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Valorization of corn husk waste: Xylooligosaccharides as a sustainable prebiotic enhance growth, gut health, and fillet quality in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Xylooligosaccharides (XOS) are advantageous prebiotics that promote sustainable aquaculture. The growth performance, gut microbiota, and fillet composition of juvenile Nile tilapia (*Oreochromis niloticus*) were investigated in relation to the prebiotic effects of corn husk-derived XOS, extracted using alkaline extraction and autohydrolysis. Following 2 weeks of acclimatization, 270 fish were split into three groups: control, 0.5% XOS, and 1% XOS, with three replicates per group. For 56 days, fish were fed twice a day. Final weight (83.9 g in 1% XOS vs. 44.5 g in control, $p < 0.001$, Cohen's $d = 2.1$) and survival rate (94% in 1% XOS vs. 70% in control, $p < 0.001$) were both considerably increased by XOS supplementation. The feed conversion ratio dropped significantly from 2.69 (control) to 0.99 (1%

XOS, $p = 0.003$). The nutritional quality of the fillet increased, with calcium levels more than doubling (2.6%–6.7%, $p < 0.001$) and protein content increasing by 35.6% (14.9% in control to 20.2% in 1% XOS, $p < 0.001$). A 36-fold increase in lactic acid bacteria (833 CFU/g in 1% XOS vs. 23 CFU/g in the control group, $p < 0.05$) and a decrease in coliforms were observed in the gut microbiota study, suggesting improved gut health. These results demonstrate that XOS derived from corn husks can serve as a sustainable feed additive improving fillet quality, shaping a favorable gut microbiota, enhancing fish development, and providing a viable substitute for antibiotic growth promoters in aquaculture.

KEYWORDS

fillet nutritional composition, growth performance, gut microbiota modulation, Nile tilapia, prebiotics in aquaculture, sustainable fish feed additives, Xylooligosaccharides

1 | INTRODUCTION

A very important farmed fish worldwide is the Nile tilapia (*Oreochromis niloticus*). Nile tilapia is one of the most significant aquaculture species in the world, with an annual production of more than 6.4 million metric tons, or around 10% of the global aquaculture output (FAO, 2023). China is the world's largest producer of Nile tilapia, with over 1.6 million tons, followed by significant producers Egypt, Indonesia, Thailand, and Brazil. Nile tilapia aquaculture, valued at USD $4.3 billion per year, is an essential source of protein for food security in poor countries and makes a significant contribution to rural economies (FAO, 2023). Their global use emphasizes the crucial need for sustainable feed additives that improve production efficiency while reducing environmental impact and antibiotic dependency. It provides adequate funds and food for aquaculture. According to a 2024 assessment, *O. niloticus* is responsible for most of Zimbabwe's fish production, which is in the top 10 in the Southern Sahara (Mabika & Utete, 2024). Even though *O. niloticus* production is critical, concerns such as poor intestinal health, insufficient feed, and inferior fillets persist. When infections spread, and a large quantity of manure is added, the problems worsen. Antibiotic growth promoter limits have resulted in lower yields and higher illness rates. It is critical to identify sustainable feed additives that improve *O. niloticus* growth performance, gut health, and product quality.

Prebiotics such as xylooligosaccharides (XOS) have demonstrated potential in this area. According to recent research, XOS can improve fillet quality, change the makeup of the gut microbiota, and boost growth performance in a range of fish species (Ajeniyi & Solomon, 2014; Li et al., 2021; Reverter et al., 2017; Ringo et al., 2010). According to Quin et al. (2013), Lui et al. (2017), and Li et al. (2020), XOS increases the growth rate, feed efficiency, and survival of Nile tilapia. It is commonly recognized that XOS has a prebiotic role. Because it is more resistant to stomach digestion than other oligosaccharides, it can enter the hindgut and serve as a selective substrate for helpful bacteria like *Lactobacillus* and *Bifidobacterium* (Zheng et al., 2018). Fermentation then produces short-chain fatty acids (SCFAs), which lower intestinal pH, inhibit bacterial growth, and support nutrient absorption (Legrand et al., 2019).

Corn husk is especially well-suited for sustainable aquaculture applications in potential agricultural waste streams for XOS production because of its multiple benefits. Corn husk is one of the most common agricultural byproducts in the world, accounting for 20%–25% of corn biomass and frequently being underutilized or incinerated in environmentally hazardous ways (Anarado et al., 2022). Compared to other sources like rice husks (24%) or wheat straw (29%), it has greater XOS conversion rates because of its composition, which includes about 35% xylan by dry weight (Contiero & Brienzo, 2022; Samanta et al., 2015). Additionally, because of its unique polymerization profile, which is mostly made up of xylobiose and xylotriose, which are both utilized by good gut bacteria, corn husk-derived XOS exhibits higher prebiotic action (Chen et al., 2020). In Zimbabwe, where corn is the main agricultural crop and annual production exceeds 700,000 tons, corn husk is a readily accessible, inexpensive, and locally sustainable feedstock for XOS synthesis. This adheres to the principles of the circular economy while reducing reliance on foreign feed additives.

People are becoming more interested in obtaining useful chemicals, such as XOS, from inexpensive agricultural waste streams to make aquaculture more sustainable. Corn husk is a plentiful, renewable byproduct of corn farming that can be used in new ways to reduce waste and the environmental problems that come with it. The hemicellulose component of lignocellulosic materials is a significant source of xylan, which can be converted into XOS via various methods (Aachary & Prapulla, 2011; Samanta et al., 2015). XOS derived from corn husks has received considerable attention because it is easy to obtain and lasts a long time (Mabika & Utete, 2024), yet there remains a significant gap in understanding. There is a dearth of extensive investigations into the generation and effects of corn husk-derived XOS, specifically on Nile tilapia fingerlings, despite a growing body of evidence supporting XOS use in aquaculture. Consequently, our study aimed to address this need by examining the possibilities of this locally derived, sustainable prebiotic. The main objectives of this work are to (1) extract and characterize XOS from corn husk; and (2) examine how nutritional supplementation with XOS produced from corn husk affects the growth performance, gut microbiota, and fillet composition of fingerlings of Nile tilapia. To promote a more sustainable future for Zimbabwe's aquaculture industry, this study's findings aim to provide a scientific basis for converting agricultural waste into a valuable feed ingredient.

