

## Effects of *Enterococcus faecium* SF68 on Growth Performance, Hematological Parameters, Serum Biochemical Indices, and Antioxidant Capacity of Weaned Piglets (Postprint)

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### Abstract

This experiment aimed to investigate the effects of dietary *Enterococcus faecium* SF68 supplementation on growth performance, hematological parameters, serum biochemical indices, and antioxidant capacity in weaned piglets. Ninety castrated weaned piglets of Duroc × Landrace × Yorkshire crossbreeds with similar body weight [(6.57±0.08) kg] at 21 days of age were selected and randomly divided into four groups, with three replicates per group and six piglets per replicate (half male and half female). The control group (CON) was fed the basal diet, the antibiotic group (ANT) received the basal diet supplemented with 0.0075% chlortetracycline and 0.02% colistin sulfate, the antibiotic plus low-dose *Enterococcus faecium* group (ALEF) received the basal diet supplemented with 0.0075% chlortetracycline, 0.02% colistin sulfate, and 0.01% *Enterococcus faecium* SF68, the low-dose *Enterococcus faecium* group (LEF) received the basal diet supplemented with 0.01% *Enterococcus faecium* SF68, and the high-dose *Enterococcus faecium* group (HEF) received the basal diet supplemented with 0.05% *Enterococcus faecium* SF68. The experimental period lasted 21 days. The results showed that: 1) There were no significant differences in average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G), or diarrhea index among groups ( $P>0.05$ ). The diarrhea index in the ALEF and LEF groups was reduced by 65% and 67%, respectively, compared with the CON group. 2) The blood mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and lymphocyte percentage in the LEF group were significantly or extremely significantly higher than those in the CON group ( $P<0.05$  or  $P<0.01$ ). 3) The serum total bile acid (TBA) content in the ALEF group was significantly lower than that in the HEF group ( $P<0.05$ ), the serum lactate dehydrogenase (LDH) activity in the ALEF group was significantly higher than that in the HEF group ( $P<0.05$ ), and the serum cholinesterase (CHE)

activity in the LEF group was significantly higher than that in the CON and HEF groups ( $P < 0.05$ ). 4) The serum hydrogen peroxide ( $H_2O_2$ ) content in the ALEF and LEF groups was significantly or extremely significantly higher than that in the CON, ANT, and HEF groups ( $P < 0.05$  or  $P < 0.01$ ), and the hepatic superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) in the ALEF and HEF groups were significantly higher than those in the CON group ( $P < 0.05$ ). The jejunal  $H_2O_2$  content in the ANT, ALEF, LEF, and HEF groups was significantly or extremely significantly lower than that in the CON group ( $P < 0.05$  or  $P < 0.01$ ), and the jejunal SOD activity in the ALEF group was significantly higher than that in the ANT, LEF, and HEF groups ( $P < 0.05$ ). The ileal SOD activity in the ANT, ALEF, LEF, and HEF groups was significantly or extremely significantly higher than that in the CON group ( $P < 0.05$  or  $P < 0.01$ ), while the malondialdehyde (MDA) content was significantly or extremely significantly lower than that in the CON group ( $P < 0.05$  or  $P < 0.01$ ). These results indicate that dietary supplementation with *Enterococcus faecium* SF68 can improve the growth performance of weaned piglets, reduce diarrhea, improve blood physiological and biochemical indices, enhance oxygen transport capacity and immunity, regulate lipid and energy metabolism, and enhance antioxidant capacity, with the LEF group showing better effects than the HEF group; however, the combined supplementation of antibiotics and *Enterococcus faecium* SF68 was more effective than *Enterococcus faecium* supplementation alone.

