

Article

Enhancing the GABA Content and Sleep-Promoting Potential of the Baihe Dihuang Decoction Through Lactic Acid Bacteria Fermentation

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Abstract

γ -aminobutyric acid (GABA) is crucial in neural inhibition and sleep regulation. This study screened lactic acid bacteria isolated from breast milk and infant fecal samples for their GABA-producing ability; their acid tolerance, bile salt resistance, and growth performance were evaluated. Based on TLC-HPLC analysis, *Lactobacillus gasseri* F002, *Lactiplantibacillus plantarum* R7, and *Lacticaseibacillus rhamnosus* B2-1 were identified as promising GABA-producing strains. *L. gasseri* F002 was selected for liquid-state fermentation of the Baihe Dihuang decoction. During fermentation, *L. gasseri* F002 utilized carbohydrates in the decoction matrix, increased GABA accumulation, with a peak of 0.063 mg/mL at 24 h, and reduced total saponin content, suggesting that lactic acid bacterial fermentation induced compositional shifts of major functional constituents in the Baihe Dihuang decoction. The sleep-promoting effect of the fermented decoction was assessed using a caffeine-induced zebrafish insomnia model. The fermented Baihe Dihuang decoction significantly prolonged sleep bout duration, while reducing wakefulness and total locomotor activity. Moreover, correlative changes altered sleep-related molecular and biochemical indicators in the zebrafish model. Correlative changes in the expression of *gabra1*, *htr1aa*, *drd2a*, *dbh*, and *th*, as well as in GABA, melatonin (MT), and monoamine oxidase (MAO) levels, suggest a potential association with the observed sleep-improving phenotypes. Therefore, fermentation with GABA-producing lactic acid bacteria may enhance the sleep-promoting potential of the Baihe Dihuang decoction and provide preliminary experimental support for the development of fermented medicinal–food homologous products aimed at improving sleep.

Keywords: γ -aminobutyric acid; lactic acid bacteria; *Lactobacillus gasseri*; Baihe Dihuang decoction; fermentation; sleep regulation; zebrafish



Academic Editors: Talha Demirci and Enes Dertli

Received: 18 May 2026

Revised: 25 June 2026

Accepted: 30 June 2026

Published: 1 July 2026

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1. Introduction

γ -aminobutyric acid (GABA) is a key inhibitory neurotransmitter in the mammalian brain, contributing to the regulation of sleep, emotion, stress adaptation, and neuronal activ-

ity [1]. GABA production by food-grade microorganisms has emerged as a significant area of research within the fields of functional foods and microecological products. Early studies mainly isolated GABA-producing lactic acid bacteria from fermented foods, dairy products, and intestinal samples, and improved yields by optimizing culture conditions [2–4]. In recent years, research has gradually expanded beyond yield improvement to include strain safety, probiotic properties, compatibility with fermented food matrices, and the evaluation of neuroregulatory functions. Lactic acid bacteria can enrich GABA in fermented dairy products through the glutamate decarboxylase (GAD) pathway and may, therefore, confer potential health benefits, such as anxiolytic, antihypertensive, and sleep-improving effects [5]. After culture conditions were optimized, Matta et al. reported GABA production of 19.67 ± 0.28 g/L by *Levilactobacillus brevis* LAB6 and 20.77 ± 0.14 g/L by *Limosilactobacillus fermentum* LAB19 [6]. Bai et al. reported that dairy-derived *Lactiplantibacillus plantarum* SY1 could produce GABA and exhibited acid tolerance, bile salt resistance, antioxidant activity, and neuromodulatory potential [7]. Clinical evidence from a randomized, double-blind, placebo-controlled trial showed that six weeks of daily *Lactiplantibacillus plantarum* Lp815 supplementation at 5×10^9 CFU alleviated insomnia symptoms, anxiety scores, and objective sleep duration, while increasing urinary GABA levels, suggesting a verifiable association between GABA-producing probiotics and sleep regulation [8].

The *L. brevis* strains LBG-29, LBG-24, and LBD-14, which are isolated from lychee juice, have been reported to produce GABA at concentrations greater than 300 mg/L [9]. Moreover, lactic acid bacteria-mediated fermentation has recently gained attention for processing Chinese medicinal materials for both therapeutic and dietary purposes. Lactic acid bacterial fermentation can improve the release and utilization of functionally active components in medicinal–food homologous substrates through cell-wall disruption, enzymatic transformation, and the structural modification of bioactive compounds; therefore, antioxidant, hypoglycemic, and anti-inflammatory bioactivities may be enhanced [10]. These findings highlight the potential of high GABA-producing lactic acid bacteria in functional fermented foods and medicinal–food homologous products.

The Baihe Dihuang decoction is a classic traditional Chinese medicine formula composed of Baihe and Sheng Dihuang, traditionally used to nourish yin, clear heat, and calm the spirit for relieving restlessness, palpitations, and insomnia caused by yin deficiency and internal heat [11]. Its major active constituents include polysaccharides and saponins, which have been reported to exert sedative, antidepressant, and neuroprotective effects, supporting its potential application in the intervention of sleep disorders, as well as in the development of medicinal–food homologous functional products [12]. The standard preparation of the Baihe Dihuang decoction uses a 1:1 ratio of *Lilii Bulbus* and *Radix Rehmanniae* Recens. This ratio is based on the original prescription from the *Synopsis of Prescriptions of the Golden Chamber* and has been validated in numerous modern pharmacological studies. Insomnia is closely related to disturbances in the sleep–wake rhythm, abnormal neurotransmitter metabolism, and increased central nervous system excitability [13]. As the main inhibitory neurotransmitter in the central nervous system, γ -aminobutyric acid (GABA) exerts sedative and hypnotic effects by suppressing neuronal firing, and impaired GABAergic signaling is a core pathological hallmark of insomnia [14]. Beyond the GABAergic system, serotonin (5-HT) and dopamine are pivotal monoamine neurotransmitters that modulate sleep–wake transitions. 5-HT plays a dual regulatory role: it acts as the biosynthetic precursor of melatonin to promote sleep initiation, while its receptor subtypes mediate distinct physiological functions. Specifically, the 5-HT_{1A} receptor (encoded by *htr1aa*) primarily transduces inhibitory signals with anxiolytic and sedative effects, whereas the 5-HT_{2A} receptor (encoded by *htr2aa*) participates in excitatory neurotransmission and sustains wakefulness [15,16]. Dopamine is a key excitatory neurotransmitter that drives arousal

and locomotor hyperactivity. Tyrosine hydroxylase (encoded by *th*) is the rate-limiting enzyme in dopamine biosynthesis, and dopamine β -hydroxylase (encoded by *dbh*) further catalyzes the conversion of dopamine to norepinephrine [17]. The dopamine D2 receptor (encoded by *drd2a*) functions as a presynaptic autoreceptor exerting negative feedback on dopamine release, and its downregulation is associated with heightened wakefulness [18]. Melatonin (MT), a critical circadian hormone synthesized from 5-HT, coordinates the sleep–wake cycle and preserves circadian stability [19]. Monoamine oxidase (MAO) is the principal rate-limiting enzyme mediating the oxidative degradation of monoamine neurotransmitters, including dopamine and 5-HT; aberrant upregulation of MAO activity accelerates monoamine depletion, triggering neurotransmitter imbalance and insomnia-like phenotypes [20]. Accordingly, evaluating the sleep-promoting effects of the Baihe Dihuang decoction and its fermented products by integrating behavioral observations, sleep-related gene expression, and key biochemical factors can provide experimental evidence for its sleep-improving function.

Zebrafish larvae are characterized by short developmental cycles, high fecundity, transparent bodies, low maintenance costs, convenient drug administration, and sensitive behavioral responses. Their circadian rhythm and sleep-like behaviors are relatively stable, making them an important experimental model for constructing sleep-disorder models and screening functional bioactive substances [21]. Compared with traditional mammalian models, zebrafish models allow multi-dose and multi-index observations to be completed within a relatively short period, facilitating the integrated evaluation of behavioral, molecular, and biochemical indicators [22]. Caffeine enhances wakefulness and induces increased locomotor activity and abnormal sleep bouts in zebrafish and is, therefore, commonly used to establish zebrafish insomnia-like models [23].

Notably, the research framework combining LAB strain screening, herbal fermentation, and multi-index efficacy evaluation in zebrafish has been well established in the functional food field. However, no systematic study has yet characterized the impact of lactic acid bacterial fermentation on the sleep-related bioactivity of the Baihe Dihuang decoction, and most existing GABA-producing LAB isolates applied in herbal fermentation are derived from fermented foods rather than human sources with higher biosafety potential.

Accordingly, this study selected lactic acid bacteria isolated from human breast milk and infant feces as screening targets and identified high GABA-producing strains using TLC combined with HPLC. Growth performance, acid tolerance, and bile salt tolerance were evaluated to select the optimal fermentation strain. This study further characterized dynamic changes in the main functional constituents of the Baihe Dihuang decoction during lactic acid bacterial fermentation. Finally, a caffeine-induced zebrafish sleep disorder model was applied to compare the sleep-regulating effects of the Baihe Dihuang decoction before and after fermentation, integrating behavioral phenotypes, sleep-related gene expression, and key biochemical indicators, including GABA, MT, and MAO. This work provides incremental experimental data for the fermentation-based modification of medicinal–food homologous materials and lays a preliminary foundation for subsequent in-depth mechanistic research.

