

## Effects of Bacillus subtilis Zinc on Growth Performance, Organ Indices, Nutrient Utilization Rate, and Trace Element Content in Organs of Congenitally Zinc-Deficient Rats: Postprint

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### Abstract

This experiment aimed to investigate the effects of Bacillus subtilis zinc on growth performance, organ indices, nutrient utilization, and trace element (zinc, copper, iron) contents in organs of congenitally zinc-deficient rats, in order to determine the efficacy of Bacillus subtilis zinc and the feasibility of reducing trace element supplementation. Pregnant dams were selected to establish a congenitally zinc-deficient rat model, i.e., model group rats were fed a zinc-deficient diet (zinc level 13.00 mg/kg) from day 10 of pregnancy, while control group rats were fed a normal diet (zinc level 38.00 mg/kg) during the same period, continuing until the end of lactation. After successful model establishment, 18 normal 24-day-old rat pups from the control group of the model establishment experiment were selected as the normal group (Group I, zinc level 13.00 mg/kg) and fed a normal diet; additionally, 90 congenitally zinc-deficient 24-day-old rat pups from the model group of the model establishment experiment were randomly divided into 5 experimental groups: zinc-deficient group (Group II, zinc level 13.00 mg/kg), zinc sulfate (ZnSO<sub>4</sub>) group (Group III, zinc level 38.00 mg/kg), and low- (Group IV, zinc level 15.00 mg/kg), medium- (Group V, zinc level 30.00 mg/kg), and high-dose Bacillus subtilis zinc groups (Group VI, zinc level 45.00 mg/kg), all fed a zinc-deficient diet, with 3 replicates per group and 6 rats per replicate. The experimental period lasted 5 weeks. The results showed: 1) Compared with Group I, Group II exhibited significantly or extremely significantly decreased body weight (BW) and average daily feed intake (ADFI) ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups IV, V, and VI showed significantly or extremely significantly increased BW and ADFI ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group V exhibited significantly increased BW, average daily gain (ADG), and ADFI ( $P < 0.05$ ). 2) Compared with Group I, Group II showed

significantly decreased liver index ( $P < 0.05$ ). Compared with Group II, Groups V and VI exhibited significantly or extremely significantly increased heart and liver indices ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group VI showed significantly increased heart index ( $P < 0.05$ ), Group V exhibited significantly or extremely significantly increased heart and kidney indices ( $P < 0.05$  or  $P < 0.01$ ), and Group IV showed significantly increased kidney index ( $P < 0.05$ ). 3) Compared with Group I, Group II exhibited significantly or extremely significantly decreased utilization rates of crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium, and zinc ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups V and VI showed significantly or extremely significantly increased utilization rates of CP, CF, EE, NDF, ADF, calcium, and zinc ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group IV exhibited significantly increased EE utilization rate ( $P < 0.05$ ), Group V showed extremely significantly increased utilization rates of EE, CF, NDF, ADF, zinc, and calcium ( $P < 0.01$ ), and Group VI exhibited significantly or extremely significantly increased utilization rates of EE, CF, NDF, ADF, and zinc ( $P < 0.05$  or  $P < 0.01$ ). 4) Compared with Group I, Group II exhibited significantly or extremely significantly decreased nitrogen intake, nitrogen retention, and nitrogen utilization rate ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups V and VI showed significantly or extremely significantly increased nitrogen intake, nitrogen retention, and nitrogen utilization rate ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group IV exhibited significantly decreased nitrogen intake and nitrogen retention ( $P < 0.05$ ), while Group V showed significantly increased nitrogen intake and nitrogen retention ( $P < 0.05$ ). 5) Compared with Group I, Group II showed significantly or extremely significantly decreased zinc content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups V and VI exhibited significantly or extremely significantly increased zinc content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group IV showed significantly decreased zinc content in heart and kidney ( $P < 0.05$ ), while Groups IV and VI exhibited significantly or extremely significantly decreased zinc content in liver ( $P < 0.05$  or  $P < 0.01$ ). 6) Compared with Group I, Group II exhibited significantly decreased copper content in heart, brain, and kidney ( $P < 0.05$ ). Compared with Group II, Groups IV, V, and VI showed significantly increased copper content in heart ( $P < 0.05$ ). Compared with Group III, Groups IV and VI exhibited significantly decreased copper content in brain ( $P < 0.05$ ), while Group V showed significantly increased copper content in heart ( $P < 0.05$ ). 7) Compared with Group I, Group II showed significantly or extremely significantly decreased iron content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups IV, V, and VI exhibited significantly or extremely significantly increased iron content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Groups IV, V, and VI showed significantly or extremely significantly decreased iron content in liver ( $P < 0.05$  or  $P < 0.01$ ). In conclusion, *Bacillus subtilis* zinc can promote the growth and development of congenitally zinc-deficient rats, increase liver index, heart index, and nutrient utilization, regulate trace element distribution, demonstrate superior efficacy compared to  $ZnSO_4$ , and reduce the amount of

zinc supplementation in the diet.