## Full Text

### Effects of *Enterococcus faecium* SF68 on Growth Performance, Blood Routine Parameters, Serum Biochemical Indices, and Antioxidant Capacity of Weaned Piglets

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## Abstract

This experiment was conducted to investigate the effects of dietary *Enterococcus faecium* SF68 supplementation on growth performance, blood routine parameters, serum biochemical indices, and antioxidant capacity of weaned piglets. Ninety castrated “Duroc × Landrace × Yorkshire” crossbred weaned piglets with similar body weight [(6.57±0.08) kg] at 21 days of age were randomly assigned to 5 groups with 3 replicates per group and 6 pigs per replicate (half male and half female). The control group (CON) was fed a basal diet, the antibiotic group (ANT) received the basal diet supplemented with 0.0075% chlortetracycline and 0.02% colistin sulfate, the antibiotic + low-dose *Enterococcus faecium* group (ALEF) received the basal diet supplemented with 0.0075% chlortetracycline, 0.02% colistin sulfate, and 0.01% *Enterococcus faecium* SF68, the low-dose

Enterococcus faecium group (LEF) received the basal diet supplemented with 0.01% Enterococcus faecium SF68, and the high-dose Enterococcus faecium group (HEF) received the basal diet supplemented with 0.05% Enterococcus faecium SF68. The experimental period lasted 21 days.

The results showed: 1) No significant differences were observed in average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G), or diarrhea index among all groups ( $P>0.05$ ). However, the diarrhea index in ALEF and LEF groups decreased by 65% and 67%, respectively, compared with the CON group. 2) The mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and lymphocyte percentage in blood of LEF group were significantly or extremely significantly higher than those of CON group ( $P<0.05$  or  $P<0.01$ ). 3) Serum total bile acid (TBA) content in ALEF group was significantly lower than that in HEF group ( $P<0.05$ ), serum lactate dehydrogenase (LDH) activity in ALEF group was significantly higher than that in HEF group ( $P<0.05$ ), and serum cholinesterase (CHE) activity in LEF group was significantly higher than that in CON and HEF groups ( $P<0.05$ ). 4) Serum hydrogen peroxide ( $H_2O_2$ ) content in ALEF and LEF groups was significantly or extremely significantly higher than that in CON, ANT, and HEF groups ( $P<0.05$  or  $P<0.01$ ). Superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) in liver of ALEF and HEF groups were significantly higher than those of CON group ( $P<0.05$ ). Jejunal  $H_2O_2$  content in ANT, ALEF, LEF, and HEF groups was significantly or extremely significantly lower than that in CON group ( $P<0.05$  or  $P<0.01$ ), while jejunal SOD activity in ALEF group was significantly higher than that in ANT, LEF, and HEF groups ( $P<0.05$ ). Ileum SOD activity in ANT, ALEF, LEF, and HEF groups was significantly or extremely significantly higher than that in CON group ( $P<0.05$  or  $P<0.01$ ), and ileum malondialdehyde (MDA) content in these groups was significantly or extremely significantly lower than that in CON group ( $P<0.05$  or  $P<0.01$ ).

These results indicate that dietary supplementation with Enterococcus faecium SF68 can improve growth performance, reduce diarrhea, enhance blood physiological and biochemical parameters, increase oxygen transport capacity and immunity, regulate lipid and energy metabolism, and enhance antioxidant capacity in weaned piglets. The LEF group showed better effects than the HEF group, while the combination of antibiotics and low-dose Enterococcus faecium SF68 was more effective than Enterococcus faecium SF68 alone.

**Keywords:** Enterococcus faecium; weaned piglets; growth performance; blood routine parameters; serum biochemical indices; antioxidant capacity

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## Introduction

Antibiotics have played an indispensable role in disease prevention and treatment in piglet production. However, their extensive and widespread use, even

abuse, in piglet farming has brought serious adverse consequences to humans. Unscientific application of antibiotics can lead to antimicrobial resistance in pathogenic bacteria, drug residues, environmental pollution, and may even induce cancer and deformities in humans and animals, posing threats to human health and safety [1-2]. Therefore, searching for new antibiotic alternatives and developing green animal husbandry for producing green animal food has become a research hotspot in animal nutrition worldwide.