2. Materials and Methods

2.1. Materials and Reagents

Powdered *Lilii Bulbus* and *Rehmanniae Radix Recens* were supplied by Beijing Tong Ren Tang Co., Ltd. (Beijing, China). The medicinal–food homologous raw materials met enterprise quality standards and were stored under cool, dry conditions (15–20 °C and 45–75% relative humidity). De Man–Rogosa–Sharpe (MRS) broth and agar were obtained from Beijing Land Bridge Technology Co., Ltd. (Beijing, China). The GABA standard,

L-monosodium glutamate, porcine bile salts, and caffeine were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Hydrochloric acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol was obtained from Merck (Darmstadt, Germany); acetonitrile was obtained from Shanghai Xingke (Shanghai, China); and formic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). Total sugar assay kits were obtained from Beijing Hezi Biotechnology Co., Ltd. (Beijing, China); total saponin assay kits were obtained from Hubei Pumei Biotechnology Co., Ltd. (Hubei, China); and ELISA kits for GABA, MT, and MAO were obtained from Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China).

2.2. Experimental Bacterial Strains

A total of 10 lactic acid bacteria strains were used in this study, which were isolated from two healthy human sources: infant feces and human breast milk. These sources were selected because lactic acid bacteria derived from the human gastrointestinal tract and breast milk generally exhibit excellent probiotic properties, safety, and adaptability to the human body.

The strains were preserved at $-80\text{ }^{\circ}\text{C}$ in MRS broth supplemented with 20% (*v/v*) sterile glycerol for long-term storage. Before each experiment, the strains were activated by streaking on MRS agar plates and incubated anaerobically at $37\text{ }^{\circ}\text{C}$ for 24 h. Single colonies were then inoculated into 10 mL of sterile MRS broth and incubated at $37\text{ }^{\circ}\text{C}$ for 18 h to prepare seed cultures. Detailed information on the bacterial strains and their origins is provided in Table 1.

Table 1. Bacterial strains and their origins.

Strain No.	Species	Source	Preservation Institution
SYB02M21	<i>Limosilactobacillus fermentum</i>	Infant feces	Sanyuan Foods, Beijing, China
R7	<i>Lactiplantibacillus plantarum</i>	Human breast milk	Sanyuan Foods, Beijing, China
R8	<i>Lactiplantibacillus plantarum</i>	Human breast milk	Sanyuan Foods, Beijing, China
R11	<i>Lactiplantibacillus plantarum</i>	Human breast milk	CGMCC, Beijing, China
F002	<i>Lactobacillus gasseri</i>	Infant feces	Sanyuan Foods, Beijing, China
F003	<i>Lactobacillus gasseri</i>	Infant feces	Sanyuan Foods, Beijing, China
F024	<i>Lactobacillus gasseri</i>	Infant feces	Sanyuan Foods, Beijing, China
B2-1	<i>Lacticaseibacillus rhamnosus</i>	Human breast milk	CGMCC, Beijing, China
B1-27	<i>Limosilactobacillus reuteri</i>	Human breast milk	CGMCC, Beijing, China
B1-29	<i>Lactobacillus gasseri</i>	Human breast milk	Sanyuan Foods, Beijing, China

2.3. Preparation of De Man–Rogosa–Sharpe (MRS) Broth

Bacterial strains were propagated in commercially available MRS broth (Beijing Luqiao Technology Co., Ltd., Beijing, China, Cat. No. CM187). Following the manufacturer's

protocol, 55.2 g of dehydrated MRS powder was suspended in 1 L of deionized water and heated with gentle stirring until fully dissolved. The medium pH was pre-calibrated by the supplier to 6.2 ± 0.2 post-sterilization, eliminating the need for further adjustment. Aliquots of 20 mL were transferred into 50 mL glass Erlenmeyer flasks and sterilized by autoclaving at $121\text{ }^{\circ}\text{C}$ for 15 min. Once cooled to ambient temperature, the sterile broth was stored in the dark at $4\text{ }^{\circ}\text{C}$ until use. All MRS broth utilized in this investigation was formulated solely for laboratory animal dosing and was never designed for human ingestion.

2.4. Screening of High GABA-Producing Lactic Acid Bacteria by TLC and HPLC

Activated strains were added to 1% L-monosodium glutamate-containing medium at a 2% (*v/v*) inoculation level. Shaking culture was performed at $37\text{ }^{\circ}\text{C}$ and 180 rpm for 48 h. After incubation, the fermentation broth was centrifuged at 6000 rpm for 15 min at $4\text{ }^{\circ}\text{C}$, and the resulting supernatant was used for TLC analysis. To verify the presence of γ -aminobutyric acid (GABA), culture supernatants were obtained by centrifugation under conditions of $8000\times g$ at $4\text{ }^{\circ}\text{C}$ for 5 min. Two microliters of each filtered supernatant were applied to silica gel 60 F₂₅₄ TLC plates (Merck, Darmstadt, Germany; Cat. No. 1.05554). The chromatographic run was carried out with a solvent system of n-butanol:acetic acid:water (5:2:2, *v/v/v*). For visualization, the plates were sprayed with 2% ninhydrin solution and heated at $105\text{ }^{\circ}\text{C}$ for 5 min, following the method from Edalatian Dovom et al. [24].

An aliquot of fermentation broth was diluted in an ethanol–water–triethylamine mixture, followed by the addition of ethanol–water–triethylamine–phenyl isothiocyanate (PITC) derivatization reagent. The reaction mixture was heated in a boiling water bath at $100\text{ }^{\circ}\text{C}$ for 10 min, then removed and allowed to cool naturally to room temperature, where it was incubated for an additional 20 min to complete the derivatization process. After filtration through a $0.22\text{ }\mu\text{m}$ organic membrane filter, the samples were analyzed using a high-performance liquid chromatography (HPLC) system (Alliance e2695, Waters, Milford, MA, USA) equipped with a Hypersil ODS C18 column. The γ -aminobutyric acid (GABA) content in the samples was quantified via an external standard method, with calculations based on a calibration curve generated using authentic GABA reference standards [25].

2.5. Determination of Growth Curves and Probiotic Properties

2.5.1. Growth Curve Determination

Three-generation activated lactic acid bacteria were adjusted to 10^8 CFU/mL and added to MRS broth at 2% (*v/v*), then incubated at $37\text{ }^{\circ}\text{C}$. OD₆₀₀ values were measured every 2 h over 24 h to monitor strain growth under shaking culture conditions.

2.5.2. Determination of Acid Tolerance

Acid tolerance was determined by measuring viable colony counts of lactic acid bacteria under low-pH conditions [26]. The bacterial suspension was diluted to 10^8 CFU/mL and centrifuged. The cells were then resuspended in media adjusted to pH 1.5, 2.0, and 3.0, or in the original medium as the control. Samples were collected after 1 h and 3 h of incubation, and viable counts were recorded. The survival rate was calculated according to the following formula:

$$\text{Survival rate (\%)} = N_0/N_1 \times 100$$

where N1 represents the viable cell count in the control group (CFU/mL), and N0 represents the viable cell count in the experimental group after 1 h or 3 h of treatment (CFU/mL).

2.5.3. Determination of Bile Salt Tolerance

Bile salt resistance was tested in sterile MRS broth containing 0.3% porcine bile salts [27]. The bacterial suspension was diluted to 10^8 CFU/mL, centrifuged, and re-

suspended in sterile MRS broth with or without 0.3% bile salts. Samples were collected at 0, 1, and 3 h, and viable counts were recorded. The control group consisted of viable bacteria in medium without bile salts; survival was calculated using the same equation.

2.6. Liquid-State Fermentation of Baihe Dihuang Decoction

The Baihe Dihuang decoction (BDD) is a traditional Chinese herbal preparation made by boiling *Lilii Bulbus* and *Rehmanniae Radix Recens* in water to extract active ingredients. Following the approach described by Miao et al. [28], *Lilii Bulbus* powder and *Rehmanniae Radix Recens* powder mixed at a ratio of 1:1. In brief, the Baihe Dihuang culture medium was prepared by placing 0.5 g each of *Lilii Bulbus* powder and *Rehmanniae Radix Recens* powder into an Erlenmeyer flask, after which 20 mL of MRS medium was added. Subsequently, a 2% inoculum of *L. gasseri* F002 was added, and the culture was maintained at 37 °C, with shaking at 180 rpm for 72 h. The blank control consisted of the Baihe Dihuang culture medium without *L. gasseri* F002 inoculation.

2.7. Determination of Total Sugar, GABA, and Total Saponins During Fermentation

The levels of total sugars, GABA, and total saponins were measured using commercial detection kits according to the protocols provided by the manufacturers.

For total sugar determination, 100 µL of appropriately prepared sample was added to each sample well of a microplate, with three replicate wells per sample. Samples were diluted or pretreated according to the kit instructions to ensure that the measured values fell within the standard curve range. A series of standards ranging from 0 to 100 µg/mL was prepared and loaded into the corresponding wells. The reaction reagent was then introduced, and the plate was incubated for 30 min at 37 °C. The colorimetric solution was subsequently added, and the reaction was carried out in the dark at room temperature for 10 min. Once the reaction was stopped, the absorbance at 490 nm was recorded with a microplate reader, and total sugar levels were quantified based on the calibration curve.

