Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134855 2026, 25(1) 105-126

Research Article

Probiotics containing *Streptomyces* spp. improved water quality, survival, growth, and fillet quality of striped catfish under intensive farming

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Keywords

Streptomyces,
Survival,
Growth performance,
Fillet quality,
Water quality

Article info

Received: June 2025
Accepted: September 2025
Published: January 2026

Abstract

This study evaluated the benefits of *Streptomyces spp.* in striped catfish under intensive farming in the Mekong Delta, Vietnam. The lyophilized biomass of two strains, *Streptomyces kunmingensis* XK9 and *Streptomyces angustmyceticus* XK22 (10^9 CFU/g), was incorporated into the catfish pellets at 1 g per kg and administered daily to fish throughout the seven-month trial. *Streptomyces* supplementation maintained water quality within optimal ranges for temperature, pH, dissolved oxygen, ammonia, and turbidity, whereas control ponds deviated after five months, potentially impairing fish performance. After seven months, fish receiving XK9 and XK22 showed significantly improved survival rate, growth performance, and feed conversion ratio compared with the control (p<0.05). Fillet quality was also enhanced in fish fed *Streptomyces*-supplemented diets, with significant improvements in proximate composition, amino acid profile, and fatty acid profile (p<0.05). The finding revealed the potential of XK9 and XK22 to improve water quality, enhance catfish growth performance, and enrich the nutritional value of striped catfish under intensive culture. The beneficial effects of these *Streptomyces* strains make them a promising alternative strategy for sustainable catfish production.

Introduction

Vietnam is the world's third-largest exporter of aquatic products, with striped catfish (*Pangasianodon hypophthalmus*) accounting for more than 75% of the global output of this species (FAO, 2022). Since the country entered the international market in 2000, Vietnamese striped catfish has been exported to 149 countries and territories (Nguyen and Curtis, 2020). Over the past two decades, global catfish production has grown at an average annual rate of 4.5%, the highest among inland aquaculture industries. The rapid development of catfish production not only generates substantial foreign exchange and provides the livelihood for farmers in many Asian countries, including Vietnam, but also contributes to global food security (Chan *et al*., 2017). These achievements have largely been driven by intensive farming methods, which offer high yields, optimized land use, efficient use of water and human resources, and minimized environmental impacts (Kumar *et al*., 2022). However, the rapid expansion of intensive aquaculture has also raised concerns, as it can lead to environmental degradation and disease outbreaks, often exacerbated by poor farming practices and insufficient hygiene (Michelle *et al*., 2008; Giang *et al*., 2008).

Many biosafety prophylaxis measures have been applied in the fish farming industry to control environmental pollution, prevent diseases, promote immune responses, improve growth, and enhance feed efficiency, all while avoiding the emergence of drug resistance or residues in the cultured environment and in processed products. A wide range of solutions has been deployed in aquaculture, including biological control, vaccines, and immunostimulants; among them, probiotics, prebiotics, symbiotics, and synbiotics consumed as additives in the feeding diets of cultivated aquatic animals are illustrated to be advantageous (Anandan *et al*., 2016; Nakhei *et al*., 2023).

Streptomyces has been shown to synthesize a broad spectrum of secondary metabolites, including enzymes, antibiotics, antifungals, antivirals, immunostimulants, and siderophores (Salwan and Sharma, 2020; Sharma *et al*., 2021). Owing to these properties, *Streptomyces* has been confirmed as a probiotic that offers multiple benefits to aquaculture, including enhancing growth performance, increasing survival rates, resisting disease, competitively eliminating pathogens, altering gastrointestinal microflora, and improving water quality (Tan *et al*., 2016; Butt *et al*., 2024). *Streptomyces* can be incorporated into probiotic formulations as individual strains, in the symbiosis between multiple strains of *Streptomyces*, or in combination with other microorganisms and prebiotics to prepare multifunctional biological products. Such formulations have demonstrated broad effectiveness in a variety of aquatic animals, cultured under saline, brackish, and freshwater conditions (Milagro *et al*., 2017; Sîrbu *et al*., 2022).

Two indigenous strains, *Streptomyces kunmingensis* XK9 (abbreviated as *S. kunmingensis* XK9 or XK9) and *Streptomyces angustmyceticus* XK22 (abbreviated as *S. angustmyceticus* XK22 or XK22), were isolated from sediments of catfish ponds in An Giang Province and shrimp ponds in Nam Dinh Province, Vietnam, respectively. These strains had previously been selected through both *in vitro* and *in vivo* methods to verify the following characteristics: (1) antibacterial activity; (2) extracellular enzymes production (amylase, cellulase, and protease); (3) safety for aquatic species such as striped catfish, black tiger shrimp, and whiteleg shrimp; (4) adaptation to the host's digestive tract; (5) growth promotion; (6) immune enhancement; (7) resistance to pathogenic microorganisms; (8) water quality improvement; and (9) stability of probiotic activity during formulation and storage (Tam *et al.,* 2024). In the present study, two strains, XK22 and XK9, were further evaluated for their effectiveness in intensive striped catfish farming, using parameters such as water quality, survival rate, growth performance, fillet quality, and the amino acid and fatty acid profiles of the fish fillets. The findings are expected to provide more substantial evidence for the efficacy of dietary *Streptomyces* supplementation as a practical strategy to promote the sustainable development of the striped catfish industry.

Materials and methods

The study was approved by the Animal Ethics Committee of Ha Noi Open University (protocol code 101/TB-HĐTVĐĐĐV, dated March 18, 2021).

