





Article

Microencapsulated Sodium Butyrate Alleviates Immune Injury and Intestinal Problems Caused by *Clostridium Perfringens* through Gut Microbiota

Ting Yang ^{1,†}, Yaowei Sun ^{1,†}, Zhenglie Dai ¹, Jinsong Liu ², Shiping Xiao ², Yulan Liu ², Xiuxi Wang ¹, Shenglan Yang ¹, Ruiqiang Zhang ¹, Caimei Yang ² and Bing Dai ^{1,*}

¹ College of Animal Science and Technology, College of Veterinary Medicine, Key Laboratory of Applied Technology on Green-Eco-Healthy Animal Husbandry of Zhejiang Province, Zhejiang Provincial Engineering Laboratory for Animal Health Inspection and Internet Technology, Zhejiang Agricultural and Forestry University, Hangzhou 311300, China; yangtingly@126.com (T.Y.); 19550177705@163.com (Y.S.); mmdaa0323@163.com (Z.D.); 15261825191@163.com (X.W.); yangshenglan7@163.com (S.Y.); zrq1034@163.com (R.Z.)

² Zhejiang Vegamax Biotechnology Co., Ltd., Huzhou 313300, China; huijialjs@163.com (J.L.); xiao1974920@126.com (S.X.); yulanflower@126.com (Y.L.); yangcaimei2012@163.com (C.Y.)

* Correspondence: daibing2008@126.com

† These authors contributed equally to this work.

Simple Summary: Necrotic enteritis is an enterotoxemic disease caused by *Clostridium perfringens*, leading to diarrhea or necrotizing lesions in the intestines of animals, with severe cases leading to death. The butyrate attenuated the inflammatory response and improved intestinal health in piglets challenged with pathogenic bacteria, but it was absorbed by the anterior segment of the gastrointestinal tract. Microencapsulation is a simple and effective method to prevent butyrate from absorption. Thus, we evaluate the effectiveness to two butyrate alleviates clostridium perfringens infections. Our results indicate that dietary supplementation with sodium butyrate or microencapsulated sodium butyrate improves the immune status and morphology of intestinal villi, increases the production of VFAs, and modulates cecal microbiota in chickens challenged with *Clostridium perfringens*. Moreover, microencapsulated sodium butyrate contains less butyrate than sodium butyrate. These findings indicate that microencapsulated sodium butyrate was more effective than sodium butyrate with the same butyrate supplemental amount.

Abstract: Microencapsulated sodium butyrate (MS-SB) is an effective sodium butyrate additive which can reduce the release of sodium butyrate (SB) in the fore gastrointestinal tract. In this study, we assess the protective effects and mechanisms of MS-SB in *Clostridium perfringens* (*C. perfringens*)-challenged broilers. Broiler chickens were pre-treated with SB or MS-SB for 56 days and then challenged with *C. perfringens* three times. Our results indicate that the addition of MS-SB or SB before *C. perfringens* infection significantly decreased the thymus index ($p < 0.05$). Serum IgA, IgY, and IgM concentrations were significantly increased ($p < 0.05$), while pro-inflammatory IL-1 β , IL-6, and TNF- α were significantly decreased ($p < 0.05$) under MS-SB or SB supplementation. Compared with SB, MS-SB presented a stronger performance, with higher IgA content, as well as a lower IL-1 β level when normal or *C. perfringens*-challenged. While *C. perfringens* challenge significantly decreased the villus height ($p < 0.05$), MS-SB or SB administration significantly increased the villus height and villus height/crypt depth (V/C ratio) ($p < 0.05$). Varying degrees of SB or MS-SB increased the concentrations of volatile fatty acids (VFAs) during *C. perfringens* challenge, where MS-SB presented a stronger performance, as evidenced by the higher content of isovaleric acid and valeric acid. Microbial analysis demonstrated that both SB or MS-SB addition and *C. perfringens* infection increase variation in the microbiota community. The results also indicate that the proportions of *Bacteroides*, *Faecalibacterium*, *Clostridia*, *Ruminococcaceae*, *Alistipes*, and *Clostridia* were significantly higher in the MS-SB addition group while, at same time, *C. perfringens* infection increased the abundance of *Bacteroides* and *Alistipes*. In summary, dietary supplementation with SB or MS-SB improves the



Citation: Yang, T.; Sun, Y.; Dai, Z.; Liu, J.; Xiao, S.; Liu, Y.; Wang, X.; Yang, S.; Zhang, R.; Yang, C.; et al. Microencapsulated Sodium Butyrate Alleviates Immune Injury and Intestinal Problems Caused by *Clostridium Perfringens* through Gut Microbiota. *Animals* **2023**, *13*, 3784. <https://doi.org/10.3390/ani13243784>

Academic Editor: Janghan Choi

Received: 3 November 2023

Revised: 23 November 2023

Accepted: 30 November 2023

Published: 8 December 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

immune status and morphology of intestinal villi, increases the production of VFAs, and modulates cecal microbiota in chickens challenged with *C. perfringens*. Moreover, MS-SB was more effective than SB with the same supplemental amount.