2 | MATERIALS AND METHODS

2.1 | Materials, chemicals, and strains

The corn husk was obtained from the Department of Research and Specialist Services (DR&SS) and research fields, then chopped into small pieces. These pieces were dried in a vacuum oven until they became fully dry, as shown by a consistent weight value. The corn husk was ground to uniform-sized powder particles and stored in an airtight container. Corn husk was first dried in an oven at 60°C until constant weight was achieved, then milled to a fine powder in a ball mill. Sodium hydroxide, absolute ethanol, and acetic acid were purchased and stored appropriately. Media such as plate count agar, de Man, Rogosa, and Sharpe (MRS) agar, MacConkey agar, and Trypticase Soy Agar with Bacitracin and Xylitol (TBX) agar were purchased and stored at room temperature in a closed, dark, dry room.

2.2 | Research design

This study was carried out in three phases: (1) XOS extraction and characterization; (2) feed formulation and dietary integration; and (3) an 8-week feeding experiment with growth performance and microbiota evaluation. Three dietary treatments (control, 0.5% XOS, and 1% XOS) with three replicates each were used in a fully complete randomized design (CRD). Throughout the experiment, water quality indicators (pH, electrical conductivity, and dissolved oxygen) were routinely monitored, and the growth performance of juvenile Nile tilapia, their gut microbiota, fillet nutritional values, and blood residues (urea and creatinine) were assessed. Although there was no blinding, bias was minimized by using established measuring procedures.

2.3 | Sample size determination

The sample size was determined using a power analysis based on differences in fish development rates. For each group, about 44 fish fingerlings were required using a 95% confidence level, 80% statistical power, a standard deviation of 5.0, and a minimum detectable difference of 3.0 g. About 132 fish were needed for the investigation, and they were split into three groups: control, 0.5% XOS, and 1% XOS. A total of 132 fish were found to be the minimum needed based on a power study. A total of 270 fish were used in the feeding trial to boost the study's robustness and account for probable death.

2.4 | Alkaline extraction of Xylan from corn husk

Fifty grams of the powdered corn husk was mixed with 500 mL of 15% w/v sodium hydroxide solution in heat-resistant bottles. The mixture was autoclaved for 20 min at 121°C and 15 psi. After cooling, the mixture was filtered through a muslin cloth, and the solids were discarded. The liquid was then neutralized with glacial acetic acid to adjust the pH of the filtered hydrolyses to 5.0. The pH-adjusted hydrolysates were then precipitated overnight with cold ethanol in a 1:3 ratio, and the precipitated xylan was collected by centrifugation at 6000 g for 20 min at 4°C. The xylan was washed with alcohol 4 times and dried for 24 h at 60°C in an oven (Xu et al., 2006).

2.5 | Production of XOS from corn husk using oven-assisted autohydrolysis

In this study, dried xylan (hemicellulose) powder was used to produce XOS via auto-hydrolysis during oven hydrothermal treatment. It is expected that this process will turn 22% of the xylan into XOS (Samanta et al., 2016). First, the xylan was mixed with water at a 1:10 ratio and heated to 160°C for 60 min to initiate autohydrolysis. After that, the mixtures were spun at 11,000 rpm for 20 min at 4°C. Then, the supernatant was mixed with 95% ethanol in a 1:3 ratio and left to settle overnight. The precipitate was air-dried and put in a desiccator after being spun at 11,000 rpm for 10 min at 4°C. To optimize XOS output from maize husk, preliminary investigations were carried out by varying important parameters, such as alkali content (5%–20% w/v NaOH), extraction temperature (100–130°C), and hydrolysis length (15–45 min). Autoclaving 15% NaOH for 20 min at 121°C produced the maximum xylan extraction (33.5%). During the autohydrolysis stage, temperature (140–180°C) and reaction time (30–90 min) were closely observed. The optimal temperature for producing XOS was 160°C for 60 min, which produced 24.15% XOS and very little xylose monomer. The ideal ratio of XOS (DP2-DP6) to monomeric xylose and other degradation products was found using these variables. The reaction pH was maintained at 5.0 throughout neutralization to ensure efficient precipitation with ethanol while preventing additional XOS breakdown. The phenol-sulfuric acid method was used to test yields against xylose standards, and each optimization step was performed in triplicate. This optimization approach followed methodologies established by Samanta et al. (2016) and Chen et al. (2020), with adjustments specific to Zimbabwean corn husk composition.

2.6 | Determination of XOS profile using TLC

We mixed the XOS sample and the standard, which had X1: xylose (DP1), X2: xylobiose (DP2), X3: xylotriose (DP3), X4: xylotetraose (DP4), X5: xylopentaose (DP5), and X6: xylohexaose (DP6), in methanol to make a solution with 1 mg/mL. To establish a uniform baseline, ethyl acetate: acetic acid: water (3:1:1) was used to pre-develop a 20 × 20 cm silica gel TLC plate (McDonald et al., 2010). The XOS sample and the standard solution were spotted in 2 μL on the TLC plate, about 1 cm from the bottom edge. The TLC plate was developed in a solvent mixture of ethyl acetate, acetic acid, and water for 15 min in a TLC chamber. After development, the TLC was air-dried, sprayed with phenol-sulfuric acid (1% phenol, 2% sulfuric acid in ethanol), and heated at 100–120°C for 5–10 min. The retention factors of the XOS sample components in replicates were compared with standards to identify and profile the XOS molecule present in the sample.

2.7 | In vivo analysis (feeding trial)

2.7.1 | Feed formulation

The feed was formulated, and the proximate analysis was conducted using AOAC methods for Fertilizer and Farm Feeds Analysis (Table 1) following the basal nutritional requirements of Nile tilapia.

2.7.2 | Experimental design and fish feeding

A total of 275 *O. niloticus* fingerlings were purchased from Mazowe Research Institute and were acclimatized for 2 weeks at the Fisheries Department, Mazowe Research Institute rearing ponds, being fed with a commercial feed (control diet). After acclimatization, five fingerlings were sacrificed for health checks using microscopy and the cultivation of gills and organs. The five fish sacrificed were found to be healthy, with no bacterial pathogens isolated from

TABLE 1 The formulation and proximate composition of experimental diets for feeding 270 juveniles.