Study location

The research was conducted in An Giang province, Vietnam, which is recognized as the "cradle" of intensive catfish farming in the Mekong Delta (De Silva and Phuong, 2011). Six ponds were selected for the study, each with an area of 3,000 m^2 and a depth of 4 m. Water was supplied directly from the Mekong River, with a daily exchange equivalent to 20% of the pond volume. The water level was consistently maintained at least 0.5 m below the pond bank. The ponds were divided into two groups: (1) the control group, including 03 ponds, where catfish were fed commercial feed (nutritional components are listed in Table 1), and (2) the experimental group, including 03 ponds, where catfish were fed the same commercial feed supplemented with *Streptomyces*.

Table 1: The nutritional components of the pellets for striped catfish.

Contents	Dry weight (%)
Moisture	11.0
Crude protein and lipid	For fish weighing 20 g-200 g on average: 28% protein and 5% lipid For fish weighing 200 g-500 g on average: 26% protein and 4% lipid For fish weighing over 500 g on average: 22% protein and 3% lipids
Crude fiber	8.0
Calcium	1.0
Total phosphorus	1.0
Total lysine	1.2
Methionine and cysteine (total)	0.8

Experimental fish

Catfish were obtained from a hatchery farm in An Giang, Vietnam. Healthy individuals with bright coloration, well-proportioned bodies, and undamaged intact arcs and rays were selected. At stocking, the average size was 18-20 fish/kg, with a stocking density of 15-20 fish/m^3.

Preparation of Streptomyces biomass

The strains *S. kunmingensis* XK9 (Genebank code OR122317) and *S. angustmyceticus* XK22 (Genebank code OR122322) were provided by Hanoi Open University. To activate the strains, mycelium samples previously stored in glycerol 40% (v/v) at -80°C were cultured on TSB (Trypticase Soy Broth, HiMedia). Cultures were grown in 250 mL baffled Erlenmeyer flasks containing 50 mL of broth, incubated at 28°C for 72 hours on a shaker at 250 rpm.

The fermentation of *Streptomyces* was performed in a 150 L fermenter following the medium composition and parameters described by Tahir *et al*. (2023), with minor modifications. The fermentation medium contained 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 4% glucose. The inoculum was introduced at 4% (v/v). Fermentation conditions were maintained at 200 rpm agitation, 20% dissolved oxygen (DO), pH 6.57-6.8, 0.75 vvm aeration, and 28°C. After 96 h of culture, *Streptomyces* cell suspensions were harvested by tangential flow filtration (TFF) using polyvinylidene difluoride (PVDF) microfiltration membrane with a pore size of 0.22 µm (Cai *et al.,* 2015). From an initial volume of 100 L of fermented biomass, the culture was concentrated to 2 L. The concentrated biomass was freeze-dried by lyophilization following the method of Tuyen and Lan (2023) and then blended with talc powder to achieve a density of 10^9 CFU/g. The density of *Streptomyces* cells was counted using the dilution and plating method as outlined in the Vietnam National Standard for the enumeration of *Streptomyces* spp. by colony count method (TCVN 14172:2024).

The dietary preparation

The present study adopted the *Streptomyces* dosage reported by Hien *et al*. (2023). The lyophilized XK9 and XK22 were incorporated into the pellets at a concentration of 1 g/kg, then coated with squid liver oil (1 L squid liver oil per 100 kg feed) and left to stand for 30 minutes to allow *Streptomyces* to adhere to the feed before being administered to the fish. Weekly, the fish were fed on demand for six days (twice daily, at 8:00 am and 7:00 pm; for the experimental ponds, the 8:00 am diet contained *Streptomyces*) and then starved for one day. The trials were conducted over a period of seven months.

Water quality

The water quality parameters monitored include temperature, pH, dissolved oxygen (DO), ammonia (NH3), and turbidity. Temperature, pH, and DO were measured using a water quality checker (AZ Instrument, Taiwan); NH3 was measured using a Checker®HC HI715; turbidity was measured using a Hanna HI 98703 (Hana Instruments). The measurements were recorded daily for 15 consecutive days, between 10:00 am and 11:00 am, at depths of 100 cm to 120 cm below the water surface.

Survival rate, growth performance, and feed conversion ratio

Survival rate (SR%); growth performance includes weight gain rate (WGR), specific growth rate (SGR), and feed conversion ratio (FCR) calculated as follows equations (Heriansah *et al.,* 2022):

$$\text{SR (\%)} = \frac{\text{Total number of fish at final}}{\text{Total number of fish at initial}} \times 100$$

$$\text{WGR} = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{SGR} = \frac{\text{Log final body weight} - \text{Log initial body weight}}{\text{experiment duration}} \times 100$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Total weight gain of fish at harvest (g)}}$$

Fillets quality

The quality of catfish fillets is assessed at harvest, when the average body weight of the fish ranges from 950 to 1,100 g/fish. The fillet quality parameters were measured, including proximal composition, amino acid profile, and fatty acid profile.

Proximal composition

The proximate composition of catfish fillets was evaluated according to the standard AOAC method (AOAC, 2004). The moisture was measured gravimetrically after complete drying of the finely ground fillets. The ash content was determined by carbonizing the fillets in a muffle furnace at 550°C for eight hours. The crude lipid content was determined by the Soxhlet extraction method, and the crude protein was measured by the Kjeldahl method.

Amino acid profile

Freeze-dried catfish fillets were hydrolyzed with 6M HCl containing 1% phenol at 110°C for 24 h under anaerobic conditions. The hydrolyzed samples were derivatized with a dansyl chloride reagent and analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1100 system equipped with a Zorbax Extend C18 column (4.6 x 250 mm) and a UV detector at 250 nm. Amino acid content was expressed in g per 100 g (%) of dry weight of fillets. Tryptophan could not be detected by acid hydrolysis and was therefore not measured.

