicrographs were taken using an Amscope MU 1403 (Irvine, CA). Three sections for each fish sample were evaluated under a light microscope for microscopic pathology, variation in mucosal cellular components, general architecture, and presence of organisms.

2.6 | 16S rRNA sequencing

Mucosal samples from the stomach, midgut, and hindgut sections of the GIT were extracted using a QIAamp PowerFecal Pro DNA kit according to the manufacturer's instructions (Betiku, Yeoman, Gaylord, Americus, et al., 2018). Amplification of extracted DNA was achieved with universal 341F (5-CCT ACG GGN GGC WGC AG-3) and 785R (5-GAC TAC HVG GGT ATC TAA TCC-3) primers pairs, targeting the V3-V4 hypervariable portions of the 16S ribosomal RNA genes. Amplicons were generated using a two-stage PCR amplification protocol (Naqib et al., 2018). The primers contained common sequences 1 and 2, CS1 and CS2 (Moonsamy et al., 2013). First-stage PCR amplifications involved 10 μL reactions in 96-well plates, using MyTaq HS 2X master mix (Bioline). The PCR conditions were 95°C for 5 min, followed by 28 cycles of 95°C for 30″, 55°C for 45″ and 72°C for 60″. Afterward, a second PCR amplification was performed in 10 μL reactions in 96-well plates. A master mix was made using MyTaq HS 2X master mix, which was used for the entire plate. Each well was a separate primer pair, a unique Illumina Access Array for 1a 0-base barcode library (Fluidigm, South San Francisco, CA; Item# 100–4876). These Access Array primers contained the CS1 and CS2 linkers at the 3′ ends of the oligonucleotides. Cycling conditions were 95°C for 5 min, followed by eight cycles of 95°C for 30″, 60°C for 30″, and 72°C for 30″. Samples were then pooled in equal volumes using an EpMotion5075 liquid handling robot (Eppendorf, Hamburg, Germany) based on the distribution of reads per barcode. The pooled library was purified using an AMPure XP cleanup protocol (0.6X, vol/vol; Agencourt, Beckmann-Coulter). To remove fragments smaller than 300 bp, the pooled libraries, with a 20% phiX spike-in, were loaded onto an Illumina MiniSeq mid-output flow cell (2 × 153 paired-end reads). Fluidigm sequencing primers targeting the CS1 and CS2 linker regions were used to initiate sequencing. De-multiplexing of reads was performed on the instrument.

2.7 | Data analysis

The obtained proximate, fatty, and amino acids data were analyzed using JMP Pro software (version 16.2, SAS Institute Inc.) to determine significant differences between the fish groups. Means separation was determined by Tukey's honesty significant difference (HSD) test, and significance is reported as $p \leq 0.05$. GraphPad Prism was used to generate graphs by groups. The sequence reads obtained was merged using PEAR (Zhang et al., 2014) and trimmed based on the quality threshold of $p = 0.01$ to remove ambiguous nucleotides and primer sequences. Reads that lack primer sequence were removed. The USEARCH algorithm, compared with the Silva v132 reference sequence database (Edgar, 2010; Glöckner et al., 2017), was used to identify removed chimeras. Amplicon sequence variants (ASVs) were identified using DADA2 (Callahan et al., 2016). For each ASV, the representative sequences were then annotated taxonomically using the Naïve Bayesian classifier included with DADA2 using the Silva v132 training set (Callahan et al., 2016; Glöckner et al., 2017). A square root transformation was applied to the ASV abundance data to obtain a resemblance matrix based on Bray-Curtis, while the environmental variables were log-transformed (Clarke & Gorley, 2015) to reduce the influence of dominant taxa. The diversity index within groups using the Kruskal-Wallis test was estimated with the vegan package in R, while diversity between groups was tested using Permutational multivariate analysis of variance (PERMANOVA) in PRIMER E software (Plymouth, UK). Analysis of similarities (ANOSIM) using 999 permutations and a significance level of $p < 0.001$ were used to partition the variation in the microbial community structure in the gut locations. Microbial genera data were log-transformed, and correlation analysis to determine the relationship with muscle nutrient (proximate and amino acids) compositions was conducted using the Kendall method, and adjusted *p*-values and plots were generated with ggplot2 in R software.

3 | RESULTS

3.1 | Fish biomass and condition indices

Physical observation of the fish showed a healthy status with no deformities or any symptoms of diseases. Reproductive organs in some of the fish were not present or well-defined as either male or female. Beluga growth differed

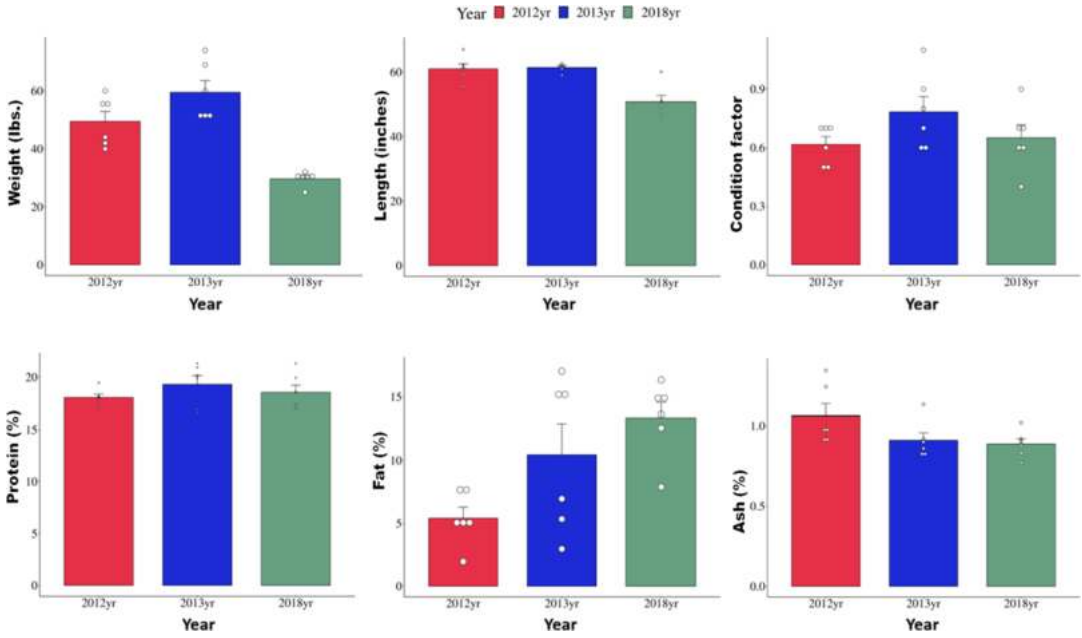

FIGURE 2 Growth parameters and proximate compositions of the three populations Beluga sturgeon.

significantly ($p < 0.00001$) between the 2012, 2013, and 2018 age groups. However, the fish from the 2013 group had the highest weight among the three populations (Figure 2). Likewise, fat tissue content was significantly different ($p = 0.0133$) in the three fish populations, while protein and ash contents were not different. The fat content in the 2013 Beluga group was significantly lower than in the 2012 and 2018 populations. The condition factor ranged from 0.61 to 0.78 for all the age groups, with values far from 1, indicating a nonoptimal condition. However, the 2013 group had a higher condition factor than Beluga in the 2012 and 2018 groups.

The VSI was significantly ($p = 0.0009$) higher in the 2018 and 2012 population groups than in the 2013 group, while the HSI ($p = 0.0001$) was significantly higher in the 2018 group than in the 2012 and 2013 groups (Figure 3). Although the fatty acids profile of the diets fed to the fish is unknown, the tissue compositions of the main class of fatty acids and fatty acid profiles varied between 2012, 2013, and 2018 age groups (Table 1).

Table 2 shows the lipid quality indices of the fish fillets, which have similar values for TI, HH, and AI. Amino acid compositions in this study were not significantly different by age group, except for histidine, which was slightly higher in the 2013 age group (Table 3).

3.2 | Histopathological analysis

The results of the H&E stain are shown in Figure 4. Overall, the intestine tissue samples revealed mild anatomic differences across the groups. The differences include the presence or absence of exocrine pancreatic tissue, lymphoid follicles, and coelomic adipose/connective tissue. Also, there is mild variation between groups in the glandular number, size, and submucosal thickness. The variation in these parameters could be a function of fish age and pathological change associated with underlying disease. A low level of mucosal hyperplasia and inflammation is consistent throughout the age groups. Chronic antigenic stimulation can be a function of mucosal exposure to antigenic dietary proteins or ingesting foreign material. It is difficult to ascertain whether the variation in microscopic lesion severity is

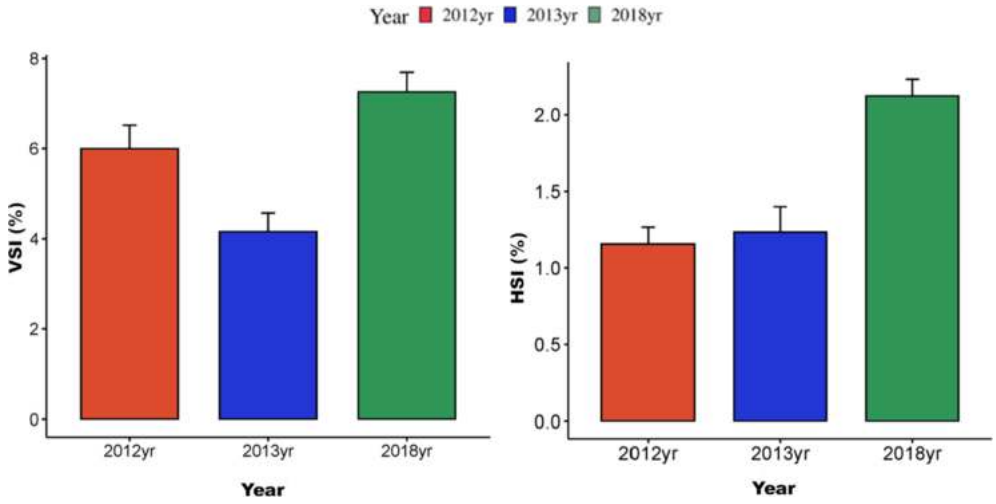

FIGURE 3 Percent viscerasomatic and hepatosomatic indexes of the three populations Beluga sturgeon.

a factor of antigenic stimulation from parasitic presence, ingestion of foreign antigen, or antigenic stimulation because of feed components, feed size, or a combination of the former.

