



# International Journal of Multidisciplinary Research and Growth Evaluation.

## Effects of Dietary Supplementation with Lactic Acid Bacteria on Growth Performance, Digestive Enzyme Activity, Histomorphology, Serum Biochemistry, and Antioxidant Capacity of ricefield eel (*Monopterus albus*)

Yumeng Ban <sup>1</sup>, Lei Zheng <sup>2</sup>, Yingbing Su <sup>3\*</sup>, Daiqin Yang <sup>4</sup>

<sup>1-4</sup> College of Animal Science and Technology, Yangtze University, Jingzhou 434025, Hubei Province, China

\* Corresponding Author: Yingbing Su

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### Article Info

**ISSN (Online):** 2582-7138  
**Impact Factor (RSIF):** 8.04  
**Volume:** 07  
**Issue:** 02  
**March-April 2026**  
**Received:** 12-02-2026  
**Accepted:** 10-03-2026  
**Published:** 08-04-2026  
**Page No:** 653-658

### Abstract

This experiment aimed to investigate the effects of dietary supplementation with different concentrations of lactic acid bacteria on the growth performance, digestive enzyme activity, serum biochemical parameters, and antioxidant capacity of ricefield eel *Monopterus albus*. A total of 150 healthy ricefield eel with an initial average weight of (5±0.5) g were selected and randomly divided into 5 groups: the control group (A1) was fed a basal diet, while the experimental groups (A2, A3, A4, A5) were fed basal diets supplemented with 1.0×10<sup>5</sup>, 1.0×10<sup>6</sup>, 1.0×10<sup>7</sup>, and 1.0×10<sup>8</sup> CFU/g of lactic acid bacteria, respectively. The experimental period lasted 6 weeks. The results showed that adding 1.0 × 10<sup>7</sup> CFU/g of lactic acid bacteria to the feed (Group A4) significantly improved the growth performance, intestinal lysozyme activity, intestinal superoxide dismutase (SOD) and catalase (CAT) activity, as well as serum high-density lipoprotein (HDL) levels, while simultaneously significantly reducing serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, as well as malondialdehyde (MDA) content of ricefield eel. Based on a comprehensive analysis of these indicators, the optimal addition level of lactic acid bacteria in the diet is 1.0 × 10<sup>7</sup> CFU/g. This dosage effectively optimizes the growth performance, digestive function, and health status of ricefield eel, providing a theoretical basis for the application of probiotics in ricefield eel farming.

**Keywords:** Ricefield eel, Lactic acid bacteria, Growth performance, Digestive enzymes, Serum biochemistry, Antioxidant capacity

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### 1. Introduction

*Monopterus albus* is an important characteristic freshwater aquaculture species in China, with advantages including tolerance to hypoxia and adaptability to shallow water and swamp environments, and a short culture cycle. It is characterized by tender flesh, high nutritional value and an absence of intermuscular bones, making it valuable for both edible and tonic purposes. Given its favorable market price, this species show great potential for industrial development <sup>[1]</sup>. With the development of intensive aquaculture, it is crucial to explore safe and effective feed additives to improve growth performance and health status. *Lactic acid bacteria* (LAB), as generally recognized probiotics, has received extensive attention in aquaculture due to their beneficial effects, including improving intestinal health, enhancing immunity, promoting growth, and increasing feed utilization efficiency <sup>[2]</sup>. Studies have shown that LAB exert probiotic effects by regulating intestinal microbial balance, enhancing digestive enzyme activity, improving intestinal morphology, increasing antioxidant capacity, and modulating immune responses <sup>[3]</sup>. To date, the application of LAB has been reported in various aquatic animals, including tilapia <sup>[4,5]</sup>, largemouth bass <sup>[6]</sup>, crucian carp <sup>[7]</sup>, and prawn <sup>[8,9]</sup>, with positive effects observed. However, studies on the application of LAB in *Monopterus albus* culture,

especially its systematic effects on growth performance, digestive physiology, tissue health, and antioxidant capacity, still remain scarce.

The present study aimed to systematically evaluate the effects of dietary supplementation with different concentrations of LAB on growth performance, digestive enzyme activities, intestinal and liver histomorphology, serum biochemical indices, and antioxidant capacity of ricefield eel *Monopterus albus*. The optimal supplemental level of LAB in eel feed was determined, so as to provide a theoretical basis for the scientific application of LAB in healthy culture of *Monopterus albus*.