Fatty acid profile

Total lipids were extracted from freeze-dried catfish fillet, followed by methyl esterification according to the ISO 12966-2:2017 method. The fatty acid profile was determined using a Thermo Scientific™

TRACE™ GC Ultra gas chromatograph equipped with a flame ionization detector (FID) attached to a TRACETM TR-FAME GC column (100 m×0.25 mm×0.20 µm; Thermo Scientific). Fatty acids were determined by comparing the samples' retention times of fatty acid methyl esters (FAMEs) with the 37-component FAMEs standard mix.

Statistical analysis

Datasets were analyzed for statistical significance using the Student's t-test (SPSS 22.0 software) to determine differences between dietary treatments. The results were expressed as the mean±standard error (SE), and differences were considered statistically significant at $p<0.05$.

Results

Water quality

The data in Figure 1 shows that pond water temperature fluctuated proportionally to weather temperature. However, during the experiment, the water temperature in catfish ponds that fed the diets containing XK9 and XK22 was substantially lower than that in the control group. The average temperature in ponds treated with *Streptomyces* was 27.55°C to 31.86°C, whereas in control ponds, the average temperature was 27.57°C to 33.35°C.

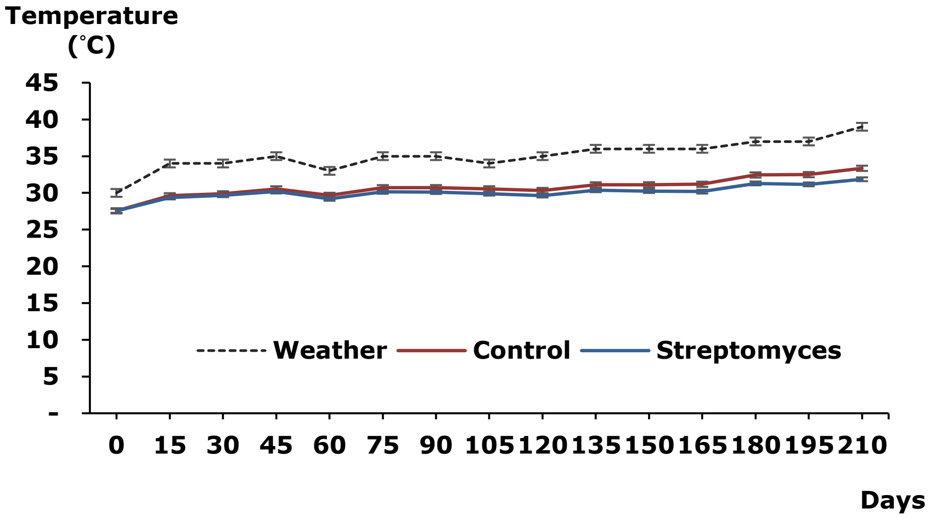

Figure 1: Variation of temperature in catfish ponds in intensive farming during the experiment. Data are presented as mean ± SE. Abbreviations: *Streptomyces*: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; control: ponds stocked with catfish were fed commercial pellets without *Streptomyces.*

Compared to the beginning of stocking, ponds in which fish were fed with a diet containing XK9 and XK22 maintained relatively stable water quality. pH ranged from 7.51 to 7.74, and NH_3 concentrations ranged from 0.03 to 0.05 mg/L, while turbidity (Nephelometric Turbidity Units, NTU) increased slightly, ranging from 31.33 to 87.67. In contrast, control ponds exhibited more pronounced fluctuations, with pH ranging from 7.50 to 8.96, NH_3 from 0.03 to 0.11 mg/L, and turbidity from 30.67 NTU to 151.67 NTU (Figs. 2-4). These differences were statistically significant, indicating that dietary supplementation with *Streptomyces* stabilized pond water quality. Furthermore, the water quality in the treated ponds remained within an optimal range for catfish compared to the control.

The dissolved oxygen (DO) in catfish ponds tended to decrease during the cultivation period. In ponds where fish were fed diets supplemented with XK9 and XK22, DO slightly decreased from 6.30 mg/mL at the start of stocking to 5.57 mg/mL at the end of the experiment. In contrast, DO in the control ponds declined more sharply, from 6.32 mg/mL to 4.01 mg/mL (Fig. 5).

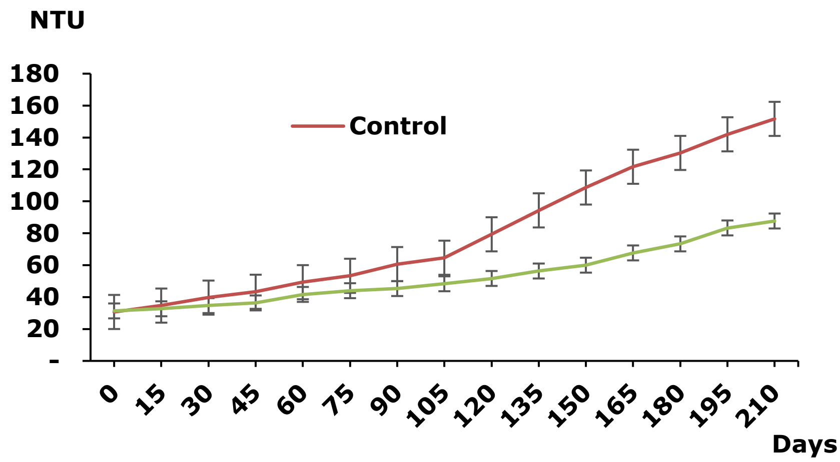

Figure 2: Variation in turbidity of catfish ponds in intensive farming during the experiment. Data are presented as mean ± SE. Abbreviations: NTU: Nephelometric Turbidity Units. *Streptomyces*: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; control: ponds stocked with catfish were fed commercial pellets without *Streptomyces.*