## Full Text

### Effects of Bacillus subtilis-Zinc on Growth Performance, Organ Index, Nutrient Utilization and Trace Element Content in Organs of Congenital Zinc Deficiency Rats

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#### Abstract

This experiment was conducted to investigate the effects of Bacillus subtilis-zinc on growth performance, organ index, nutrient utilization, and trace element (zinc, copper, iron) content in organs of congenital zinc deficiency rats, and to determine the efficacy of Bacillus subtilis-zinc and the feasibility of reducing trace element supplementation. Pregnant rats were selected to establish a congenital zinc deficiency model. The model group was fed a zinc-deficient diet (13.00 mg/kg zinc) from day 10 of pregnancy through lactation, while the control group received a normal diet (38.00 mg/kg zinc). After successful modeling, eighteen 24-day-old normal juvenile rats from the control group were selected as the normal group (Group I, 13.00 mg/kg zinc) and fed a normal diet. Additionally, ninety 24-day-old congenital zinc-deficient juvenile rats from the model group were randomly divided into five experimental groups: zinc deficiency group (Group II, 13.00 mg/kg zinc), zinc sulfate (ZnSO<sub>4</sub>) group (Group III, 38.00 mg/kg zinc), and low- (Group IV, 15.00 mg/kg zinc), medium- (Group V, 30.00 mg/kg zinc), and high-dose Bacillus subtilis-zinc groups (Group VI, 45.00 mg/kg zinc), all fed zinc-deficient diets. Each group had 3 replicates with 6 rats per replicate. The trial lasted for 5 weeks.

The results showed: (1) Compared with Group I, Group II exhibited significantly or extremely significantly decreased body weight (BW) and average daily feed intake (ADFI) ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups IV, V, and VI showed significantly or extremely significantly increased BW and ADFI ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group V demonstrated significantly increased BW, average daily gain (ADG), and ADFI ( $P < 0.05$ ). (2) Compared with Group I, Group II showed significantly decreased liver index ( $P < 0.05$ ). Compared with Group II, Groups V and VI exhibited significantly or extremely significantly increased heart and liver indices ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group VI showed significantly increased heart index ( $P < 0.05$ ), Group V displayed significantly or extremely significantly increased heart and kidney indices ( $P < 0.05$  or  $P < 0.01$ ), and

Group IV had significantly increased kidney index ( $P < 0.05$ ). (3) Compared with Group I, Group II showed significantly or extremely significantly decreased utilization of crude protein (CP), crude fat (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium, and zinc ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups V and VI demonstrated significantly or extremely significantly increased utilization of CP, CF, EE, NDF, ADF, calcium, and zinc ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group IV showed significantly increased EE utilization ( $P < 0.05$ ), Group V exhibited extremely significantly increased utilization of EE, CF, NDF, ADF, zinc, and calcium ( $P < 0.01$ ), and Group VI displayed significantly or extremely significantly increased utilization of EE, CF, NDF, ADF, and zinc ( $P < 0.05$  or  $P < 0.01$ ). (4) Compared with Group I, Group II showed significantly or extremely significantly decreased nitrogen intake, deposited nitrogen, and nitrogen utilization ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups V and VI demonstrated significantly or extremely significantly increased nitrogen intake, deposited nitrogen, and nitrogen utilization ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group IV showed significantly decreased nitrogen intake and deposited nitrogen ( $P < 0.05$ ), while Group V exhibited significantly increased nitrogen intake and deposited nitrogen ( $P < 0.05$ ). (5) Compared with Group I, Group II showed significantly or extremely significantly decreased zinc content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups V and VI demonstrated significantly or extremely significantly increased zinc content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Group IV showed significantly decreased zinc content in heart and kidney ( $P < 0.05$ ), while Groups IV and VI exhibited significantly or extremely significantly decreased liver zinc content ( $P < 0.05$  or  $P < 0.01$ ). (6) Compared with Group I, Group II showed significantly decreased copper content in heart, brain, and kidney ( $P < 0.05$ ). Compared with Group II, Groups IV, V, and VI demonstrated significantly increased heart copper content ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Groups IV and VI showed significantly decreased brain copper content ( $P < 0.05$ ), while Group V exhibited significantly increased heart copper content ( $P < 0.05$ ). (7) Compared with Group I, Group II showed significantly or extremely significantly decreased iron content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group II, Groups IV, V, and VI demonstrated significantly or extremely significantly increased iron content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ). Compared with Group III, Groups IV, V, and VI showed significantly or extremely significantly decreased liver iron content ( $P < 0.05$  or  $P < 0.01$ ).

In conclusion, *Bacillus subtilis*-zinc can promote the growth and development of congenital zinc deficiency rats, improve liver index, heart index, and nutrient utilization, regulate trace element distribution, demonstrate superior effects compared to  $ZnSO_4$ , and reduce dietary zinc supplementation levels.

**Keywords:** *Bacillus subtilis*-zinc; congenital zinc deficiency; growth and development; nutrient utilization; trace elements

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## Introduction

Zinc serves as a catalyst for numerous enzymes in the human body and participates importantly in cell division, playing a crucial role in the growth and development of animals, pregnant women, and infants, particularly for pregnant women and babies. Zinc deficiency during pregnancy can lead to miscarriage, intrauterine growth retardation, malformation, and even stillbirth. Infants with congenital zinc deficiency may exhibit anorexia, 注意力不集中 (attention deficits), emaciation, and suffer from growth retardation, reduced intelligence, and compromised immunity. Wang Decai et al. found that zinc-deficient or high-zinc rats had lower body weight and feed efficiency than normal controls, affecting related organ indices. Wedekind et al. and Wang Daigang demonstrated that dietary zinc supplementation significantly affected zinc content in tissues and organs and serum alkaline phosphatase (AKP) activity. Additionally, Cui Zhiying et al. reported that animals showed higher bioavailability of organic zinc than inorganic zinc.

