EVALUATING POND AND CAGE-IN-POND CULTURE SYSTEMS FOR IMPROVING MUSCLE NUTRITIONAL QUALITY IN *SPINIBARBUS SINENSIS*

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Abstract. This study investigated the effects of traditional pond culture (CT) and the cage-in-pond system (JY) on the muscle nutritional composition and fatty acid profiles of *Spinibarbus sinensis*. The results showed that crude protein, crude lipid, total amino acids, essential amino acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and n-3 polyunsaturated fatty acids (n-3 PUFAs) in the muscle were significantly higher in the CT group than in the JY group ($P < 0.05$), whereas ash content was significantly lower. Fatty acid analysis revealed that the CT group exhibited a broader fatty acid spectrum, with significantly higher levels of total saturated fatty acids (∑SFA), total monounsaturated fatty acids (∑MUFA), and total polyunsaturated fatty acids (∑PUFA), particularly n-3 PUFAs and long-chain polyunsaturated fatty acids (LC-PUFAs), especially EPA and DHA. These improvements contributed to markedly lower IA, IT, and HI indices in the CT group, indicating a greater potential for reducing cardiovascular risks. The superior nutritional quality of the CT group may be attributed to the availability of natural food organisms in pond environments, whereas the JY group was limited by higher stocking density and a reliance on formulated diets with relatively simple lipid sources. Overall, the pond culture system enhanced the nutritional quality of *S. sinensis* muscle, while the cage-in-pond system offered advantages in environmental sustainability and management efficiency. Further improvements in the cage-in-pond system could be achieved by optimizing stocking density and dietary lipid composition, thereby enhancing fatty acid deposition and overall nutritional value. These findings provide new insights into the optimization of aquaculture models for improving the flesh quality of cultured fish.
Keywords: *amino acid composition, fatty acid profile, pond culture, cage-in-pond system, muscle quality indices*

Abbreviations. The following abbreviations are used in this manuscript:

∑AA	Total amino acids
∑EAA	Total essential amino acids
∑DAA	Total Delicious Amino Acids
∑NEAA	Total non-essential amino acids
BCAA	Branched-Chain Amino Acids
AAA	Aromatic Amino Acids
∑SFA	Total saturated fatty acids
∑MUFA	Total monounsaturated fatty acids
∑PUFA	Total polyunsaturated fatty acids
∑PUFA n-3	Total n-3 polyunsaturated fatty acids
∑PUFA n-6	Total n-6 polyunsaturated fatty acids
∑UFA	Total unsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
HI	Hypercholesterolaemic index
IA	index of Atherogenic
IT	index of Thrombogenic

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Introduction

Aquaculture has become one of the fastest growing sectors in global agriculture and food production. In terms of output, China possesses the largest aquaculture industry worldwide (Zhou et al., 2024). In 2024, the freshwater pond culture area in China reached 2.6246 million hectares, with a production of 25.214 million tons, accounting for 49.26% of the total freshwater aquaculture area and 71.38% of the total freshwater production, respectively (Agriculture and Rural Development Ministry's Fisheries and Fishery Administration Bureau, n.d.). However, traditional pond culture faces severe challenges, including excessive water consumption, water quality deterioration, and discharge of polluted effluents, which seriously restrict its sustainable development (Liu et al., 2021; Liu, 2024). To address these issues, China has developed and promoted a variety of novel facility-based aquaculture models (Cui et al., 2024), such as land-based recirculating aquaculture systems (Guo et al., 2023), container-based cultures (Liu et al., 2022), in-pond raceway system (Liu et al., 2022), and cage-in-pond culture systems (He and Hou, 2021; Deng et al., 2024). Among these, the cage-in-pond model, characterized by high solid waste removal efficiency, low environmental disturbance, and significant overall benefits, was recognized by the Ministry of Agriculture and Rural Affairs as a key leading agricultural technology in both 2019 and 2020 (He and Hou, 2021).

Freshwater fish are an important source of high-quality protein for humans and play an increasingly prominent role in the global food supply system (Naylor et al., 2021). The muscle nutrient composition of fish, including crude protein, crude lipid, amino acid profiles, and fatty acid composition, not only determines their commercial value and sensory quality but also contributes to human health benefits, particularly cardiovascular health (Yu et al., 2025). Among these components, the content of long-chain n-3 polyunsaturated fatty acids (LC-n-3PUFAs), such as EPA and DHA, is considered a key indicator (Van Dael, 2021). Previous studies have demonstrated that culture models significantly affect muscle quality and nutrient composition in aquaculture species (Liu et al., 2022; Qiu et al., 2022; Su et al., 2025). For example, compared with pond culture, intensive factory-based systems have been shown to improve crude protein, total amino acids, C20:5n3, and saturated fatty acid contents in *Opsariichthys bidens* (Qiu et al., 2022); cage-in-reservoir systems markedly enhanced the nutritional balance, flavor compound richness, and textural properties of *Micropterus salmoides* (Su et al., 2025); and land-based circular tank culture improved lipid content and functional fatty acid levels in hybrid snakehead "Male Snakehead No. 1" (Yu et al., 2025). However, systematic comparisons of fish muscle nutritional value between traditional pond culture and cage-in-pond systems remain scarce. Elucidating the effects of different culture models and their management practices on muscle quality parameters is of great significance for optimizing aquaculture practices, enhancing product competitiveness, and meeting consumer demand for highly nutritious aquatic products.