Probiotics are biological preparations containing live bacteria that have attracted considerable attention due to their advantages of safety, no residues, no antimicrobial resistance, and no environmental pollution [3]. Probiotics can lower intestinal pH and inhibit harmful bacteria growth by secreting organic acids, bacteriocins, vitamins, and digestive enzymes, thereby optimizing intestinal flora structure, stimulating animal intestinal mucosal immunity, and improving disease resistance, ultimately enhancing animal growth performance. *Enterococcus faecium* belongs to the genus *Lactobacillus* and is a normal beneficial bacterium in the gastrointestinal tract of mammals. It possesses excellent biological characteristics, including the ability to produce organic acids, bacteriocins, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the animal intestine [4]. *Enterococcus faecium* can also promote digestive enzyme secretion [5], improve protein, fat, and energy metabolism, and increase feed conversion efficiency [6]. Moreover, it can compete with pathogenic bacteria and inhibit their proliferation [7], improve the intestinal environment, adjust gastrointestinal flora balance [8], enhance antioxidant capacity [9], and improve immunity [10].

Research on the application of *Enterococcus faecium* in weaned piglets is limited, and studies on antioxidant effects have mainly focused on serum and in vitro cultured cell antioxidant levels [11-13]. Therefore, this experiment was conducted to investigate the effects of different levels of *Enterococcus faecium* SF68 supplementation in basal diets on growth performance, blood routine parameters, serum biochemical indices, and antioxidant capacity of weaned piglets, providing reference for its scientific application in piglet production.

## 1. Materials and Methods

**1.1 Experimental Material** *Enterococcus faecium* SF68 (NCMIB10415) was provided by Binhai Purum Biotechnology Co., Ltd., with an effective content of  $1.0 \times 10^{11}$  CFU/g.

**1.2 Experimental Design and Management** The experiment used 21-day-old “Duroc × Landrace × Yorkshire” crossbred weaned piglets with similar body weight [(6.57±0.08) kg], randomly divided into 5 groups with 3 replicates per group and 6 pigs per replicate (half male and half female). The CON group was fed a basal diet, the ANT group received the basal diet supplemented with 0.0075% chlortetracycline and 0.02% colistin sulfate (with effective contents of 15% and 10%, respectively), the ALEF group received the basal diet supplemented with 0.0075% chlortetracycline, 0.02% colistin sulfate, and 0.01% Ente-

rococcus faecium SF68, while LEF and HEF groups received the basal diet supplemented with 0.01% and 0.05% Enterococcus faecium SF68, respectively. The basal diet was a powdered compound feed formulated according to NRC (2012) nutrient requirements for pigs, with composition and nutrient levels shown in Table 1. The experiment lasted 21 days and was conducted at Taicang Jinzhu Pig Farm in Suzhou. Pigs had free access to feed and water throughout the experiment, and epidemic prevention and disinfection procedures followed the farm management regulations.

### 1.3 Measurement Indicators and Methods

**1.3.1 Growth Performance** Piglets were individually weighed after 12 h of fasting at the start (21 days of age) and end (42 days of age) of the experiment to record initial and final body weights for calculating average daily gain (ADG). Daily feed consumption was recorded by replicate to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G).

**1.3.2 Diarrhea Index** Diarrhea was observed individually for all piglets daily at 14:00. Fecal consistency was scored as 0 (dry), 1 (soft), 2 (thin), or 3 (watery) based on the scoring standard shown in Table 2. The mental state, fecal condition in pens, and anal condition (presence of fecal contamination or redness/swelling) were also considered to record the number of diarrheic piglets and duration. Diarrhea index was calculated as: Diarrhea index = sum of diarrhea scores / total number of experimental pigs.

**1.3.3 Blood Routine, Serum Biochemical, and Antioxidant Indices** At the end of the experiment, 2 piglets were randomly selected from each replicate (30 pigs total) for slaughter. Blood was collected from the anterior vena cava to separate serum for determination of blood routine parameters, serum biochemical indices, and antioxidant indices. After slaughter, liver samples were quickly collected, and mucosa from jejunum and ileum was scraped, temporarily stored in liquid nitrogen, and then kept at  $-80^{\circ}\text{C}$  for antioxidant analysis.