3.3 | Bacterial community

A total of 2,807,161 high-quality sequences were generated from the V3-V4 region of the 16S ribosomal gene, as earlier described (Betiku, Yeoman, Gaylord, Americus, et al., 2018), using a Miniseq flow cell (Illumina, San Diego, CA). A total of 1473 amplicon sequence variant (ASVs) were identified in the stomach, midgut, and hindgut sections, 99% of which belong to bacteria. The Shannon-Weiner index estimates of species evenness showed a significant difference within the gut locations ($p = 0.0272$, $F = 3.901$) but not in age ($p = 0.216$, $F = 1.587$); likewise, species richness using Chao 1 index revealed a similar difference in gut locations ($p = 0.0433$, $F = 3.363$), but not in age ($p = 0.4030$, $F = 0.9270$) (Table 4).

Figure 5 shows the alpha diversity measures for both Chao 1 and Shannon indices for the stomach, midgut, and hindgut. The Chao 1 species richness was highest in the midgut and hindgut for 2018, while 2012 had the highest species richness in the stomach than the rest of the age groups. The species evenness (species richness and abundance) by Shannon was highest in the 2018 group in the stomach and midgut sections, while similar evenness estimates were found in the 2012 and 2018 groups. These results indicate more species diversity in the 2018 group than in 2012 and 2013.

The dissimilarity analysis (beta diversity estimates) by PERMANOVA showed a significant difference in species-level diversity by gut location ($p = 0.002$, Pseudo-F = 3.4858) but not by age ($p = 0.033$, Pseudo-F = 1.7909). In addition, the global test of analyses of similarity by ANOSIM showed a significant difference by gut location ($p = 0.003$, R = 0.22) but not by age ($p = 0.111$, R = 0.0558). The comparison between the gut locations showed that the stomach significantly differs from the midgut and hindgut ($p = 0.035$, $R = 0.14$; $p = 0.005$, $R = 0.30$), respectively. Likewise, a significant difference was observed between the midgut and hindgut ($p = 0.026$, $R = 0.19$). Further, the significant pairwise comparisons of the diversity between the stomach, midgut, and hindgut by the age groups are shown in Table 4. There is a distinct significant variation in stomach samples from 2012 and midgut and hindgut from both 2012 and 2018. Similarly, the stomach samples from 2013 differ significantly from 2018. In addition, diversity in the hindgut from 2018 varied significantly from the 2013 midgut and 2012 hindgut.

TABLE 1 Tissue compositions of fatty acids of the three groups of Beluga sturgeon.

	Beluga populations			
Fatty acid	**Year 2012**	**Year 2013**	**Year 2018**	***p*-value**
C10:0	1.68 ± 1.31	Nd	0.46 ± 1.12	0.3733
C12:0	2.10 ± 0.40	1.64 ± 0.63	1.65 ± 0.78	0.3247
C13:0	0.91 ± 0.48	0.69 ± 0.46	0.47 ± 0.49	0.3647
C14:0	3.98 ± 0.52	4.04 ± 0.29	4.34 ± 0.53	0.3966
C15:0	1.02 ± 0.11	0.90 ± 0.16	0.92 ± 0.18	0.5772
C16:0	21.90 ± 0.77	21.46 ± 1.71	22.46 ± 2.10	0.3701
C17:0	2.10 ± 0.29	1.75 ± 0.48	1.74 ± 0.63	0.1064
C18:0	5.88 ± 0.74	5.27 ± 1.35	4.13 ± 1.74	0.3344
C24:0	0.09 ± 0.22	0.35 ± 0.28	0.25 ± 0.36	0.9970
Total SFA	39.65 ± 5.76	36.10 ± 5.68	36.43 ± 5.90	0.0655
C14:1	0.78 ± 1.26	2.05 ± 0.78	0.69 ± 0.98	0.5959
C16:1	4.44 ± 0.97	4.87 ± 0.96	4.96 ± 0.84	0.3743
C17:1	4.29 ± 0.78	3.39 ± 1.24	3.42 ± 1.55	0.3358
C18:1	10.33 ± 4.52	15.78 ± 6.96	13.87 ± 6.94	0.0727
C18:1 n-9	1.29 ± 0.76	0.36 ± 0.32	1.31 ± 1.02	0.4141
C20:1 n-9	1.15 ± 0.49	1.70 ± 0.86	1.72 ± 1.02	$p = 0.0271$
C22:1 n-9	2.20 ± 0.11^{b}	2.23 ± 0.18^{ab}	2.60 ± 0.38^{a}	0.9364
Total MUFA	24.48 ± 3.29	30.38 ± 4.92	28.56 ± 4.33	0.3952
C18:2 n-6	2.85 ± 1.44	3.74 ± 1.52	4.52 ± 2.87	0.2725
C18:3 n-6	1.94 ± 0.13	1.91 ± 0.26	1.74 ± 0.27	0.3835
C18:3 n-3	3.36 ± 0.59	2.70 ± 0.92	2.77 ± 1.07	0.0585
C20:4 n-6 (ARA)	6.00 ± 1.47	5.07 ± 1.63	3.75 ± 1.36	0.1774
C20:3 n-6	1.33 ± 0.15	1.24 ± 0.26	1.06 ± 0.28	0.3460
C20:3 n-3	1.48 ± 0.27	1.17 ± 0.39	1.23 ± 0.45	0.9244
C20:5 n-3 (EPA)	8.41 ± 1.58	8.76 ± 3.26	8.96 ± 2.05	0.2306
C22:6 n-3 (DHA)	10.49 ± 2.02	8.93 ± 1.75	10.99 ± 2.40	0.9853
Total PUFA	35.87 ± 3.58	33.52 ± 3.29	35.00 ± 3.75	0.3733

Note: The values represent mean ± SD of three replicates, expressed as percent of total fatty acids detected in tissue samples from the three Beluga age groups. Superscript indicates Tukey's groupings.

3.4 | Correlation analysis

Because age was not a strong factor on the microbial community as highlighted in Table 4, the correlation analysis was focused on the relationship between the microbiota and the gut locations (stomach, midgut, and hindgut). Figures 6–8 shows the relation between microbial genera in the three gut locations (stomach, midgut, and hindgut) and tissue nutrient compositions (proximate and amino acids). The asterisks in the figure denote significant correlation coefficients.

The genus *Aeromonas* from Proteobacteria phylum was positively and significantly ($r = 0.9661$, $p = 0.0074$) correlated with essential amino acids in the hindgut. A negative correlation ($r = -0.4661$, $p = 0.2722$) was shown between the genus *Paenibacillus* (phylum Firmicutes) and nonessential amino acids in the stomach (Figure 6). There was no significant correlation between the genera and protein, fat, and ash contents. In the midgut, genus

TABLE 2 Nutritional quality indices of lipid contents in Beluga populations.

Lipid contents	Year 2012	Year 2013	Year 2018
MUFA/SFA	0.62	0.84	0.78
PUFA/SFA	0.90	0.93	0.96
n-3/*n*-6	1.96	1.80	2.16
ARA/EPA	0.71	0.58	0.42
EPA + DHA	18.90	17.69	19.95
DHA/EPA	1.25	1.02	1.23
AI	0.66	0.61	0.65
TI	0.35	0.35	0.33
H/H	1.25	1.16	1.21

Abbreviations: ARA, arachidonic acid; AI, atherogenic index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; H/H, hypocholesterolemia/hypercholesterolemia ratio; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenic index.

TABLE 3 Tissue compositions of amino acids of the three groups of Beluga sturgeon (g 100 g^{-1}).

Amino acids	Year 2012	Year 2013	Year 2018	*p*-value
Essential AA				
Histidine	0.42 ± 0.0	0.51 ± 0.0	0.47 ± 0.0	$p = 0.0085$
Isoleucine	0.78 ± 0.0	0.77 ± 0.0	0.83 ± 0.0	$p = 0.2382$
Leucine	1.34 ± 0.0	1.31 ± 0.0	1.42 ± 0.0	$p = 0.2258$
Lysine	1.58 ± 0.0	1.53 ± 0.0	1.63 ± 0.0	$p = 0.3011$
Phenylalanine	0.70 ± 0.0	0.67 ± 0.0	0.70 ± 0.0	$p = 0.2141$
Tyrosine	0.55 ± 0.0	0.56 ± 0.0	0.61 ± 0.0	$p = 0.1990$
Threonine	0.83 ± 0.0	0.81 ± 0.0	0.82 ± 0.0	$p = 0.6041$
Arginine	1.27 ± 0.0	1.24 ± 0.0	1.13 ± 0.0	$p = 0.4949$
Valine	0.84 ± 0.0	0.84 ± 0.0	0.90 ± 0.0	$p = 0.1135$
Nonessential AA				
Aspartic acid	1.80 ± 0.4	1.75 ± 0.4	1.80 ± 0.4	$p = 0.6785$
Glutamic acid	2.76 ± 0.0	2.61 ± 0.0	2.69 ± 0.0	$p = 0.2918$
Serine	0.87 ± 0.0	0.86 ± 0.0	0.82 ± 0.0	$p = 0.6646$
Glycine	1.76 ± 0.3	1.72 ± 0.3	1.13 ± 0.3	$p = 0.3864$
Alanine	1.26 ± 0.1	1.24 ± 0.1	1.09 ± 0.1	$p = 0.4582$
Proline	1.1 ± 0.2	1.0 ± 0.2	0.79 ± 0.2	$p = 0.3628$
TAA	17.86 ± 0.60	17.42 ± 0.56	16.83 ± 0.57	
ΣEAA	8.31 ± 0.39	8.24 ± 0.36	8.51 ± 0.38	
ΣNEAA	9.55 ± 0.68	9.18 ± 0.64	8.32 ± 0.73	

Note: The values represent mean ± SD of three replicates.
Abbreviations: AA, amino acids; EAA, essential AA; NEAA, nonessential AA.

Paenibacillus was negatively correlated with arginine in the stomach. The genus *Haloimpatients* from phylum Firmicutes showed a positive and significant correlation with phenylalanine ($r = 0.8563$, $p = 0.0238$) and threonine ($r = 0.8281$, $p = 0.0217$) in the stomach for the essential amino acids (Figure 7).