Therefore, in this study, *Spinibarbus sinensis* was selected as the research species to systematically compare the differences in muscle nutritional composition between traditional pond culture and cage-in-pond systems. Specifically, we analyzed the basic nutritional components (moisture, crude protein, crude lipid, and ash), amino acid profiles and contents, as well as fatty acid profiles and contents. The objective of this study was to scientifically evaluate the effects of cage-in-pond culture on the muscle nutritional quality of *S. sinensis*, elucidate its nutritional differences from traditional pond-cultured fish, and provide theoretical and data support for the optimization and wider application

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of this culture model, thereby promoting the healthy and sustainable development of facility-based aquaculture.

Materials and methods

Fish management and sample collection

The experiment was conducted at the production and research base of the Hunan Fisheries Research Institute and Aquatic Products Seed Stock Station, located in Changsha, Hunan Province, China. The cage-in-pond culture system (JY) was newly constructed in 2022. The system is mainly composed of tanks, aeration, and sewage discharge modules. The tank is made of polyethylene. The upper half of the tank is a cylinder with a diameter of 4 meters and a height of 2 meters, and the lower half is an inverted cone with a depth of 1 meter. The traditional pond (CT) was located adjacent to the cage-in-pond system, with both ponds having identical depth, size, shape, and water source. A schematic diagram comparing the two systems is presented in *Figure 1*. The average water temperature, dissolved oxygen (DO), pH, and ammonia nitrogen (NH_4^+-N) were comparable between the CT and JY systems throughout the experimental period ($P > 0.05$). Overall mean values were approximately 28.7 °C, 5.2 mg/L, 7.8, and 0.2 mg/L, respectively.

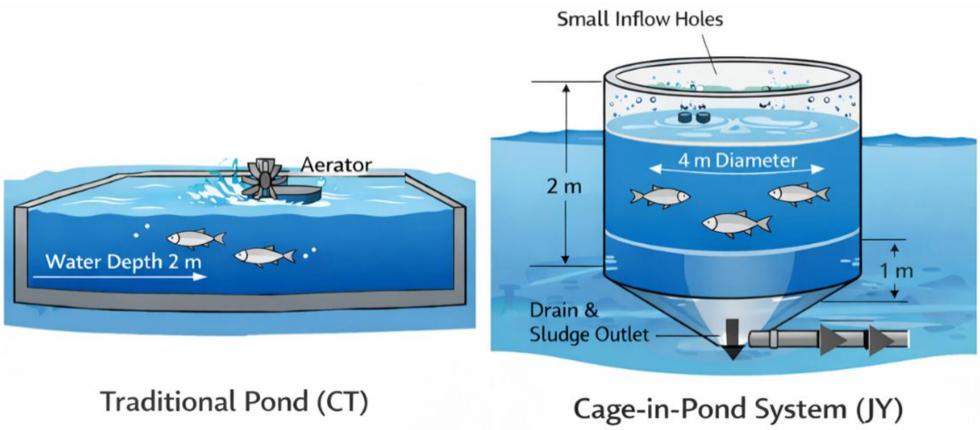

Figure 1. *Schematic of the traditional pond (CT) and cage-in-pond system (JY). The JY system consists of a cylindrical tank (2 m height, 4 m diameter) with a conical bottom (1 m height), top-side inflow holes, and a bottom drain/outlet. Arrows indicate water flow direction*

Experimental fish (*S. sinensis*) were obtained from the same batch of juveniles reared by the project team. The initial body weight and body length of the fish were 130.19 ± 12.35 g and 18.5 ± 0.8 cm, respectively. According to the stocking density reported by Lu et al. (2022), the cage-in-pond system was stocked with 2000 fish per cage (approximately 100 fish/m^3), whereas the traditional pond followed a conventional density of 2000 fish per 667m^2 (approximately 3 fish/m^3). Three replicates were set up for each culture system. During the experimental period, fish were fed extruded feed twice daily (08:00 and 17:00) at a ration of 3% of body weight, equivalent to 3.9 g per fish per day. Based on stocking density, the total daily feed supplied was approximately 7.8 kg per cage in the JY system and 7.8 kg per pond in the CT system. The feeding rate was adjusted weekly according to the average body weight of the fish. The diet, manufactured