Ingredient	Control (kg)	0.5% XOS (kg)	1% XOS (kg)
Fish meal	0.52	0.52	0.52
Corn meal	2.13	2.13	2.13
Soybean meal	2.25	2.25	2.25
Wheat flour	0.15	0.15	0.15
wheat bran	1.0	1.0	1.0
Soybean oil/fish oil	0.25	0.25	0.25
Vitamin mix	0.05	0.05	0.05
Mineral mix	0.05	0.05	0.05
MCP	0.05	0.05	0.05
Salt	0.05	0.05	0.25
XOS	0	0.05	0.1
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)			
Protein	35.80	36.73	36.90
Fat	5.10	5.30	5.07
Ash	7.01	6.79	7.10
Salt	0.37	0.44	0.66
Phosphorus	1.15	0.62	0.66
Moisture content	9.43	12.52	11.63
Fiber	1.72	1.70	1.7
Calcium	0.22	0.26	0.20

Abbreviations: MCP, monocalcium phosphate; XOS, xylooligosaccharides.

the gills, organs, or muscles. The remaining 270 fish were randomly distributed into three feeding groups, with three replicates each, using a completely randomized design across nine concrete fishponds. The fish were selected based on uniform size (16.0 ± 0.01 g) and good health. Any fish that deviated from their size, had signs of disease or deformities, or showed abnormal swimming behavior were excluded. The fish were fed basal feed supplemented with 0.5% or 1% XOS, or commercial feed without any supplements (control group). The fingerlings were kept in fish-harp nets in nine concrete ponds for 56 days (8 weeks), fed twice a day in accordance with a natural photoperiod. Fish were closely monitored for abnormal behavior or any signs of a disease, while mortality and feed consumption were recorded daily. Water quality parameters (pH, temperature, dissolved oxygen, and electrical conductivity) were monitored daily using a multi-parameter water quality meter (Hanna Instruments, HI98194). Throughout the 56-day feeding trial, water quality parameters were kept within the following acceptable ranges for optimal Nile tilapia growth and health: temperature 26–30°C (mean: 28.2 ± 0.7°C), dissolved oxygen >5.0 mg/L (mean: 6.3 ± 0.4 mg/L), pH 6.5–8.5 (mean: 7.4 ± 0.3), and electrical conductivity 150–500 µS/cm (mean: 312 ± 45 µS/cm). According to Boyd (2017) and Meurer et al. (2025), the ranges fall within the accepted criteria for *O. niloticus* farming. The corrective measures, that is, increased aeration and partial water exchanges (10%–15% of total volume), were implemented immediately when pond values reached the limits of these ranges (e.g., dissolved oxygen falling below 5.5 mg/L during peak feeding periods). Daily monitoring logs of the ponds confirmed that all measured parameters remained within allowable limits throughout the trial, ensuring that observed effects could be attributed to the food interventions rather than to variations in water quality.

2.8 | Growth parameters

2.8.1 | Sampling strategy

For growth parameters, all fish in each tank were counted and bulk-weighed every 2 weeks. At the end of the trial, three fish per tank ($n = 9$ per treatment) were randomly selected for fillet composition analysis. Another three fish per tank were anesthetized for blood collection via caudal venipuncture for biochemical analysis. Subsequently, the same fish were euthanized for gut microbiota sampling and the calculation of health indices (fish hepatosomatic index [HIS] and viscerosomatic index[VSI]). On day 56, all the fish were weighed, and feed consumption was measured by recording the amount of feed provided and the amount consumed (the amount consumed by each feeding group was noted). The measured parameters were used to calculate fish growth parameters, as described by Hussain et al. (2021), using the following formulae:

$$\text{Weight gain (WG)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{Specific growth rate (SGR)} = \text{natural logarithm of WG/feeding duration (days)} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake (g)/weight gain (g)}$$

$$\text{Survival (\%)} = (\text{number of fish at harvest/number of fish stocked}) \times 100.$$

$$\text{Protein Efficiency Ratio (PER)} = \text{Weight gain per fish (g)} \times 100/\text{Protein intake (g)} \times 100$$

$$\text{Mean daily weight gain (MDWG)} = (\text{final weight of fish} - \text{initial weight of fish})/\text{days of the experiment}.$$

$$\text{Feed efficiency (FE)} = 100 \times (\text{final weight of fish} - \text{initial weight of fish})/\text{feed consumed}$$

2.8.2 | Fillet nutritional value of the experimental diets

Fillets of 3 fish per cage were collected and tested for crude protein, crude fat, ash, dry matter, calcium, and phosphorus using McDonald et al. (2010). The protein content was calculated using the following formulae:

$$\%\text{Nitrogen} = (\text{mLs of } 0.25\text{N } H_2SO_4 - \text{Titre reading } 0.25\text{N NaOH})(0.35)$$
$$\%\text{Protein} = \%\text{Nitrogen} \times 6.25$$
$$0.35 = \text{nitrogen factor and } 6.25 = \text{protein factor}$$

The phosphorus percentage was calculated using the following method:

$$\%\text{phosphorus} = \left(\frac{\text{UV reading} \times \text{dilution factor}}{1{,}000{,}000}\right)100$$

Moisture content was calculated using the following method:

$$\%\text{moisture} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}}100$$

Ash content was measured using the following method:

$$\%\text{Ash} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}}100$$

Calcium and salt content in the feed were determined by titration, and fat content was determined by Soxhlet extraction.

2.8.3 | Biochemical parameters

At 8 weeks, serum from three fish per tank was analyzed for biochemical parameters, including blood urea nitrogen, creatinine, albumin, globulin, total protein, and cholesterol. Samples were processed using a fully automatic chemical analyzer (Cobas C 311).

2.8.4 | Health indices

HSI and VSI were calculated using the following formulae:

$$\text{HSI}(\%) = (\text{liver weight}/\text{fish weight}) \times 100$$

$$\text{VSI}(\%) = (\text{visceral weight}/\text{fish weight}) \times 100$$

2.8.5 | Gut microbiome sampling

After 56 days, the fish were starved for 24 h before sample collection (McDonald et al., 2010). Then, three fish from each cage (9 per feeding group/3 replicates) were randomly sampled and anesthetized, and hindgut contents were collected aseptically using scalpel blades and placed in different Falcon tubes. Finally, the samples were put on ice and stored at −80°C before processing.