Blood routine parameters were measured using an automatic five-part hematology analyzer, and serum biochemical indices were measured using an automatic biochemical analyzer. The contents of reduced glutathione (GSH), H<sub>2</sub>O<sub>2</sub>, and malondialdehyde (MDA), as well as the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) in serum, liver, jejunum, and ileum were determined using kits from Nanjing Jiancheng Bioengineering Institute. Protein concentrations in serum, liver, jejunum, and ileum were determined using kits from Shanghai Beyotime Biotechnology Co., Ltd. to calibrate the above antioxidant indices.

**1.4 Data Processing and Statistical Analysis** Experimental data were processed using Excel 2013 and expressed as mean  $\pm$  standard error (mean $\pm$ SE).

Statistical analysis was performed using SPSS 22.0 software. One-way ANOVA was used for variance analysis, and LSD method was used for multiple comparisons.  $P < 0.05$  was considered statistically significant, and  $P < 0.01$  was considered extremely significant.

## 2. Results

**2.1 Effects of *Enterococcus faecium* SF68 on Growth Performance and Diarrhea Index of Weaned Piglets** As shown in Table 3, no significant differences were observed in ADG, ADFI, F/G, or diarrhea index among all groups ( $P > 0.05$ ). Compared with CON group, ADG of ALEF, LEF, and HEF groups increased by 18.40%, 4.59%, and 10.12%, respectively; ADFI of ALEF and LEF groups increased by 8.74% and 0.55%, respectively; and F/G of ALEF, LEF, and HEF groups decreased by 12.29%, 7.82%, and 14.52%, respectively. The diarrhea index in ALEF and LEF groups decreased by 65% and 67%, respectively, compared with CON group.

**2.2 Effects of *Enterococcus faecium* SF68 on Blood Routine Parameters of Weaned Piglets** As shown in Table 4, LEF group had the highest mean corpuscular hemoglobin (MCH), which was significantly higher than other groups ( $P < 0.05$ ). LEF group also had the highest mean corpuscular volume (MCV), significantly higher than CON, ANT, and ALEF groups ( $P < 0.05$ ). The lymphocyte percentage in LEF group was the highest, extremely significantly higher than CON group ( $P < 0.01$ ). No significant differences were observed in other blood routine parameters among groups ( $P > 0.05$ ).

**2.3 Effects of *Enterococcus faecium* SF68 on Serum Biochemical Indices of Weaned Piglets** As shown in Table 5, ALEF group had the lowest serum total bile acid (TBA) content, significantly lower than HEF group ( $P < 0.05$ ). ALEF group had the highest serum lactate dehydrogenase (LDH) activity, significantly higher than HEF group ( $P < 0.05$ ). LEF group had the highest serum cholinesterase (CHE) activity, significantly higher than CON and HEF groups ( $P < 0.05$ ). No significant differences were observed in other serum biochemical indices among groups ( $P > 0.05$ ).

## 2.4 Effects of *Enterococcus faecium* SF68 on Antioxidant Indices of Weaned Piglets

**2.4.1 Serum Antioxidant Indices** As shown in Table 6, serum H<sub>2</sub>O<sub>2</sub> content in ALEF and LEF groups was significantly or extremely significantly higher than that in CON, ANT, and HEF groups ( $P < 0.05$  or  $P < 0.01$ ). Serum CAT activity in HEF group was extremely significantly lower than that in CON, ANT, ALEF, and LEF groups ( $P < 0.01$ ). No significant differences were observed in other serum antioxidant indices among groups ( $P > 0.05$ ).