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by Fujian Aolong Aquatic Science and Technology Co., Ltd., contained ≥40% crude protein, ≥5% crude lipid, ≤8% crude fiber, ≤15% crude ash, ≥1.2% total phosphorus, ≥2.2% lysine, and ≤10% moisture. The trial lasted from June 26 to September 6, 2023. At the end of the experiment, the average body weight and total length of the fish were 176 ± 15 g and 21 ± 0.9 cm in the CT group, and 172 ± 14 g and 20.8 ± 0.8 cm in the JY group, respectively. Fish were fasted for 24 h before sampling. Nine fish were randomly selected from each group (three per replicate), anesthetized with MS-222, descaled, and skinned. Approximately 10 g of dorsal muscle was collected from each individual for subsequent analyses of muscle nutrient composition and fatty acid profiles.

Determination and evaluation methods

The proximate composition of muscle was determined according to the methods specified in the Chinese national food safety standards: crude protein (GB 5009.5-2016), crude lipid (GB 5009.6-2016), moisture (GB 5009.3-2016), and ash (GB 5009.4-2016). Amino acid composition was analyzed using the hydrolysis method following GB 5009.124-2016. Fatty acid composition was determined by the internal standard method (Method I) described in GB 5009.168-2016.

Amino acid nutritional value was evaluated based on the FAO/WHO scoring pattern, and amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) were calculated according to the formulas described by Yu et al. (2025). The index of atherogenicity (IA) and index of thrombogenicity (IT) were calculated using the formulas of Chen and Liu (2020), while the hypercholesterolemic index (HI) was calculated according to Lou et al. (2016). The specific formulas are as follows:

$$IA = \frac{C12{:}0 + (4 \times C14{:}0) + C16{:}0}{\sum UFA} \quad \text{(Eq.1)}$$

where C12:0 (lauric acid), C14:0 (myristic acid), and C16:0 (palmitic acid) are individual saturated fatty acids, and ∑UFA represents the total unsaturated fatty acids.

$$IT = \frac{(C14{:}0 + C16{:}0 + C18{:}0)}{(0.5 \times \sum MUFA + 0.5 \times \sum n-6PUFA + 3 \times \sum n-3PUFA + (n-3/n-6)} \quad \text{(Eq.2)}$$

where C18:0 is stearic acid, ∑MUFA represents the total monounsaturated fatty acids, ∑n-6 PUFA and ∑n-3 PUFA are the total n-6 and n-3 polyunsaturated fatty acids, respectively, and n-3/n-6 is the ratio of n-3 to n-6 polyunsaturated fatty acids.

$$HI = C14{:}0 + C16{:}0 \quad \text{(Eq.3)}$$

Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD). The data were tested for normality and homogeneity of variance. Data between the two groups were compared using the Student's T-test. Statistical significance was then analyzed using SPSS 18.0, with a P-value $<$ 0.05 considered statistically significant. Figures were generated using GraphPad Prism 8.0.1 software.

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Results

Basic nutritional composition

The muscle composition and contents of *S. sinensis* under the two culture systems are presented in *Table 1*. The results showed that crude protein and crude lipid levels were significantly higher in the CT group than in the JY group ($P < 0.05$), whereas ash content was significantly lower ($P < 0.05$). There were no significant differences in moisture content between the CT and JY groups ($P > 0.05$).

Table 1. *Comparison of muscle nutritional composition and content of S. sinensis under different culture modes*

Components	Content (g/100g)	
	CT group	**JY group**
Crude protein	20.23±0.07^{a}	19.13±0.11^{b}
Crude lipids	2.12±0.02^{a}	1.88±0.03^{b}
Moisture	77.03±0.15	77.70±0.60
Ash	1.42±0.05^{a}	1.55±0.04^{b}

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences ($P < 0.05$, Student's t-test)

Amino acid composition and nutritional evaluation

The amino acid composition and contents of *S. sinensis* under the two culture systems are presented in *Table 2* and *Figure 2*. Among the 18 commonly detected amino acids, seven were essential amino acids (EAA) and four were Delicious amino acids (DAA). The total amino acids (∑AA), total essential amino acids (∑EAA), and total flavor amino acids (∑DAA) in the CT group were significantly higher than those in the JY group ($P < 0.05$). No significant differences were observed between the two culture systems in the ratios of ∑EAA/∑AA, ∑EAA/∑NEAA, or the ratio of branched-chain to aromatic amino acids (F) ($P > 0.05$). The proportion of flavor amino acids relative to total amino acids was significantly higher in the CT group than in the JY group ($P < 0.05$).