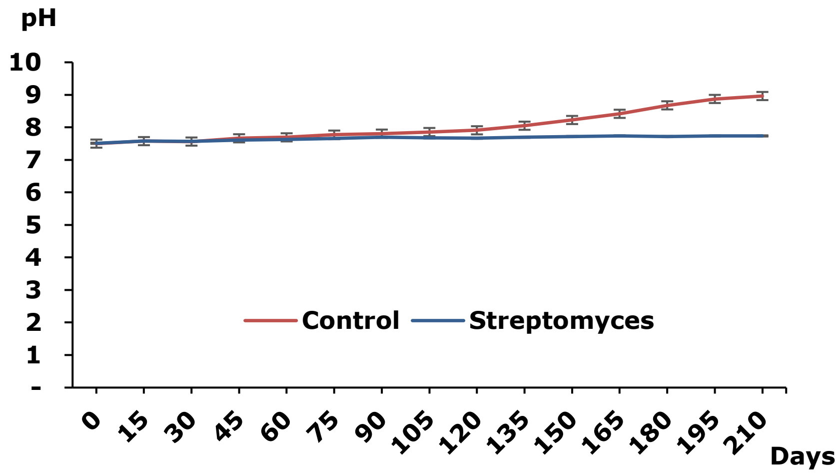

Figure 3: Variation of pH in catfish ponds in intensive farming during the experiment. Data are presented as mean ± SE. Abbreviations: *Streptomyces*: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; control: ponds stocked with catfish were fed commercial pellets without *Streptomyces.*

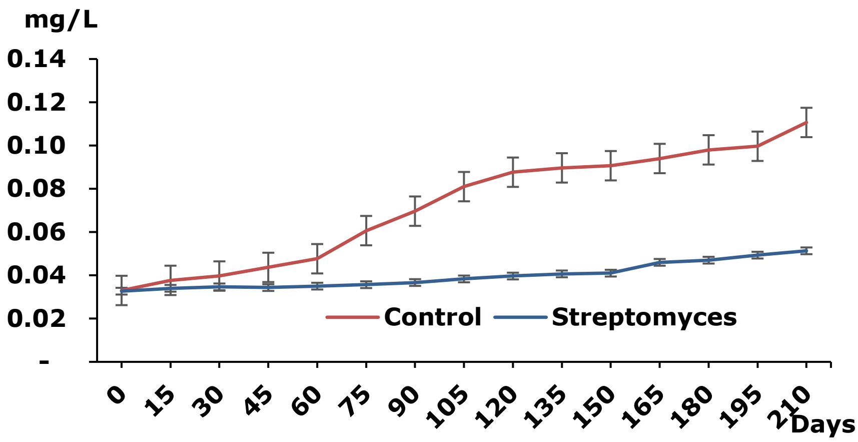

Figure 4: Variation of ammonia concentration in catfish ponds in intensive farming during the experiment. Data are presented as mean ± SE. Abbreviations: *Streptomyces*: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; control: ponds stocked with catfish were fed commercial pellets without *Streptomyces.*

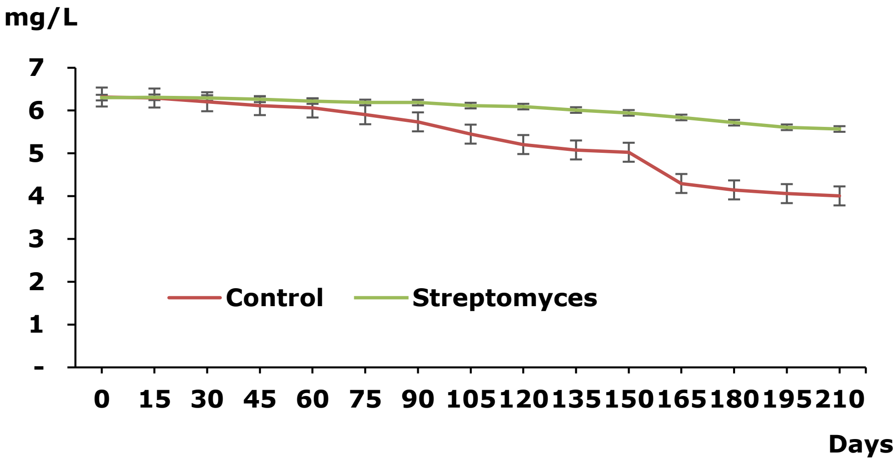

Figure 5: Variation of DO concentration in catfish ponds in intensive farming during the experiment. Data are presented as mean ± SE. Abbreviations: *Streptomyces*: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; control: ponds stocked with catfish were fed commercial pellets without *Streptomyces.*

Survival rate, growth, and feed conversion ratio

Data in Table 2 shows that *S. kunmingensis* XK9 and *S. angustmyceticus* XK22 significantly improved catfish growth performance and survival ($p<0.05$). Compared to control ponds, the WGR, SGR, and SR of catfish fed the diet supplemented with XK9 and XK22 increased by 1.19 times, 1.04 times, and 1.2 times, respectively. Conversely, the feed conversion ratio showed a decreasing trend; at the end of the experiment, when all of the catfish were harvested, the FCR of the group treated with *Streptomyces* was significantly lower than in the control ponds ($p<0.05$), showing a 1.06 times reduction (Table 2).

Table 2: Growth performance, survival rate, and feed conversion ratio of catfish under intensive farming.