2.9 | Total viable bacterial count (TBC)

TBC was determined according to the method described by Quin et al. (2013). Colonies above 30 were counted, and the TBC per milliliter of water (CFU/mL) or gram of the gut sample (CFU/g) was calculated using the following formula:

$$\text{TBC}\left(\frac{\text{CFU}}{\text{g}}\right) = \text{total number of colonies} \times \text{reciprocal dilution factor} \times \text{amount of material used}$$

2.10 | Total coliform count (TCC)

TCC was measured using the method described by Quin et al. (2013), and the TCC per gram of the gut sample (CFU/g) was calculated using the following formula:

$$\text{Total coliform count}\left(\frac{\text{CFU}}{\text{g}}\right) = \text{total number of colonies} \times \text{reciprocal dilution factor} \times \text{amount of material used}$$

2.11 | Total lactic acid bacteria (LAB) enumeration

The LAB enumeration was determined following the method described by McDonald et al. (2010), using per gram of the gut sample (CFU/g) using the following formula:

$$\text{total LABcount}\left(\frac{\text{CFU}}{\text{g}}\right) = \text{total number of colonies} \times \text{reciprocal dilution factor} \times \text{amount of material used}$$

Colonies were randomly chosen and tested using Gram staining and biochemical tests to confirm the presence of LAB.

2.12 | Isolation and identification of LAB

We then isolated and identified some LAB in the fish hindguts using MRSA, Gram staining, and biochemical testing. We performed this after observing this interesting pattern of bacterial loads. Separate colonies from the MRS agar plates were subcultured to obtain pure isolates. These isolates were characterized by Gram staining, the catalase test, and carbohydrate fermentation patterns using API 50 CHL strips (bioMérieux) according to the manufacturer's instructions.

2.13 | In vitro fermentation of XOS by the isolated bacteria

The isolated bacteria were evaluated for their ability to ferment XOS following the procedure described in our previous work (Gufe et al., 2021). Briefly, the isolated bacteria were subcultured onto MRS agar and incubated anaerobically (BBL® Gas Pak anaerobic system envelopes, Becton, Dickinson) for 24 h at 37°C. The 2× MRS broth without a carbon source was prepared and sterilized by autoclaving at 121°C for 15 min. Carbohydrate solutions (2% XOS and 2% glucose) were prepared in sterile distilled water and sterilized with 0.22 μm Millipore membrane filters (Millipore, Billerica, MA, USA). In 24-well microplates, 0.005 mL (1%) of each isolated bacterium (10^7 CFU/mL/ ~1 OD600) was added to 0.5 mL of modified MRS medium (1% XOS + 0.5 g (w/v) L-cysteine, pH 6.6) and incubated under anaerobic conditions at 37°C for 72 h. Bacterial growth was manually measured at 24, 48, and 72 h by measuring the optical density (OD) at 600 nm. All bacteria were tested in triplicate, and the average optical densities were calculated.

2.14 | Data analysis

R (v4.3.1) was used for all statistical analyses. Before testing the hypotheses, the normality of the data was assessed using the Shapiro–Wilk test, and the data's homogeneity of variances was assessed using Levene's test. When significant differences (at $\alpha = 0.05$) were found in a one-way analysis of variance (ANOVA), a Tukey's Honestly Significant Difference (HSD) post hoc test was used for pairwise comparisons. A linear regression analysis was used to evaluate the dose-dependent relationship between the XOS and observed outcomes in the fish. To investigate potential connections between growth characteristics and microbiota composition, Pearson's correlation coefficients were also calculated. All data are presented as mean ± standard deviation (SD), and for clarity, tables also display the standard error of the mean (SEM). The level of statistical significance was set at $p = 0.05$.

2.15 | Declaration of AI and AI-assisted technologies in the writing process

During the manuscript preparation, the authors used Grammarly and QuillBot, artificial intelligence tools, solely for proofreading and language correction to improve clarity, grammar, and scientific English usage. There was no part of the manuscripts' intellectual content, including study design, data analysis, interpretation of results, or drafting of core scientific arguments, that was generated by artificial intelligence. The authors reviewed and edited all AI-assisted text and take full responsibility for the accuracy and integrity of the final manuscript. The AI tools used are not listed as authors, nor did they fulfill any authorship roles, in accordance with Wiley's policy on artificial intelligence in scholarly publishing.

3 | RESULTS

The impacts of dietary cornhusk-derived XOS are described in the following order: production yield and characterization, growth performance and health indices, fillet nutritional content, and gut microbiome change.

3.1 | Production and characterization of XOS

The alkaline extraction method yielded 33.5% xylan from corn husk, which was subsequently converted to 24.15% XOS via oven-assisted autohydrolysis. This yield is close to that reported by Xu et al. (2006) for a similar alkaline extraction procedure, which yielded 28.4% xylan. We discovered that xylobiose (X2) and xylotriose (X3), which are recognized for their prebiotic qualities, made up the majority of the XOS profile using thin-layer chromatography (TLC) (Figure 1). The lack of xylose (X1) in the TLC analysis suggests that the XOS generated is unlikely to host pathogenic microorganisms. It is therefore a useful prebiotic for aquaculture. The yield was the same in all repeats, with a

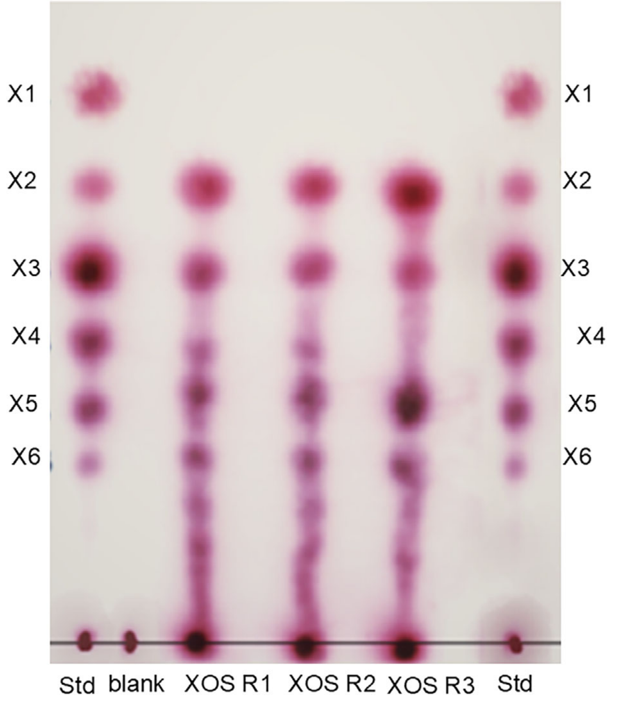

FIGURE 1 Qualitative analysis of corn husk-derived XOS by TLC. X1: Xylose (DP1), X2: Xylobiose (DP2), X3: Xylotriose (DP3), X4: Xylotetraose (DP4), X5: Xylopentaose (DP5), X6: Xylohexaose (DP6).