Current research on microbial enrichment of trace metal elements primarily focuses on yeast adsorption of metal ions such as copper ( $\text{Cu}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), and cadmium ( $\text{Cd}^{2+}$ ) from the environment. Zhao Jiaying et al. showed that *Bacillus subtilis* can enrich zinc ions and convert inorganic zinc to organic zinc. As a novel zinc supplement additive, *Bacillus subtilis*-zinc has been relatively understudied both domestically and internationally, and research on its intervention in congenital zinc deficiency rats remains unexplored. This study used congenital zinc deficiency rats as subjects to investigate the effects of *Bacillus subtilis*-zinc on growth performance, organ index, nutrient utilization, and zinc, copper, and iron content in organs, aiming to determine its efficacy and the feasibility of reducing zinc supplementation levels.

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### 1.1 Experimental Materials

*Bacillus subtilis*-zinc was provided by the National Waterfowl Industrial Technology System Nutrition and Feed Function Laboratory, containing  $3 \times 10^8$  CFU/g viable *Bacillus subtilis* and 2,511.55 mg/kg zinc content. Food-grade zinc sulfate ( $\text{ZnSO}_4$ ) was purchased from Hubei Jinmingzhou Chemical Co., Ltd. (analytical grade,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  content 99.5%). AKP, metallothionein (MT), and copper-zinc superoxide dismutase (Cu-Zn SOD) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

### 1.2 Main Instruments

Equipment included a high-speed large-capacity refrigerated centrifuge (Xiangyi Centrifuge Instrument Co., Ltd.), ultra-low temperature freezer

(DW-86L388J, Qingdao Haier Special Electric Appliance Co., Ltd.), super constant temperature water bath (DKB-501, Shanghai Jinghong Experimental Equipment Co., Ltd.), automatic microplate reader (Multiskan MK3), electronic balance (AR1140, Ohaus International Trading Co., Ltd.), automatic calorimeter (WZR-1T), inductively coupled plasma emission spectrometer (ICP, PerkinElmer, USA), vacuum dryer (FD-1A-50, Beijing Boyikang Experimental Instrument Co., Ltd.), Kjeldahl nitrogen analyzer (FOSS TECATOR), fat analyzer (SZC-C, Shanghai Fiber Inspection Instrument Co., Ltd.), and fiber analyzer (ANKOM TECHNOLOGY).

### 1.3 Experimental Diets

The basal diet was formulated using corn starch, soybean protein isolate, sucrose, and other ingredients. Analysis revealed the basal diet contained 13.00 mg/kg zinc, qualifying as a zinc-deficient diet. The normal diet was prepared by adding ZnSO to the basal diet to achieve 38.00 mg/kg zinc. The composition and nutrient levels of the basal diet are shown in Table 2 .

**Table 2 Composition and nutrient levels of the basal diet (air-dry basis)**

Items	Content
<b>Ingredients</b>	
Corn starch	
Soybean-extracted protein	
Cellulose	
Casein	
Soybean oil	
CaCO	
CaHPO	
DL-Met	
Choline	
Cane sugar	
NaCl	
Trace elements <sup>1)</sup>	
Multivitamin <sup>2)</sup>	
<b>Total</b>	
<b>Nutrient levels<sup>3)</sup></b>	
ME/(MJ/kg)	
CP	
CF	
EE	
AP	
Zinc/(mg/kg)	

<sup>1)</sup> Trace elements (without zinc) provided per kg of diet: MgO 272.7273 g, FeSO<sub>4</sub> · 7H<sub>2</sub>O 25 g, K<sub>2</sub>SO<sub>4</sub> 612.8817 g, MnSO<sub>4</sub> · H<sub>2</sub>O 16.95 g, KI 0.07 g, NaSeO<sub>3</sub> 0.0222 g, CuSO<sub>4</sub> · 5H<sub>2</sub>O 2 g, CaCO<sub>3</sub> 70.36 g.

<sup>2)</sup> Multivitamin provided per kg of diet: VA 14,000 IU, VD 1,500 IU, VE 120 IU, VK 5 mg, VB<sub>1</sub> 12 mg, VB<sub>2</sub> 12 mg, nicotinic acid 60 mg, VB<sub>6</sub> 12 mg, biotin 0.2 mg, pantothenate 24 mg, folic acid 6 mg.

<sup>3)</sup> Zinc and ME were measured values, while other nutrient levels were calculated values.

## 1.4 Experimental Design

Healthy SD rats were used in two experiments: establishment of a congenital zinc deficiency model in juvenile rats and postnatal intervention in zinc-deficient rats.

**1.4.1 Establishment of Congenital Zinc Deficiency Model in Juvenile Rats** Seventy-two pregnant female SD rats were selected and divided into two groups: model group and control group, with 6 replicates per group and 6 rats per replicate. The trial began on day 10 of pregnancy, with the model group receiving the zinc-deficient diet and the control group receiving the normal diet throughout pregnancy and lactation.

After model establishment, 12 juvenile rats from each group were fasted for 12 hours, then serum and liver samples were collected. Growth indices, liver zinc content, and serum biochemical markers were measured to verify successful model establishment.