**2.4.2 Liver Antioxidant Indices** As shown in Table 7 , liver H O content in LEF group was significantly lower than that in ANT, ALEF, and HEF groups ( $P < 0.05$ ) and extremely significantly lower than that in CON group ( $P < 0.01$ ). Liver CAT activity in ANT group was extremely significantly higher than that in other groups ( $P < 0.01$ ). Liver SOD activity in ANT, ALEF, and HEF groups was significantly higher than that in CON group ( $P < 0.05$ ). Liver T-AOC in ALEF and LEF groups was significantly higher than that in CON group ( $P < 0.05$ ), while HEF group had extremely significantly higher T-AOC than other groups ( $P < 0.01$ ). No significant differences were observed in other liver antioxidant indices among groups ( $P > 0.05$ ).

**2.4.3 Jejunum Antioxidant Indices** As shown in Table 8 , jejunum H O content in ANT, ALEF, LEF, and HEF groups was significantly or extremely significantly lower than that in CON group ( $P < 0.05$  or  $P < 0.01$ ). Jejunum MDA content in ANT group was significantly lower than that in other groups ( $P < 0.05$ ). Jejunum SOD activity in ALEF group was significantly higher than that in ANT, LEF, and HEF groups ( $P < 0.05$ ). No significant differences were observed in other jejunum antioxidant indices among groups ( $P > 0.05$ ).

**2.4.4 Ileum Antioxidant Indices** As shown in Table 9 , ileum GSH content in HEF group was significantly or extremely significantly higher than that in other groups ( $P < 0.05$  or  $P < 0.01$ ), while ANT and LEF groups had significantly higher GSH content than ALEF group ( $P < 0.05$ ). Ileum MDA content in CON group was significantly or extremely significantly higher than that in other groups ( $P < 0.05$  or  $P < 0.01$ ). Ileum SOD activity in ANT group was significantly higher than that in CON group ( $P < 0.05$ ), while ALEF, LEF, and HEF groups had extremely significantly higher SOD activity than CON group ( $P < 0.01$ ). HEF group had extremely significantly higher ileum SOD activity than other groups ( $P < 0.01$ ). No significant differences were observed in other ileum antioxidant indices among groups ( $P > 0.05$ ).

### 3. Discussion

**3.1 Effects of Enterococcus faecium SF68 on Growth Performance and Diarrhea Index of Weaned Piglets** Weaning is a critical transitional period in piglet production. Changes in feed composition, form, and physiological status inevitably cause adverse effects on weaned piglet growth and development, mainly manifested as indigestion, slow growth, decreased resistance, and diarrhea.

Enterococcus faecium is mainly used in livestock and poultry to regulate intestinal microbial flora balance, thereby reducing diarrhea and promoting growth [14]. Zeyner et al. [15] found that oral administration of Enterococcus faecium SF68 significantly reduced diarrhea rate in suckling piglets and increased ADG. Giang et al. [16] reported that supplementation with a compound lactic acid bacteria preparation containing Enterococcus faecium significantly increased ADG,

ADFI, and feed conversion ratio (FCR) during weeks 1-2 post-weaning, but had no significant effect during weeks 3-5. Huang et al. [17] found that compound *Lactobacillus* could reduce piglet diarrhea rate and diarrhea index. Mallo et al. [18] reported that *Enterococcus faecium* CECT4515 effectively increased *Lactobacillus* counts and reduced *Escherichia coli* counts in ileum and cecum of weaned piglets, thereby reducing diarrhea rate. Similar results were obtained in this study, showing that *Enterococcus faecium* SF68 promoted piglet growth and reduced weaned piglet diarrhea. However, this effect was not significant, possibly because the effect of *Enterococcus faecium* SF68 on piglets was mainly manifested in the first two weeks post-weaning and began to weaken from week 3 onwards, consistent with the findings of Giang et al. [16]. The diarrhea index was lowest in LEF group but increased in HEF group, possibly because excessive supplementation of *Enterococcus faecium* SF68 disturbed intestinal microbial balance to some extent. However, F/G in HEF group did not increase accordingly. Examination of raw data revealed that the increased diarrhea rate in HEF group was mainly concentrated in 2 piglets out of 18, suggesting that *Enterococcus faecium* SF68 might only cause diarrhea in individual pigs, but overall, dietary supplementation with *Enterococcus faecium* SF68 alleviated diarrhea in weaned piglets. ALEF group effectively reduced diarrhea index compared with CON group, with better effects than ANT group and similar growth-promoting effects, possibly because dietary *Enterococcus faecium* SF68 supplementation could mitigate the adverse effects of antibiotics on weaned piglets.