For GABA determination, standards and samples were added to the microplate according to the kit instructions. The plate was first incubated at room temperature for 60 min, then washed five times. Next, the HRP-labeled secondary antibody was applied for 30 min, followed by another five washing cycles. After 15 min of TMB-based color development, the reaction was stopped, and absorbance was read at 450 nm. The GABA concentration was then calculated from the calibration curve.

For saponin quantification, 100 µL of each sample was loaded into the microplate and mixed with the prepared reaction reagent. The microplate was kept at 70 °C for 40 min, followed by a 10 min color development step. After the reaction was stopped, the absorbance was measured at 475 nm, and total saponin levels were derived from the standard curve.

2.8. Animal Experiments

2.8.1. Experimental Animals

The experimental animals were wild-type AB zebrafish. The rearing temperature was maintained at approximately 28 °C, under a 14:10 h light–dark cycle, with the light phase from 09:00 to 23:00 and the dark phase from 23:00 to 09:00 the next day to simulate the natural circadian rhythm. Adult zebrafish were fed brine shrimp thrice daily at fixed intervals. Embryos were obtained by placing male and female zebrafish in divided mating tanks overnight at a 1:1 ratio. The next morning, after the light was turned on, the divider was removed, and the zebrafish spawned naturally. Fertilized embryos were collected approximately 60 min later and transferred to 10 cm culture dishes containing 25 mL of 1×10^3 medium supplemented with 0.3 ppm methylene blue (NaCl, KCl, CaCl₂, and MgSO₄). No more than 100 embryos were placed in each dish. The dishes were kept

at 28.5 °C under a 14:10 h light–dark cycle in an artificial climate incubator. Normally developed larvae at 5 days post-fertilization (5 dpf) were selected under a stereomicroscope for subsequent experiments.

2.8.2. Generation of the Zebrafish Insomnia Model

The animal experiments complied with welfare and ethical standards, as well as the 3R principles, under approval no. SR20260105. Zebrafish larvae at 5 dpf were randomly divided into the control group (Ctrl), the model group (Model), the positive drug group (GABA), the non-fermented Baihe Dihuang decoction group (BDNF), and the fermented Baihe Dihuang decoction group (BDF). Each group contained three replicates, with 10 larvae per replicate. The control group of zebrafish was treated with E3 medium. The model group was treated with 200 µmol/L caffeine for 24 h to establish a zebrafish insomnia model. The GABA group received 50 µmol/L GABA in addition to the treatment given to the model group. The BDNF group received the unfermented Baihe Dihuang decoction in addition to the treatment given to the model group. The BDF group received the fermented Baihe Dihuang decoction in addition to the treatment given to the model group.

2.8.3. Evaluation of the Maximum Tolerated Concentration in Zebrafish Larvae

After model induction, the BDNF and BDF groups received their respective samples at 1, 10, 50, 100, or 1000 µg/mL for 24 h. Mortality, heart rate, and malformation rate were then recorded, and representative malformations were photographed. The maximum tolerated concentration (MTC) was defined as the highest dose producing no mortality or deformities. The subsequent experimental concentrations were set at MTC, 1/2 MTC, and 1/4 MTC, corresponding to 1000, 500, and 250 µg/mL, respectively.

2.8.4. Drug Intervention and Sleep Behavioral Assay in Zebrafish Insomnia Model

The 5 dpf model larvae were exposed to 200 µmol/L caffeine for 24 h to induce insomnia-like symptoms. The zebrafish in the positive drug group (GABA) were co-treated with 50 µmol/L GABA on the basis of model induction [29]. The BDNF1 group (250 µg/mL), BDNF2 group (500 µg/mL), BDNF3 group (1000 µg/mL), BDF1 group (250 µg/mL), BDF2 group (500 µg/mL), and BDF3 group (1000 µg/mL) were co-treated with the corresponding concentrations for 24 h after model induction. Each group contained eight larvae, and a total of nine groups were included. At 6 dpf, behavioral changes were recorded for 1 h. The immobility time per minute was analyzed; when the immobility duration was greater than or equal to 60 s, the zebrafish was considered to be in a sleep period. Sleep parameters, including bout frequency, bout length, and latency to sleep onset, were recorded.

2.8.5. Detection of Sleep-Related Gene Expression in Zebrafish

At 6 dpf, larvae from each group (20 larvae per replicate) were harvested and rinsed three times with fresh embryo medium to remove residual treatment solution. The total RNA was extracted using the TRIzol reagent (Muri, Suzhou, China; Cat. No. MR208-01), according to the manufacturer's protocol. The concentration and purity of the total RNA were verified by spectrophotometry, and 1 µg of the total RNA was reverse-transcribed into cDNA, using a reverse transcription kit (Lanjieke, Beijing, China; Cat. No. BL696A) China; Cat. No. BL696A). Quantitative real-time PCR (qRT-PCR) was performed in technical triplicate, using the SYBR Green qPCR Master Mix (Adlai, Beijing, China; Cat. No. PC6201) on an ABI 7500 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The amplification procedure was set as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s. The *β-actin* gene was used as the endogenous reference for normalization, and

the relative expression level of each target gene was calculated using the $2^{-\Delta\Delta C_t}$ method. The expression levels of *gabra1*, *htr1aa*, *htr2aa*, *th*, *drd2a*, and *dbh* were then detected. The sequences of all primers used in this study are listed in Table 2.

Table 2. Primer sequences used for quantitative real-time PCR.

Gene Name	Primer Sequence (5' → 3')	Amplicon Length (bp)
<i>gabra1</i>	F: TCCGGGTGAGTCAGAGACAA R: ACTTCGGTTACACGCTCTCC	234
<i>htr1aa</i>	F: CTACTCAACTTTTCGGGGCGT R: CACCGCCAAGCATTATCCG	145
<i>htr2aa</i>	F: ACAGAACTGCTGTCATCGCA R: GGGAAAGGCCATGAGTAACCATA	182
<i>th</i>	F: TGGAGAGCTGGTGCATTCTC R: GAACCGCACAGAAAACGGTC	218
<i>drd2a</i>	F: TGTGATTGCGAATCCTGCCT R: CGGGATGGGTGCATTCTTT	173
<i>dbh</i>	F: GTGTTCAATGCTCCCCTCA R: AGTCCCTCCGCCCTGATAAA	241

2.8.6. Determination of Key Sleep-Related Factors in Zebrafish

At 6 dpf, zebrafish larvae were collected and homogenized. ELISA kits were used to detect the contents of GABA, MT, and MAO.

2.9. Software and Data Processing

The results are shown as the mean $\pm$ standard deviation of triplicate measurements. GraphPad Prism 8.0.2 was applied for data analysis and statistics.

Statistical significance was defined as $p \leq 0.05$, with stronger significance indicated at $p < 0.01$ and $p < 0.001$. Image data were quantified using ImageJ software (version 1.53t, National Institutes of Health, Bethesda, MD, USA).

3. Results

3.1. Selection of Lactic Acid Bacteria with High GABA Yield

As shown in Figure 1A, TLC analysis was performed on fermentation supernatants from different strains. After color development, all fermentation supernatants showed spots at the same position as the GABA standards of different concentrations. Compared with the other strains, *Lc. rhamnosus* B2-1, *Lp. plantarum* R7, *L. gasseri* F002, *L. gasseri* F003, *Lp. plantarum* R8, and *Lp. Plantarum* R11 showed darker spots. In particular, *Lc. rhamnosus* B2-1 and *Lp. plantarum* R7 showed spots similar to those of the 0.5 mg/mL GABA standard. Therefore, these six strains were preliminarily screened for strong GABA-producing capacity, and HPLC was subsequently used for further screening.

The quantitative results for the fermentation supernatants of the 10 lactic acid bacterial strains are shown in Figure 1B. All 10 strains were able to synthesize GABA, but the production differed among strains. Some strains showed relatively high GABA-producing capacity. Among them, *L. gasseri* F002 had the highest GABA yield, at 0.81 ± 0.01 mg/mL, followed by *Lp. plantarum* R7 at 0.74 ± 0.04 mg/mL, and *Lc. rhamnosus* B2-1 at 0.67 ± 0.02 mg/mL. In contrast, the other strains had lower GABA yields. Therefore, these three strains were selected for subsequent experiments.