**1.4.2 Postnatal Intervention in Zinc-Deficient Rats** Following successful modeling, eighteen 24-day-old normal juvenile rats from the control group were selected as the normal group (Group I) and fed the normal diet. Additionally, ninety 24-day-old congenital zinc-deficient juvenile rats from the model group were randomly divided into five experimental groups: zinc deficiency group (Group II), ZnSO<sub>4</sub> group (Group III), and low- (Group IV), medium- (Group V), and high-dose Bacillus subtilis-zinc groups (Group VI), all fed zinc-deficient diets. Each group comprised 3 replicates with 6 rats per replicate. Zinc sources were administered via gavage: Groups I and II received deionized water, Group III received ZnSO<sub>4</sub> solution, and Groups IV, V, and VI received Bacillus subtilis-zinc (diluted with deionized water) at 0.2 mL per 10 g body weight daily. The trial lasted 5 weeks. Group feeding details during the intervention period are shown in Table 1.

**Table 1 Group feeding situation during the trial intervention**

Groups	Diet type	Zinc level (mg/kg)	Gavage type
Normal group (I)	Normal diet	13.00	Deionized water

Groups	Diet type	Zinc level (mg/kg)	Gavage type
Zinc deficiency group (II)	Zinc-deficient diet	13.00	Deionized water
ZnSO <sub>4</sub> group (III)	Zinc-deficient diet	38.00	ZnSO <sub>4</sub> solution
Low-dose Bacillus subtilis-zinc group (IV)	Zinc-deficient diet	15.00	Low-dose Bacillus subtilis-zinc
Mid-dose Bacillus subtilis-zinc group (V)	Zinc-deficient diet	30.00	Mid-dose Bacillus subtilis-zinc
High-dose Bacillus subtilis-zinc group (VI)	Zinc-deficient diet	45.00	High-dose Bacillus subtilis-zinc

### 1.5 Feeding Management

During the trial, rats had ad libitum access to feed and deionized water. The environment was maintained at  $(22\pm 2)^{\circ}\text{C}$  with  $(55\pm 5)\%$  relative humidity and good ventilation. Daily observations recorded mental status, activity, feed intake, and water consumption, with body weight measured weekly.

### 1.6 Animal Slaughter and Sampling

After 5 weeks, rats were fasted for 12 hours, then weighed and measured for body length. Following ether anesthesia, blood was collected via orbital puncture and centrifuged at 3,000 r/min for 15 minutes; serum was stored at  $-80^{\circ}\text{C}$ . After euthanasia, body length was measured and organs (heart, liver, spleen, kidney, etc.) were isolated, washed in cold physiological saline, dried with filter paper, and weighed.

### 1.7 Measurement Indicators

**1.7.1 Growth Performance Indices** Rats were fasted (water available) and weighed at 08:00 on the final day of each week. At trial completion, BW, ADG, ADFI, and feed-to-gain ratio (F/G) were calculated. After dissection, heart, liver, spleen, kidney, and other organs were washed with physiological saline and weighed wet to calculate organ index:

**Organ index (%) = 100 × organ wet weight (g) / body weight (g)**

**1.7.2 Nutrient Utilization Measurement** Test diets were ground through a 40-mesh sieve, mixed uniformly, and stored at low temperature after drying. Feces were dried at 65-75°C in an oven, conditioned for 24 hours at room temperature to prepare air-dry samples, then pulverized using a small universal grinder.

Nitrogen and CP content were determined by Kjeldahl nitrogen analyzer. EE content was measured by ether extraction. CF, NDF, and ADF contents were determined by acid-detergent fiber method. Zinc and calcium contents were measured by inductively coupled plasma emission spectrometer (ICP).

Nutrient utilization formulas: - Nitrogen utilization (%) = [(feed nitrogen content - excreta nitrogen content) / feed nitrogen content] × 100 - CP utilization (%) = [(feed CP content - excreta CP content + endogenous CP content) / feed CP content] × 100 - EE utilization (%) = [(feed EE content - excreta EE content) / feed EE content] × 100 - CF utilization (%) = [(feed CF content - excreta CF content) / feed CF content] × 100 - NDF utilization (%) = [(feed NDF content - excreta NDF content) / feed NDF content] × 100 - ADF utilization (%) = [(feed ADF content - excreta ADF content) / feed ADF content] × 100 - Zinc utilization (%) = [(feed zinc content - excreta zinc content) / feed zinc content] × 100 - Calcium utilization (%) = [(feed calcium content - excreta calcium content) / feed calcium content] × 100

### **1.7.3 Determination of Zinc, Iron, and Copper Content in Organs**

Organ tissues were dried to constant weight and digested by wet method: 0.5 g of organ tissue was accurately weighed into a triangular flask, moistened with a small amount of ultrapure water, then 10 mL nitric acid and 2 mL perchloric acid were added, mixed, covered with a watch glass, and left overnight. The mixture was heated on an adjustable electric furnace until nearly dry, then transferred without loss to a 100 mL volumetric flask, diluted to volume with ultrapure water, mixed, and stored for analysis with blank correction.

### **1.8 Statistical Analysis**

Data were analyzed using one-way ANOVA (LSD) and t-test methods in SPSS 17.0 software. Results are expressed as means with standard error of the mean (SEM). P < 0.05 and P < 0.01 were considered significant and extremely significant differences, respectively.