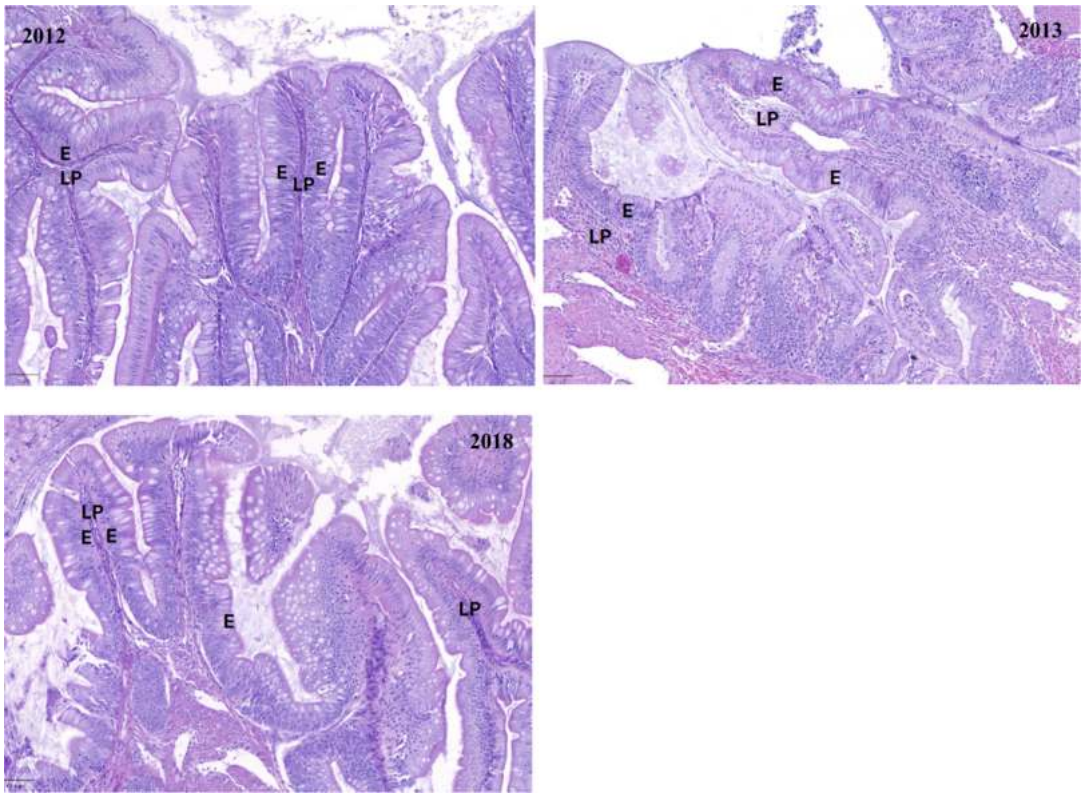

FIGURE 4 Histological sections of the three populations Beluga sturgeon. 2012—normal mucosa, 2013—mild lymphocytic infiltration, 2018. E, epithelium; LP, lamina propria.

Further, genera *Gallicola* ($r = 0.7877$, $p = 0.0321$), *Haloimpatients* ($r = 0.7877$, $p = 0.0320$), and *Staphylococcus* ($r = 0.7877$, $p = 0.0320$), all from phylum Firmicutes, were positively correlated with valine, while *Staphylococcus was* positively correlated with histidine ($r = 0.8281$, $p = 0.0217$), in the hindgut. However, both *Paenibacillus* ($r = -0.7454$, $p = 0.0441$; $r = -0.8280$, $p = 0.0217$; $r = -0.7454$, $p = 0.0441$) and *Gallicola* ($r = -0.4472$, $p = 0.2270$; $r = -0.5521$, $p = 0.1259$; $r = -0.4472$, $p = 0.2270$) negatively correlated with isoleucine, leucine, and tyrosine, respectively, in the hindgut. In the midgut, a positive correlation was found between *Enterococcus* and *Vagococcus* ($r = 0.8081$, $p = 0.0217$; $r = 0.8666$, $p = 0.0166$; $r = 0.8081$, $p = 0.0217$) genera and isoleucine, total lysine, and tyrosine. However, a negative correlation was shown between *Clostridium* and valine (Figure 7).

The genus *Lactococcus* negatively correlated with glycine ($r = -0.2000$, $p = 0.7194$) and proline ($r = -0.2000$, $p = 0.7194$), which are nonessential amino acids in the midgut; likewise, *Paenibacillus* ($r = -0.4667$, $p = 0.2722$) was negatively correlated with alanine in the stomach (Figure 8).

Predominantly, phylum Firmicutes (>90%) was more abundant in all three GIT sections (stomach, midgut, and hindgut sections) (Figure 9). By contrast, the abundance of the Fusobacteria phylum increased along the intestinal sections, which showed the hindgut having the most abundant community than the stomach and midgut of the fish. By age groups, bacteria were mainly from the phyla Firmicutes, Fusobacteriota, and Proteobacteria (Figure 10). However, they differed in relative abundance between the age groups and intestinal sections. The hindgut had a higher abundance of Firmicutes in all groups, and the phylum was more abundant in the hindgut of younger Beluga, 2018 (43%) and 2013 (31%) age groups than the 2012 age group (29%). Further, the relative abundance of the phylum Proteobacteria decreased with the age of fish; the hindgut of the 2018 age group had the most abundant composition and was almost inexistent in the 2012 and 2013 age groups. Figure 11 show the genus-level compositions

TABLE 4 Bacterial diversity indices of Beluga.

Samples	Alpha diversity				
By gut locations	**Stomach**	**Midgut**	**Hindgut**	***F*-values**	***p*-values**
Shannon	1.48[b]	1.48[b]	2.27[a]	3.901	0.0272
Chao 1	0.34[a]	0.36[a]	0.52[a]	3.363	0.0433
By age groups	2012	2013	2018		
Shannon	1.82	1.41	1.99	1.587	0.2160
Chao 1	0.44	0.34	0.43	0.9270	0.4030
	Beta diversity				
	Pseudo-*F*	***p*-value**			
Gut location	3.4858	0.002			
Age	1.7907	0.033			
Location × Age	1.3193	0.112			
a. Gut locations	***t***	***p*-value**	b. Age group	***t***	***p*-value**
Stomach vs. Midgut	1.8226	0.005	Y18 vs. Y12	1.1206	0.2220
Stomach vs. Hindgut	2.2745	0.001	Y18 vs. Y13	1.3680	0.0650
Midgut vs. Hindgut	1.4184	0.054	Y12 vs. Y13	1.0194	0.3780
	Significant pairwise comparison				
Groups	**R statistic**	***p*-value**			
S_Y12 vs. H_Y12	0.368	0.04			
S_Y12 vs. M_Y12	0.344	0.043			
S_Y12 vs. H_Y18	0.838	0.008			
S_Y12 vs. M_Y18	0.42	0.008			
S_Y13 vs. H_Y18	0.524	0.01			
H_Y18 vs. M_Y13	0.607	0.005			
H_Y12 vs. M_Y13	0.283	0.052			
H_Y18 vs. H_Y12	0.338	0.056			

Note: A species-level alpha and beta diversity estimates to determine richness and evenness in the Beluga samples. Chao 1 and Shannon indices were used for alpha diversity, while permutational multivariate analysis of variance was used for the Beta diversity. Groups representations are S_(stomach), M_ (midgut), and H_ (hindgut), while Y12, Y13, and Y18 represent (12-, 13-, and 18-year age groups), respectively. Superscripts a and b indicate significant differences among the gut locations.

across the three age groups. Further, more genera were found in the midgut and hindgut sections of the 2018 age group than in the 2012 and 2013 age groups. High level of *Clostridium* dominated the three GIT sections, but the abundance was highest in the 2012 group and reduced along the digestive tract as age increased. In addition, *Lactococcus* and *Lactobacillus* genera were abundant in the lower and increased in the hindgut section of the fish from all the age groups. Further, the abundance of genus *Lactobacillus* was more in the young (2018 age group) than the older Beluga.

4 | DISCUSSION

Beluga is a valuable fish due to its production of caviar and meat. The available literature about its nutritional requirements is scarce, making it challenging for farmers to raise the fish optimally. The proximate fat content in the

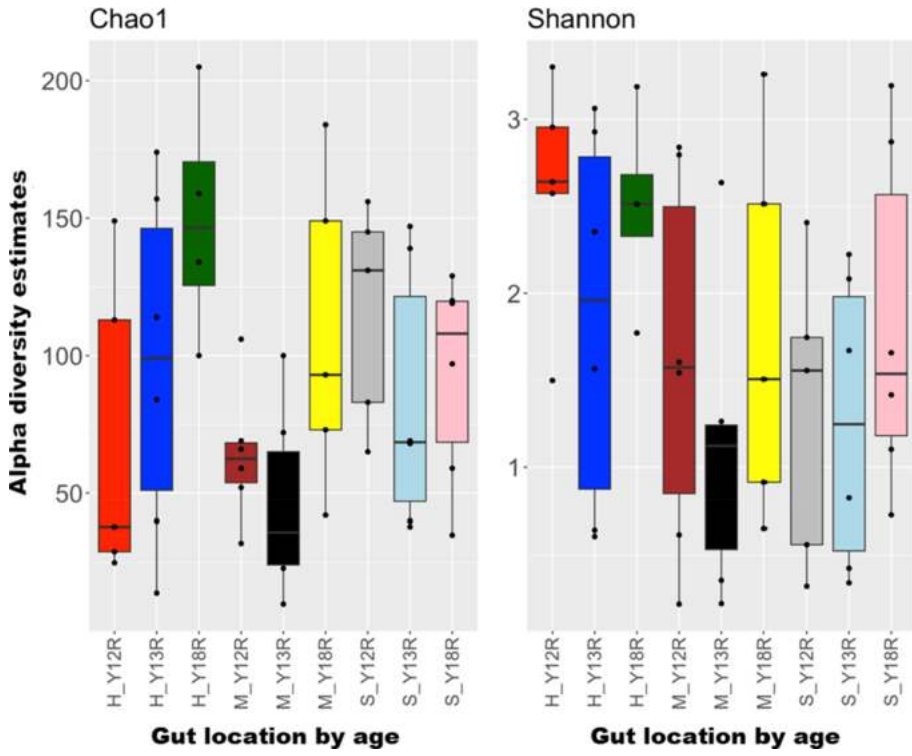

FIGURE 5 Alpha diversity estimates in the gut of Beluga sturgeon. H, hindgut; M, midgut; S, stomach.