The amino acid evaluation based on the FAO/WHO reference pattern and the whole egg protein pattern for both culture systems is shown in *Table 3*. No significant differences were found in amino acid score (AAS) or chemical score (CS) between traditional pond and cage-in-pond systems. In both systems, only Val showed an AAS below 1.00, while the other amino acids had scores ≥ 1.00, with Lysine scores of 1.66 and 1.68 in the CT and JY groups, respectively. For CS, only Lysine had a value greater than 1, while all other amino acids scored below 1.

Fatty acid composition and nutritional evaluation

The muscle fatty acid composition and contents of *S. sinensis* under the two culture systems are presented in *Table 4*. In the CT group, 18 fatty acids were detected, including five saturated fatty acids (SFA), six monounsaturated fatty acids (MUFA), and seven polyunsaturated fatty acids (PUFA). In the JY group, 15 fatty acids were detected, including three SFA, five MUFA, and seven PUFA. Among the detected fatty acids, only palmitic acid (C16:0) was significantly higher in the JY group than in the CT group, whereas the contents of all other fatty acids were significantly higher in the CT group ($P < 0.05$).

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Table 2. *Muscle amino acid composition of S. sinensis under different culture modes*

Amino Acid	Content (g/100g)	
	CT group	**JY group**
Aspartic (Asp)	2.05±0.00[a]	1.92±0.02[b]
Threonine (Thr)	0.81±0.01[a]	0.74±0.02[b]
Serine (Ser)	0.69±0.01[a]	0.64±0.02[b]
Glutamic (Glu)	2.93±0.01[a]	2.69±0.00[b]
Glycine (Gly)	0.93±0.03[a]	0.78±0.02[b]
Alanine (Ala)	1.14±0.01[a]	1.03±0.01[b]
Valine (Val)	0.86±0.01	0.84±0.01
Methionine (Met)	0.53±0.02	0.50±0.00
Isoleucine (Ile)	0.83±0.01[a]	0.79±0.00[b]
Leucine (Leu)	1.43±0.02[a]	1.34±0.01[b]
Tyrosine (Tyr)	0.63±0.02	0.60±0.01
Phenylalanine (Phe)	0.73±0.02[a]	0.69±0.00[b]
Histidine (His)	0.65±0.02	0.60±0.00
Lysine (Lys)	1.82±0.02[a]	1.75±0.01[b]
Arginine (Arg)	1.20±0.00[a]	1.10±0.00[b]
Proline (Pro)	1.13±0.01	1.11±0.00
Total amino acids (∑AA)	18.38±0.08[a]	17.14±0.07[b]
Total essential amino acids(∑EAA)	7.02±0.10[a]	6.66±0.02[b]
Total Delicious Amino Acids（∑DAA）	7.05±0.03[a]	6.42±0.03[b]
Total non-essential amino acids（∑NEAA）	11.36±0.04[a]	10.49±0.05[b]
∑EAA/∑NEAA	0.62±0.01	0.63±0.00
∑EAA/∑AA/%	38.21±0.40	38.82±0.07
∑DAA/∑AA/%	38.38±0.30[a]	37.49±0.03[b]
Essential amino acid index（EAAI）/%	83.55±1.08	83.83±0.20
F	2.30±0.03	2.29±0.01

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences ($P < 0.05$, Student's t-test). F is the ratio of Branched-Chain Amino Acids (BCAAs; Leu, Ile, and Val) to Aromatic Amino Acids (AAAs; Phe and Tyr)

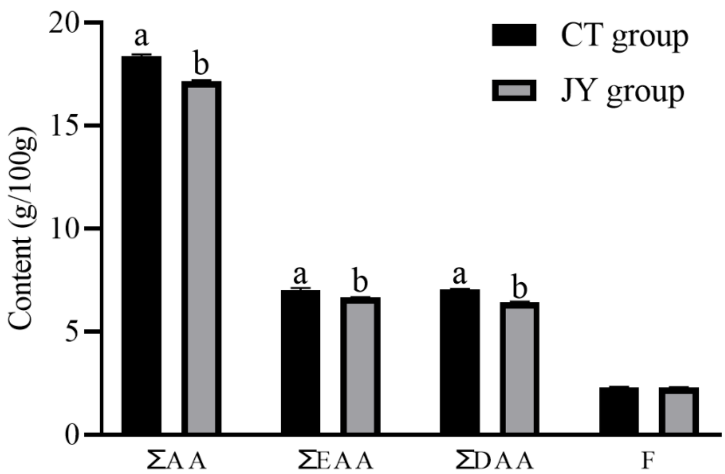

Figure 2. *Analysis of Amino Acid Composition in S. sinensis under Different Culture Mode. Bars with different letters above them are significantly different (P ＜0.05). The same convention applies to the following figures*