## Results

### 2.1 Establishment of Zinc Deficiency Model in Juvenile Rats

As shown in Table 3 , after zinc-deficient diet intervention, the model group exhibited significantly lower body weight and body length than the control group ( $P < 0.05$ ), along with significantly reduced liver zinc content, serum MT content, and AKP and Cu-Zn SOD activities ( $P < 0.05$ ). These results confirmed successful establishment of the juvenile rat zinc deficiency model.

**Table 3 Effects of low-zinc diet on growth and blood parameters of young rats**

Items	Control group	Model group	P-value
Body weight (BW, g)	51.62	39.54	<0.001
Body length (BL, cm)	20.30	18.36	<0.001
Liver zinc content (mg/kg)	42.98	32.65	<0.001
AKP (King' s units/dL)	50.86	38.41	<0.001
MT (ng/mL)	10.12	7.48	<0.001
Cu-Zn SOD (U/mL)	203.67	170.67	<0.001

In the same row, values with the same small or no letter superscripts mean no significant difference ( $P > 0.05$ ), while adjacent small letter superscripts indicate significant difference ( $P < 0.05$ ), and alternate small letter superscripts indicate extremely significant difference ( $P < 0.01$ ). The same as below.

### 2.2 Effects of Bacillus subtilis-Zinc on Growth and Development of Congenital Zinc Deficiency Rats

**2.2.1 Effects on Growth Performance** As shown in Table 4 , compared with Group I (normal group), Group II (zinc deficiency group) showed extremely significantly decreased body weight ( $P < 0.01$ ) and significantly decreased ADFI ( $P < 0.05$ ), with no significant differences in F/G or ADG ( $P > 0.05$ ). Compared with Group II, Groups IV, V, and VI (low-, medium-, and high-dose Bacillus subtilis-zinc) showed no significant differences in F/G ( $P > 0.05$ ). Group IV exhibited significantly increased body weight and ADFI ( $P < 0.05$ ) with no significant ADG difference ( $P > 0.05$ ). Groups V and VI showed extremely significantly increased body weight and ADFI ( $P < 0.01$ ) and significantly increased ADG ( $P < 0.05$ ). Compared with Group III (ZnSO<sub>4</sub> group), Groups IV, V, and VI showed no significant F/G differences ( $P > 0.05$ ). Group IV had significantly decreased body weight and ADFI ( $P < 0.05$ ) with no significant ADG difference ( $P > 0.05$ ). Groups V and VI showed significantly increased body weight and ADG ( $P < 0.05$ ), with Group V also demonstrating significantly increased ADFI ( $P < 0.05$ ) while Group VI showed no significant ADFI difference ( $P > 0.05$ ).

These results indicate that congenital zinc deficiency significantly reduces feed intake, slows growth and development, and decreases body weight. *Bacillus subtilis*-zinc promotes growth and development in zinc-deficient rats, with medium and high doses showing superior effects compared to ZnSO<sub>4</sub>.

**Table 4 Effects of *Bacillus subtilis*-zinc on growth performance of congenital zinc deficiency rats**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Body weight (BW, g)	146.16	132.73	144.69	140.52	155.72	153.87	<0.001	<0.001	
ADG (g)	3.18	3.07	3.20	3.16	3.74	3.68	<0.001		
ADFI (g)	9.04	8.60	9.09	8.91	9.53	9.17	<0.001	<0.001	
F/G									

**2.2.2 Effects on Organ Index** As shown in Table 5, compared with Group I, Group II showed no significant differences in heart, spleen, kidney, or lung indices ( $P > 0.05$ ) but significantly decreased liver index ( $P < 0.05$ ). Compared with Group II, Groups IV, V, and VI showed no significant differences in spleen, kidney, or lung indices ( $P > 0.05$ ) but significantly increased liver index ( $P < 0.05$ ). Group VI showed significantly increased heart index ( $P < 0.05$ ), Group V showed extremely significantly increased heart index ( $P < 0.01$ ), and Group IV showed no significant heart index difference ( $P > 0.05$ ). Compared with Group III, Groups IV, V, and VI showed no significant differences in liver, spleen, or lung indices ( $P > 0.05$ ). Group VI showed significantly increased heart index ( $P < 0.05$ ) with no significant kidney index difference ( $P > 0.05$ ). Group V exhibited extremely significantly increased heart index ( $P < 0.01$ ) and significantly increased kidney index ( $P < 0.05$ ). Group IV showed no significant heart index difference ( $P > 0.05$ ) but significantly increased kidney index ( $P < 0.05$ ).

These results demonstrate that congenital zinc deficiency significantly affects liver development. *Bacillus subtilis*-zinc had no significant effect on spleen, kidney, or lung development but significantly promoted liver development, with medium and high doses significantly promoting heart development. Medium and high doses showed superior heart repair effects compared to ZnSO<sub>4</sub>, while low and medium doses showed superior kidney repair effects.