Parameters	*Streptomyces* group	Control group
SR (%)	$88.55^{a} \pm 0.63$	$73.81^{b} \pm 0.65$
SGR	$2.23^{a} \pm 0.03$	$2.14^{b} \pm 0.02$
WGR	$2075.30^{a} \pm 63.19$	$1744.43^{b} \pm 55.32$
FCR	$1.61^{a} \pm 0.03$	$1.71^{b} \pm 0.02$

Data are presented as mean ± SE. Abbreviations: *Streptomyces* group: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; Control group: ponds stocked with catfish were fed commercial pellets without *Streptomyces*; SR, survival rate; SGR, specific growth rate; WGR, weight gain rate; FCR, feed conversion ratio. Different letter notations indicate a statistical difference between dietary treatments.

Catfish fillets quality

The quality of catfish fillets was evaluated in terms of proximal composition, amino acid, and fatty acid profiles. The fillet moisture did not differ significantly between the groups ($p>0.05$), with a mean value of 72.49% in the *Streptomyces* -treated group and 72.10% in the control group. Crude protein and ash contents were significantly higher in the fillets from catfish fed a diet supplemented with *Streptomyces* compared to the control group ($p<0.05$), with crude protein levels of 63.20% and 57.28%, and ash contents of 4.14% and 3.89%, respectively. Conversely, crude lipid content was significantly lower in catfish fillets of the *Streptomyces* group (26.20%) than in the control group (28.02%) ($p<0.05$) (Table 3).

Table 3 Proximal composition (% of dried weight) of catfish fillets under intensive farming.

Parameters	*Streptomyces* group	Control group
Moisture	$72.49^{a} \pm 0.15$	$72.10^{a} \pm 0.19$
Crude protein	$63.20^{a} \pm 0.34$	$57.28^{b} \pm 0.23$
Crude lipid	$26.20^{a} \pm 0.52$	$28.02^{b} \pm 0.72$
Ash	$4.14^{a} \pm 0.02$	$3.89^{b} \pm 0.01$

Data are presented as mean ± SE. Abbreviations: *Streptomyces* group: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; Control group: ponds stocked with catfish were fed commercial pellets without *Streptomyces*. Different letter notations indicate a statistical difference between dietary treatments.

The results presented in Table 4 display that catfish fillets contain both essential amino acids (threonine, histidine, valine, leucine, phenylalanine, isoleucine, methionine, and lysine) and non-essential amino acids (arginine, glutamate, alanine, asparagine, serine, proline, cysteine, tyrosine, aspartate, and glycine). Overall, the amino acid content in fillets from catfish fed a diet supplemented with XK9 and XK22 was consistently higher than that of fish in control ponds ($p<0.05$). The data further revealed that the concentration of amino acids followed the same trend in both groups. Among the essential amino acids, leucine, and lysine were the most predominant (4.79%, 4.10% and 4.28%, 2.92%, respectively in the *Streptomyces* and control group, followed by valine, isoleucine, phenylalanine, and methionine, while histidine was the least abundant (1.02% and 0.60%, respectively in the *Streptomyces* and control group). For the non-essential amino acids, glutamate and aspartate counted for the highest (concentrations in the *Streptomyces* and control group were 6.93%, 5.76% and 6.15%, 4.54%, respectively), followed by glycine, alanine, arginine, proline, tyrosine; asparagine and cysteine were present in the lowest proportions (Table 4).

The fatty acid profile of catfish fillets is presented in Table 5. The total saturated fatty acids (SFA) were significantly higher in fillets from the control group than in those from fish fed the diet containing XK9 and XK22 ($p<0.05$). Among the saturated fatty acids, palmitic acid and stearic acid were predominant in both groups, with concentrations of 18.68% and 10.89% in the *Streptomyces*-fed group, and 19.73% and 13.42% in the control group, respectively. In contrast, the proportions of unsaturated fatty acids, including both monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), were significantly higher in fillets from fish fed the diet supplemented with XK9 and XK22 ($p<0.05$). Among these, oleic acid, docosahexaenoic acid (DHA), and linoleic acid were the most abundant, accounting for 21.23%, 13.09%, and 8.66% in the *Streptomyces* group, compared to 17.93%, 11.87%, and 8.97% in the control group, respectively.

Table 4: Amino acid profile of freeze-dried fillets of catfish under intensive farming.

Amino acids (%)	*Streptomyces* group	Control group
Essential amino acid		
Threonine	$2.16^{a} \pm 0.01$	$1.36^{b} \pm 0.02$
Histidine	$1.02^{a} \pm 0.03$	$0.60^{b} \pm 0.01$
Valine	$3.69^{a} \pm 0.03$	$1.83^{b} \pm 0.00$
Leucine	$4.79^{a} \pm 0.01$	$4.10^{b} \pm 0.02$
Phenylalanine	$2.23^{a} \pm 0.11$	$1.88^{b} \pm 0.00$
Isoleucine	$2.90^{a} \pm 0.03$	$2.32^{b} \pm 0.02$
Methionine	$1.93^{a} \pm 0.01$	$0.91^{b} \pm 0.01$
Lysine	$4.28^{a} \pm 0.06$	$2.92^{b} \pm 0.06$
Non-essential amino acid		
Arginine	$3.09^{a} \pm 0.03$	$2.64^{b} \pm 0.02$
Glutamate	$6.93^{a} \pm 0.01$	$5.76^{b} \pm 0.03$
Alanine	$3.19^{a} \pm 0.01$	$2.93^{b} \pm 0.01$
Asparagine	$0.03^{a} \pm 0.00$	$0.13^{b} \pm 0.00$
Serine	$1.67^{a} \pm 0.02$	$1.55^{b} \pm 0.00$
Proline	$2.54^{a} \pm 0.01$	$1.83^{b} \pm 0.01$
Cysteine	$0.82^{a} \pm 0.01$	$0.47^{b} \pm 0.00$
Tyrosine	$1.05^{a} \pm 0.01$	$0.84^{b} \pm 0.01$
Aspartate	$6.15^{a} \pm 0.01$	$4.54^{b} \pm 0.03$
Glycine	$3.20^{a} \pm 0.00$	$2.82^{b} \pm 0.01$

Data are presented as mean ± SE. Abbreviations: *Streptomyces* group: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; Control group: ponds stocked with catfish were fed commercial pellets without *Streptomyces*. Different letter notations indicate a statistical difference between dietary treatments.