2013 age group was lower than earlier reported values for cultivated and wild Beluga in similar age groups (Fazli et al., 2020). Lower condition factors observed in age groups confirm the concerns of irregular growth patterns observed by the farmers in this population of farmed Beluga. Because the condition factor shows the physiological status of the fish (Fazli et al., 2020; Kumolu-Johnson & Ndimele, 2010), our speculation for the irregular growth could be linked to an imbalance nutrient profile in the commercial diets, which may have limited nutrients available to the fish for normal growth and development. Because the dietary data and water quality over the feeding periods are unavailable to better understand the result of this study, a subsequent study to properly monitor the feeding regime of the Beluga in the commercial setting is warranted. It should be noted that limited information is available on nutrient requirements of farm-raised Beluga. The practice of formulating commercial feed for Beluga based on nutritional information on other sturgeon species like sterlet or other fishes such as rainbow trout (Amrkolaie et al., 2013; Hung & Deng, 2002; Williot et al., 2001) may be another challenge to the industry. In addition, suitable environmental conditions and stocking density have been reported to enhance condition factor values or growth performance in Beluga (Dediu et al., 2021), but there was no detailed record of the water quality parameters of the recirculating culturing system used on the farm for the fish over the period investigated. The reason for the higher values for the body condition indexes, particularly VSI and HSI observed for all age groups, especially in the 2018 age group compared with previous studies, is not yet known (Abtahi et al., 2013; Ebrahimi & Zare, 2006; Falahatkar et al., 2018) but could be attributed to abnormal lipid utilization or imbalance nutrient compositions in the diet.

Fatty acid compositions, especially contents of highly unsaturated fatty acids in the diet, are important for growth and gonad development. A diet with less than 300 g kg^{-1} of lipid is deemed fit for Beluga, while a diet with high lipid content will induce lipid deposition in the liver and viscera of the fish (Amrkolaie et al., 2013; Falahatkar et al., 2018). Beluga from the 2012 population had higher percentages of total saturated fatty acid but a lower mono-unsaturated fatty acid than those in the 2013 and 2018 groups, which contrasts with previous results obtained in

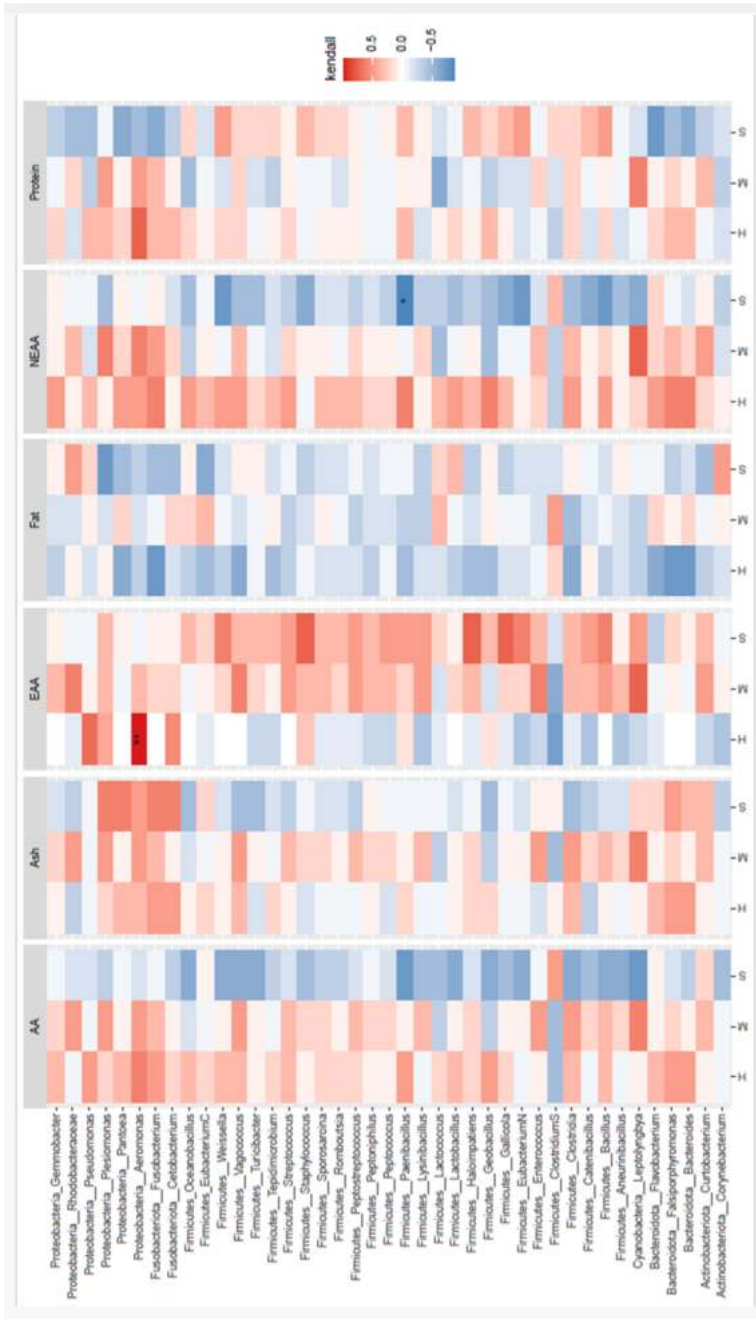

FIGURE 6 Correlation of bacteria genera with proximate compositions and major class of amino acids using Kruskal method, significant values are based on * = $p < 0.05$ and ** = $p < 0.01$. The samples are AA = amino acids; EAA = essential amino acids; NEAA = nonessential amino acids.

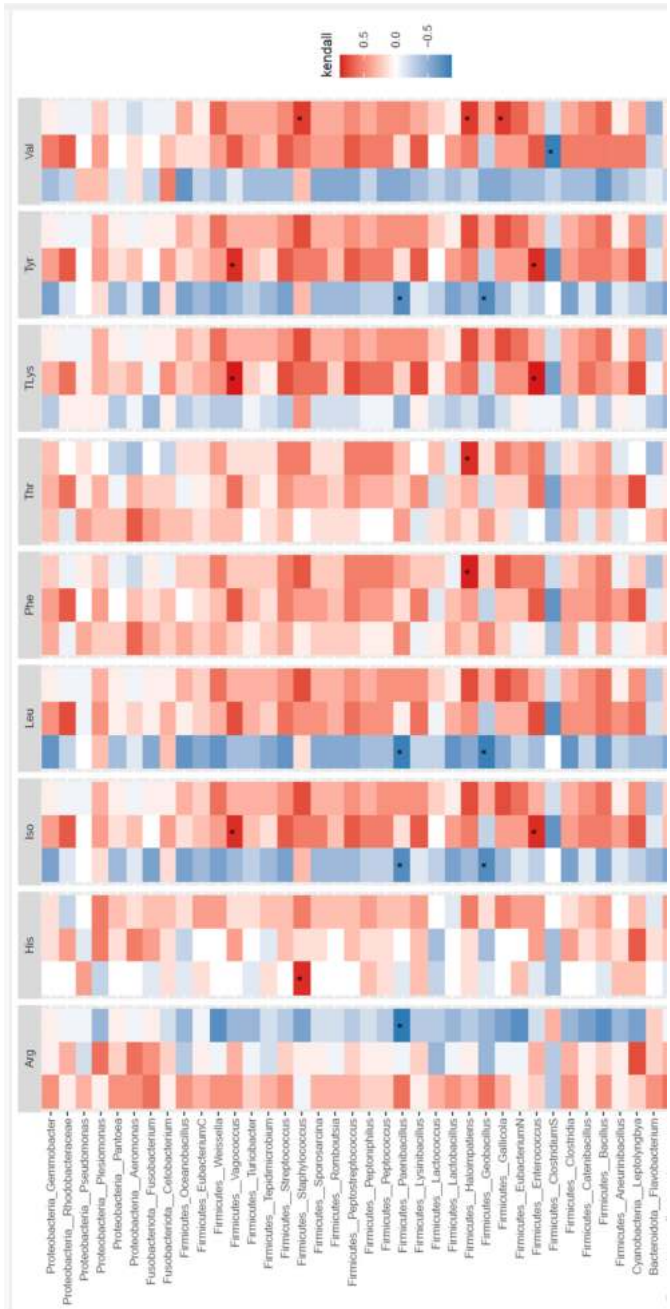

FIGURE 7 Correlation of bacteria genera with essential amino acid compositions using Kruskal method, significant values are based on * = $p < 0.05$ and ** = $p < 0.01$. The samples are Arg, arginine; His, histidine; Leu, leucine; Iso, isoleucine; Phe, phenylalanine; Thr, threonine; TLys, total lysine; Try, tyrosine; Val, valine.

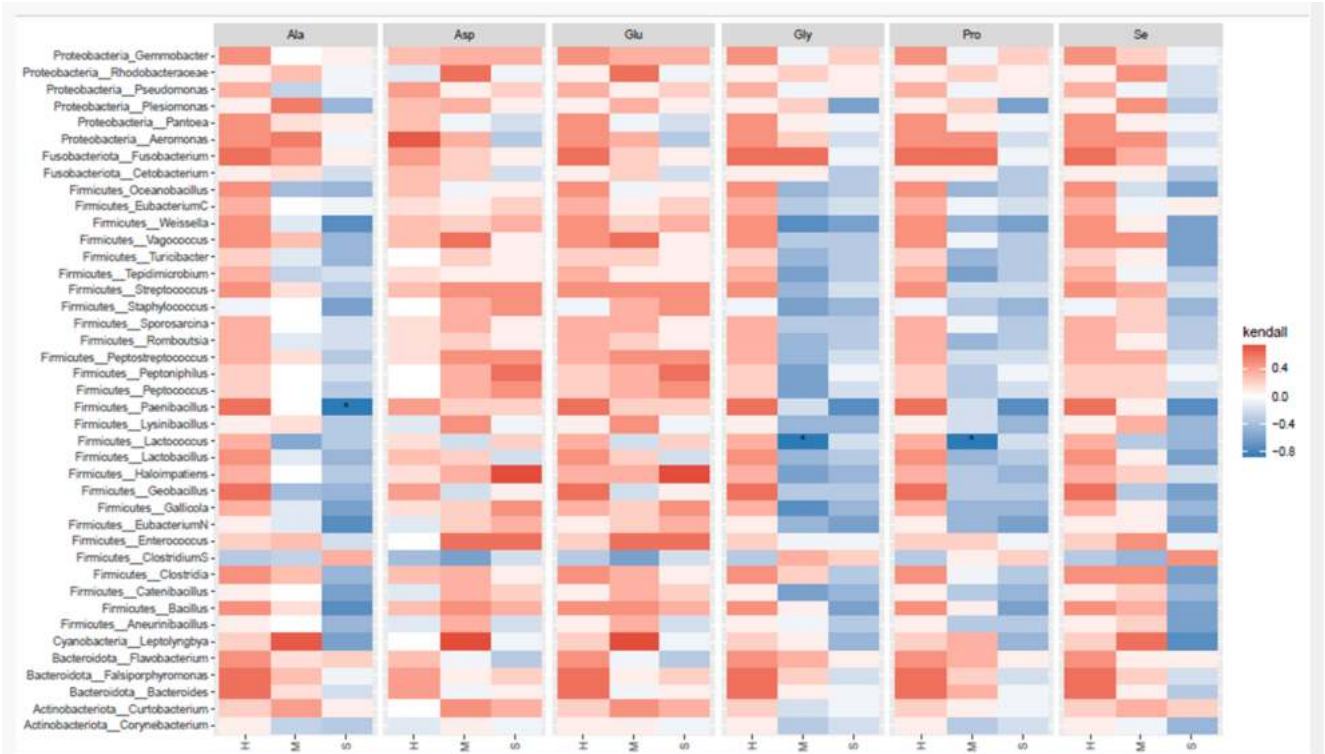

FIGURE 8 Correlation of bacteria genera with nonessential amino acid compositions using Kruskal method, significant values are based on * = $p < 0.05$ and ** = $p < 0.01$. The samples are Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Pro, proline; Se, serine.