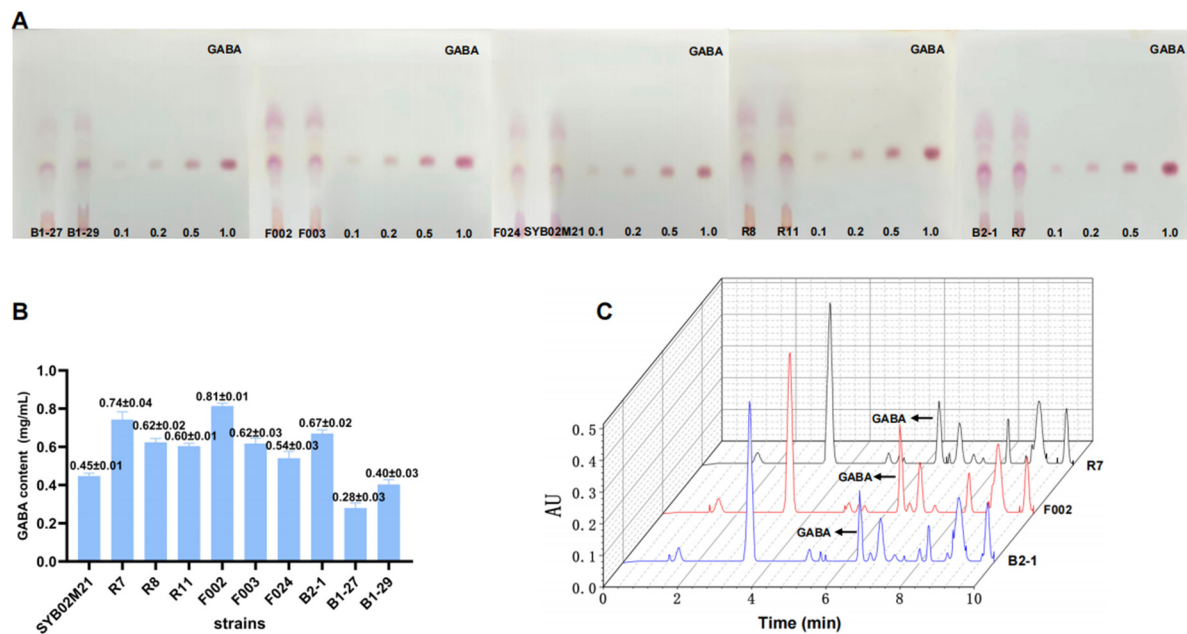


Figure 1. (A) TLC analysis for screening GABA-producing lactic acid bacteria (B). The GABA content in the LAB fermentation supernatants measured by UHPLC. (C) The GABA levels in the supernatants of three LAB strains were analyzed, showing an approximate retention time of 6.40 min.

As shown in Figure 1C, the retention time of GABA was 6.40 min. Characteristic peaks were detected at the same retention time in the supernatants of *Lp. plantarum* R7, *L. gasseri* F002, and *Lc. rhamnosus* B2-1, indicating that these strains produced GABA after fermentation.

3.2. Growth Curve Characteristics and Probiotic Properties Analysis

As shown in Figure 2A, when *Lc. rhamnosus* B2-1, *L. gasseri* F002, and *Lp. plantarum* R7 were cultured for approximately 12 h, all three strains gradually entered the logarithmic growth phase. *L. gasseri* F002 grew faster than the other two strains, with a rapid increase in OD₆₀₀ between 12 and 16 h. *Lp. plantarum* R7 exhibited the second-highest growth rate, whereas *Lc. rhamnosus* B2-1 showed a relatively smaller increase and slower growth. As culture time approached 20 h, the three strains gradually entered the stationary phase, and OD₆₀₀ values tended to stabilize. At this stage, *L. gasseri* F002 had the highest OD₆₀₀ value (1.66), indicating the greatest biomass, *Lp. plantarum* R7 followed with an OD₆₀₀ value of 1.52, and *Lc. rhamnosus* B2-1 had the lowest OD₆₀₀ value (1.08), indicating relatively weaker growth. Overall, the three strains showed similar growth trends under the same culture conditions, but differences in growth rate and final biomass were observed. *L. gasseri* F002 showed the strongest growth ability, followed by *Lp. plantarum* R7, whereas *Lc. rhamnosus* B2-1 showed relatively weak growth. Growth phase analysis of lactic acid bacteria helped determine suitable culture times for strain characterization [30].

Acid resistance is essential for assessing lactic acid bacteria, as their benefits depend on survival in the gastrointestinal tract [31]. In this study, three pH conditions (pH 1.5, 2.0, and 3.0) were used to assess the survival of each strain at different time points. As shown in Figure 2B, after 1 h of culture at pH 1.5, the survival rates of *L. gasseri* F002 and *Lc. rhamnosus* B2-1 were 33.16% and 31.40%, respectively, whereas R7 showed very low survival and almost no viable growth. After 3 h, the survival rates of all three strains decreased to nearly zero. After 1 h at pH 2.0, *L. gasseri* F002 showed the highest survival rate (61.30%), followed by *Lc. rhamnosus* B2-1 (53.60%), whereas *Lp. plantarum* R7 showed poor survival. At pH 3.0, all strains survived after 1 h of culture, with *L. gasseri* F002

showing the highest survival rate (74.67%). After 3 h under these conditions, the survival rates of all three strains decreased: the survival rates of *Lc. rhamnosus* B2-1 and *L. gasseri* F002 were 37.16% and 38.33%, respectively, whereas that of *Lp. plantarum* R7 was the lowest at 32.14%. These results indicate that *L. gasseri* F002 had the highest survival rate and the strongest adaptability to low-pH environments.

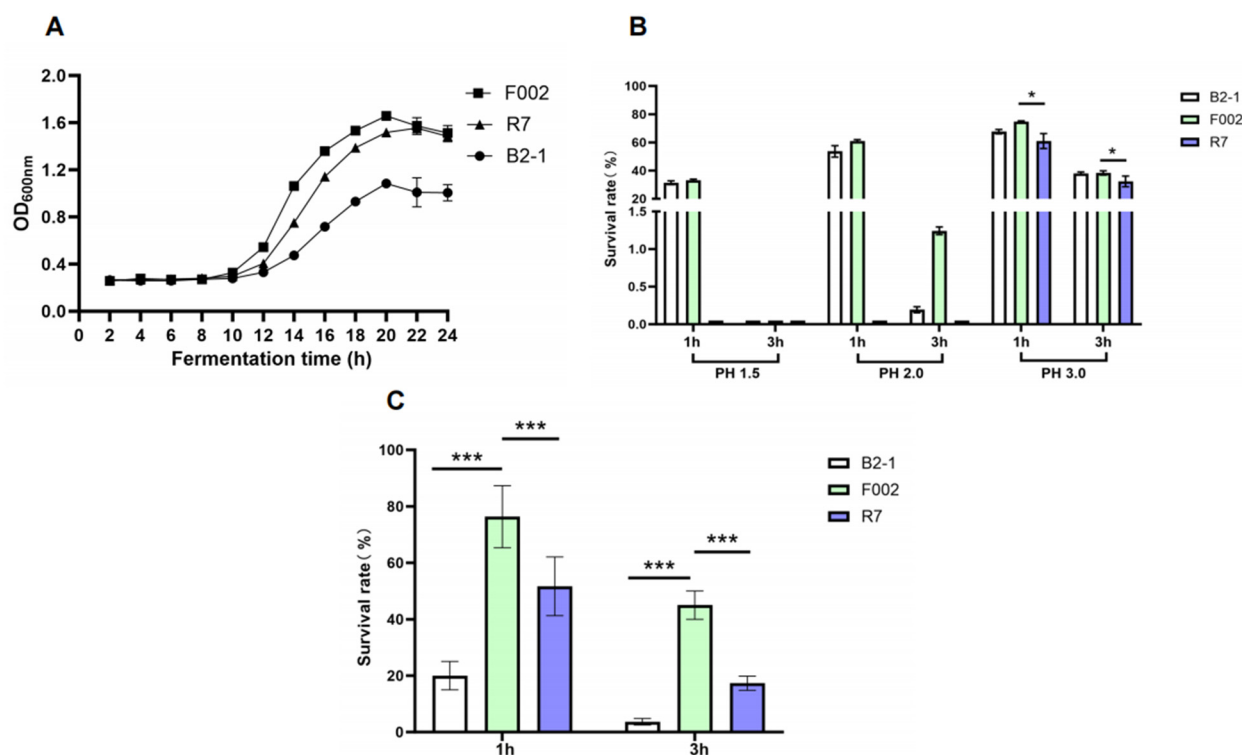


Figure 2. (A) Growth curves of *Lp. plantarum* R7, *L. gasseri* F002, and *Lc. rhamnosus* B2-1. (B) Acid tolerance. (C) Bile salt tolerance (“*” means $p < 0.05$; “***” means $p < 0.001$).

Bile salt tolerance is a fundamental probiotic trait, reflecting the ability of microorganisms to survive harsh gastrointestinal conditions [32]. As shown in Figure 2C, after 1 h of culture in MRS medium containing porcine bile salts, the survival rate of *L. gasseri* F002 was 74.60%, significantly higher than that of *Lp. plantarum* R7 (50.78%) and *Lc. rhamnosus* B2-1 (19.97%). After 3 h of incubation, all three strains showed reduced survival, but *L. gasseri* F002 retained the highest viability at 43.52%. These results indicate that *L. gasseri* F002 had the strongest bile salt tolerance and was therefore selected for subsequent experiments.

3.3. Changes in Key Components During Lactic Acid Bacterial Fermentation of Baihe Dihuang Decoction

Lactic acid bacteria can utilize carbohydrates in substrates for fermentation, and the total sugar content can reflect substrate consumption and fermentation progress [33]. As shown in Figure 3A, during fermentation of the Baihe Dihuang decoction using *L. gasseri* F002, the total sugar content decreased rapidly within the first 12 h, from 7.59 mg/mL to 3.67 mg/mL. Thereafter, the total sugar content remained at approximately 3.77 mg/mL until 72 h of fermentation. This finding suggests that the strain consumed carbohydrates from the Baihe Dihuang decoction during fermentation.