## 2. Materials and Methods

### 2.1. Experimental Materials

**Experimental Materials.** A total of 150 ricefield eel fry, with an initial average weight of (5±0.5) g, free of external injuries, and in good health, were purchased from the local farm in Jianli City, Hubei Province. Prior to the experiment, the fry was temporarily reared in plastic aquaculture tanks and subjected to a 24-hour fasting treatment. The lactic acid bacteria *Lactobacillus* preparation (live bacterial count:  $3.0 \times 10^9$  CFU/mL) was supplied by Hubei Hunan Bio-Technology Co., Ltd. The basal feed (crude protein: 42%) was purchased from Hubei Zhanxiang Agricultural Technology Co., Ltd. Major equipment included a low-temperature tissue homogenizer, a low-temperature high-speed centrifuge, a constant-temperature incubator, a UV spectrophotometer, a fully automatic microplate reader, a water bath, and a microscope. Assay kits for superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lysozyme (LZM), and high-density lipoprotein (HDL) were all purchased from Nanjing Jiancheng Biological Engineering Research Institute.

### 2.2. Experimental Design

The experiment included a control group (A1) fed a basal diet, as well as four treatment groups (A2, A3, A4, and A5) to which *Lactobacillus spp.* were added at concentrations of  $1.0 \times 10^5$ ,  $1.0 \times 10^6$ ,  $1.0 \times 10^7$ , and  $1.0 \times 10^8$  CFU/g, respectively. The *Lactobacillus spp.* solution was uniformly mixed into the feed in a stepwise manner and then stored at 4°C; A total of 150 *Monopterus albus* were randomly divided into 5 groups, with 3 replicates per group and 10 eels per replicate. Each group was housed in a separate plastic tank. The experiment lasted 6 weeks, with feed administered twice daily at fixed times.

### 2.3. Sample Collection and Index Determination

**Growth Performance Assessment:** At the end of the experiment, weigh and count the total number of *Monopterus albus* in each tank, and calculate the survival rate (SR), weight gain rate (WGR), specific growth rate (SGR), and condition factor (CF).

The formulas are as follows:

$SR (\%) = (\text{Number of fish at the end of the experiment} / \text{Initial number of fish}) \times 100;$

$WGR (\%) = [(\text{Final average weight} - \text{Initial average weight}) / \text{Initial average weight}] \times 100;$

$SGR (\%/d) = [(\text{Ln Final average weight} - \text{Ln Initial average weight}) / \text{Number of experimental days}] \times 100;$

$CF (\%) = \text{Final body weight} / \text{Final body length}^3 \times 100.$

After the experiment, 9 *Monopterus albus* were randomly selected from each group, with 3 eels per replicate anesthetized and dissected. The intestines, liver, and blood were quickly collected. The remaining tissues were weighed, mixed with pre-chilled saline at a 1:9 (w/v) ratio, ground at low temperature for 120 s, and centrifuged at 2500 rpm for 10 min at 4°C. The supernatant was collected to measure LZM, TRY, SOD, CAT, MDA, and other parameters; blood was allowed to stand to separate the serum, after which biochemical parameters such as TP, AST, ALT, and HDL were measured. All measurements were performed according to the kit instructions.

### 2.4. Data Statistics and Analysis

The experimental data were preliminarily organized using Excel 2019, statistically analyzed using SPSS 26.0, and plotted using GraphPad Prism 9.0. Results are expressed as “mean ± standard deviation” (Mean ± SD).

## 3. Results

### 3.1. Effects of Lactic Acid Bacteria on Growth Performance of *Monopterus albus*

As shown in Table 1, the addition of lactic acid bacteria to the diet had a significant effect on the growth performance of *Monopterus albus* ( $P < 0.05$ ). Group A4 ( $1.0 \times 10^7$  CFU/g) exhibited significantly higher final body weight, weight gain rate, and specific growth rate than all other groups ( $P < 0.05$ ), with a 22.8% increase in weight gain rate and a 7.9% increase in specific growth rate compared to group A1. Survival rates were 100% across all groups, with no significant differences ( $P > 0.05$ ). The condition factor (CF) of Group A4 was also significantly higher than that of group A1 ( $P < 0.05$ ). These results indicate that dietary supplementation with  $1.0 \times 10^7$  CFU/g LAB most effectively promotes the growth of ricefield eel *Monopterus albus*.