Table 5: Fatty acid profile of free-dried fillets of catfish in intensive farming.

Fatty acids (% total lipid)		*Streptomyces* group	Control group
SFA			
C14:0	Myristic acid	2.18 ^{a} ± 0.05	2.55 ^{b} ± 0.12
C16:0	Palmitic acid	18.68 ^{a} ± 0.16	19.73 ^{b} ± 0.34
C18:0	Stearic acid	10.89 ^{a} ± 0.22	13.42 ^{b} ± 0.09
C20:0	Arachidic acid	0.64 ^{a} ± 0.03	0.93 ^{b} ± 0.05
C24:0	Lignoceric acid	0.56 ^{a} ± 0.03	0.79 ^{b} ± 0.02
Σ SFA		32.96 ^{a} ± 0.34	37.43 ^{b} ± 0.38
MUFA			
C16:1	Palmitoleic acid	4.57 ^{a} ± 0.04	5.09 ^{b} ± 0.11
C18:1n9c	Oleic acid	21.23 ^{a} ± 0.15	17.93 ^{b} ± 0.15
C18:1n7c	Vaccenic acid	4.26 ^{a} ± 0.08	3.63 ^{b} ± 0.12
C20:1	Eicosenoic acid	0.72 ^{a} ± 0.01	0.89 ^{b} ± 0.04
C22:1n9	Erucic acid	3.53 ^{a} ± 0.33	2.84 ^{b} ± 0.06
C24:1n9	Nervonic acid	1.10 ^{a} ± 0.03	2.04 ^{b} ± 0.04
Σ MUFA		35.41 ^{a} ± 0.28	32.40 ^{a} ± 0.25
PUFA			
C18:3n3	α-Linolenic acid	4.60 ^{a} ± 0.06	4.51 ^{a} ± 0.02
C20:5n3	Eicosapentaenoic acid	1.16 ^{a} ± 0.03	1.08 ^{a} ± 0.06
C22:6n3	Docosahexaenoic acid	13.09 ^{a} ± 0.26	11.87 ^{b} ± 0.16
C18:2n6	Linoleic acid	8.66 ^{a} ± 0.10	8.97 ^{a} ± 0.10
C18:3n6	γ-Linolenic acid	0.19 ^{a} ± 0.04	0.42 ^{b} ± 0.02
C20:3n6	Eicosatrienoic acid	0.41 ^{a} ± 0.04	0.72 ^{b} ± 0.03
C20:4n6	Arachidonic acid	3.53 ^{a} ± 0.14	2.93 ^{b} ± 0.09
Σ PUFA		31.63 ^{a} ± 0.22	30.50 ^{b} ± 0.30
UFA		67.04 ^{a} ± 0.32	62.90 ^{b} ± 0.05
n3 PUFA		18.85 ^{a} ± 0.33	17.46 ^{b} ± 0.20
n6 PUFA		12.78 ^{a} ± 0.12	13.04 ^{a} ± 0.10
n3/n6		1.47 ^{a} ± 0.04	1.34 ^{b} ± 0.01
PUFA/FSA		0.96 ^{a} ± 0.01	0.82 ^{b} ± 0.01
MUFA/FSA		1.08 ^{a} ± 0.02	0.86 ^{b} ± 0.00
UFA/FSA		1.04 ^{a} ± 0.01	1.23 ^{b} ± 0.02

Data are presented as mean ± SE. Abbreviations: *Streptomyces* group: ponds stocked with catfish were fed commercial pellets supplemented with XK9 and XK22; Control group: ponds stocked with catfish were fed commercial pellets without *Streptomyces*; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids, including both monounsaturated fatty acids and polyunsaturated fatty acids. Different letter notations indicate a statistical difference between dietary treatments.

Discussion

The striped catfish is a commercially important species and one of the most widely farmed aquaculture species in Asia (Ngor, 1999; Van *et al.,* 2002; Soem *et al.,* 2023). In intensive aquaculture systems, high stocking densities and the extensive use of feed throughout the culture cycle can lead to environmental pollution and increase the risk of disease outbreaks (Okamura *et al*., 2011; Pulkkinen *et al*., 2010; Bartie *et al*., 2023; Oanh and Phu, 2022). Environmental surveys of intensive striped catfish culture ponds in certain localities of the Mekong Delta have revealed that concentrations of ammonia, nitrite (NO_2^-), phosphate (PO_4^{3-}), biochemical oxygen demand (BOD), and hydrogen sulfide (H_2S) consistently exceeded the permissible thresholds for aquaculture water quality (Giang *et al.,* 2008; Nguyen *et al*., 2014a; Nguyen *et al*., 2014b). Practice of sustainable industrial striped catfish farming requires the implementation of appropriate environmental management measures. The strategies for controlling water quality in striped catfish culture include the use of sedimentation ponds, daily water exchange, aeration, sludge removal and treatment, and the application of probiotics to maintain environmental parameters within optimal thresholds, thereby ensuring fish health and productivity. Lauzon *et al.* (2014) indicated that administering probiotics in rearing water is considered the most suitable approach, applicable across all developmental stages, from larvae to marketable size. However, the effectiveness of probiotics in environmental control varies; while some commercial products are highly effective, others may be ineffective or even exert detrimental effects on fish (Hai, 2015).