standard deviation of ±1.2%, indicating high reproducibility. The densitometric analysis of the TLC plate showed that xylobiose (X2) and xylotriose (X3) accounted for over 80% of the profile, confirming a significant proportion of low-degree polymerization oligomers favored by beneficial bacteria.

3.2 | Effects of XOS on growth performance

The growth performance of Nile tilapia fed XOS-supplemented diets showed significant and dose-dependent improvements compared to the control group (Figure 2). The final weight in the 1% XOS group (83.9 g, 95% confidence interval (CI) [82.7, 85.1]) was 88% greater than that of the control group (44.5 g, 95% CI [43.4, 45.6]), with a mean difference of 39.4 g (95% CI for the difference [37.9, 4.9], $p < 0.001$, Cohens $d = 2.1$). The 0.5% XOS group also showed substantial improvements (72.6 g, 95% CI [71.2, 74.0]).

Feed efficiency was significantly improved by the addition of XOS. The feed conversion ratio was greater in the control group (2.69, 95% CI [2.60, 2.78], $p = 0.003$) than in the 1% XOS group (0.99, 95% CI [0.96, 1.02]). The overall nutritional utilization was confirmed by the protein efficiency ratio, which increased from 1.32 (95% CI [1.27, 1.37]) in the control group to 2.57 (95% CI [2.52, 2.62]) in the 1% XOS group ($p < 0.001$). Fish health and survival rates also greatly improved; survival increased from 70% (95% CI [67.7, 72.3]) in the control group to 94% (95% CI [92.7, 95.3]) in the 1% XOS group ($p < 0.001$), showing greater disease resistance. A specific growth rate of 3.3%/day (95% CI [3.27, 3.33]) in the 1% XOS group and 2.6%/day (95% CI [2.57, 2.63]) in the control group demonstrated quicker growth kinetics ($p = 0.01$). Changes in the gut microbiota coincided with these growth increases, which were fuelled by increased feed efficiency. The results of this study are consistent with those of Wangkahart et al. (2024), who

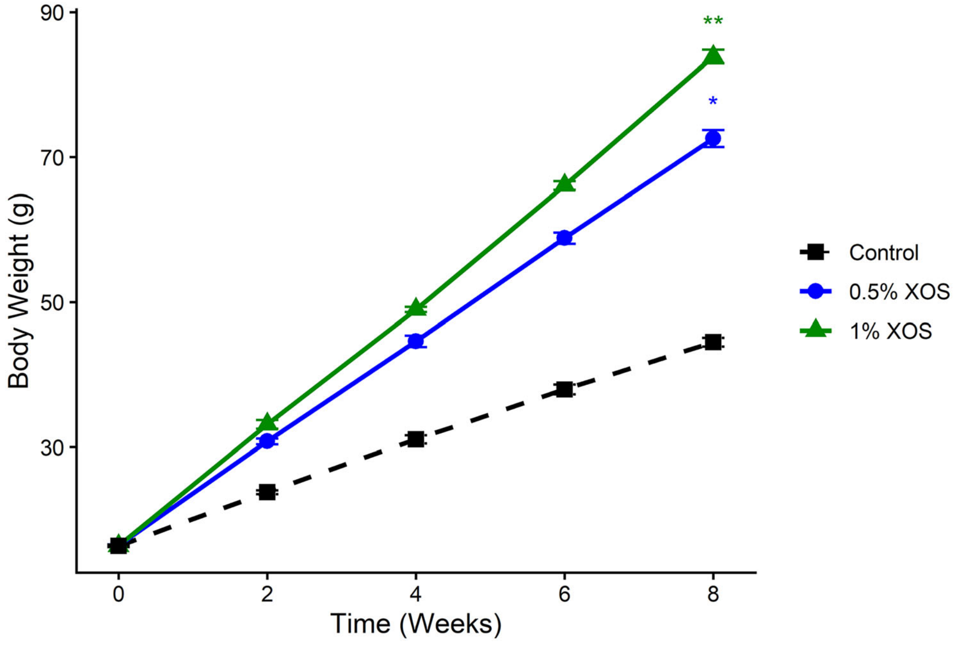

FIGURE 2 Average weight progression of *Oreochromis niloticus* supplemented with XOS over 8 weeks. The initial weight was 16.0 g for all groups. The 1% XOS group exhibited a significant final weight gain compared to the control (mean difference: 39.4 g, $p < 0.001$), while the 0.5% XOS showed improvement ($p < 0.05$). The error bars represent the standard error of the mean (SEM) with $n = 9$ per group. Statistical significance denoted as: **$p < 0.001$; *$p < 0.05$. XOS, xylooligosaccharides.

found that feeding *O. niloticus* an XOS-based diet derived from agricultural waste resulted in comparable growth gains.

3.3 | Effects of XOS on survival rates and health indicators

Fish survival rates were significantly better in the XOS groups (93% in 0.5% XOS and 94.44% in 1% XOS vs. 70% in control, $p < 0.001$), suggesting improved immune function and illness resistance (Table 2). Blood urea nitrogen (BUN), creatinine, and cholesterol levels were among the health indicators of Nile tilapia that were within acceptable limits for fish in good health (Table 3). All readings were within the normal range (0.5–5.0 mg/L) published by Martínez Steele and Monteiro (2017); however, the BUN levels in the XOS groups (3.2 mg/L) were somewhat higher than in the control group (2.8 mg/L). Compared with the control group (157 mg/dL), the cholesterol levels in the XOS groups were lower (126 mg/dL), suggesting a potential health advantage. There were no negative effects on liver or visceral health, as shown by the HSI and VSI, both of which were within normal limits. The results are consistent with Zulfiqar's observation that XOS supplementation does not affect hepatic or renal function in Nile tilapia.