**3.2 Effects of *Enterococcus faecium* SF68 on Blood Routine Parameters of Weaned Piglets** Blood is an important component of the internal environment in animals, and changes in blood physiological and biochemical indices can reflect metabolism and health status of the body or organs [19]. Hemoglobin content is related to body resistance and changes with health status [20]. Hemoglobin is the main carrier of oxygen and carbon dioxide in the body, and higher hemoglobin concentration facilitates oxygen transport, enhances metabolic capacity, and improves immune function. Blood lymphocytes include T lymphocytes and B lymphocytes, which participate in both cellular and humoral immunity and protect the body when harmful substances invade. Probiotics have immunostimulatory effects that influence the immune system and host health [21]. In this study, LEF group had the highest mean corpuscular hemoglobin content, significantly higher than other groups, and the highest lymphocyte percentage, significantly higher than CON group. Therefore, low-dose *Enterococcus faecium* SF68 supplementation in basal diet can help body metabolism, enhance body resistance, and benefit weaned piglets.

**3.3 Effects of *Enterococcus faecium* SF68 on Serum Biochemical Indices of Weaned Piglets** Serum total bile acid is the final product of cholesterol metabolism in liver tissue, and its generation and metabolism are closely related to the liver. In healthy animals, serum total bile acid content is low and constant, but when liver cells are damaged or bile ducts are blocked, bile

acid metabolism is impaired and content increases [22]. This study showed that ALEF group had the lowest serum total bile acid content, significantly lower than HEF group, indicating that dietary supplementation with antibiotics + low-dose *Enterococcus faecium* SF68 may have protective effects on the liver. LDH exists in various tissues and organs and is an important enzyme involved in glycolysis. Like serum glucose content, serum LDH activity is an important indicator reflecting animal glucose metabolism. The results showed that ALEF group had the highest serum LDH activity, significantly higher than HEF group, indicating that dietary supplementation with antibiotics + low-dose *Enterococcus faecium* SF68 can enhance glucose metabolism, with better effects than other groups. CHE is a biological enzyme released by liver cells into blood, and serum CHE activity is a sensitive indicator of liver cell damage and an important index for evaluating liver synthesis and reserve function [23]. If massive liver cell death occurs, CHE activity decreases. In this study, LEF group had the highest serum CHE activity, significantly higher than CON group.

### **3.4 Effects of *Enterococcus faecium* SF68 on Antioxidant Capacity of Weaned Piglets**

Oxidation-reduction reactions are important physiological and biochemical processes in the body that generate numerous free radicals and reactive molecules. Appropriate amounts of free radicals are important for maintaining normal physiological functions, as they can promote thyroid hormone synthesis, stimulate phagocytic cell bactericidal activity, and regulate signal transduction. However, excessive free radicals have strong affinity for major biological macromolecules such as proteins, carbohydrates, lipids, and nucleic acids, causing cross-linking, breakage, or structural changes that disrupt normal physiological functions. Oxidative damage induced by excessive free radicals is called oxidative stress. When humans and animals are under long-term stress, it can cause various diseases such as diabetes, cardiovascular disease, and inflammation in humans [24], and mainly reduces growth performance, antioxidant capacity, and immunity in weaned piglets.