**Table 1:** Effects of lactic acid bacteria on growth performance of *Monopterus albus*

Items	groups				
	A1	A2	A3	A4	A5
IBW/g	5.26±0.03c	5.15±0.03a	5.24±0.02c	5.17±0.02ab	5.23±0.04bc
FBW/g	18.76±0.03a	18.93±0.03b	20.35±0.02d	21.75±0.03e	19.77±0.02c
SR/%	100.00±0.00d	100.00±0.00d	100.00±0.00d	100.00±0.00d	100.00±0.00d
WGR/%	259.18±1.16a	264.49±0.58b	289.96±0.11d	318.23±0.88e	279.08±1.01c
SGR(%/d)	6.2±0.01a	6.25±0.01b	6.47±0.01d	6.69±0.01e	6.37±0.01c

**Note:** Values in the same row with different lowercase superscripts indicate significant difference ( $P < 0.05$ ), while the same or no superscripts indicate no significant difference ( $P > 0.05$ ). The same applies to the following figures.

### 3.2. Effects of LAB on digestive enzyme activities in liver and intestine of *Monopterus albus*

As shown in Figure 1, differences in lactic acid bacteria *Lactobacillus* levels in the feed resulted in significant differences ( $P < 0.05$ ) in lysozyme activity in the livers and intestines of *Monopterus albus*. Regarding liver lysozyme activity, Group A5 had the highest measured value. Although no statistically significant difference was observed between this group and Group A4 ( $P > 0.05$ ), it was significantly higher than that of groups A1, A2, and A3 ( $P < 0.05$ ). Furthermore, groups A1, A2, A3, and A5 showed significant differences among these groups ( $P < 0.05$ ). Regarding intestinal lysozyme activity, Group A4 was significantly

higher than groups A1, A2, A3, and A5 ( $P < 0.05$ ), and significant differences were observed among all experimental groups ( $P < 0.05$ ). As shown in Figure 2, the level of LAB in the diet had a significant effect on triglyceride content in the intestines and liver of ricefield eel ( $P < 0.05$ ). In addition, intestinal triglyceride levels were significantly higher in group A1 than in group A4 ( $P < 0.05$ ) and group A1 differed significantly from all other groups ( $P < 0.05$ ). Hepatic triglyceride content was highest in Group A3, which showed no significant differences from groups A2, A4, and A5 ( $P > 0.05$ ), but was significantly higher than that of group A1 ( $P < 0.05$ ). Furthermore, there were differences in triglyceride content between the intestine and liver within the same group.

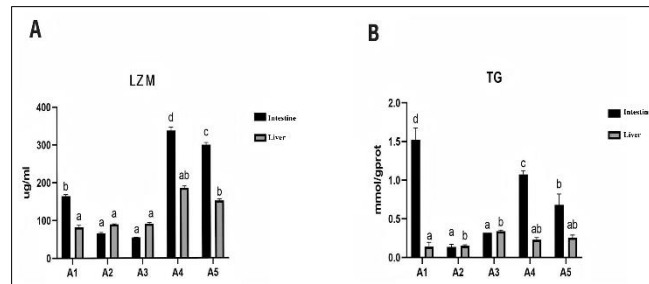


Fig 1: Effects of LAB on digestive enzyme activities in liver and intestine of *Monopterus albus*.

### 3.3. Effects of LAB on serum biochemical indices of *Monopterus albus*

As shown in Figure 2, regarding the effects of LAB on serum biochemical indices of *Monopterus albus*, ALT activity in groups A1 and A2 was significantly higher than that in groups A3 and A4, and group A5 was intermediate between groups A2, A3 and A4. AST activity was highest in group

A1, followed by group A2, and both were significantly higher than those in groups A3, A4 and A5 ( $P < 0.05$ ). For HDL content, group A3 was the highest and significantly higher than groups A2 and A5 ( $P < 0.05$ ), while groups A2 and A5 were significantly higher than groups A1 and A4 ( $P < 0.05$ ). TP content showed no significant difference among all groups ( $P > 0.05$ ).