**Table 5 Effects of *Bacillus subtilis*-zinc on organ index of congenital zinc deficiency rats (%)**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Heart in-dex	0.45	0.43	0.44	0.43	0.54	0.49	<0.001		
Liver in-dex	3.03	2.85	3.07	3.14	3.12	3.02			
Spleen in-dex									
Kidney in-dex	0.87	0.90	0.87	0.98	0.97	0.93			
Lung in-dex	0.80	0.76	0.76	0.73	0.82	0.78			

### 2.3 Effects of Bacillus subtilis-Zinc on Nutrient Utilization in Congenital Zinc Deficiency Rats

As shown in Table 6, compared with Group I, Group II showed extremely significantly decreased utilization of CP, EE, CF, NDF, ADF, and calcium ( $P < 0.01$ ) and significantly decreased zinc utilization ( $P < 0.05$ ). Compared with Group II, Groups IV, V, and VI showed extremely significantly increased EE and zinc utilization ( $P < 0.01$ ). Groups V and VI exhibited extremely significantly increased CF, NDF, and calcium utilization ( $P < 0.01$ ), while Group IV showed significantly increased CF, NDF, and calcium utilization ( $P < 0.05$ ). Group IV showed no significant difference in CP or ADF utilization ( $P > 0.05$ ), Group V showed extremely significantly increased CP and ADF utilization ( $P < 0.01$ ), and Group VI showed significantly increased CP and ADF utilization ( $P < 0.05$ ). Compared with Group III, Groups IV, V, and VI showed no significant difference in CP utilization ( $P > 0.05$ ). Group IV showed significantly increased EE utilization ( $P < 0.05$ ) but significantly decreased NDF and calcium utilization ( $P < 0.05$ ), with no significant differences in CF, ADF, or zinc utilization ( $P > 0.05$ ). Group V exhibited extremely significantly increased utilization of EE, CF, NDF, ADF, zinc, and calcium ( $P < 0.01$ ). Group VI showed extremely significantly increased EE and CF utilization ( $P < 0.01$ ), significantly increased NDF, ADF, and zinc utilization ( $P < 0.05$ ), with no significant calcium utilization difference ( $P > 0.05$ ).

These findings indicate that congenital zinc deficiency severely impairs nutrient utilization. Low-dose Bacillus subtilis-zinc significantly increased EE, CF, NDF, zinc, and calcium utilization, while medium and high doses significantly improved utilization of EE, CP, ADF, CF, NDF, zinc, and calcium. Low-dose

Bacillus subtilis-zinc produced higher EE, NDF, and calcium utilization than the ZnSO group, while medium and high doses yielded higher EE, CF, NDF, ADF, zinc, and calcium utilization than ZnSO .

**Table 6 Effects of Bacillus subtilis-zinc on nutrient utilization of congenital zinc deficiency rats (%)**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
CP	0.52	0.47	0.50	0.48	0.51	0.50			
EE	48.05	43.29	44.89	46.90	51.02	51.83	<0.001	<0.001	
CF	19.23	14.58	17.18	17.43	20.49	21.24	<0.001	<0.001	
NDF	47.81	44.46	47.92	46.44	55.58	49.50	<0.001		
ADF	58.22	56.30	56.07	56.96	58.18	57.93			
Zinc	50.06	47.85	50.98	50.96	53.65	52.17	<0.001	<0.001	
Calcium	48.63	43.72	48.08	45.44	52.41	48.45	<0.001		

#### 2.4 Effects of Bacillus subtilis-Zinc on Nitrogen Utilization in Congenital Zinc Deficiency Rats

As shown in Table 7 , compared with Group I, Group II showed extremely significantly decreased nitrogen intake and nitrogen utilization ( $P < 0.01$ ) and significantly decreased deposited nitrogen ( $P < 0.05$ ) with significantly increased fecal nitrogen ( $P < 0.05$ ). Compared with Group II, Groups IV, V, and VI showed no significant differences in fecal nitrogen ( $P > 0.05$ ). Group IV showed significantly increased nitrogen intake ( $P < 0.05$ ) with no significant differences in deposited nitrogen or nitrogen utilization ( $P > 0.05$ ). Group V exhibited extremely significantly increased nitrogen intake, deposited nitrogen, and nitrogen utilization ( $P < 0.01$ ). Group VI showed extremely significantly increased nitrogen intake ( $P < 0.01$ ) and significantly increased deposited nitrogen and nitrogen utilization ( $P < 0.05$ ). Compared with Group III, Groups IV, V, and VI showed no significant differences in fecal nitrogen or nitrogen utilization ( $P > 0.05$ ). Group IV showed significantly decreased nitrogen intake and deposited nitrogen ( $P < 0.05$ ), while Group V exhibited significantly increased nitrogen intake and deposited nitrogen ( $P < 0.05$ ). Group VI showed no significant differences in nitrogen intake or deposited nitrogen ( $P > 0.05$ ).

These results demonstrate that congenital zinc deficiency reduces nitrogen utilization and increases fecal nitrogen excretion. Bacillus subtilis-zinc promotes nitrogen intake, reduces nitrogen excretion, and increases nitrogen deposition, thereby improving nitrogen utilization. Low and medium doses enhanced nitrogen intake, deposition, and utilization more effectively than ZnSO .