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Table 3. *Nutritional evaluation of amino acids in the muscle of S. sinensis under different culture modes*

Amino acid	FAO/WHO Standard pattern content (mg/g, as N)	Protein content of whole egg (mg/g, as N)	CT group			JY group		
			Content （mg/g， as N）	AAS	CS	Content （mg/g， as N）	AAS	CS
Threonine (Thr)	250	292	250.68±2.49	1.00±0.01	0.86±0.01	244.62±5.42	0.98±0.02	0.84±0.02
Valine (Val)	310	410	266.17±2.58	0.86±0.01	0.65±0.01	272.92±4.61	0.88±0.01	0.67±0.01
Isoleucine (Ile)	250	331	257.61±4.03	1.03±0.02	0.78±0.01	257.74±0.76	1.03±0.00	0.78±0.00
Leucine (Leu)	440	534	441.96±5.56	1.00±0.01	0.83±0.01	439.05±1.87	1.00±0.00	0.82±0.00
Lysine (Lys)	340	441	563.48±7.06	1.66±0.02	1.28±0.02	571.48±1.84	1.68±0.01	1.30±0.00
Phenylalanine + Tyrosine (Phe＋Tyr)	380	565	419.16±9.42	1.10±0.02	0.74±0.02	424.02±0.99	1.12±0.00	0.75±0.00

Note: Values are expressed as mean ± SD (n = 3). AAS, amino acid score; CS, chemical score. Different superscript letters within the same row indicate significant differences (*P* < 0.05, Student's t-test)

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Table 4. *Muscle fatty acid composition of S. sinensis under different culture modes*

Fatty acid	Content (g/100g)	
	CT group	**JY group**
Myristic acid (C14:0)	0.061±0.000[a]	0.035±0.000[b]
Myristoleic acid (C14 :1n5)	0.016±0.000[a]	0.007±0.000[b]
Pentadecanoic acid (C15:0)	0.003±0.000	
Palmitic acid (C16:0)	0.046±0.001[a]	0.173±0.001[b]
Palmitoleic acid (C16 :1n7)	0.046±0.000[a]	0.025±0.000[b]
Heptanoic acid (C17:0)	0.004±0.000	
Stearic acid (C18:0)	0.096±0.000[a]	0.063±0.000[b]
oleic acid (C18 :1n9c)	0.461±0.001[a]	0.271±0.000[b]
linoleic acid (C18:2n6c)	0.293±0.001[a]	0.190±0.001[b]
Arachidonic acid (C20 :1)	0.023±0.000[a]	0.013±0.000[b]
α-linolenic acid (C18 :3n3)	0.032±0.000[a]	0.021±0.000[b]
Eicosadienoic acid (C20:2)	0.012±0.000[a]	0.009±0.000[b]
Scolic acid (C20 :3n6)	0.018±0.000[a]	0.013±0.000[b]
Erucic acid (C22 :1n9)	0.007±0.000[a]	0.004±0.000[b]
Arachidonic acid (C20:4n6)	0.038±0.000[a]	0.029±0.000[b]
EPA (C20 :5n3)	0.009±0.000[a]	0.006±0.000[b]
Nervonic acid C24 :1n9	0.005±0.000	
DHA (C22:6n3)	0.064±0.000[a]	0.050±0.000[b]

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences ($P < 0.05$, Student's t-test)

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Further analysis showed that total SFA (∑SFA), total MUFA (∑MUFA), and total PUFA (∑PUFA) in the CT group were significantly higher than those in the JY group ($P < 0.05$). The ∑n-3 PUFA and ∑n-6 PUFA in the CT group were also significantly higher than those in the JY group ($P < 0.05$), although the n-3/n-6 ratio did not differ significantly ($P > 0.05$). The sum and ratio of EPA and DHA were significantly higher in the CT group than in the JY group ($P < 0.05$). Conversely, the ∑PUFA/∑SFA ratio, hypercholesterolemic index (HI, *Eq.1*), index of atherogenicity (IA, *Eq.2*), and index of thrombogenicity (IT, *Eq.3*) were all significantly higher in the JY group compared with the CT group ($P < 0.05$) (*Table 5*). *Figure 3* provides a graphical representation of key fatty acid parameters, including ∑SFA, ∑MUFA, ∑PUFA, EPA+DHA. *Figure 4* illustrates the health-related lipid indices (IT, IA, and HI).