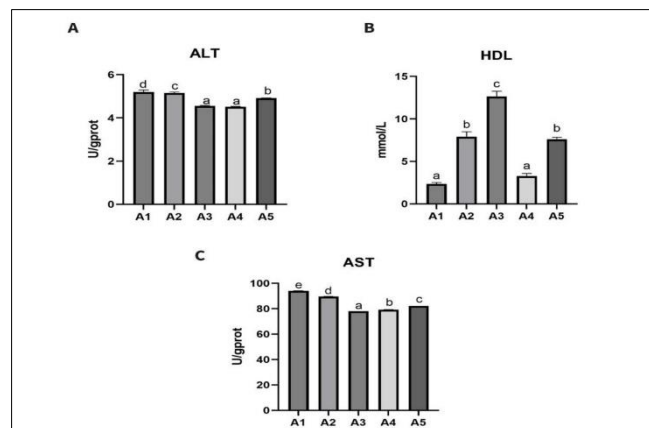


Fig 2: Effects of LAB on serum biochemical indices of *Monopterus albus*

### 3.4. Effects of LAB on antioxidant capacity of *Monopterus albus*

As shown in Figure 5, SOD activity in the intestine was significantly higher in groups A1, A3, and A4 than in group A5 ( $P < 0.05$ ), and group A5 showed higher activity than group A2 ( $P < 0.05$ ); liver SOD activity was lowest in the A1 group, while the A2 and A4 groups were similar and showed significant differences from the other three groups ( $P < 0.05$ ). Additionally, CAT activity in the intestine was highest in the A4 group, followed by group A5, and both were significantly higher than those in groups A1, A2, and A3 ( $P < 0.05$ ). In liver, group A4 had the lowest activity, while groups A1, A2,

and A3 were comparable and higher than group A4 ( $P < 0.05$ ), and group A5 was higher than groups A1, A2, and A3 ( $P < 0.05$ ) (Figure 6). As shown in Figure 7, the MDA content in the intestine was highest in group A2, followed by group A4, with both significantly higher than groups A1, A3, and A5 ( $P < 0.05$ ), while groups A1 and A3 were comparable and higher than group A5 ( $P < 0.05$ ); in the liver, group A3 had the highest MDA content, significantly higher than all other groups except group A4 ( $P < 0.05$ ); group A4 was higher than groups A1, A2, and A5 ( $P < 0.05$ ); group A2 was higher than groups A1 and A5 ( $P < 0.05$ ); and group A1 was significantly higher than group A5 ( $P < 0.05$ ).

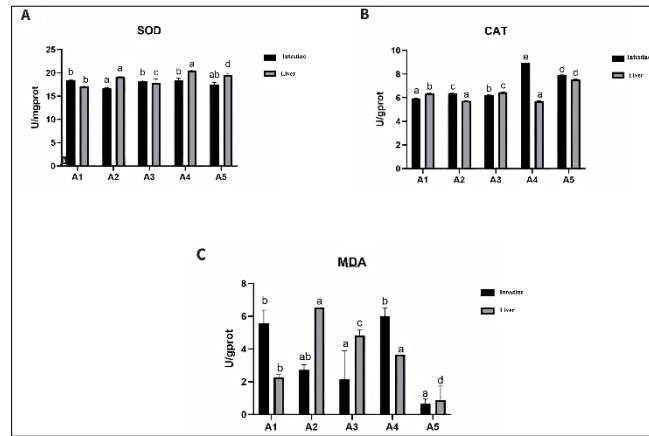


Fig 3: Effects of LAB on antioxidant capacity in liver and intestine of *Monopterus albus*

## 4. Discussion

### 4.1. Effects of LAB on Growth Performance of *Monopterus albus*

Microbial preparations are live bacterial feed additives that can enhance immune function<sup>[10]</sup>, improve gut microbiota<sup>[11]</sup>, and increase survival rates<sup>[12]</sup> and growth performance<sup>[13]</sup>. Previous studies have demonstrated that *Monocytogenes titanica* significantly improves growth performance of juvenile turbot, whereas *Halophilus halophilus* markedly enhances immune function and the expression of related immune genes in this species<sup>[14]</sup>. Similar observations have been reported that *Clostridium butyricum* can improve the growth performance of juvenile flathead flounder, enhance their intestinal structure, regulate the composition of the gut microbiota, and promote intestinal health<sup>[15]</sup>. Jiang *et al.*(2023) indicated that *Clostridium butyricum* can improve the intestinal tissue morphology and digestive absorption function of hybrid grouper, enhance intestinal antioxidant capacity, increase the relative abundance of probiotics, strengthen the effective utilization of carbon sources by the intestinal microbiota, and improve intestinal immune balance, thereby playing a positive role in the intestinal health of hybrid grouper<sup>[16]</sup>. The present study indicated that dietary supplementation with lactic acid bacteria *Lactobacillus* effectively promoted the growth in ricefield eel, which was consistent with previous reports. Using weight gain rate and specific growth rate as evaluation indices, the optimal supplemental level of LAB in ricefield eel feed was determined to be  $1.0 \times 10^7$  CFU/g.