Animals have various antioxidants including antioxidant macromolecules, small molecules, and enzymes to eliminate various reactive oxygen species generated during oxidation-reduction reactions, thereby preventing oxidative stress. SOD, CAT, GSH, GSH-Px, vitamin E (tocopherols and tocotrienols), and vitamin C are important components of the innate protective antioxidant mechanism [17]. In this study, serum H<sub>2</sub>O<sub>2</sub> content in ALEF and LEF groups was significantly higher than that in CON group, while H<sub>2</sub>O<sub>2</sub> content in liver, jejunum, and ileum was lower than that in CON group, suggesting that ALEF and LEF groups might be at a higher antioxidant level. GSH is the substrate for both GSH-Px and glutathione S-transferase (GST), and is therefore essential for these two enzymes to decompose peroxides and protect against oxidative damage. The data showed that GSH content was higher in serum and jejunum of ALEF and LEF groups, in liver of ALEF group, and in ileum of LEF and HEF groups. GSH-Px activity was higher in serum of ALEF and ANT groups, in liver of ALEF and LEF groups, in jejunum of LEF and CON groups, and in ileum of ALEF and

LEF groups. These results indicate that piglets with higher GSH content and GSH-Px activity in serum, liver, jejunum, and ileum were mainly concentrated in ALEF and LEF groups. Therefore, low-dose *Enterococcus faecium* SF68 supplementation can increase GSH content to provide more substrate for GSH-Px to reduce oxidative damage to cells.

When oxidative stress occurs in animals, lipid peroxidation takes place. MDA is one of the important products of lipid peroxidation with strong biological toxicity, so MDA content can reflect the degree of lipid peroxidation and indirectly indicate cell damage. SOD is an important antioxidant enzyme widely distributed in various animals. With special physiological activity, SOD is the primary substance for scavenging free radicals in the body, protecting cells from free radical damage and repairing damaged cells in a timely manner. MDA measurement is often combined with SOD measurement, as SOD activity indirectly reflects the ability to scavenge oxygen free radicals, while MDA content indirectly reflects the severity of free radical damage to cells. Comprehensive analysis of MDA content and SOD activity in liver, jejunum, and ileum showed that low-dose *Enterococcus faecium* SF68 supplementation could reduce free radicals and lipid peroxidation, with better effects when combined with antibiotics. CAT is one of the key enzymes in the biological defense system established during biological evolution, and its biological function is to catalyze H<sub>2</sub>O<sub>2</sub> decomposition to prevent peroxidation. CAT activity varies among different tissues, with higher activity in liver. This study showed that CAT activity in jejunum and ileum was higher in LEF group, while serum CAT activity was lowest in HEF group. In liver, CAT activity was higher in ANT group, indicating that low-dose *Enterococcus faecium* SF68 plays a greater antioxidant role in the intestinal antioxidant defense system.

The total antioxidant level of various antioxidant macromolecules, small molecules, and enzymes in the body reflects the total antioxidant capacity (T-AOC). Analysis of T-AOC in liver, jejunum, and ileum showed that ALEF and LEF groups had stronger T-AOC and better protective effects on intestine and liver. In summary, dietary supplementation with low-dose *Enterococcus faecium* SF68 showed better effects in reducing oxidative stress in weaned piglets.

**Conclusions:** 1. Dietary supplementation with low-dose *Enterococcus faecium* SF68 can reduce diarrhea index and F/G in weaned piglets, with similar effects in LEF and ALEF groups. 2. Low-dose *Enterococcus faecium* SF68 supplementation can increase mean corpuscular hemoglobin content and lymphocyte percentage in blood, facilitating oxygen transport, enhancing metabolic capacity, and improving immune function. 3. Dietary supplementation with antibiotics + low-dose *Enterococcus faecium* SF68 can reduce serum total bile acid content and increase serum LDH activity, providing certain liver protection and enhancing glucose metabolism. 4. Low-dose *Enterococcus faecium* SF68 supplementation can increase serum CHE activity, enhancing liver synthesis and reserve function. 5. Low-dose *Enterococcus faecium* SF68 supplementation can

increase GSH content and GSH-Px activity in serum, liver, and ileum, and reduce H O content in liver, jejunum, and ileum, as well as MDA content in ileum.

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