3.4 | Effects of XOS on the fillet nutritional composition

In a dose-dependent manner, XOS significantly enhanced the nutritional quality of Nile tilapia fillets (Table 4), suggesting potential benefits for aquaculture. Crude protein content increased by 35.6%, from 14.9% (95% CI [14.2,

TABLE 2 Growth performance parameters of *O. niloticus* fish fed diets supplemented with 0.5% and 1% XOS in comparison to the control group.

Parameter	Control (mean ± 95% CI)	0.5% XOS (mean ± 95% CI)	1% XOS (mean ± 95% CI)	*p*-Value
Initial weight (g)	16.0 [15.8, 16.2]	16.0 [15.8, 16.2]	16.0 [15.8, 16.2]	–
Final weight (g)	44.5 [43.4, 45.6]	72.6 [71.2, 74.0]	83.9 [82.7, 85.1]	<0.001
Weight gain (g)	28.5 [27.5, 29.5]	56.6 [55.2, 58.0]	67.8 [66.6, 69.0]	<0.001
Feed conversion ratio	2.69 [2.60, 2.78]	1.15 [1.12, 1.18]	0.99 [0.96, 1.02]	0.003
Specific growth rate	2.6 [2.57, 2.63]	3.1 [3.07, 3.13]	3.3 [3.27, 3.33]	0.01
Survival rate (%)	70 [67.7, 72.3]	93 [91.7, 94.3]	94 [92.7, 95.3]	<0.001
Mean daily weight gain	0.51 [0.49, 0.53]	1.01 [0.99, 1.03]	1.21 [1.19, 1.23]	<0.001
Protein efficiency ratio	1.32 [1.27, 1.37]	2.23 [2.18, 2.28]	2.57 [2.52, 2.62]	<0.001

Note: $n = 9$ per treatment group for all weight-related parameters.
Abbreviations: CI, confidence interval; XOS, xylooligosaccharides.

TABLE 3 Effects of dietary XOS on the health of *O. niloticus* indices.

	Control	0.5% XOS	1% XOS
Urea, mg/L	2.8	3.2	3.2
Creatinine, mmol/L	0.0105	0.0103	0.0102
VSI%	4.6	4.8	4.7
HIS%	2.6	2.7	2.7
Cholesterol, mg/dL	157	126	126
Albumin, g/dL	3.3	3.4	3.4
Proteins, g/dL	6.4	6.8	6.9
Globulin, g/dL	3.0	3.4	3.5

Abbreviations: HIS, hepatosomatic index; VIS, viscerosomatic index; XOS, xylooligosaccharides.

15.6]) in the control group to 20.2% (95% CI [19.7, 20.7]) in the 1% XOS group ($p < 0.001$). The mineral composition was enhanced, shown by a fillet calcium content more than double from 2.6% (95% CI [2.2, 3.0]) to 6.7% (95% CI [6.0, 7.4]) in the 1% XOS group ($p < 0.001$). The moisture content significantly increased from 75.3% (95% CI [73.7, 76.9]) to 83.9% (95% CI [82.5, 85.3]). The total ash content (2.0%–2.7%, $p = 0.02$) and crude fat (2.6%–3.1%, $p = 0.03$) both showed significant increases. These findings indicated that XOS supplementation can improve nutrient deposition in fish muscle. The results are consistent with Quin et al. (2013) and Guerreiro et al. (2017), who found that prebiotics significantly improved fillet quality in fish species.

3.5 | Effects of XOS on gut microbiota modulation

The study shows that XOS supplementation significantly improves the gut microbiota of Nile tilapia (Figure 3), highlighting both prebiotic effects and changes in bacterial populations. Groups given XOS had notably lower TBC and TCC compared to the control group, suggesting a healthier gut environment. LAB counts rose sharply, especially in the 1% XOS group, with a 36-fold increase over the control's 23 CFU/g ($p < 0.05$, 95% CI [780, 890]). This

TABLE 4 Effects of dietary XOS on the nutritional composition of *O. niloticus* fillets demonstrate their impact on key dietary aspects.

Parameter (%)	Control (mean ± 95% CI)	0.5% XOS (mean ± 95% CI)	1% XOS (mean ± 95% CI)	*p*-Value
Protein content	14.9 [14.2, 15.6]	18.4 [17.6, 19.2]	20.2 [19.7, 20.7]	<0.001
Calcium content	2.6 [2.2, 3.0]	4.1 [3.8, 4.4]	6.7 [6.0, 7.4]	<0.001
Crude fat content	2.6 [2.5, 2.7]	2.7 [2.3, 3.1]	3.1 [2.8, 3.4]	0.03
Moisture content	75.3 [73.7, 76.9]	82.1 [80.2, 84.0]	83.9 [82.5, 85.3]	<0.001
Ash content	2.0 [1.7, 2.3]	2.3 [1.9, 2.7]	2.7 [2.3, 3.1]	0.02

Abbreviations: CI, confidence interval; XOS, xylooligosaccharides.