Figure 3B shows no marked change in GABA content during the first 6 h of fermentation. This may have been because the strain was in the lag phase, with low viable cell counts and limited GABA production. During 6–24 h of fermentation, the GABA content increased and reached a maximum of 0.063 mg/mL. Combined with the growth curve

results, this indicates that viable lactic acid bacterial cells increased rapidly during this stage, and proteins in the Baihe Dihuang decoction were hydrolyzed to release glutamate, which served as the substrate for GABA synthesis [34].

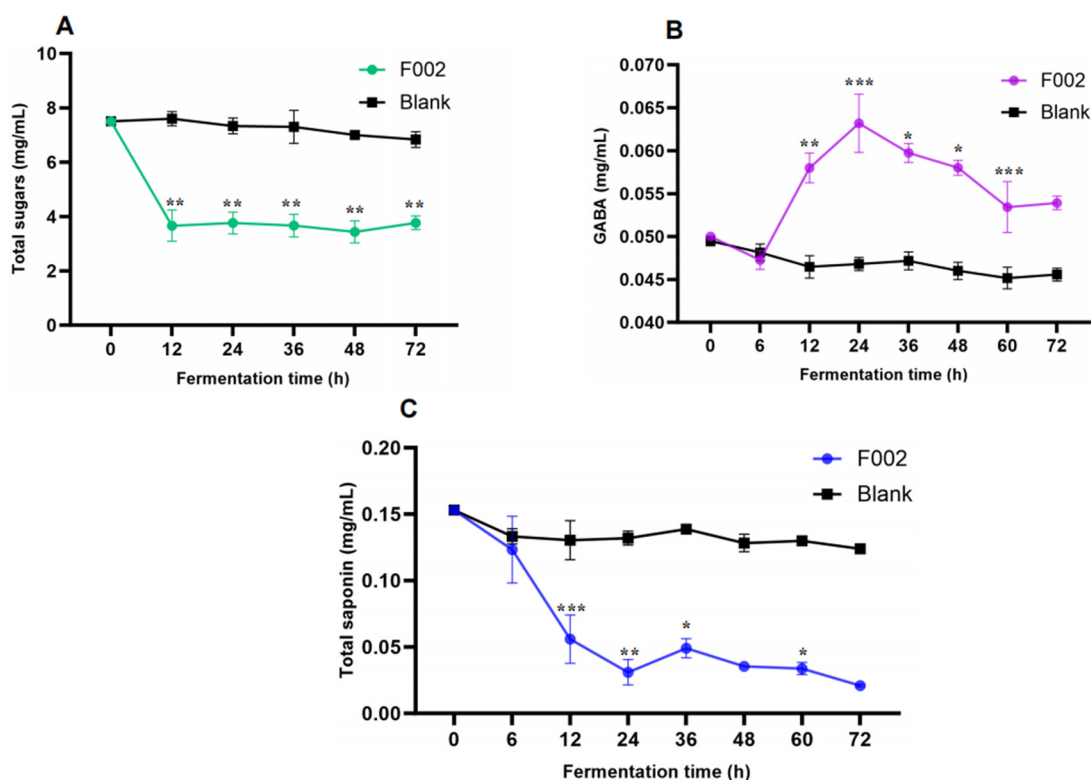


Figure 3. (A) Changes in total sugar content, (B) GABA content, and (C) total saponin content during fermentation (“*” means $p < 0.05$; “**” means $p < 0.01$; and “***” means $p < 0.001$).

Notably, the absolute GABA content in the fermented decoction remained relatively modest. This is primarily because the Baihe Dihuang decoction matrix only provides endogenous glutamate from endogenous protein hydrolysis, without exogenous glutamate supplementation. Studies have shown that sufficient exogenous monosodium glutamate substrate can significantly enhance GAD catalytic efficiency, increasing GABA production by approximately tenfold compared with conditions without glutamate addition [35]. Therefore, GABA production in this herbal fermentation system was approximately tenfold lower than that observed under glutamate-supplemented optimized culture conditions. This is consistent with the general rule observed in other fermented food matrices, such as GABA-rich soy sauce, where endogenous substrate content and fermentation parameters are key determinants of the final GABA yield [36]. Optimization of fermentation conditions and exogenous glutamate supplementation to further improve GABA accumulation will be the focus of our follow-up research.

As fermentation progressed from 24 to 72 h, the GABA content gradually decreased. This was likely caused by nutrient exhaustion and organic acid buildup, which decreased pH and weakened bacterial metabolism. The bacterial population also declined accordingly, resulting in a reduced GABA production rate. In addition, GABA itself may be further metabolized and degraded by lactic acid bacteria during late fermentation, with the degradation rate possibly exceeding the production rate, leading to a decline in GABA content over time [37].

The saponins in the Baihe Dihuang decoction are mainly steroidal saponins, which have been shown to have effects in improving depression, relieving anxiety, and treating

neurological disorders [38]. As shown in Figure 3C, during the first 12 h of fermentation using *L. gasseri* F002, the total saponin content showed a marked decreasing trend. The initial content was 0.15 mg/mL and decreased to 0.03 mg/mL by 24 h of fermentation. During fermentation, β -glucosidase from lactic acid bacteria hydrolyzes glycosides, promoting aglycone formation. In addition, lactic acid produced during fermentation reduces the pH of the system, and the acidic environment may further promote the decomposition of saponin aglycones [39].

Compared with the non-fermented Baihe Dihuang decoction, fermentation using *L. gasseri* F002 increased the GABA content and reduced the total saponin content. Overall, 24 h was determined to be the optimal fermentation time.

3.4. Maximum Tolerated Concentration of Baihe Dihuang Decoction in Zebrafish

To determine the MTC of the two test samples under caffeine-induced model conditions, at 5 dpf, WT (AB) larvae were randomly assigned to the Ctrl, Model, BDNF, and BDF cohorts; the treatment groups were exposed to 1, 10, 50, 100, or 1000 μ g/mL samples. As shown in the figures, the sample used in Figure 4 is the unfermented Baihe Dihuang decoction, while the sample used in Figure 5 is the fermented Baihe Dihuang decoction. After 24 h of treatment, zebrafish in the Ctrl group developed normally, with a typical straight body axis that was continuous and symmetrical from head to tail. No obvious malformations were observed in the Model group or in any sample group (Figures 4A and 5A). No deaths occurred in the Ctrl, Model, or any concentration group in the two samples, and the survival rate was 100% (Figures 4B and 5B). The heart rates remained stable without obvious fluctuations, ranging from 42 to 52 beats/20 s across replicate wells (Figures 4C and 5C).

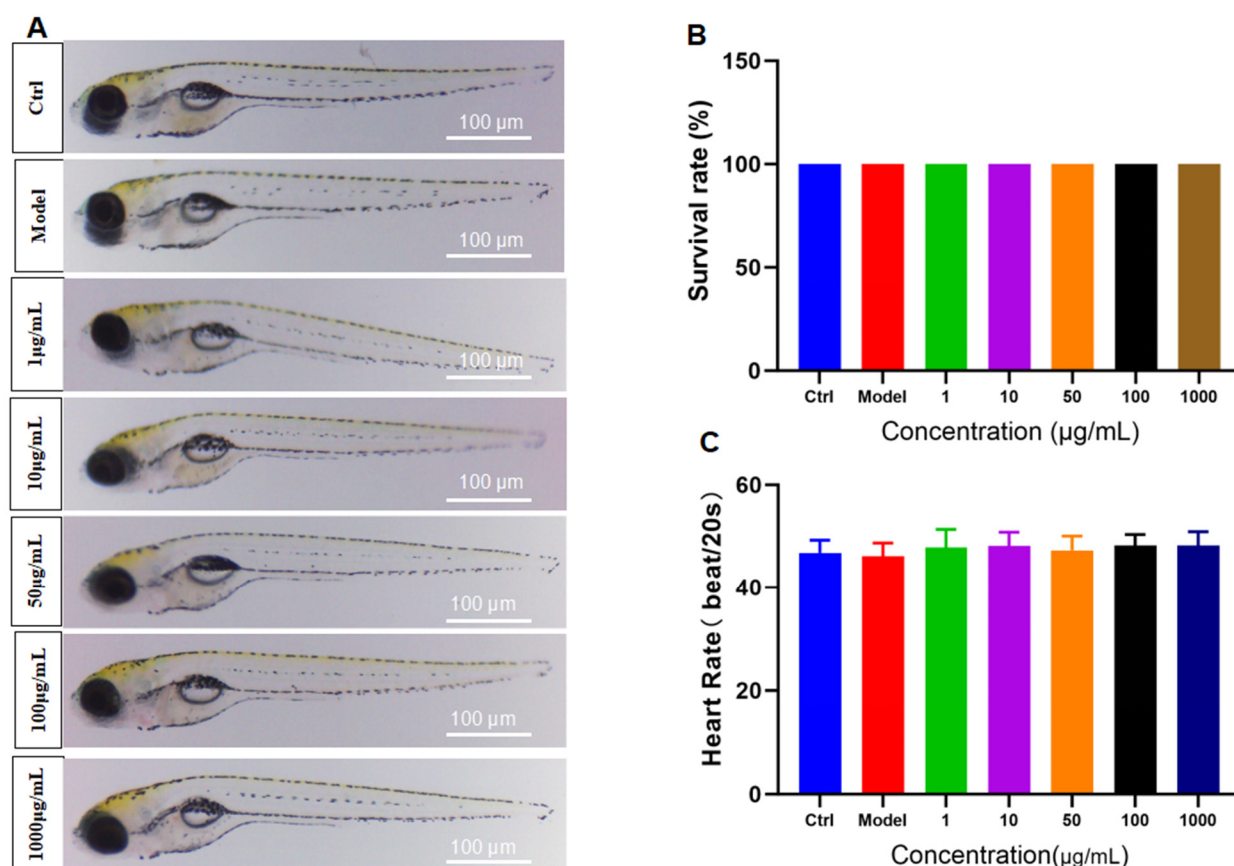


Figure 4. Treatment with unfermented Baihe Dihuang decoction. (A) Changes in zebrafish phenotype. (B) Zebrafish survival rate. (C) Zebrafish heart rate.