Aquatic animals closely interact with the external environment, and both environmental conditions and feeding practices have a substantial impact on the host. Probiotics intended for aquaculture must satisfy criteria of biosafety and functionality, including: (1) being non–harmful to the host; (2) the ability to survive passage through the host's gastrointestinal tract; (3) the capacity to colonize and proliferate within the host; (4) absence of virulence or antibiotic resistance genes; (5) promotion of host growth; (6) production of antagonistic compounds; (7) enhancement of nutrient digestion; (8) improvement of water quality; (9) stimulation of immune responses; and (10) competition for nutrients (Hai, 2015; Defoirdt *et al*., 2007; Martínez Cruz *et al*., 2012).

Streptomyces have been demonstrated to be beneficial in aquaculture, exhibiting strong probiotic properties and possessing specific characteristics that enable them to tolerate harsh environmental conditions (Tan *et al*., 2016). This allows them to maintain a longer shelf life in ponds before being consumed (McBride and Ensign, 1987). Some reports have highlighted the limitations of *Streptomyces* when applied as probiotics in aquaculture, as certain strains may be toxic to the host or produce off-flavor compounds, such as geosmin and 2-methylisoborneol (MIB), which impair the sensory quality of aquaculture products (Auffret *et al*., 2011).

Striped catfish is a tropical species, with a normal temperature range of 25-35°C for optimal physiological adaptation (Howerton, 2001). Islam *et al*. (2019) reported that the optimal temperature range for the growth performance of striped catfish cultured in Bangladesh was 28°C to 32°C, while De Silva and Phuong (2011) noted that temperatures up to 34°C provided the best thermal conditions for culturing this species in Vietnam. Furthermore, Tucker and Schrade (2019) demonstrated that off-flavors caused by geosmin and 2-MIB are purged relatively quickly from fish in warm water. Depending on fish size, fat content, and the initial concentration of geosmin and 2-MIB, these compounds can be reduced below detectable levels within a few days in clean water at temperatures above 25°C. Because catfish are fed daily in warm weather, the occurrence of off-flavors in their processing products is rare. In addition, intensive striped catfish farming typically involves a considerable amount of daily water exchange, which not only helps maintain water quality but also effectively mitigates the development of off-flavors in water and fish.

The present study employed two strains, XK9 and XK22, which had previously been confirmed under laboratory conditions to possess desirable probiotic traits for aquaculture applications and to be safe for striped catfish, black tiger shrimp, and whiteleg shrimp. This was evidenced by the absence of mortality and histopathological lesions in tissues such as the intestine, liver, kidney, spleen, and gills of fish, as well as the hepatopancreas and gills of shrimp, when administered at a dietary dose of 10^{11} CFU/g (Tam *et al.,* 2024). Collectively, these findings indicate that the use of *Streptomyces* strains as probiotics is safe and feasible, alleviating concerns regarding their application in striped catfish culture.

The results of using *Streptomyces* in intensive striped catfish farming indicate that the water quality was significantly improved in ponds where catfish were fed a diet supplemented with XK9 and XK22 compared to the control. Throughout the experiment, the parameters such as temperature, turbidity, pH, NH_3, DO in the experimental ponds remained within optimal thresholds for fish (Boyd, 1990; Mohanty *et al*., 2014), whereas in the control ponds, specific parameters such as NH_3, DO, pH, and temperature fluctuated in waysthat adversely affected the catfish after five months of stocking. These findings are consistent with previous studies reporting that dietary supplementation with probiotics effectively improved water quality (Padmavathi *et al*., 2012; Ngan *et al*., 2016; Ahmed *et al*., 2022). Rahayu *et al.* (2024) reported that probiotics not only stimulate the synthesis of digestive enzymes in the fish gastrointestinal tract, enhancing nutrient digestion, but also play an important role in decomposing excess feed and residual organic compounds in the culture environment. Earlier, Boyd and Gross (1998) demonstrated that probiotics can effectively degrade various organic materials, uneaten feed, and organic salts such as phosphate, CO_2, and nitrate. In line with these findings, XK9 and XK22 exhibit a strong capacity to produce extracellular enzymes with high catalytic activity, which may contribute to improving aquaculture water quality by degrading organic compounds derived from fish waste and excess feed, thereby mitigating eutrophication and sludge accumulation while maintaining stable physicochemical parameters of the aquatic environment to support the physiological activities of catfish.

Growth performance and survival rates of aquatic animals are influenced not only by genetic quality, feed intake, or stocking density, but also by abiotic factors such as temperature, dissolved oxygen (DO), pH, nutrients, and the organic fraction of water (Boyd and Tucker, 1998; Sikoki and Veen, 2004; Bhatnagar and Devi, 2013; Imsland *et al*., 2017). In the present study, the ability of strains XK9 and XK22 to regulate water quality and maintain it within the optimal range for catfish likely contributed to the superior growth performance and significantly higher survival rates observed in probiotic-supplemented ponds compared to control ponds. Beyond their capacity to synthesize extracellular enzymes, these *Streptomyces* strains have been shown to enhance immune responses and confer resistance against bacterial pathogens (Tam *et al*., 2024), further supporting improved fish survival. The findings are consistent with previous reports by Rahman *et al*. (2019) and Jagannathan *et al*. (2021), who demonstrated that *Streptomyces* synthesize enzymes that facilitate digestion and nutrient absorption, thereby optimizing feed utilization for promoting growth. Similarly, the results align with those of Ravikumar *et al*. (2012) and Anandan *et al*. (2016), which indicated that *Streptomyces* supplementation can enhance both survival and growth of host organisms.