Table 5. *Nutritional evaluation of fatty acids in S. sinensis under different culture modes*

Indicators	Content (g/100g)	
	CT group	JY group
Total saturated fatty acids ∑SFA	0.440±0.001[a]	0.271±0.001[b]
Total monounsaturated fatty acids ∑MUFA	0.559±0.001[a]	0.320±0.000[b]
Total polyunsaturated fatty acids ∑PUFA	0.467±0.001[a]	0.318±0.001[b]
Total n-3 polyunsaturated fatty acids ∑PUFA n-3	0.105±0.000[a]	0.077±0.000[b]
Total n-6 polyunsaturated fatty acids ∑PUFA n-6	0.350±0.001[a]	0.232±0.002[b]
Total unsaturated fatty acids ∑UFA	1.026±0.001[a]	0.638±0.001[b]
EPA+DHA	0.073±0.000[a]	0.056±0.000[b]
EPA/DHA	0.141±0.001[a]	0.120±0.001[b]
n-3/n-6	0.300±0.001	0.332±0.000
∑UFA/∑SFA	2.332±0.001	2.354±0.001
∑PUFA /∑SFA	1.061±0.000[a]	1.173±0.001[b]
HI (Hypercholesterolaemic index)	0.107±0.000[b]	0.208±0.001[a]
IA (index of Atherogenic)	0.283±0.001[b]	0.491±0.002[a]
IT (index of Thrombogenic)	0.311±0.001[b]	0.511±0.001[a]

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences ($P < 0.05$, Student's t-test)

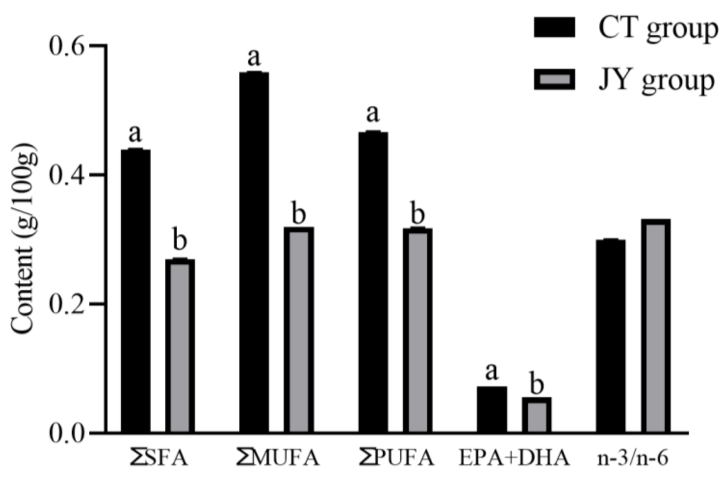

Figure 3. *Nutritional evaluation of fatty acids in S. sinensis under different culture modes*

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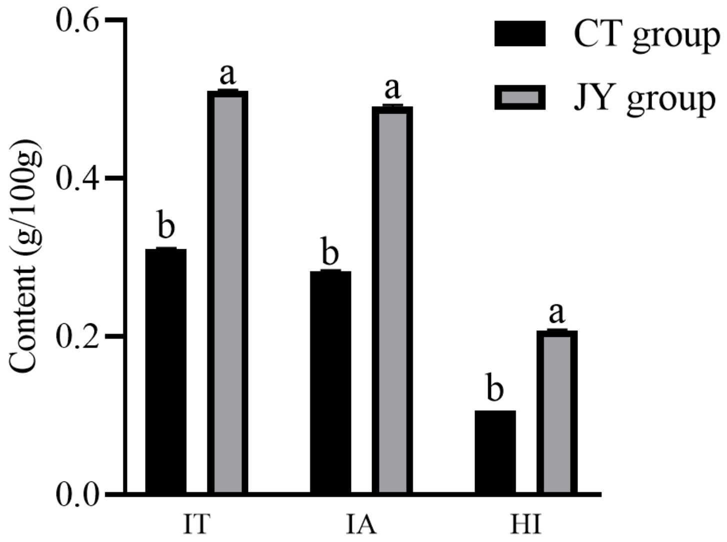

Figure 4. *Muscular health risk indices in S. sinensis under Different Culture Modes*

Discussion

Muscle is the main edible portion of cultured fish, and its nutritional composition directly reflects the quality and nutritional value of the fish (He et al., 2020). Culture system is one of the key factors influencing muscle nutrient composition (Qiu et al., 2022; Su et al., 2025; Yu et al., 2025). With the rapid development of facility-based aquaculture in China, a variety of modern culture systems have emerged, including cage-in-pond culture (Cui et al., 2024). However, studies comparing the effects of traditional pond culture and cage-in-pond systems on muscle quality of cultured fish remain limited. In this study, we systematically evaluated the effects of traditional pond and cage-in-pond culture on the muscle nutritional value of *S. sinensis*, providing direct scientific evidence for optimizing culture practices and enhancing the nutritional quality of aquaculture products.