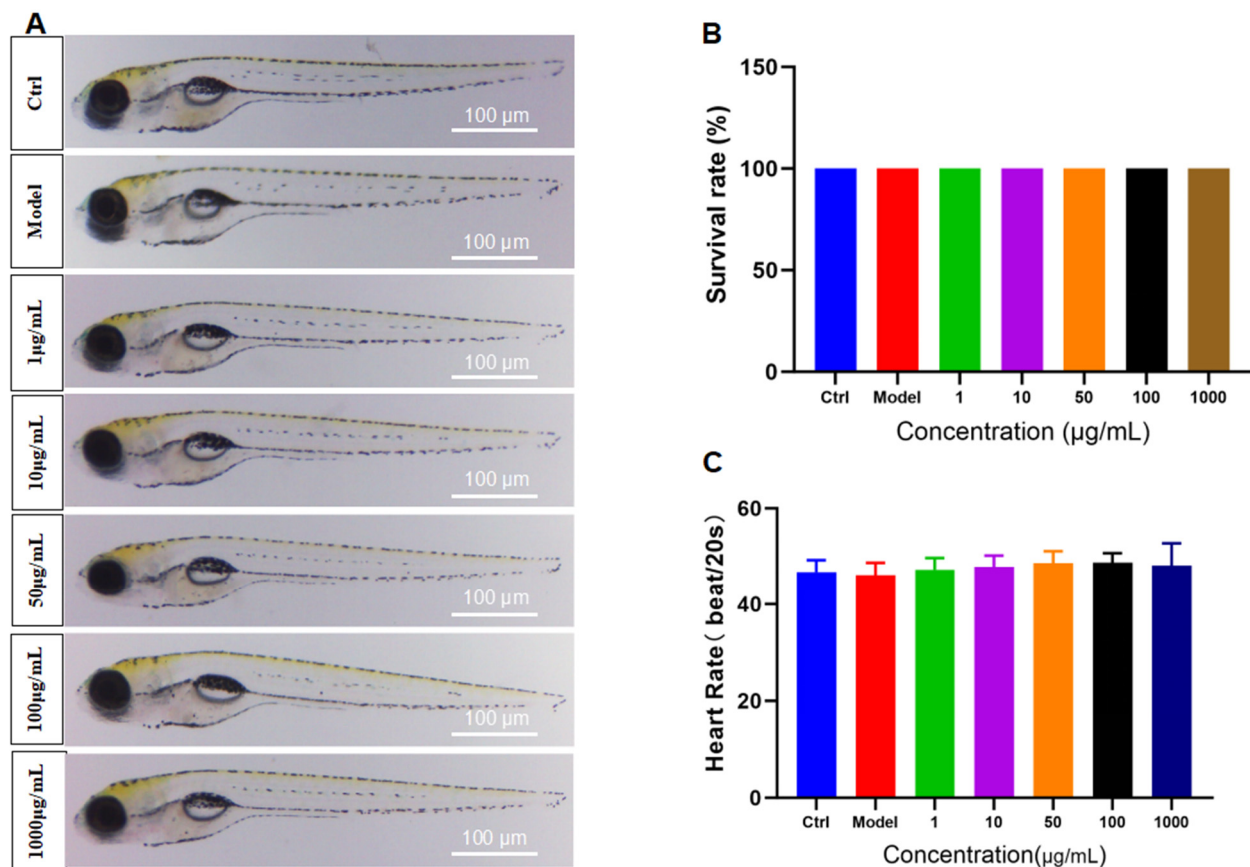


Figure 5. Treatment with fermented Baihe Dihuang decoction. (A) Changes in zebrafish phenotype. (B) Zebrafish survival rate. (C) Zebrafish heart rate.

These results indicate that both the Baihe Dihuang decoction and the fermented Baihe Dihuang decoction had no teratogenic effects on zebrafish larvae at 5 dpf. Therefore, the MTC was determined to be 1000 μg/mL.

3.5. Zebrafish Behavioral Analysis

As illustrated in Figure 6, the Model group exhibited a significantly shorter sleep bout duration than the Ctrl group ($p < 0.05$), a significantly increased sleep bout number ($p < 0.001$), significantly increased wakefulness duration ($p < 0.01$), and significantly increased total locomotor activity ($p < 0.01$). These results indicate sleep fragmentation, decreased sleep quality, and hyperarousal in zebrafish, confirming successful establishment of the insomnia model (Figure 6A–D). The sleep bout number was significantly lower in the GABA group than in the Model group ($p < 0.001$), as well as wakefulness duration ($p < 0.05$) and total locomotor activity ($p < 0.05$), indicating that GABA significantly improved insomnia-like symptoms in zebrafish (Figure 6B–D).

Compared with the Model group, BDF3 prolonged sleep bout duration ($p < 0.05$; Figure 6A) and reduced bout number, wakefulness time, and locomotor activity ($p < 0.01$; Figure 6B–D). Relative to the Model group, the BDF2 group showed a significantly reduced sleep bout number ($p < 0.01$; Figure 6B), wakefulness duration, and total locomotor activity ($p < 0.05$; Figure 6C,D). The BDNF2 group showed significantly reduced wakefulness duration and total locomotor activity. These indices declined significantly relative to the Model group ($p < 0.05$; Figure 6C,D), and BDNF3 further reduced total locomotor activity ($p < 0.01$; Figure 6D).

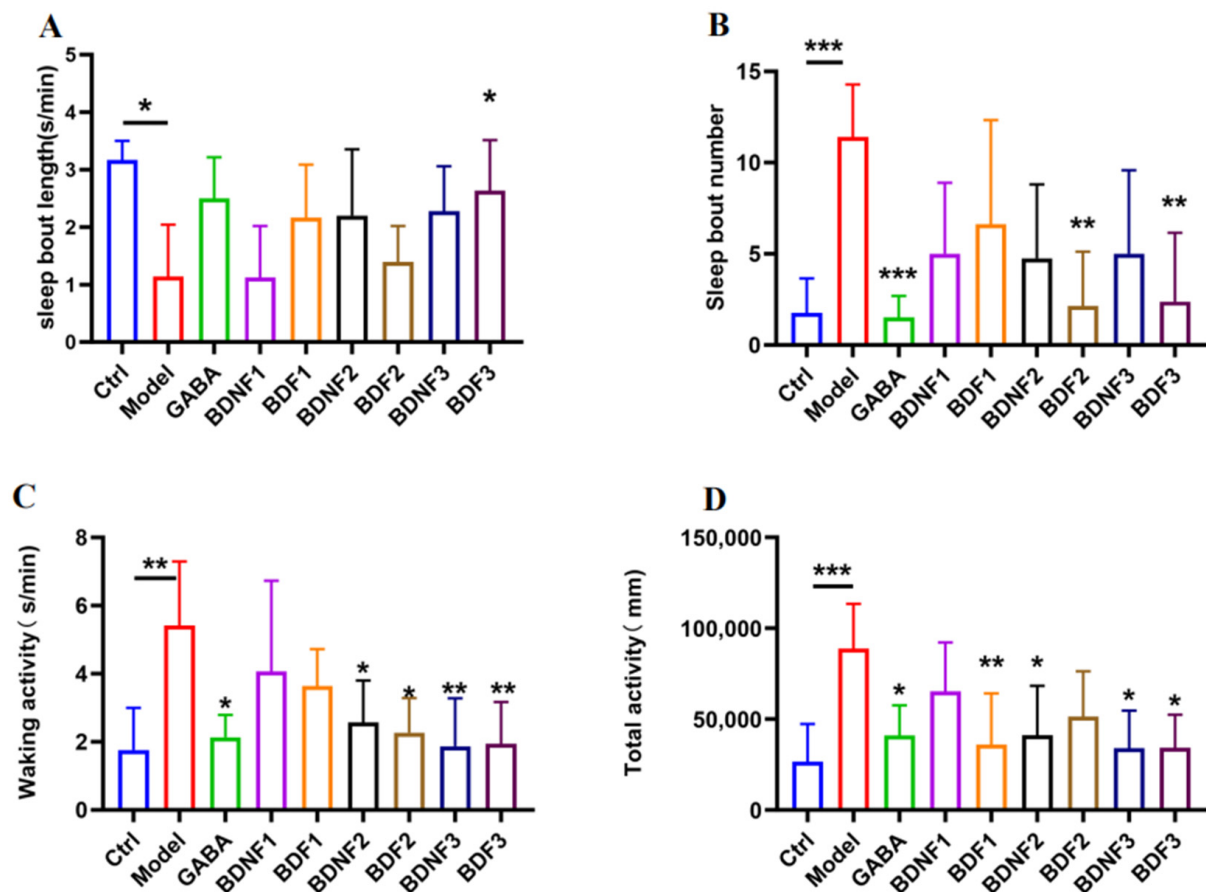


Figure 6. (A) Sleep bout length. (B) Sleep bout number. (C) Total sleep time. (D) Total locomotor activity (“*” means $p < 0.05$; “**” means $p < 0.01$; and “***” means $p < 0.001$).