**Table 7 Effects of Bacillus subtilis-zinc on nitrogen utilization of congenital zinc deficiency rats**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Nitrogen intake (g/d)	2.01	2.86	3.02	2.96	3.17	3.05	<0.001		
Nitrogen excretion (g/d)	1.44	1.52	1.50	1.42	1.54	1.52			
Nitrogen deposition (g/d)	1.57	1.34	1.52	1.54	1.62	1.52			
Nitrogen utilization (%)	0.52	0.47	0.50	0.48	0.51	0.50			

## 2.5 Effects of Bacillus subtilis-Zinc on Trace Element Content in Organs of Congenital Zinc Deficiency Rats

**2.5.1 Effects on Zinc Content in Organs** As shown in Table 8, compared with Group I, Group II showed significantly decreased zinc content in heart and kidney ( $P < 0.05$ ) and extremely significantly decreased liver zinc content ( $P < 0.01$ ), with no significant brain zinc difference ( $P > 0.05$ ). Compared with Group II, Group IV showed no significant differences in heart, liver, brain, or kidney zinc content ( $P > 0.05$ ). Group V exhibited significantly increased zinc content in heart, brain, and kidney ( $P < 0.05$ ) and extremely significantly increased liver zinc content ( $P < 0.01$ ). Group VI showed significantly increased zinc content in heart, liver, and kidney ( $P < 0.05$ ) with no significant brain zinc difference ( $P > 0.05$ ). Compared with Group III, Groups IV, V, and VI showed no significant brain zinc differences ( $P > 0.05$ ). Groups V and VI showed no significant heart or kidney zinc differences ( $P > 0.05$ ), while Group IV exhibited significantly decreased heart and kidney zinc content ( $P < 0.05$ ). Group IV showed extremely significantly decreased liver zinc content ( $P < 0.01$ ), Group V showed significantly increased liver zinc content ( $P < 0.05$ ), and Group VI showed significantly decreased liver zinc content ( $P < 0.05$ ).

These results indicate that congenital zinc deficiency affects zinc deposition in liver, heart, and kidney. Medium-dose Bacillus subtilis-zinc rapidly increased zinc content in heart, brain, kidney, and liver, with comparable efficacy to ZnSO

but at reduced zinc supplementation levels.

**Table 8 Effects of Bacillus subtilis-zinc on organ zinc content of congenital zinc deficiency rats (mg/kg)**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Heart	27.02	20.98	26.68	21.89	26.58	26.24	<0.001		
Liver	37.01	31.29	36.29	31.77	37.24	34.13	<0.001		
Brain	23.44	21.66	23.11	22.24	23.58	22.79			
Kidney	34.65	30.58	33.94	31.35	34.17	34.24	<0.001		

**2.5.2 Effects on Copper Content in Organs** As shown in Table 9 , compared with Group I, Group II showed significantly decreased copper content in heart, brain, and kidney ( $P < 0.05$ ) but extremely significantly increased liver copper content ( $P < 0.01$ ). Compared with Group II, Group IV showed significantly increased heart copper content ( $P < 0.05$ ) and significantly decreased liver copper content ( $P < 0.05$ ), with no significant brain or kidney copper differences ( $P > 0.05$ ). Group V exhibited extremely significantly increased heart copper content ( $P < 0.01$ ), extremely significantly decreased liver copper content ( $P < 0.01$ ), and significantly increased brain and kidney copper content ( $P < 0.05$ ). Group VI showed significantly increased heart copper content ( $P < 0.05$ ), extremely significantly decreased liver copper content ( $P < 0.01$ ), with no significant brain or kidney copper differences ( $P > 0.05$ ). Compared with Group III, Groups IV, V, and VI showed no significant kidney copper differences ( $P > 0.05$ ). Groups IV and VI showed no significant heart copper differences ( $P > 0.05$ ) but significantly decreased brain copper content ( $P < 0.05$ ). Group V showed significantly increased heart copper content ( $P < 0.05$ ) with no significant brain copper difference ( $P > 0.05$ ). Group IV showed significantly increased liver copper content ( $P < 0.05$ ), while Groups V and VI showed no significant liver copper differences ( $P > 0.05$ ).

These findings demonstrate that congenital zinc deficiency reduces copper deposition in heart, brain, kidney, and liver. Low-dose Bacillus subtilis-zinc promoted copper deposition in heart while reducing liver copper accumulation. Medium-dose Bacillus subtilis-zinc promoted copper deposition in brain and kidney. Bacillus subtilis-zinc showed superior repair effects on heart copper content compared to ZnSO .

**Table 9 Effects of Bacillus subtilis-zinc on organ copper content of congenital zinc deficiency rats (mg/kg)**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Heart	3.67	3.50	3.68	3.71	3.79	3.69	<0.001		

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Liver	3.14	4.46	3.14	4.13	3.24	3.10	<0.001	<0.001	
Brain	2.34	2.02	2.37	2.07	2.51	2.01			
Kidney	5.23	5.17	5.21	5.16	5.24	5.22			

**2.5.3 Effects on Iron Content in Organs** As shown in Table 10, compared with Group I, Group II showed significantly decreased heart iron content ( $P < 0.05$ ) and extremely significantly decreased liver and kidney iron content ( $P < 0.01$ ), with no significant brain iron difference ( $P > 0.05$ ). Compared with Group II, Groups IV, V, and VI showed significantly or extremely significantly increased iron content in heart, liver, and kidney ( $P < 0.05$  or  $P < 0.01$ ), with no significant brain iron differences ( $P > 0.05$ ). Compared with Group III, Groups IV, V, and VI showed no significant brain iron differences ( $P > 0.05$ ) but significantly or extremely significantly decreased liver iron content ( $P < 0.05$  or  $P < 0.01$ ). Groups V and VI showed significantly increased kidney iron content ( $P < 0.05$ ), while Group IV showed significantly decreased kidney iron content ( $P < 0.05$ ). Groups IV and VI showed no significant heart iron differences ( $P > 0.05$ ), while Group V exhibited significantly increased heart iron content ( $P < 0.05$ ).