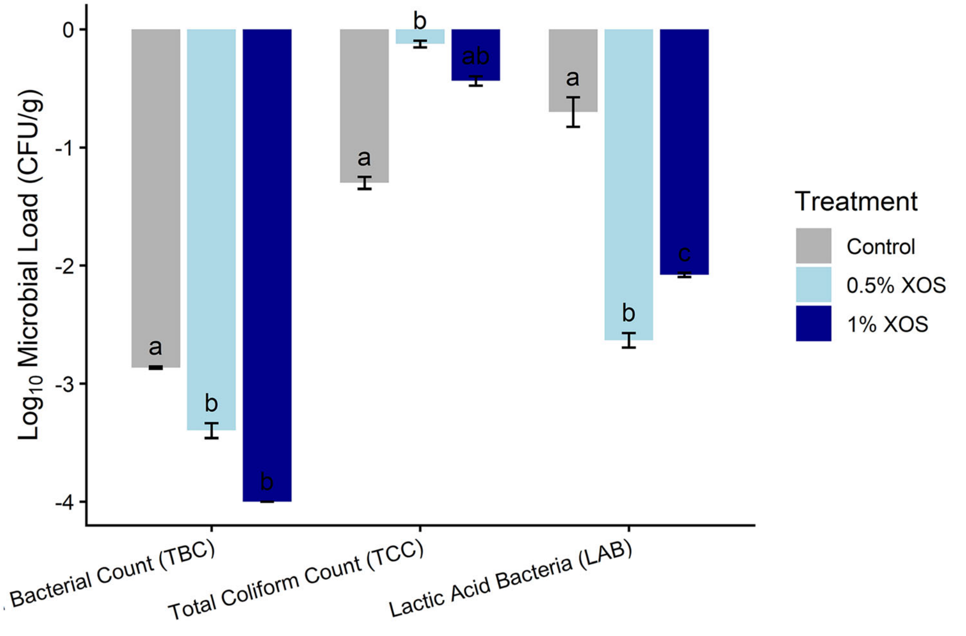

FIGURE 3 The effects of XOS derived from corn husk on the total bacterial count (TBC), total coliform count (TCC), and lactic acid bacteria (LAB) of the fish hindgut. The microbial load of the fish (CFU/g) is expressed as log10 ± SEM. Statistically significant differences in the treatment groups ($p < 0.005$) are indicated by the letters a, b, and c above the bars. The SEM is shown by error bars. All three microbiological parameters are compared between the control group (gray), the 0.5% XOS group (light blue), and the 1% XOS group (dark blue).

underscores the dose-dependent effects of XOS, indicating it may positively alter gut microbiota, thereby enhancing fish health and growth performance.

The 1% XOS fish group had the highest LAB counts (c), the 0.5% XOS fish group had intermediate levels (b), and the control fish group had the lowest levels (a). Similarly, the XOS-diet-supplemented groups had significantly lower TBC and TCC levels than the control fish group. A substantial prebiotic effect on the fish was seen by the high levels of beneficial microbial organisms, for example, *Bacillus*, *Lactobacillus*, and *Lactococcus* species, in the XOS-fed Nile tilapia. The findings of the study further align with Zheng et al. (2018), who found that fish fed with XOS exhibited significant increases in lactic acid bacteria and coliform reductions. Selective cultivation of beneficial microorganisms and overall inhibition of potential pathogenic organisms demonstrated that XOS derived from corn husks was useful as a prebiotic for aquaculture feed.

TABLE 5 Linear regression of XOS dose–response relationships.

Outcome	R^2	β_1 (slope)	*p*-Value
Final weight (g)	0.92	33.9	<0.001
Weight gain (g)	0.89	39.3	<0.001
Feed conversion ratio	0.75	−0.3	0.003
Specific growth rate	0.85	0.7	<0.001
Survival rate (%)	0.78	24.4	0.001

Note: The regression equation is $y = \beta 0 + \beta 1x$, where y is the outcome variable, and x is the percentage of XOS. At 1% XOS, the final weight (83.9 g) was higher than the linear prediction (49.9 g), indicating nonlinear synergies.
Abbreviation: XOS, xylooligosaccharides.

TABLE 6 Pearson's correlation between gut microbiota and growth parameters.

Microbiota parameter	Final weight (*r*)	Weight gain (*r*)	SGR (*r*)	*p*-Value
Total bacterial count	−0.91	−0.89	−0.85	<0.001
Total coliforms	−0.83	−0.80	−0.78	0.002
Lactic acid bacteria	+0.76	+0.72	+0.69	0.008

3.6 | Correlation and dose–response relationships

Growth performance and dietary supplementation with XOS derived from corn husks showed a strong correlation (Table 5). The Nile tilapia FCR decreased significantly ($\beta = -0.3$, $p = 0.003$), while the weight at day 56 increased by 33.9 g for each 1% rise in XOS ($y = 16.0 + 33.9x$, $R^2 = 0.92$, $p < 0.001$). The specific growth rate of the fish improved by 0.7% per day per 1% XOS ($p < 0.001$), while the final survival rate increased linearly with higher XOS levels ($\beta = 24.4$, $p = 0.001$). The final weight of 83.9 g for Nile tilapia exceeded the model-predicted 49.9 g at 1% XOS supplementation, suggesting nonlinear effects and possible synergistic interactions at higher dosages.

Growth outcomes and the makeup of the gut microbiota were clearly related (Table 6). TCC and TBC showed negative relationships with SGR ($r = -0.85$, $p < 0.001$) and ultimate weight ($r = -0.91$, $p < 0.001$). On the other hand, fish weight gain was favorably correlated with the quantity of LAB ($r = 0.72$, $p = 0.008$), indicating a link between probiotic treatment and quicker fish development.

3.7 | In vitro fermentation of XOS

XOS was effectively fermented in vitro by isolated lactic acid bacteria, including *Bacillus*, *Lactobacillus*, and *Lactococcus* species (Figure 4). Bacterial growth was much greater with XOS than with glucose during a 72-h period, suggesting that XOS supplementation especially promotes the growth of beneficial gut bacteria. Additionally, fermenting XOS favored the growth of probiotic bacteria in vitro, according to Gufe et al. (2021). The growth kinetics also showed that the isolated LAB utilized XOS as a primary carbon source effectively, with optical density values comparable to or exceeding those in glucose media over 72 h, indicating its significant fermentative capacity for this prebiotic.