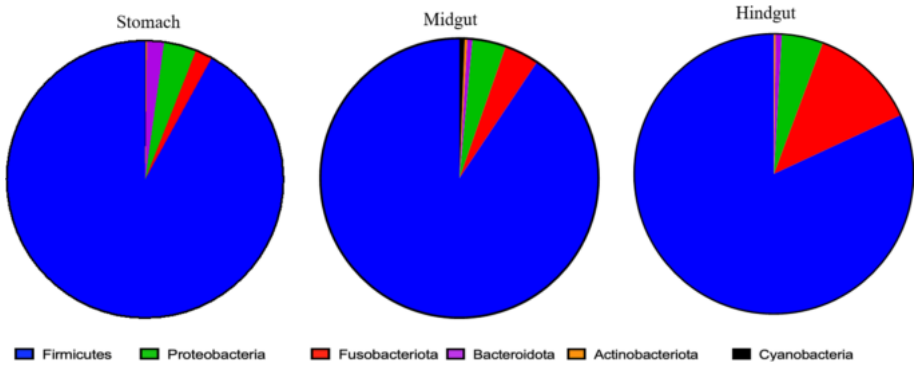

FIGURE 9 Overall phylum compositions of Beluga sturgeon.

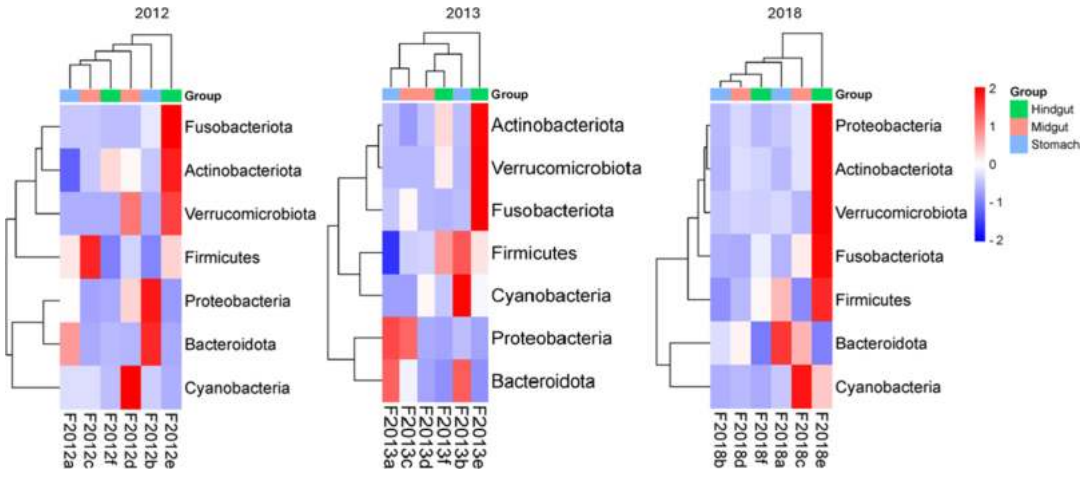

FIGURE 10 Heatmap showing the phylum-level compositions in the stomach, midgut, and hindgut in the three age groups of Beluga sturgeon.

tissue and caviar samples of 10 years farm Beluga (Abbas & Hrachya, 2015). In all the age groups, the main fatty acids were palmitic acid, stearic acid, and myristic acid in decreasing order. Earlier studies on farm-raised and wild Beluga sturgeon (Barimani et al., 2021; Hosseini et al., 2010) as well as in 10 years old farm-raised Beluga (Abbas & Hrachya, 2015) reported palmitic acid as the major saturated fatty acid in the fish tissues. Further, high contents of the three saturated fatty acids have been identified in farm-raised finfish from within and outside the US (Cladis et al., 2014; Sissener et al., 2018; Stejskal et al., 2011).

The current study shows a high possibility of erucic acid in the oil used in manufacturing of the diet fed to the fish. Beluga from the 2018 age group had a significantly higher content of C22:1n-9 (erucic acid) than the 2012 and 2013 groups (Table 1), and oleic acid (C18:1n-9) was the lowest percentage fatty acid among the omega-9 fatty acids for all age groups. Because a high content of erucic acid in feeds does not correlate with a high concentration of the acid in fish tissue, as reported in some farmed fishes (Caballero et al., 2002; Memon et al., 2011; Sharma et al., 2010), we proposed a further study to understand lipid sources and erucic tissue metabolism in sturgeon. Erucic acid has been identified as a natural toxicant with the potential to contribute to myocardial lipidosis and heart lesions in laboratory studies (Knutsen et al., 2017; Vetter et al., 2020). Despite the possible impact of erucic acid and its isomeric on human health, this fatty acid has constantly been identified in fish fillets, including farm-raised

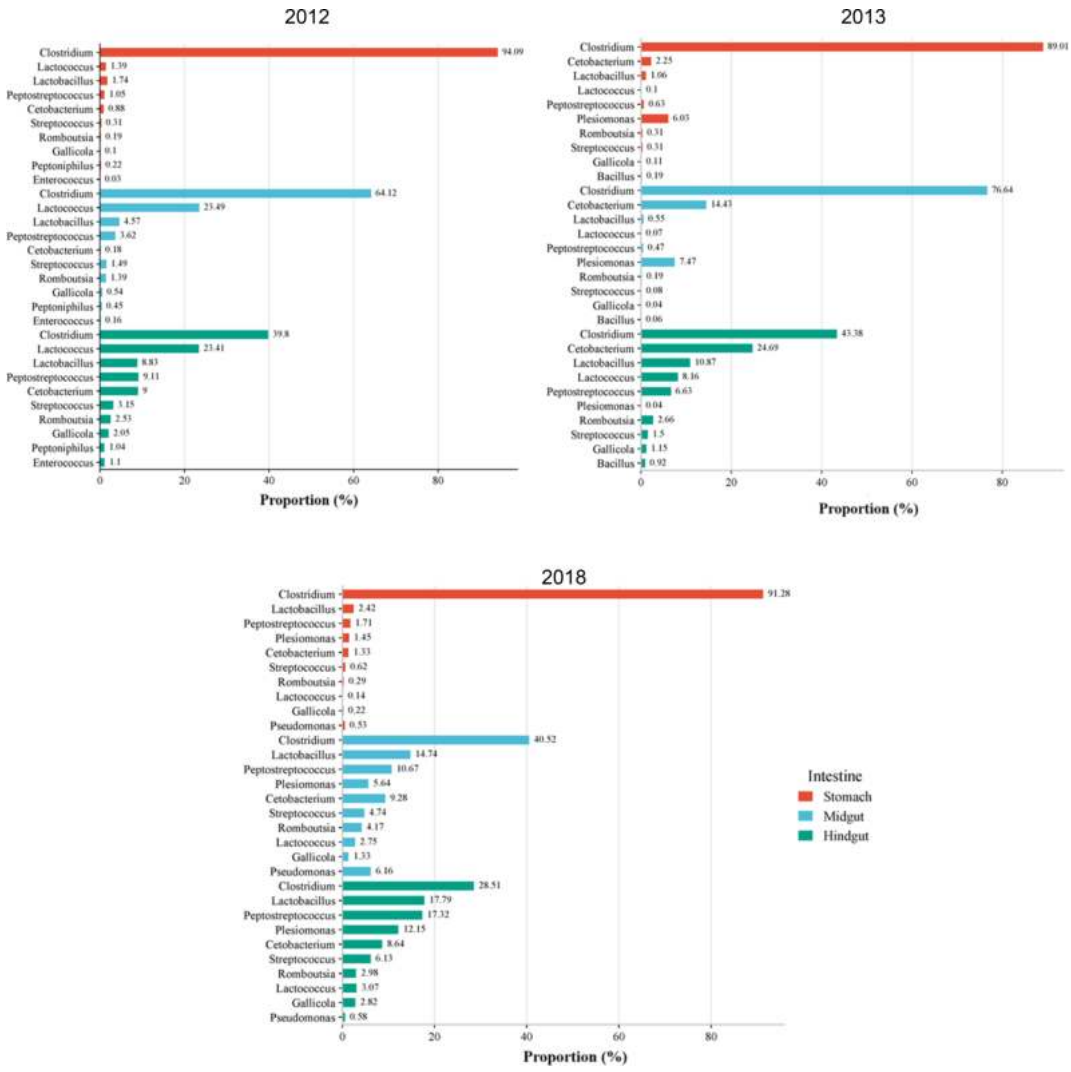

FIGURE 11 Major genera compositions in the stomach, midgut, and hindgut in the three age groups of Beluga sturgeon.

and wild salmon (Sissener et al., 2018). The observed TI, HH, and AI values are within the limits reported for wild and culture Beluga, with little differences between the age group fillets (Badiani et al., 1996; Barimani et al., 2021; Kenari et al., 2009). The values of AI and TI >1 have been suggested to affect human health (Moussavi Javardi et al., 2020), which is not the case in the present study as the values obtained (Table 2) were <1, making the fish beneficial to humans as a source of quality fish food.

The amino acid levels observed in the present study are lower compared with previous studies from wild and cultured Beluga (Barimani et al., 2021; Hamzeh et al., 2015). Likewise, the values observed for total, essential, and nonessential amino acids were lower than previously reported. The discrepancies in amino acid values are expected due to possible differences in nutrient compositions of the diet in the studies. It is noteworthy that Beluga, like most sturgeon species, does not have commercial diets formulated to meet the nutritional requirements of the fish, especially for different developmental stages. As observed in this study, farmers opted for a lower crude protein diet to save cost since it takes several years to reach maturity, which may not be a sustainable nutritional option for

the fish to attain optimal performance. The importance of amino acids in fish nutrition is well documented in cultured fishes (Kaushik & Seiliez, 2010; McLean et al., 2024; Nunes et al., 2014). However, limited data are available for farm sturgeon species. The limited availability of fishmeal in the aquafeed industry has increased the usage of alternative protein sources such as plant proteins for cultured fishes. One caveat with using plant protein ingredients as an alternative to fishmeal is the imbalance amino acid profile, which makes it a necessity to supplement such diets with synthetic amino acids (Betiku et al., 2023; Betiku, Yeoman, Gaylord, Americus, et al., 2018; Gaylord & Barrows, 2009; Hoseini et al., 2022). In Beluga, most of the diets used by fish farms are formulated for other fishes, and the quantitative requirements of the essential amino acids are not tailored to meet the muscle requirements of the fish, which may have accounted for the low values of the amino acid compositions observed in the present study.