Proximate composition is widely recognized as a reliable indicator of physiological status and is essential for routine analysis of fisheries (Cui and Wootton, 2006). On average, fish carcasses contain 75% water, 16% protein, 6% lipid, and 3% ash, with moisture being the major component, typically ranging from 60% to 80% across various fish species (Love, 1970; Murra and Burt, 2001). The findings of the study, catfish fed diets supplemented with XK9 and XK22 exhibited significantly higher crude protein and ash content, but lower lipid content, compared to the control group. These findings are consistent with previous studies reporting enhanced protein levels and reduced lipid content in fish fed probiotic-supplemented diets (Mary *et al.*, 2019; El-Haroun *et al*., 2006; Bagheri *et al*., 2008; Azarin *et al*., 2015; Hassaan *et al*., 2018). The increased protein content in the fillet of treated catfish may be attributed to the direct contribution of proteins from the probiotic, as well as improved feed digestion stimulated by the extracellular enzymes of these strains, which facilitate efficient conversion of dietary nutrients into structural proteins (Lara-Flores and Olvera-Novoa, 2013; Mehrabi *et al*., 2012). In addition, referring to Gallagher *et al*. (1991), who reported a positive correlation between ash content in fish fillets and the concentrations of calcium, potassium, zinc, iron, and magnesium, it can be inferred that catfish is a good source of these minerals. In the probiotic-fed group, the content of these minerals was higher, which could be attributed to more efficient nutrient absorption promoted by *Streptomyces* strains.

The amino acid profiles of catfish fillets were significantly enhanced by dietary supplementation with XK9 and XK22, with both essential and non-essential amino acids showing higher concentrations compared to the control group. These findings are consistent with previous reports highlighting the effectiveness of probiotics in improving fish fillet quality (Cao *et al*., 2019; Ringø *et al*., 2022). Among the essential amino acids, lysine and leucine were particularly elevated in catfish fillets from fish that received *Streptomyces, which* correlated strongly with improved growth performance. Lysine is an essential amino acid critical for protein synthesis in all animals (Ball *et al.,* 2007; Tomé and Bos, 2007), while elevated leucine levels are indicative of enhanced stimulation of muscle protein synthesis (Etzel, 2004). Analysis of non-essential amino acids further confirmed the benefits of XK9 and XK22. Fillets from treated catfish exhibited significantly higher concentrations of glutamate and aspartate, which are closely associated with improved growth performance and survival. While glutamate plays a role in transamination reactions and is essential for the synthesis of key molecules, such as glutathione, which is necessary for the removal of highly toxic products, aspartate is the precursor of methionine, threonine, isoleucine, and lysine, and regulates the synthesis of essential hormones (Guoyao, 2013). The high arginine content in catfish fillets is also a growth-promoting factor in fish because this amino acid plays a vital role in cell division, wound healing, ammonia removal, immune function, and hormone release (Mohanty *et al*., 2014). The findings of this study demonstrate that supplementation with XK9 and XK22 not only improves the quality of catfish fillets by enhancing amino acid compositions but also promotes growth and increases survival, thereby enhancing the efficiency of catfish farming.

The fatty acid profile of catfish fillets revealed the composition of 18 fatty acids. Dietary supplementation with XK9 and XK22 significantly influenced the fatty acid composition. Specifically, the total saturated fatty acids (SFA) in fillets from catfish fed a diet containing probiotics were significantly lower than in the control group; in contrast, total unsaturated fatty acids (including monounsaturated fatty acids-MUFA, and polyunsaturated fatty acids-PUPA) were significantly higher in the group of fish fed daily with *Streptomyces*. Among the unsaturated fatty acids, docosahexaenoic acid (DHA), oleic acid, and linoleic acid were particularly elevated, the concentrations of these fatty acids in fillets from probiotic-fed catfish exceeded those reported for the muscle of *Clarias gariepinus* (Mmandu and Clement, 2020) and were higher than in several freshwater fish species such as *Clarias gariepinus*, *Cyprinus carpio*, *Siluris glanis*, and *Tinca tinca* (Yesim *et al*., 2007). DHA, linoleic acid, is an omega-3 polyunsaturated fatty acid, a well-known nutrient source that benefits human visual and brain development (Durmus, 2019). Oleic acid, an omega-9 monounsaturated fatty acid, has proven advantages for cardiovascular health, metabolism related to insulin, and the absorption function of the gastrointestinal system (Igor, 2014). These findings indicate that supplementation with *Streptomyces* not only enhances fish fillet quality but also increases the content of fatty acids that are beneficial for human nutrition.

Conclusion

This study shows that *S. kunmingensis* XK9 and *S. angustmyceticus* XK22 bring positive effects in intensive catfish farming by improving water quality, growth performance, feed efficiency, survival rate, and the quality of catfish fillets. All these benefits of the two *Streptomyces* strains, when supplemented in the diet of intensively farmed catfish, can enhance production efficiency and are feasible for promoting the sustainable development of the catfish industry. However, further studies on application protocols in intensive catfish farming are needed, particularly regarding dosage, frequency, duration of probiotic use, and the withdrawal period before harvest, in order to optimize economic efficiency.

Acknowledgments

This study is funded by project code MHN2025-01.01 of Hanoi Open University. The authors sincerely thank Mr.

Lam Thanh Pha, Party Committee Secretary and Chairman of Long Hoa commune, Phu Tan district, An Giang province, for his dedicated support in selecting intensive catfish ponds for the experiments. The authors would also like to thank Mr. Nguyen Van Dung, the owner of the catfish ponds in Long Hoa 2 hamlet, Long Hoa commune, Phu Tan district, An Giang province, for his strict collaboration during the trial and for assessing the impacts of *Streptomyces* on the water quality and farming efficiency. His experience in catfish farming has been a valuable reference for this study.

Conflicts of interest

The authors declare that there is no conflict of interest regarding financial support or personal relationships.

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