The nutritional value of fish muscle is closely related to crude protein, crude lipid, amino acid composition, and fatty acid composition (Guan et al., 2022). Protein is an essential component of the human diet, and its content is one of the most important indicators for evaluating the nutritional value of food (Wu, 2016). In this study, we compared the differences in muscle nutrient composition between two culture systems. The results showed that crude protein and crude lipid contents were significantly higher in the pond-cultured (CT) group than in the cage-in-pond (JY) group, while ash content was significantly lower in the CT group ($P < 0.05$), indicating that pond-cultured *S. sinensis* has higher nutritional value. The higher crude protein content in the CT group may be attributed to the more diverse natural food resources available in the pond culture system, whereas the cage-in-pond system relies solely on formulated feed (He et al., 2021). Similar results were reported by Gao et al. (2025), who found differences in muscle quality of bighead carp cultured in rice fields, ponds, and reservoirs (Gao et al., 2025). The lower crude lipid content in the JY group may result from continuous aeration in the cage-in-pond system, which increases fish activity and energy expenditure. This finding is consistent with the observation by Qiu et al. (2022) that wild *Opsariichthys bidens* had significantly lower crude protein content than pond-cultured fish.

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Fish muscle is rich in various essential amino acids (EAA), particularly lysine, which is often abundant and considered an important source of high-quality protein (Mohanty et al., 2014). In this study, the contents of EAA, non-essential amino acids (NEAA), total amino acids (TAA), and flavor-enhancing amino acids (DAA) in the CT group were significantly higher than those in the JY group ($P < 0.05$), consistent with the observed differences in crude protein content. This may be attributed to the more diverse natural food resources available in the pond culture system, which could enhance the absorption and synthesis of these amino acids (He et al., 2021). According to the Food and Agriculture Organization (FAO), a ∑EAA/∑NEAA ratio greater than 0.6 indicates a high-quality protein (Yamamoto et al., 2005; Qiu et al., 2022). In this study, the ∑EAA/∑NEAA ratios were 0.62 and 0.63 for the CT and JY groups (*Table 2*), respectively, both exceeding 0.6, indicating that even under cage-in-pond culture, *S. sinensis* provides high-quality protein despite a slightly lower amino acid content than pond-cultured fish. Branched-chain amino acids (BCAA) play important roles in liver protection and cholesterol reduction (Shao et al., 2018). In the present study, the BCAA/aromatic amino acid ratios (F) were 2.30 and 2.29 in the CT and JY groups, respectively, with no significant difference ($P > 0.05$), comparable to *Pelteobagrus fulvidraco* “Huangyou No.1” (F = 2.48) (Shao et al., 2018) and *Eleutheronema tetradactylum* (F = 2.33) (Huang, 2013), suggesting that fish from both culture systems possess high nutritional value. The content and balance of essential amino acids are important indicators for evaluating muscle protein quality, reflecting not only protein quality but also culture environment, feed utilization, and metabolic status (Wang et al., 2024). Common evaluation indices include the essential amino acid index (EAAI), amino acid score (AAS), and chemical score (CS) (Qiu et al., 2022; Niu et al., 2023; Su et al., 2025; Yu et al., 2025). In this study, no significant differences were observed in EAAI, AAS, or CS between the two culture systems ($P > 0.05$). Notably, the AAS values for isoleucine (Ile), leucine (Leu), and threonine (Thr) exceeded 1.00 in both groups (*Table 3*), while lysine (Lys) content was higher than both the FAO/WHO standard (CT: 563 mg/g N, AAS = 1.66; JY: 571 mg/g N, AAS = 1.68) and whole egg protein, indicating a balanced amino acid composition and abundant lysine reserve in the muscle under both culture systems. These findings are consistent with studies on *Pagrus major* (You et al., 2016). For populations consuming predominantly cereal-based diets, lysine is typically the limiting amino acid (Kim and Lall, 2000; Gao et al., 2025;). Both pond and cage-in-pond cultured *S. sinensis* can provide sufficient lysine to meet dietary needs. Based on AAS values, valine (Val) was identified as the first limiting essential amino acid in this study, with AAS values of 0.86 (CT) and 0.88 (JY), slightly below the standard of 1.00, indicating relative deficiency. This is consistent with previous findings in bighead carp (Gao et al., 2025) and in Jian carp and Jinbian carp (Ye et al., 2020).