Previous studies show a positive relationship between diets/feed ingredient types and gut epithelial in fishes (Heikkinen et al., 2006; Sohrabnezhad et al., 2017). Dietary composition is an environmental factor that influences microbiota composition and fish performance (Nayak, 2010; Perry et al., 2020). While the specific roles of each microbe in fish GIT are yet to be understood entirely, studies have emphasized the involvement of the microbiota in nutrient digestion, energy homeostasis, health, and immunity by preventing colonization of pathogenic agents (Delzenne & Cani, 2008; Luan et al., 2023; Nie et al., 2023). In a study on rainbow trout, amino acid catabolism by gut microbes is influenced by dietary compositions (Betiku, Yeoman, Gaylord, Duff, et al., 2018). Although the information on the functions of the gut microbial community in Beluga remains limited, the same functions observed in other teleost are expected in sturgeons. This information may be vital to understanding which nutrients are important to Beluga and how they are altered by diet and abiotic factors. In the present study, a positive correlation between the genus *Aeromonas* and essential amino acids suggests the importance of these amino acids to the intestinal microbes and influences their distribution in the GIT of the host (Mardinoglu et al., 2015). *Aeromonas* spp. are pathogenic bacteria in aquaculture. During disease resistance to infection from *Aeromonas* spp. and other pathogenic bacteria, profiles of certain amino acids such as valine and leucine have been shown to increase in cultured fishes (Cao et al., 2024; Nurdalila et al., 2020). This information suggests interaction of the amino acids to fish host during identified bacterial infection.

The *Lactococcus* belongs to the genus of lactic acid bacteria (LAB), which are known to produce lactic acid from glucose fermentation (Onyeaka & Nwabor, 2022); the bacteria were significantly and negatively correlated with glycine and proline in beluga midgut from this study. Further, the involvement of *Staphylococcus* in amino acids catabolism has been reported, and the bacteria can adapt to metabolites from carbon and nitrogenous sources to proliferate in the host, particularly catabolism of pyruvate-yielding amino acids (Halsey et al., 2017). In the study by Halsey and colleagues, *Staphylococcus* prefers catabolizing other glucogenic amino acids (alanine, serine, glycine, threonine, arginine, proline, glutamate, and aspartate) over histidine, which may support the negative correlation observed between *Staphylococcus* and histidine in this study.

Another LAB observed is the *Enterococcus* genus, which showed a positive and significant association with isoleucine, total lysine, and tyrosine. *Enterococcus* is known to be associated with carbohydrate metabolism (Zhong et al., 2017). However, its involvement with these essential amino acids in Beluga is unknown, and future studies are required. Curiously, the *Gallicola* genus has shown no ability to utilize carbohydrates but depends on amino acid metabolism. The abundance of *Gallicola* was found to be associated with acetic acids (Bao et al., 2023), and *Gallicola* was found to be positively correlated with valine in this study. The specific functional role of this association is also not known, but the best speculation is that valine is being catabolized to acetic acid via the production of acetyl-CoA, which is a precursor for the citric acid cycle. Unfortunately, the formulation of commercial diets used in feeding Beluga is unavailable due to propriety status, so interpreting the association between the bacteria and the amino acids in the present study must be carried out cautiously. Recent dietary evaluation of valine in juvenile largemouth bass (LMB) shows a strong correlation with utilization for antioxidant activities during stressful conditions caused by bacterial infection (Cao et al., 2024), suggesting optimal amino acid levels to enhance the growth of LMB. Future studies focusing on meeting the essential amino acid requirements, for instance, valine, in Beluga for optimum growth and how they modulate microbiota compositions and immune functions are long overdue. This information

may help to meet the needs of cultured Beluga for faster growth and survival. In the histopathological analysis, low-level/sub-clinical inflammation observed in the GIT may decrease the ability of the fish to digest, absorb, and utilize nutrients. This has been shown in an earlier study on Beluga (Sohrabnezhad et al., 2017) and other fishes (Bonaldo et al., 2008; Pervin et al., 2020). In addition, all fish have zymogen granule depletion in the pancreas, which can indicate decreased nutrient ingestion or anorexia. Because feed intake and utilization data are unavailable, it is difficult to understand how the fish utilize nutrients in the different age groups. Amino acid requirements vary between juvenile and adult fish species, including commercially important fishes like tilapia, catfish, and salmonids (NRC, 2011). However, there is a dearth of such information on sturgeons (Hung, 2017). A possible speculation is that the nutrient requirements of these farm-raised Beluga, especially amino acids, may not have been met, limiting nutrient utilization because the formulation of the commercial feed was based on nutritional information for other fishes.

The observed phyla in the farm-raised Beluga in this study align with the gut microbial community reported in Beluga and other cultured fishes (Xu et al., 2019; Zhao et al., 2020). The differences in microbial composition and abundance by age group observed in this study are supported by previous findings. Age-related changes were associated with the gut microbiota of southern catfish (Zhang et al., 2018). Normal microbial communities and abundance have been shown to increase with age in humans (La-Ongkham et al., 2020; Wang et al., 2015; Zhao et al., 2011) and animals (Nayak, 2010; Samanta & Bandyopadhyay, 2019; Wei et al., 2018), which support changes in microbial abundance observed in this study. *Clostridium* is one of the normal gut genera, which is associated with cellulose decomposition, and its abundance was highest in the midgut and hindgut sections of the fish with the most abundance in the oldest population (2012 group) of Beluga. Although the functional roles of *Clostridium* species in aquatic animals have not been well characterized, the bacteria are well-known as spore-forming rods with the ability to produce butyrate, ethanol acetate, and D-lactate (Froidurot & Julliand, 2022).

Because the digestive system of sturgeon is structurally different from that of other fishes, studies to understand the representatives of cellulolytic microbes and their functions in Beluga may help improve their farming conditions. In addition, a consistently high percentage of *Lactococcus* and *Lactobacillus* genera observed in the midgut and hindgut sections of Beluga revealed the ability of the microbes to persist within the GIT sections and across the age groups. Although the modulatory factors influencing the abundance of *Lactococcus* and *Lactobacillus* genera in the Beluga GIT sections are unknown, mainly if the diets fed on the farm played a significant role or not, we speculate the diets provided a conducive environment that enhanced the growth of the microbes. Both *Lactococcus* and *Lactobacillus* species are probiotic candidates in aquatic species that have been isolated from cultured fishes; their supplementation in feeds and culturing water have shown to enhance fish growth and reproductive performance and a reduction in disease occurrence and mortality (Ringø et al., 2020). The application of probiotics is limited in Beluga, but a previous study isolated a strain of *Lactococcus lactis* that improved growth and disease resistance in juvenile Beluga (Yeganeh Rastekenari et al., 2021). Likewise, *L. lactis* isolates from Siberian broodstock resisted *Aeromonas* infection in a laboratory setting (Chen et al., 2023). More studies are needed to explore the benefits of these microbes as probiotics in Beluga and other cultured species.

5 | CONCLUSION

In summary, Beluga is an important fish species cultured for its caviar and flesh to meet the needs of humans for fish proteins. The farm-raised Beluga characterized in this study were in physically good condition. However, they differed in nutrients and microbial compositions across the age groups. The condition factor observed for the age groups in this study was nonoptimal, indicating the need to improve the growth through diets or feeding regimes. Because many nutrients required for optimal growth and health of Beluga are unknown, future research should be tailored to meet this need. The predominant bacteria associated with the stomach, midgut, and hindgut sections of Beluga were Firmicutes, Fusobacteriota, and Proteobacteria, and their compositions differed by age and in the GIT locations. The hindgut section contained the highest abundance of Firmicutes. *Aeromonas* and *Gallicola* were

positively correlated with essential amino acids and valine, respectively, suggesting the importance of these amino acids to the fish host under certain conditions, such as stress. More importantly, research to exploit the benefits of the resident bacteria that have the potential for enhanced nutrient utilization and reproduction should be explored in Beluga. Dietary formulation to supplement specific essential amino acids observed to have significant correlations in the present study may help optimize fish growth and enhance immunity during stressful conditions.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data used in this study are available upon request.

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REFERENCES

Abbas, E., & Hrachya, G. (2015). Fatty acid composition of caviar and liver from cultured great sturgeon (Huso huso). *International Food Research Journal*, *22*, 1083.

Abtahi, B., Yousefi, M., & Kenari, A. A. (2013). Influence of dietary nucleotides supplementation on growth, body composition and fatty acid profile of beluga sturgeon juveniles (Huso huso). *Aquaculture Research*, *44*, 254–260.

Amrkolaie, A. K., Yansari, A. T., & Khalesi, M. (2013). Calculation of protein and energy requirements in beluga sturgeon (Huso huso) using a factorial approach. *Journal of Animal Physiology and Animal Nutrition*, *97*, 485–494.

AOAC (Ed.). (2005). *Official methods of analysis* (16th ed.). AOAC.

Badiani, A., Anfossi, P., Fiorentini, L., Gatta, P., Manfredini, M., Nanni, N., Stipa, S., & Tolomelli, B. (1996). Nutritional composition of cultured sturgeon (Acipenserspp.). *Journal of Food Composition and Analysis*, *9*, 171–190.

Baeverfjord, G., & Krogdahl, Å. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, Salmo salar L., distal intestine: A comparison with the intestines of fasted fish. *Journal of Fish Diseases*, *19*, 375–387.

Bao, R., Wei, Y., Guan, R., Li, X., Lu, X., Rong, S., Zuo, X., & Yuan, H. (2023). High-solids anaerobic co-digestion performances and microbial community dynamics in co-digestion of different mixing ratios with food waste and highland barley straw. *Energy (Oxford)*, *262*, 125529.

Barimani, S., Hedayatifard, M., Motamedzadegan, A., & Bozorgnia, A. (2021). Changes of amino acids and proximate compositions in freshwater farmed beluga sturgeon (Huso huso) caviar. *Iranian Journal of Fisheries Sciences*, *20*, 230–242.