### 4.2. Effects of LAB on digestive enzyme activities in the liver and intestine of *Monopterus albus*

Digestive enzymes activity in organisms serves as an important indicator of digestive capacity. Lysozyme is a key innate enzyme involved in host defense, while triglycerides represent a lipid rather than an enzyme. Investigating these five components can provide further insight into the mechanism by which lactic acid bacteria promote growth in ricefield eel. In this experiment, the levels of triglycerides and lysozyme activity in the ricefield eel intestine, as well as the content of triglycerides and lysozyme in the liver, were significantly affected by the LAB level in the diet ( $P < 0.05$ ). The present study indicates that dietary supplementation with *Lactobacillus* significantly increased lysozyme activity and triglyceride levels in the livers and intestines of ricefield eel, suggesting that *Lactobacillus* promotes the secretion of these two substances (lysozyme and triglycerides) in the liver and

intestine. These findings are consistent with results from studies conducted in largemouth bass *Micropterus salmoides*<sup>[17]</sup>, common carp *Cyprinus carpio*<sup>[18]</sup>, and Pacific white shrimp *Litopenaeus vannamei*<sup>[19]</sup>.

### 4.3. Effects of LAB on serum biochemical indices of *Monopterus albus*

Serum biochemical indices in fish can reflect their nutritional status and are widely used to evaluate metabolic level and physiological health status<sup>[20,21]</sup>. Blood serves as the main site for nutrient exchange. When the organism is affected by external factors, hematological indicators change accordingly, which can effectively reflect physiological alterations in the body<sup>[22]</sup>. The activities of ALT, HDL, and AST can indirectly reflect hepatocyte damage<sup>[23,24]</sup>. Under normal conditions, fish serum exhibits low ALT and AST activities and high HDL activity. When liver tissue is damaged or cell permeability increases, serum ALT and AST levels rise while HDL decreases. The present study showed that lactic acid bacteria selectively improved serum biochemical indices in *Monopterus albus*, significantly reducing ALT and AST and increasing HDL, indicating a protective effect on hepatocyte integrity, regulation of lipid metabolism, and reduction of abnormal cholesterol deposition.

### 4.4. Effects of LAB on antioxidant capacity of *Monopterus albus*

Oxidative stress is one of the main causes of disease in aquatic animals. SOD, CAT and MDA are key antioxidant indices in the body, and their levels can directly reflect the antioxidant status<sup>[25,26]</sup>. Glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and other indices reflect antioxidant status. Once the animal body is injured, an imbalance between oxidation and antioxidation occurs, accompanied by excessive MDA production. Thus, MDA content indicates the degree of cellular oxidative damage<sup>[27]</sup>. Guan *et al.*<sup>[28]</sup> reported that lactic acid bacteria preparations could improve growth performance and intestinal enzyme activities, and enhance body composition in common carp, with the optimal supplemental level being 0.4%. Meanwhile, SYNSEA probiotics have the potential to improve growth performance and disease resistance in white shrimp by regulating immune responses and immune-related gene expression, as well as modulating intestinal microbiota<sup>[29]</sup>. The results of the present experiment showed that dietary supplementation with LAB significantly increased

antioxidant enzyme activities and decreased lipid peroxidation levels in the intestine and liver of *Monopterus albus*. The differential effects among treatments indicated that LAB have the potential to improve antioxidant capacity in *Monopterus albus*. By enhancing antioxidant enzyme activities and elevating oxygen free radical scavenging capacity, LAB alleviate oxidative damage and inhibit lipid peroxidation, thereby protecting the structural and functional integrity of cell membranes.

## 5. Conclusion

This study systematically investigated the effects of dietary supplementation with different concentrations of *Lactobacillus* on growth performance, digestive enzyme activities, serum biochemical indices, and antioxidant capacity of ricefield eel (*Monopterus albus*). The results showed that dietary supplementation with *Lactobacillus* significantly improved growth performance, enhanced the activities of SOD and CAT, boosted immune function as indicated by lysozyme activity, and ameliorated intestinal tissue structure. Based on the overall results, the optimal dietary supplementation level of *Lactobacillus* for ricefield eel was  $1.0 \times 10^7$  CFU/g.

## Declaration of Competing Interest

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

## Acknowledgment

This work was supported by Jingzhou Science and Technology Plan Project 2025 (No.2025EB21); Hubei Science and Technology Plan Project 2024 (No.2024EBA028); and 2025 Foreign Talent Introduction Project of Hubei Provincial Department of Science and Technology (No.2025DJC018).

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#### How to Cite This Article

Ban Y, Zheng L, Su YB, Yang D. Effects of dietary supplementation with lactic acid bacteria on growth performance, digestive enzyme activity, histomorphology, serum biochemistry, and antioxidant capacity of ricefield eel (*Monopterus albus*). *International Journal of Multidisciplinary Research and Growth Evaluation.* 2026;7(2):653-658.

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