These results indicate that congenital zinc deficiency affects iron deposition in heart, liver, and kidney. *Bacillus subtilis*-zinc rapidly replenished iron content in these organs by increasing iron absorption, with superior repair effects on liver and kidney iron content compared to ZnSO.

**Table 10 Effects of *Bacillus subtilis*-zinc on organ iron content of congenital zinc deficiency rats (mg/kg)**

Items	Group I	Group II	Group III	Group IV	Group V	Group VI	P-value	Linear	Quadratic
Heart	120.89	115.30	121.34	122.29	124.07	122.60	<0.001		
Liver	206.88	168.16	198.21	170.57	182.42	183.25	<0.001	<0.001	
Brain	32.89	31.94	31.64	31.88	31.78	31.82			
Kidney	124.46	109.14	120.46	111.50	123.41	123.69	<0.001	<0.001	

## Discussion

### 3.1 Effects of *Bacillus subtilis*-Zinc on Growth and Development of Congenital Zinc Deficiency Rats

Doboszewska et al. and Shah et al. reported that zinc deficiency during pregnancy reduces maternal immunity and feed intake, affecting fetal growth and

development and decreasing metabolism. Doboszewska et al. and Fukada et al. demonstrated that zinc deficiency causes slow growth in body weight, body length, and tibia length, reduced feed intake, and decreased feed efficiency in growing rats. This study found that congenital zinc deficiency significantly reduced feed intake, affected heart and liver development, slowed growth, and decreased body weight. Zhang Qing reported that combined zinc sources significantly increased thymus, spleen, and pancreas indices in piglets. Wang Daigang found that yeast zinc increased ADFI and ADG while decreasing F/G in zinc-deficient rats. Under our experimental conditions, *Bacillus subtilis*-zinc groups showed higher body weight, ADG, ADFI, and heart and liver indices than the zinc deficiency group, with medium and high doses demonstrating superior heart repair effects compared to ZnSO<sub>4</sub>, and low and medium doses showing superior kidney repair effects. This indicates that *Bacillus subtilis*-zinc at appropriate concentrations can promote growth and development in zinc-deficient rats with better efficacy than ZnSO<sub>4</sub>.

### **3.2 Effects of *Bacillus subtilis*-Zinc on Nutrient Utilization in Congenital Zinc Deficiency Rats**

Murugesan et al. and Feng Dingyuan et al. reported that good growth performance depends on adequate digestion, absorption, and utilization of dietary nutrients. Liu Yingli demonstrated that yeast zinc had significantly higher bioavailability than zinc sulfate. Guo Jianlai et al. found that organic zinc groups significantly improved dry matter, calcium, and phosphorus utilization in piglets compared to inorganic zinc, and extremely significantly improved crude ash utilization. This study found that *Bacillus subtilis*-zinc rapidly replenished zinc and increased nutrient utilization, consistent with its improvement of growth performance. Additionally, *Bacillus subtilis*-zinc groups showed increased nitrogen and other nutrient utilization with reduced excretion, decreasing environmental pollution and demonstrating important research significance as an eco-friendly feed additive.

### **3.3 Effects of *Bacillus subtilis*-Zinc on Trace Element Content in Organs of Congenital Zinc Deficiency Rats**

Zinc deficiency not only reduces zinc levels but also affects transport and bioavailability of other trace elements. Yuan Xiuqin et al. found that zinc deficiency significantly decreased zinc and iron content in rat serum, liver, spleen, and kidney, and reduced copper content in kidney and spleen. Zhao Changfeng et al. and Zhao et al. reported that zinc-deficient pregnant rats had significantly lower zinc and other trace element levels in tissues than supplemented and control groups. Kong Lin found no significant difference between yeast zinc and zinc sulfate on mouse growth performance and tissue zinc content under adequate zinc conditions. This study found that *Bacillus subtilis*-zinc regulated trace element content in heart, liver, brain, and kidney of congenital zinc deficiency rats. Medium-dose *Bacillus subtilis*-zinc promoted

trace element absorption, possibly due to its dual functions: zinc supplementation and promotion of nutrient digestion and absorption, increasing feed intake and improving utilization of copper, zinc, and iron, thereby increasing deposition in tissues and organs. High-dose *Bacillus subtilis*-zinc showed lower efficacy than medium dose, indicating that zinc requirements have a threshold range, and excessive zinc levels are not conducive to nutrient digestion and absorption.

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## Conclusion

1. Dietary supplementation with *Bacillus subtilis*-zinc significantly increased feed intake, improved liver and heart indices, enhanced nutrient utilization, promoted growth and development, and regulated trace element distribution in congenital zinc deficiency rats.
2. *Bacillus subtilis*-zinc demonstrated superior postnatal intervention and repair effects compared to ZnSO<sub>4</sub>.
3. The medium-dose *Bacillus subtilis*-zinc group showed better repair effects than the ZnSO<sub>4</sub> group while reducing dietary zinc supplementation levels.

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