Betiku, O. C., Barrows, F. T., Ross, C., & Sealey, W. M. (2016). The effect of total replacement of fish oil with DHA-gold (R) and plant oils on growth and fillet quality of rainbow trout (Oncorhynchus mykiss) fed a plant-based diet. *Aquaculture Nutrition*, *22*, 158–169.

Betiku, O. C., Yeoman, C. J., Gaylord, T. G., Americus, B., Olivo, S., Duff, G. C., & Sealey, W. M. (2018). Water system is a controlling variable modulating bacterial diversity of gastrointestinal tract and performance in rainbow trout. *PLoS One*, *13*, e0195967.

Betiku, O. C., Yeoman, C. J., Gaylord, T. G., Duff, G. C., Hamerly, T., Bothner, B., Block, S. S., & Sealey, W. M. (2018). Differences in amino acid catabolism by gut microbes with/without prebiotics inclusion in GDDY-based diet affect feed utilization in rainbow trout. *Aquaculture*, *490*, 108–119.

Betiku, O. C., Yeoman, C. J., Gaylord, T. G., Ishaq, S. L., Duff, G. C., & Sealey, W. M. (2023). Evidence of a divided nutritive function in rainbow trout (Oncorhynchus mykiss) midgut and hindgut microbiomes by whole shotgun metagenomic approach. *Aquaculture Reports*, *30*, 101601.

Bonaldo, A., Roem, A. J., Fagioli, P., Pecchini, A., Cipollini, I., & Gatta, P. P. (2008). Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (Sparus aurata L.) and European sea bass (Dicentrarchus labrax L.). *Aquaculture Research*, *39*, 970–978.

Caballero, M., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., & Izquierdo, M. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, Oncorhynchus mykiss. *Aquaculture*, *214*, 253–271.

Caimi, C., Renna, M., Lussiana, C., Bonaldo, A., Gariglio, M., Meneguz, M., Dabbou, S., Schiavone, A., Gai, F., & Elia, A. C. (2020). First insights on black soldier Fly (Hermetia illucens L.) larvae meal dietary administration in Siberian sturgeon (Acipenser baerii Brandt) juveniles. *Aquaculture*, *515*, 734539.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, *13*(7), 581–583.

Cao, Q., Zhao, J., He, L., Zhang, T., Feng, L., Jiang, W., Wu, P., Zhao, J., Liu, H., & Jiang, J. (2024). Evaluation of the dietary L-valine on fish growth and intestinal health after infection with Aeromonas veronii. *Aquaculture*, *580*, 740294.

Chen, D., Yang, F., Yang, L., Pei, Q., Zhang, X., Huang, X., Ouyang, P., Li, Z., & Geng, Y. (2023). Co-isolated bacteria from septicemia of female Siberian sturgeon broodstock: Lactococcus petauri resisting Aeromonas hydrophila infection. *Aquaculture*, *575*, 739763.

Cladis, D. P., Kleiner, A. C., Freiser, H. H., & Santerre, C. R. (2014). Fatty acid profiles of commercially available finfish fillets in the United States. *Lipids*, *49*, 1005–1018.

Clarke, K., & Gorley, R. (2015). *Getting started with PRIMER v7. PRIMER-E* (p. 20). Plymouth Marine Laboratory.

Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food chemistry*, *103*(3), 891–899.

Dediu, L., Docan, A., Creţu, M., Grecu, I., Mogodan, A., Maereanu, M., & Oprea, L. (2021). Effects of stocking density on growth performance and stress responses of bester and bester♀× beluga♂ juveniles in recirculating aquaculture systems. *Animals*, *11*, 2292.

Delzenne, N. M., & Cani, P. D. (2008). Gut microflora is a key player in host energy homeostasis. *Médecine Sciences*, *24*, 505–510.

Ebrahimi, E., & Zare, P. (2006). Growth and survival of beluga (Huso huso) and Persian sturgeon (Acipenser persicus) fingerlings fed by live food and artificial diet. *Journal of Applied Ichthyology*, *22*, 321–324.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461.

Falahatkar, B., Asheri, S., Safarpour Amlashi, A., & Ershad Langroudi, H. (2018). Canola oil, as a good alternative dietary lipid source in sturgeon: Effects on growth, physiology and fatty acid profile in beluga sturgeon Huso huso L. *Aquaculture Nutrition*, *24*, 1263–1273.

FAO. (2023). In FAO (Ed.), *Aquaculture transformation – Innovation and investment for sustainable intensification and expansion of aquaculture in Asia and the Pacific region*. FAO. https://doi.org/10.4060/cc4962en

Fazli, H., Tavakoli, M., Khoshghalb, M. R. B., & Moghim, M. (2020). Biological parameters and fisheries indices of beluga sturgeon in the southern Caspian Sea. *Croatian Journal of Fisheries*, *78*, 1–10.

Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Froidurot, A., & Julliand, V. (2022). Cellulolytic bacteria in the large intestine of mammals. *Gut Microbes*, *14*, 2031694.

Gaylord, T. G., & Barrows, F. T. (2009). Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, Oncorhynchus mykiss, feeds. *Aquaculture*, *287*, 180–184.

Glöckner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., & Ludwig, W. (2017). 25 years of serving the community with ribosomal RNA gene reference databases and tools. *Journal of biotechnology*, *261*, 169–176.

Gu, M., Bai, N., Zhang, Y., & Krogdahl, Å. (2016). Soybean meal induces enteritis in turbot Scophthalmus maximus at high supplementation levels. *Aquaculture*, *464*, 286–295.

Halsey, C. R., Lei, S., Wax, J. K., Lehman, M. K., Nuxoll, A. S., Steinke, L., Sadykov, M., Powers, R., & Fey, P. D. (2017). Amino acid catabolism in Staphylococcus aureus and the function of carbon catabolite repression. *MBio*, *8*(1), 10–1128. https://doi.org/10.1128/mbio.01434-01416

Hamzeh, A., Moslemi, M., Karaminasab, M., Khanlar, M., Faizbakhsh, R., Batebi Navai, M., & Tahergorabi, R. (2015). Amino acid composition of roe from wild and farmed beluga sturgeon (Huso huso). *Journal of Agricultural Science and Technology*, *17*, 357–364.

Heikkinen, J., Vielma, J., Kemiläinen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Navia-Paldanius, D., & von Wright, A. (2006). Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (Oncorhynchus mykiss). *Aquaculture*, *261*, 259–268.

Hoseini, S. M., Moghaddam, A. A., Ghelichpour, M., Pagheh, E., Haghpanah, A., Gharavi, B., Mansouri, B., & Arghideh, M. (2022). Dietary glycine supplementation modulates antioxidant and immune responses of beluga, Huso huso, juveniles. *Aquaculture Reports*, *23*, 101026.

Hosseini, S. V., Kenari, A. A., Regenstein, J. M., Rezaei, M., Nazari, R. M., Moghaddasi, M., Kaboli, S. A., & Grant, A. A. (2010). Effects of alternative dietary lipid sources on growth performance and fatty acid composition of beluga sturgeon, Huso huso, juveniles. *Journal of the World Aquaculture Society*, *41*, 471–489.

Hung, S. S. (2017). Recent advances in sturgeon nutrition. *Animal Nutrition*, *3*, 191–204.

Hung, S. S., & Deng, D.-F. (2002). Sturgeon, Acipenser spp. In *Nutrient requirements and feeding of finfish for aquaculture* (pp. 344–357). CABI Publishing.

Jobling, M. (2012). *National Research Council (NRC): Nutrient requirements of fish and shrimp: The National Academies Press*. Springer.

Karabulut, H. A., Kurtoğlu, İ. Z., & Kirtan, Y. E. (2019). Effects of the feeds containing hazelnut meal as plant protein source on growth performance and body composition of Siberian sturgeon (Acipenser baeri) and economic profitability value. *Turkish Journal of Veterinary and Animal Sciences*, *43*, 244–252.

Kaushik, S. J., & Seiliez, I. (2010). Protein and amino acid nutrition and metabolism in fish: Current knowledge and future needs. *Aquaculture Research*, *41*, 322–332.

Kenari, A. A., Regenstein, J. M., Hosseini, S. V., Rezaei, M., Tahergorabi, R., Nazari, R. M., Mogaddasi, M., & Kaboli, S. A. (2009). Amino acid and fatty acid composition of cultured beluga (Huso huso) of different ages. *Journal of Aquatic Food Product Technology*, *18*, 245–265.

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., & Frøkiær, H. (2008). Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (Salmo salar L.). *British Journal of Nutrition*, *100*, 120–129.

Knutsen, H. K., Alexander, J., Barregaard, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Nebbia, C. S., Oswald, I., Petersen, A., Rose, M., Roudot, A.-C., Schwerdtle, T., Vollmer, G., Wallace, H., … Vleminckx, C. (2017). Erucic acid in feed and food. *EFSA Journal*, *14*, 4593.

Kumar, V., Hossain, S., Ragaza, J. A., & Benito, M. R. (2020). *The potential impacts of soy protein on fish gut health. Soybean hum. Consum. Anim. Feed*. IntechOpen.

Kumolu-Johnson, C., & Ndimele, P. (2010). Length-weight relationships and condition factors of twenty-one fish species in Ologe lagoon, Lagos, Nigeria. *Asian Journal of Agricultural Sciences*, *2*, 174–179.

La-Ongkham, O., Nakphaichit, M., Nakayama, J., Keawsompong, S., & Nitisinprasert, S. (2020). Age-related changes in the gut microbiota and the core gut microbiome of healthy Thai humans. *3 Biotech*, *10*, 1–14.

Lepage, G., & Roy, C. C. (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research*, *25*, 1391–1396.

Li, H. (2020). *Effects of the soybean antinutrients saponin, isoflavonoid, and phytosterol on morphology and immune responses in the distal intestine of Atlantic salmon*. Norwegian University of Life Sciences, Ås.

Liu, H., Wu, X., Zhao, W., Xue, M., Guo, L., Zheng, Y., & Yu, Y. (2009). Nutrients apparent digestibility coefficients of selected protein sources for juvenile Siberian sturgeon (Acipenser baerii Brandt), compared by two chromic oxide analyses methods. *Aquaculture Nutrition*, *15*, 650–656.

Luan, Y., Li, M., Zhou, W., Yao, Y., Yang, Y., Zhang, Z., Ringø, E., Olsen, R. E., Clarke, J. L., & Xie, S. (2023). The fish microbiota: Research progress and potential applications. *Engineering*, *29*, 137–146.

Mardinoglu, A., Shoaie, S., Bergentall, M., Ghaffari, P., Zhang, C., Larsson, E., Bäckhed, F., & Nielsen, J. (2015). The gut microbiota modulates host amino acid and glutathione metabolism in mice. *Molecular Systems Biology*, *11*, 834.