From the perspective of effect size, the high-dose BDF3 group reduced the sleep bout number by approximately 40% relative to the Model group, with a magnitude comparable to the positive GABA control, representing a biologically meaningful improvement in sleep fragmentation. In contrast, low-dose groups showed only modest statistically significant changes in partial indicators with small effect sizes, whose direct physiological relevance remains limited.

3.6. Sleep-Related Gene Expression in Zebrafish

Figure 7 presents the expression profiles of sleep-associated genes across all groups, including the 5-HT-related genes *htr1aa* and *htr2aa*, the dopamine-related genes *dbh*, *th*, and *drd2a*, and the GABA-related gene *gabra1*.

Relative to the Ctrl group, the Model group exhibited marked increases in *dbh* and *th* expression ($p < 0.001$; Figure 7C,D) and significant downregulation of *htr1aa* and *drd2a* ($p < 0.001$; Figure 8A,E). The results demonstrate successful induction of insomnia-associated molecular changes in zebrafish. Relative to the Model group, the GABA group showed significant upregulation of *htr1aa* ($p < 0.01$; Figure 7A), *drd2a*, and *gabra1* ($p < 0.05$; Figure 7E,F), and significant downregulation of *dbh* and *th* ($p < 0.001$; Figure 7C,D), suggesting that increased GABA-related signaling improved sleep in zebrafish.

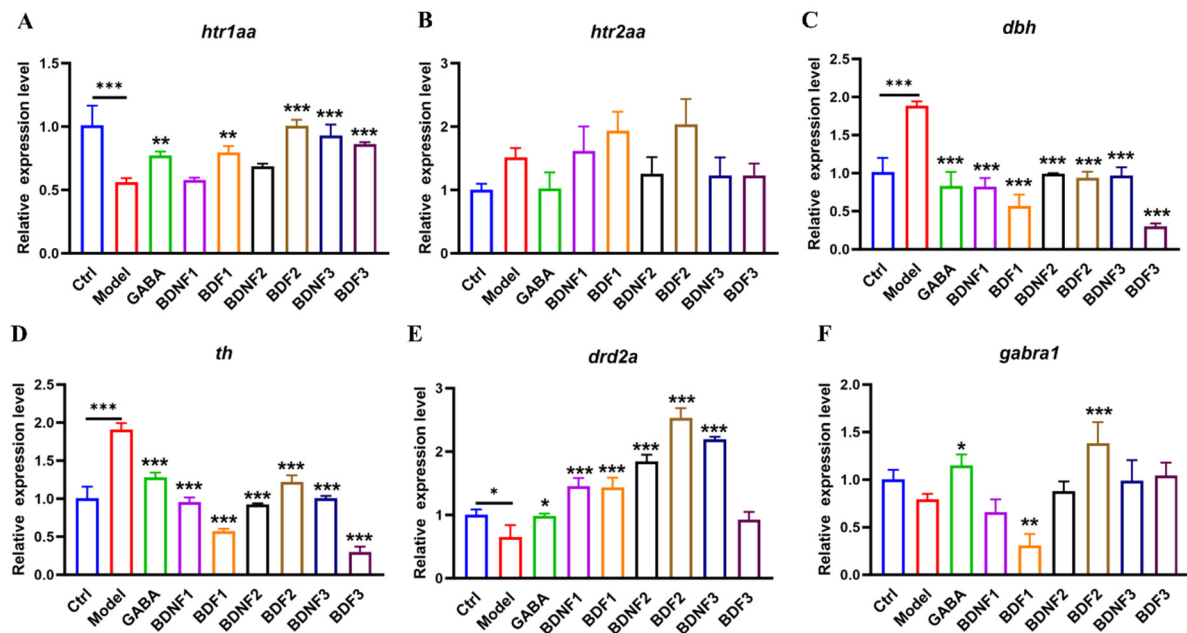


Figure 7. Expression of (A) *htr1aa*, (B) *htr2aa*, (C) *dbh*, (D) *th*, (E) *drd2a*, and (F) *gabra1*. Significance: (“” means $p < 0.05$; “”” means $p < 0.01$; and “””” means $p < 0.001$).

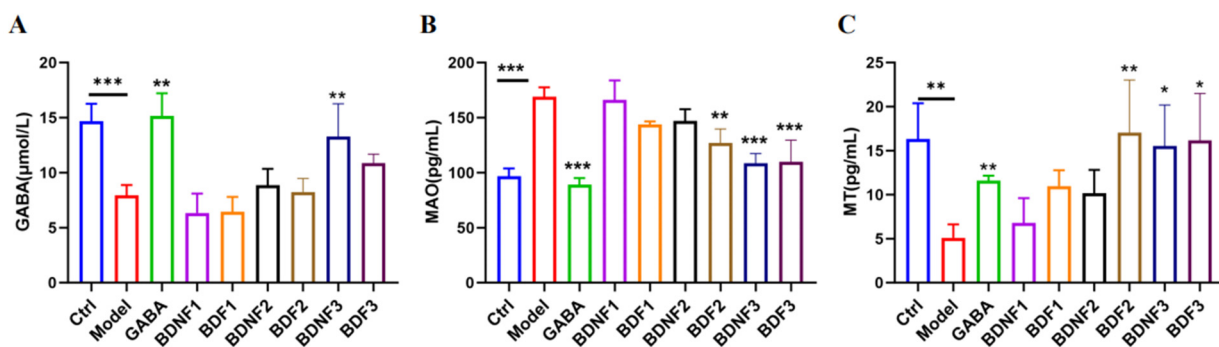


Figure 8. Changes in (A) GABA content, (B) MT content, and (C) MAO content. Significance: (“” means $p < 0.05$; “”” means $p < 0.01$; and “””” means $p < 0.001$).

Relative to the Model group, the BDNF1 group showed significant upregulation of *drd2a* ($p < 0.001$; Figure 7E) and significant downregulation of *dbh* and *th* ($p < 0.001$; Figure 7C,D). No significant changes were observed in *htr2aa* or *gabra1* expression ($p > 0.05$; Figure 7B,F). The BDF1 group showed significant upregulation of *htr1aa* ($p < 0.01$; Figure 7A) and *drd2a* ($p < 0.001$; Figure 7E). The expression of *dbh* and *th* was markedly reduced ($p < 0.001$; Figure 7C,D), along with *gabra1* ($p < 0.05$; Figure 7F), while *htr2aa* showed no significant change ($p > 0.05$; Figure 7B). The BDNF2 group showed significant upregulation of *drd2a* ($p < 0.001$; Figure 7E) and significant downregulation of *dbh* and *th* ($p < 0.001$; Figure 7C,D). The expression levels of *htr1aa*, *htr2aa*, and *gabra1* remained statistically unchanged ($p > 0.05$; Figure 7A,B,F). The BDF2 group showed significant upregulation of *htr1aa*, *th*, *drd2a*, and *gabra1* ($p < 0.001$; Figure 7A,D–F). The expression of *htr2aa* remained statistically unchanged ($p > 0.05$; Figure 7B). The BDNF3 group showed significant upregulation of *htr1aa* and *drd2a* ($p < 0.001$; Figure 7A,E), and significant downregulation of *dbh* and *th* ($p < 0.001$; Figure 7C,D). The expression of *htr2aa* and *gabra1* remained statistically unchanged ($p > 0.05$; Figure 7B,F). The BDF3 group showed significant upregulation of *htr1aa* ($p < 0.001$; Figure 7A) and significant downregulation of *dbh* and *th* ($p < 0.001$; Figure 7C,D), whereas *htr2aa*, *drd2a*, and the expression of *gabra1* remained statistically unchanged ($p > 0.05$; Figure 7B,E,F).

Collectively, these transcriptional profiles reveal that the fermented Baihe Dihuang decoction groups, particularly BDF2 and BDF3, exhibited gene expression trends associated with alleviated caffeine-induced sleep disturbance, showing a pattern concordant with multi-pathway transcriptional alterations. Specifically, reduced transcript levels of *dbh* and *th* correspond to a transcriptional profile consistent with lowered catecholaminergic synthetic capacity, while the elevated mRNA levels of *gabra1*, *drd2a*, and *htr1aa* match a pattern linked to inhibitory neurotransmitter receptor signaling. It should be noted that these are only mRNA-level observations and do not confirm actual activation or suppression of the corresponding signaling pathways. These transcriptional changes are concordant with the sedative-like behavioral phenotypes observed in this zebrafish model, but the causal link remains to be functionally verified. No significant transcriptional alteration was detected for the excitatory 5-HT_{2A} receptor gene *htr2aa* across the treatment groups.