4 | DISCUSSION

The current study demonstrated that diet supplemented with XOS derived from corn husk, an agricultural byproduct and waste, significantly enhanced the development, health, and nutritional quality of Nile tilapia (*O. niloticus*)

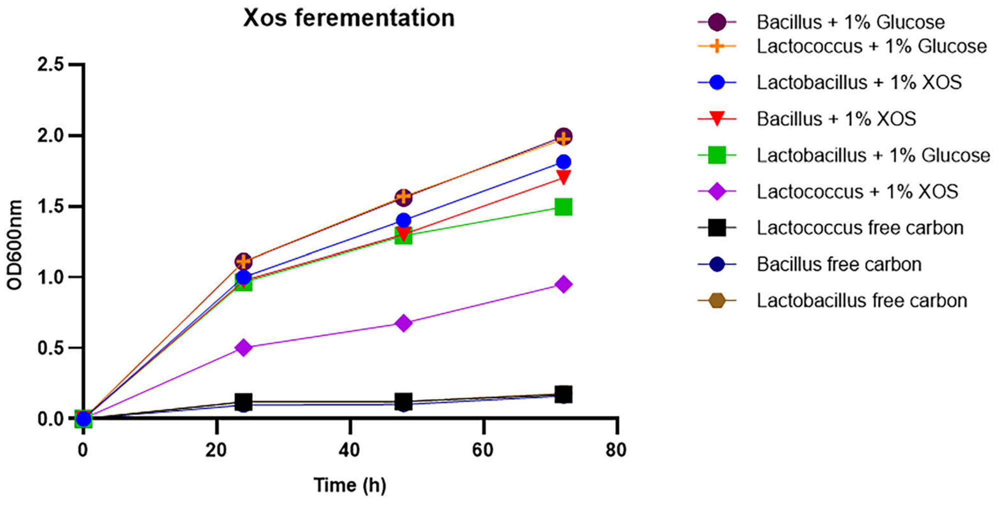

FIGURE 4 XOS fermentation by lactic acid-producing bacteria isolated from *O. niloticus* hindguts.

fingerlings. The findings offer a strong evidence base on the prebiotic advantages of this eco-friendly fish feed additive and its potential to tackle key challenges in aquaculture systems.

One of the main causes of the Nile tilapia's quick growth was the inclusion of XOS from corn husks to supplement their diet. In comparison to the control group (44.5 g, $p < 0.001$), fish administered 1% XOS gained 83.9 g, a 128% increase. The fish exhibited a superior feed conversion ratio of 0.99 when compared to the control group that only had 2.69. Aquaculture depends on this improved ability to transform feed into biomass because it lowers expenses and waste. Our findings are in line with those of Li et al. (2021), who similarly found that XOS enhanced fish nutritional utilization and gastrointestinal health. However, there is a strong correlation between changes in gut flora and other factors. The survival rates of the XOS groups (93%–94%) were significantly greater than those of the control group (70%, $p < 0.001$). These results suggest that XOS supplementation enhances overall health and disease resistance. Because it lowers losses and reliance on chemotherapy, this is a significant step towards sustainable intensification. Table 3 demonstrates that all fish health indices remained within normal ranges. This significant characteristic shows that the levels of XOS made from the used maize husks are safe.

The fish's gut microbial flora underwent significant shifts following XOS supplementation in their diet. The 1% XOS fish group showed 833 CFU/g of LAB, a 36-fold increase ($p < 0.001$) in comparison with the control fish group that had 23 CFU/g. The Nile tilapia fed XOS, the targeted enrichment of the beneficial microbial species indicated that the XOS functions not only as a food supplement but also as a potent prebiotic (Zheng et al., 2018). At the same time, we noted a significant reduction in the amount of total coliform counts within the aquaculture systems, likely because LAB producing antimicrobial substances and microbes outcompeting each other for the available resources (Quin et al., 2013). The use of agricultural waste-derived prebiotics to support a robust, healthy gut microbiota is essential for managing long-term fish health issues, effectively minimizing the overuse of antibiotics in aquaculture and enhancing environmental and fish health. The in vitro fermentation in our study provided strong evidence for this link, with XOS serving as the primary carbon source for the isolated LAB cultures, suggesting that XOS could serve as an excellent fermentable prebiotic substrate in vivo.

Supplementation with dietary XOS significantly enhanced the growth, health metrics, and the nutritional content of the Nile tilapia fish fillets. The fish fillets in the 1% XOS group had nearly double the total protein content (20.2%) compared to the control group (10.8%; $p < 0.001$). The increase in the total protein content of the Nile tilapia is likely attributable to an improved intestinal health composition and overall absorption of nutrients that were responsible for the promotion of protein synthesis and retention (Guerreiro et al., 2017). Additionally, the fish calcium levels rose significantly (6.7% vs. 2.6% in the control group), further enunciating the overall effects of the XOS supplementation on the fish. The gains observed in the fish improve their market value and boost the product quality, positioning the fish fillets as a premium, nutrient-rich alternative. The results of the study are in tandem with sustainable aquaculture management and farming objectives that focus on delivering high-quality food alternatives using the same or alternative resources. As a result, this will safeguard the nation's food security and economic benefits from the export of the Nile tilapia nutrient-rich fillets globally.

4.1 | Limitations of the study

It is important to recognize several limitations, even if the study provides strong evidence of the benefits of XOS generated from maize husks. As previously stated, we primarily relied on culture-based microbial research, which limited our understanding of Nile tilapia's gut microbiome. Metagenomic sequencing should be used in future XOS research to characterize microbial ecology fully. Second, longer-term studies are advised, as the 8-week trial duration may not have been sufficient to capture long-term health and performance outcomes, even if it was sufficient to show significant growth benefits. Lastly, further investigation is required to identify the reasons behind variations in fillet composition, especially the notable rise in protein levels in Nile tilapia through targeted metabolic and transcriptomic analyses.

Our future studies on XOS supplementation in aquaculture will use thorough metagenomic techniques to overcome these constraints. We wish to use metatranscriptomic analysis to identify active metabolic pathways affected by XOS, in conjunction with shotgun metagenomics, to describe the whole microbial community structure and functional potential in the tilapia gut. The observed growth and health advantages will be supported by the remarkable resolution of microbial interactions and host–microbe linkages made possible by these cutting-edge approaches. To demonstrate causal linkages between specific bacterial taxa and metabolic outputs, we will also employ stable isotope probing to monitor carbon flow from XOS through the microbial food web. In addition to validating our existing findings, this multi-omics method will reveal new bacterial strains with improved XOS utilization for possible probiotic production.

5 | CONCLUSION

This study found that XOS made from corn husks is a good prebiotic for Nile tilapia, encouraging better intestinal health, quicker development, and higher-quality fillets. The notable increases in weight gain, feed efficiency, and survival rates, together with modifications in gut flora and an improved nutritional profile of fillets, show how effective XOS is as a sustainable feed supplement. This method may help the environment and provide a workable substitute for the improper use of antibiotic growth promoters in agriculture by using agricultural waste. Future studies should concentrate on boosting XOS production and analyzing its long-term effects on various fish species and aquaculture systems.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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