McLean, E., Alfrey, K. B., Gatlin, D. M., III, Gaylord, T. G., & Barrows, F. T. (2024). Muscle amino acid profiles of eleven species of aquacultured animals and their potential value in feed formulation. *Aquaculture and Fisheries*, *9*(4), 642–652.

Memon, N. N., Talpur, F. N., Bhanger, M., & Balouch, A. (2011). Changes in fatty acid composition in muscle of three farmed carp fish species (Labeo rohita, Cirrhinus mrigala, Catla catla) raised under the same conditions. *Food Chemistry*, *126*, 405–410.

Merrifield, D., Dimitroglou, A., Bradley, G., Baker, R., & Davies, S. (2009). Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, Oncorhynchus mykiss (Walbaum). *Journal of Fish Diseases*, *32*, 755–766.

Moonsamy, P., Williams, T., Bonella, P., Holcomb, C., Höglund, B., Hillman, G., Goodridge, D., Turenchalk, G., Blake, L., & Daigle, D. (2013). High throughput HLA genotyping using 454 sequencing and the Fluidigm access Array™ system for simplified amplicon library preparation. *Tissue Antigens*, *81*, 141–149.

Moussavi Javardi, M. S., Madani, Z., Movahedi, A., Karandish, M., & Abbasi, B. (2020). The correlation between dietary fat quality indices and lipid profile with Atherogenic index of plasma in obese and non-obese volunteers: A cross-sectional descriptive-analytic case-control study. *Lipids in Health and Disease*, *19*, 1–9.

Naqib, A., Poggi, S., Wang, W., Hyde, M., Kunstman, K., & Green, S. J. (2018). Making and sequencing heavily multiplexed, high-throughput 16S ribosomal RNA gene amplicon libraries using a flexible, two-stage PCR protocol. In *Gene expression analysis* (pp. 149–169). Springer.

Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. *Aquaculture Research*, *41*, 1553–1573.

Nie, Z., Xu, X., Shao, N., He, J., Li, P., Xu, P., Hu, J., Qin, W., Wang, B., & Xu, G. (2023). Integrative analysis of microbiome and metabolome reveals the linkage between gut microbiota and carp growth. *Environmental Research*, *220*, 115133.

NRC. (2011). *Nutrient requirements of fish and shrimp*. The National Academies Press.

Nunes, A. J., Sá, M. V., Browdy, C. L., & Vazquez-Anon, M. (2014). Practical supplementation of shrimp and fish feeds with crystalline amino acids. *Aquaculture*, *431*, 20–27.

Nurdalila, A. w. A., Natnan, M. E., & Baharum, S. N. (2020). The effects of amino acids and fatty acids on the disease resistance of Epinephelus fuscoguttatus in response to Vibrio vulnificus infection. *3 Biotech*, *10*, 1–13.

Onyeaka, H., & Nwabor, O. (2022). Lactic acid bacteria and bacteriocins as biopreservatives. In H. Onyeaka & O. Nwabor (Eds.), *Food preservation and safety of natural products* (pp. 147–162). Academic Press.

Ortiz-Estrada, Á. M., Gollas-Galván, T., Martínez-Córdova, L. R., & Martínez-Porchas, M. (2019). Predictive functional profiles using metagenomic 16S rRNA data: A novel approach to understanding the microbial ecology of aquaculture systems. *Reviews in Aquaculture*, *11*, 234–245.

Parrish, C. C. (1999). Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In M. T. Arts & B. C. Wainman (Eds.), *Lipids in freshwater ecosystems*. Springer.

Perry, W. B., Lindsay, E., Payne, C. J., Brodie, C., & Kazlauskaite, R. (2020). The role of the gut microbiome in sustainable teleost aquaculture. *Proceedings of the Royal Society B*, *287*, 20200184.

Pervin, M. A., Jahan, H., Akter, R., Omri, A., & Hossain, Z. (2020). Appraisal of different levels of soybean meal in diets on growth, digestive enzyme activity, antioxidation, and gut histology of tilapia (Oreochromis niloticus). *Fish Physiology and Biochemistry*, *46*, 1397–1407.

Petersen, C., & Round, J. L. (2014). Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology*, *16*, 1024–1033.

Ringø, E., Van Doan, H., Lee, S. H., Soltani, M., Hoseinifar, S. H., Harikrishnan, R., & Song, S. K. (2020). Probiotics, lactic acid bacteria and bacilli: Interesting supplementation for aquaculture. *Journal of Applied Microbiology*, *129*, 116–136.

Ringø, E., Zhou, Z., Vecino, J. G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R., Dimitroglou, A., Foey, A., & Davies, S. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquaculture Nutrition*, *22*, 219–282.

Samanta, I., & Bandyopadhyay, S. (2019). *Antimicrobial resistance in agriculture: Perspective, policy and mitigation*. Academic Press.

Santos-Silva, J., Bessa, R., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. *Livestock Production Science*, *77*, 187–194.

Seibel, H., Chikwati, E., Schulz, C., & Rebl, A. (2022). A multidisciplinary approach evaluating soybean meal-induced enteritis in rainbow trout Oncorhynchus mykiss. *Fishes*, *7*(22), 1–18.

Sharma, P., Kumar, V., Sinha, A. K., Ranjan, J., Kithsiri, H., & Venkateshwarlu, G. (2010). Comparative fatty acid profiles of wild and farmed tropical freshwater fish rohu (Labeo rohita). *Fish Physiology and Biochemistry*, *36*, 411–417.

Sissener, N. H., Ørnsrud, R., Sanden, M., Frøyland, L., Remø, S., & Lundebye, A.-K. (2018). Erucic acid (22: 1n-9) in fish feed, farmed, and wild fish and seafood products. *Nutrients*, *10*, 1443.

Sohrabnezhad, M., Sudagar, M., & Mazandarani, M. (2017). Effect of dietary soybean meal and multienzyme on intestine histology of beluga sturgeon (Huso huso). *International Aquatic Research*, *9*, 271–280.

Stejskal, V., Vejsada, P., Cepak, M., Špička, J., Vacha, F., Kouril, J., & Policar, T. (2011). Sensory and textural attributes and fatty acid profiles of fillets of extensively and intensively farmed Eurasian perch (Perca fluviatilis L.). *Food Chemistry*, *129*, 1054–1059.

Ulbricht, T., & Southgate, D. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, *338*, 985–992.

Urán, P., Gonçalves, A., Taverne-Thiele, J., Schrama, J., Verreth, J., & Rombout, J. (2008). Soybean meal induces intestinal inflammation in common carp (Cyprinus carpio L.). *Fish & Shellfish Immunology*, *25*, 751–760.

Van Eenennaam, J. P., Chapman, F., & Jarvis, P. (2004). Aquaculture. In: LeBreton, G. T. O., Beamish, F. W. H., & McKinley, R. S. (Eds.), *Sturgeons and Paddlefish of North America*. Kluwer Academic Publishers, Dordrecht, pp 284–287.

Vargas-Albores, F., Martínez-Córdova, L. R., Hernández-Mendoza, A., Cicala, F., Lago-Lestón, A., & Martínez-Porchas, M. (2021). Therapeutic modulation of fish gut microbiota, a feasible strategy for aquaculture? *Aquaculture*, *544*, 737050.

Vetter, W., Darwisch, V., & Lehnert, K. (2020). Erucic acid in Brassicaceae and salmon–an evaluation of the new proposed limits of erucic acid in food. *NFS Journal*, *19*, 9–15.

Wang, F., Yu, T., Huang, G., Cai, D., Liang, X., Su, H., Zhu, Z., Li, D., Yang, Y., & Shen, P. (2015). Gut microbiota community and its assembly associated with age and diet in Chinese centenarians. *Journal of Microbiology and Biotechnology*, *25*, 1195–1204.

Wei, N., Wang, C., Xiao, S., Huang, W., Lin, M., Yan, Q., & Ma, Y. (2018). Intestinal microbiota in large yellow croaker, Larimichthys crocea, at different ages. *Journal of the World Aquaculture Society*, *49*, 256–267.

Williot, P., Sabeau, L., Gessner, J., Arlati, G., Bronzi, P., Gulyas, T., & Berni, P. (2001). Sturgeon farming in Western Europe: Recent developments and perspectives. *Aquatic Living Resources*, *14*, 367–374.

Xu, G., Xing, W., Li, T., Xue, M., Ma, Z., Jiang, N., & Luo, L. (2019). Comparative study on the effects of different feeding habits and diets on intestinal microbiota in Acipenser baeri Brandt and Huso huso. *BioMed Central Microbiology*, *19*, 1–12.

Yeganeh Rastekenari, H., Kazami, R., Shenavar Masouleh, A., Banavreh, A., Najjar Lashgari, S., Sayed Hassani, M. H., Ghorbani Vaghei, R., Alizadeh Roudposhti, M., & Hallajian, A. (2021). Autochthonous probiotics Lactococcus lactis and Weissella confusa in the diet of fingerlings great sturgeon, Huso huso: Effects on growth performance, feed efficiency, haematological parameters, immune status and intestinal morphology. *Aquaculture Research*, *52*, 3687–3695.

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614–620.

Zhang, W., Tan, B., Deng, J., Dong, X., Yang, Q., Chi, S., Liu, H., Zhang, S., Xie, S., & Zhang, H. (2021). Mechanisms by which fermented soybean meal and soybean meal induced enteritis in marine fish juvenile pearl gentian grouper. *Frontiers in Physiology*, *12*, 646853.

Zhang, Z., Li, D., Refaey, M. M., Xu, W., Tang, R., & Li, L. (2018). Host age affects the development of southern catfish gut bacterial community divergent from that in the food and rearing water. *Frontiers in Microbiology*, *9*, 495.

Zhao, L., Xu, W., Ibrahim, S. A., Jin, J., Feng, J., Jiang, J., Meng, J., & Ren, F. (2011). Effects of age and region on fecal microflora in elderly subjects living in Bama, Guangxi, China. *Current Microbiology*, *62*, 64–70.

Zhao, R., Symonds, J. E., Walker, S. P., Steiner, K., Carter, C. G., Bowman, J. P., & Nowak, B. F. (2020). Salinity and fish age affect the gut microbiota of farmed Chinook salmon (Oncorhynchus tshawytscha). *Aquaculture*, *528*, 735539.

Zhong, Z., Zhang, W., Song, Y., Liu, W., Xu, H., Xi, X., Menghe, B., Zhang, H., & Sun, Z. (2017). Comparative genomic analysis of the genus enterococcus. *Microbiological Research*, *196*, 95–105.

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