3.7. Biochemical Indicators in Each Treatment Group

Figure 8 shows the GABA, MT, and MAO levels in each group. Relative to the Ctrl group, the Model group had lower GABA and MT contents but higher MAO levels ($p < 0.001$; Figure 8A–C). These findings indicate that caffeine intervention induced insomnia-like phenotypes, such as sleep disturbance and hyperarousal, by reducing inhibitory neurotransmitter levels and disrupting circadian homeostasis, consistent with the expected model characteristics.

Relative to the Model group, GABA treatment elevated GABA and MT levels while reducing MAO content, confirming its protective effect against caffeine-induced sleep disturbance ($p < 0.01$ or $p < 0.001$; Figure 8A or Figure 8B).

Relative to the Model group, BDF2 increased MT levels ($p < 0.01$; Figure 8C) and reduced MAO content ($p < 0.05$; Figure 8B). The BDNF3 group showed significantly increased GABA content ($p < 0.01$; Figure 8A), significantly increased MT content ($p < 0.05$; Figure 8C), and significantly decreased MAO content ($p < 0.001$; Figure 8B). The BDF3 group showed significantly increased MT content ($p < 0.05$; Figure 8C) and significantly decreased MAO content ($p < 0.001$; Figure 8B).

Overall, the BDNF3 group significantly increased GABA content in zebrafish. In addition, the BDF2, BDF3, and BDNF3 groups reversed the caffeine-induced decrease in MT content, with the fermented groups showing higher levels than the non-fermented groups. These findings suggest positive regulation of the GABAergic system and melatonin rhythm pathway. By increasing inhibitory neurotransmitter and circadian hormone levels, these treatments alleviated caffeine-induced sleep dysregulation. For MAO, the results showed a trend opposite to those of GABA and MT. Treatment with BDF2, BDF3, and BDNF3 was associated with reduced MAO content compared with the Model group. This pattern suggests a potential relationship with suppressed excessive MAO activation, which may contribute to reduced monoamine neurotransmitter degradation and maintenance of monoaminergic homeostasis in this zebrafish model. These findings further indicate that the fermented Baihe Dihuang decoction may contribute to the regulation of sleep-related biochemical pathways and improve caffeine-induced sleep abnormalities from the perspective of neurotransmitter metabolism. In terms of effect magnitude, the BDF3 group restored melatonin content to approximately 75% of the control level, showing a substantial recovery effect. In contrast, the GABA content exhibited only partial recovery with a smaller effect size, which further supports the view that the sleep-improving phenotype is not solely driven by GABA elevation but likely involves multi-component synergistic effects.

4. Discussion

This study screened human-derived GABA-producing lactic acid bacteria and compared their growth and gastrointestinal tolerance capacities, then characterized component changes of a fermented Baihe Dihuang decoction and explored its sleep-modulatory trends in a caffeine-induced zebrafish insomnia model via behavioral, transcriptional, and biochemical indicators. It should be noted that the overall experimental framework followed a standard functional food research workflow, and the present findings represent incremental advances rather than groundbreaking conceptual innovations. The core contributions of this work lie in systematic data on human-origin LAB fermentation of the Baihe Dihuang decoction and multi-level preliminary evidence of its sleep-alleviating effects in larval zebrafish.

Ten human breast milk- and infant feces-derived LAB strains were screened for GABA biosynthesis via combined TLC and HPLC detection. Among the candidates, *L. gasseri* F002 exhibited the highest GABA yield, faster growth kinetics, and superior acid and bile salt resistance compared with *Lp. plantarum* R7 and *Lc. rhamnosus* B2-1, which qualified it as the optimal strain for herbal matrix fermentation. Consistent with previous LAB fermentation reports, human intestinal/breast milk isolates generally display stronger gastrointestinal adaptability than strains isolated from plant fermented foods, laying a safer foundation for subsequent food-related product development [40,41]. During 72 h liquid-state fermentation of the Baihe Dihuang decoction, the strain rapidly consumed substrate carbohydrates within the first 12 h for proliferation and glutamate production, driving a peak in GABA content at 24 h; prolonged culture led to nutrient depletion and GABA catabolism, resulting in a gradual decline of this metabolite. Meanwhile, total steroidal saponins markedly decreased, which can be attributed to β -glucosidase secreted by *L. gasseri* that hydrolyzes glycosidic bonds to generate smaller aglycone derivatives, a common biotransformation phenomenon in microbial herbal fermentation [42]. Despite these well-characterized component changes, it must be critically acknowledged that the relatively modest increase in GABA content (maximum 0.063 mg/mL) alone is insufficient to fully explain the sleep-improving phenotypes observed in the zebrafish model. It is unlikely that this limited GABA elevation accounts for all the detected behavioral, transcriptional, and biochemical alterations. Instead, the overall bioactivity is more plausibly driven by the combined action of multiple fermentation-induced changes: the biotransformation of steroidal saponins into more bioavailable aglycone forms, the generation of other unmeasured microbial metabolites, and potential synergistic interactions between GABA and these transformed constituents. The specific contribution of GABA to the overall efficacy remains to be determined through further bioactivity-guided fractionation and component verification.

Subsequent zebrafish behavioral tests demonstrated that the fermented Baihe Dihuang decoction (especially the 1000 μ g/mL BDF3 group) relieved caffeine-triggered sleep fragmentation, shortened wake duration, and reduced locomotor hyperactivity, with effects comparable to the positive GABA control group at the tested concentration. Transcriptional and biochemical measurements further provided correlative evidence for multi-pathway regulatory trends in this zebrafish model. Caffeine exposure upregulated catecholamine synthesis-related *th* and *dbh*, while suppressing inhibitory signaling genes *htr1aa* and *drd2a*. Treatment with fermented decoction reversed these transcriptional shifts: it downregulated catecholamine biosynthetic genes and elevated transcripts of inhibitory 5-HT_{1A} and D₂ autoreceptors, without significant influence on excitatory *htr2aa*. At the biochemical level, the fermented decoction increased GABA and melatonin concentrations and lowered MAO levels, which aligns with reduced monoamine neurotransmitter breakdown and restored inhibitory neural tone. It is important to clarify that these mRNA and biochemical alter-

ations only reflect correlative phenotypic links in larval zebrafish, and definitive causal pathway activation cannot be concluded solely from the present dataset.

Notably, several limitations of this research must be acknowledged. First, larval zebrafish possess substantial evolutionary divergence from humans, so all observed sleep-modulatory trends cannot be directly extrapolated to human physiological or therapeutic responses. Second, only mRNA expression was quantified; corresponding protein expression and gene functional verification experiments are absent. Third, the exact bioactive metabolites produced after the LAB fermentation of the Baihe Dihuang decoction remain unseparated and identified. Fourth, this study used statistical significance as the primary criterion for evaluation; however, the biological significance of some minor but statistically significant changes still requires further verification. Further mammalian animal trials and component isolation research are required to validate and expand the preliminary results reported here. Collectively, this work supplements basic experimental data on lactic acid bacteria-mediated modification of the Baihe Dihuang formula and offers a reference for subsequent development of sleep-improving medicinal–food homologous fermented products.

5. Conclusions

In conclusion, this study screened *L. gasseri* F002 among 10 lactic acid bacterial strains as a high GABA-producing strain with favorable acid and bile salt resistance. The strain was then used for the fermentation of the Baihe Dihuang decoction, which increased the GABA content and reduced the total saponin content. The fermented Baihe Dihuang decoction improved caffeine-induced sleep disorder in zebrafish, reduced total locomotor activity, and regulated sleep-related genes, as well as GABA, MT, and MAO levels. These findings provide preliminary experimental evidence for the sleep-modulatory effects of the fermented Baihe Dihuang decoction in a zebrafish insomnia model and lay a basic foundation for further mechanistic investigation and evaluation in mammalian models.

Author Contributions: Conceptualization, L.C.; data curation, W.Q.; formal analysis, J.Z.; funding acquisition, J.Z.; investigation, Y.Z. and J.L.; methodology, B.Y.; project administration, L.C.; resources, W.Q.; software, Q.Z.; supervision, X.L.; validation, Y.Z.; visualization, L.L.; writing—original draft preparation, Y.Z.; writing—review and editing, X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Beijing Rural Revitalisation Agricultural Technology Project (NY2601500000) and the Shounong Food Group Independent Technology Project (SNSPKJ (2024)-04).

Institutional Review Board Statement: The animal study protocol was approved by the Beijing Shenrui Ethics Review Committee (SR20260105) on 5 January 2026.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: Authors Yining Zhou, Jinqiu Luo, Xianping Li, Junying Zhao, Baoyu Yang, Quansheng Zhu, Weicang Qiao, Lu Liu, and Lijun Chen were employed by the company Beijing Sanyuan Foods Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The authors declare that this study received funding from the Beijing Rural Revitalisation Agricultural Technology Project (NY2601500000) and the Shounong Food Group Independent Technology Project (SNSPKJ (2024)-04). The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

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