Fat content is a critical factor influencing the palatability and flavor of fish meat, while its nutritional value largely depends on the fatty acid composition (Mohanty et al., 2019; Guan et al., 2022). Aquatic products are a major dietary source of unsaturated fatty acids, which play vital roles in antioxidation and reducing the risk of human diseases (Guo and Li, 2011). In the present study, 18 fatty acids were detected in the CT group, compared with only 15 in the JY group, suggesting that pond culture sustains a more diverse fatty acid profile. Further analysis showed that the levels of total saturated fatty acids (∑SFA), total monounsaturated fatty acids (∑MUFA), and total polyunsaturated fatty acids (∑PUFA) were all significantly higher in the CT group than in the JY group ($P < 0.05$). Notably, both ∑PUFA n-3 and ∑PUFA n-6 were markedly elevated in the CT group,

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while the n-3/n-6 ratio showed no significant difference between the two culture systems ($P > 0.05$). These results indicate that pond culture not only increases the overall fatty acid content but also maintains a balanced n-3/n-6 ratio. Previous studies have demonstrated that the fatty acid composition and content of feed directly affect fatty acid deposition in fish tissues (Chen et al., 2011; Niu et al., 2023). Thus, the richer fatty acid composition observed in pond-cultured *S. sinensis* may be attributed to the availability of natural dietary sources such as algae, zooplankton, and benthic organisms. In terms of functional fatty acids, the absolute contents of EPA and DHA, as well as their combined total, were significantly higher in the CT group, indicating that pond culture promotes the deposition of long-chain n-3 polyunsaturated fatty acids (LC-PUFA). EPA and DHA are widely recognized for their beneficial effects on cardiovascular protection, anti-inflammatory activity, and neurological development (Tocher, 2015; Chen et al., 2025; Liu et al., 2025). Algal species such as diatoms, red algae, and brown algae are capable of synthesizing EPA and DHA, while fish primarily accumulate these fatty acids through trophic transfer (Zhuang et al., 2010). Tocher (2015) also emphasized that the deposition of EPA and DHA in fish is strongly influenced by dietary lipid sources, with fish oil or microalgal oil supplementation significantly enhancing muscle EPA and DHA levels (Tocher, 2015). In contrast, cage-in-pond culture relies mainly on formulated feeds, which often use plant oils as the primary lipid source, potentially resulting in insufficient LC-PUFA deposition.

Atherogenic index (IA), thrombogenic index (IT), and hypercholesterolemic index (HI) are commonly used indicators to comprehensively evaluate the potential effects of dietary fatty acid profiles on cardiovascular risks, including atherosclerosis, thrombosis, and hypercholesterolemia (Chen and Liu, 2020). In the present study, IA, IT, and HI values were significantly lower in the CT group than in the JY group.

Analysis of the fatty acid composition revealed that the CT group had significantly higher ∑PUFA levels than the JY group, and these polyunsaturated fatty acids were the primary contributors to the reduction in IA, IT, and HI (Tocher, 2015; Chen and Liu, 2020). Li et al. (2025) reported that higher rearing densities significantly reduced DHA and ∑n-3 PUFAs in the dorsal muscle and C16:1n-9 and ∑n-3 PUFAs in the abdominal muscle of rainbow trout (Li et al., 2025). Therefore, the excessively high rearing density in the JY group (100 fish/m^3) may have been one of the main factors contributing to the elevated IA, IT, and HI values in muscle tissue. In addition, the availability of abundant natural food resources may have also contributed to the significantly lower IA, IT, and HI values in the CT group (Tocher, 2015). Previous studies have indicated that a reduction in dietary n-3 LC-PUFA content is the major cause of decreased n-3 LC-PUFA concentrations in the muscle of farmed fish (Turchini et al., 2009; Li et al., 2025). Thus, under the cage-in-pond culture systems, it is recommended to appropriately increase the inclusion of fish oil rich in PUFAs in the feed and reduce rearing density, in order to improve the fatty acid composition and nutritional value of cultured fish.

Conclusions

In summary, the muscle of *Spinibarbus sinensis* reared under the traditional pond culture system (CT) exhibited significantly higher levels of crude protein, crude lipid, total amino acids, essential amino acids, EPA, DHA, and n-3 PUFAs compared with fish reared in the cage-in-pond system (JY). These differences resulted in lower IA, IT, and HI values and overall superior nutritional quality in the CT group. Conversely, the cage-in-pond system was characterized by relatively higher palmitic acid (C16:0) content and higher stocking density, which may contribute to insufficient deposition of beneficial fatty acids and a slightly inferior flesh quality. Overall, the pond culture system appears more favorable for enhancing the nutritional quality of *S. sinensis*, while the cage-in-pond system offers advantages in environmental sustainability and management convenience. Optimization of stocking density and dietary lipid sources in the cage-in-pond system is expected to further improve its nutritional characteristics.

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