Keywords: microencapsulated sodium butyrate; broiler chicken; immunity; cecum microflora; *Clostridium perfringens*

1. Introduction

Antibiotic growth promoters have been used to improve animal growth and to control or prevent animal disease during rearing; however, many countries have forbidden the dietary use of antimicrobial agents to avoid the emergence of antibiotic-resistant bacteria [1]. Thus, animal nutritionists have been attempting to discover antibiotic alternatives that are low-cost, widely used, and which have an obvious effect. Butyric acid has a positive effect on the normal intestinal mucosa, which can be attributed to a major energy source for intestinal epithelial cells [2,3]. In the intestine, an appropriate concentration of butyric acid is beneficial in protecting against the invasion of micro-organisms, which may be attributed to the effects of VFAs in terms of maintaining the pH of the intestinal lumen [4]. In a previous study, butyrate significantly ameliorated the mice intestine and intestinal epithelial cell inflammatory response and intestinal epithelium barrier dysfunction caused by 2,4,6-trinitrobenzene sulphonic acid (TNBS) [5]. Butyrate also has an immunomodulatory effect; for example, butyrate attenuated steatohepatitis through restoring the dysbiosis of gut microbiota. As such, butyrate has been considered as a potential gut microbiota modulator and therapeutic substance for non-alcoholic fatty liver disease (NAFLD) [6].

In the context of animal rearing, the harsh environment, stress, harmful bacteria, and other factors may result in intestinal health damage, expressed as intestinal inflammatory responses, intestinal barrier injury, and intestinal flora imbalances. Necrotic enteritis is an enterotoxemic disease caused by *C. perfringens*, leading to diarrhea or necrotizing lesions in the intestines of livestock and humans and, in severe cases, leading to death, with mortality rates of up to 100% in affected piglets [7]. As one of the most economically important diseases affecting poultry worldwide, necrotic enteritis causes \$6 billion in annual losses globally [8]. In animals, associated lesions caused by *C. perfringens* have been found in the whole intestine, and were significantly more severe in the jejunum compared to the duodenum and ileum [9]. In healthy chickens, it is hard to find *C. perfringens* spores in the gastrointestinal tract, but the total number of vegetative *C. perfringens* cells increased when necrotic enteritis occurred, presenting a positive correlation between presence in the duodenum, jejunum, and ileum and disease severity [9,10]. In a retrospective study, *C. perfringens* was also found to cause liver morphological lesions, necrotizing hepatitis, congested lungs, and neurological diseases, manifesting as tremors, stargazing, and incoordination [11].

In a previous study, dietary supplementation with butyrate attenuated the inflammatory response and improved intestinal health in piglets challenged with enterotoxigenic *Escherichia coli* (ETEC) through inhibiting the activation of NF- κ B/MAPK and modulating the hindgut microbiota [12]. Another study used an adherent-invasive *Escherichia coli* (AIEC) challenge model and showed that butyrate augmented AIEC invasiveness, while concurrently bolstering the intestinal epithelial barrier and reducing intestinal inflammation [13]. However, to the best of our knowledge, there have been few studies on the effect of butyrate in mitigating *C. perfringens* infection in broilers. In addition, butyrate given orally is quickly absorbed and used as energy by mucosal cells, absorbed and metabolized by the bird ingluvies and throughout the whole gastrointestinal tract, limiting the amount of butyrate that reaches the hindgut and restricting its practical use in the animal production context [14]. In order to prevent butyrate from absorption and metabolization in the anterior segment of the gastrointestinal tract, encapsulation is a simple and effective

method. Previous studies have shown that microencapsulation reduces the release of contents into gastrointestinal fluid [15].

Here, we propose the hypothesis that microencapsulated sodium butyrate has a better protective effect than sodium butyrate in broilers challenged with *C. perfringens*. Consequently, we systematically assess the protective and underlying mechanisms of sodium butyrate (SB) and microencapsulated sodium butyrate (MS-SB) against *C. perfringens* infection in broilers. This study possesses considerable theoretical significance in relation to protection against the occurrence of necrotic enteritis and providing a strategy for effectively utilizing gastrointestinal-sensitive biological agents.

2. Materials and Methods

2.1. Animals and Diets

A total of 360 1-day-old male yellow feather broiler chickens were obtained from a commercial hatchery. The chickens were completely randomly allocated into 6 groups, with 6 replicates, and each replicate containing 10 chickens. The study adopted a completely randomized design with a 3×2 factorial pattern (3 kinds of diet and *C. perfringens*-challenged or not). The chickens were reared in a temperature-controlled room and maintained on a 24 h constant light schedule, and allowed ad libitum access to feed and water. The experiment last 57 days. The basal diet was formulated based on the NRC (1994) [16] and nutrient requirements for yellow chickens [17]; see Table 1. During the experimental period, chickens were fed a basal diet (basic), a basal diet with 1000 mg/kg sodium butyrate (SB), or 1000 mg/kg microencapsulated sodium butyrate (MS-SB), respectively. At 53 days old, the challenged group of chickens were challenged with 1 mL *C. perfringens* suspension (10^9 CFU/mL) via intragastric administration every other day, and the non-challenged group of chickens were given 1 mL media in the same way. The *C. perfringens* used in this study were kept in our laboratory. The SB contained 98% sodium butyrate, and was purchase from Wuhan Jiyesheng Chemical Co., Ltd. (Wuhan, China). The MS-SB was coated with a polymer enteral material and contained 40% sodium butyrate, and was provided by Zhejiang Vegamax Biotechnology Co., Ltd. (Huzhou, China).

Table 1. Ingredients and nutrient composition of base diets, as feed basis.

Ingredients (%)	Starter (Days 1–28)	Grower (Days 29–56)	Nutritional Level	Starter (Days 1–28)	Grower (Days 29–56)
Corn	54.4	53	Me (kcal/kg)	2983	3090
Soybean meal	23.6	16	CP (%)	20.4	17.2
Expanded soybean	5	3	Lysine (%)	1.18	0.96
Rice DDGS	5	8	Methionine (%)	0.55	0.44
Rice bran	/	8	Met + Cys (%)	0.90	0.74
Corn bran	/	2	Tryptophan (%)	0.22	0.20
Soybean oil	2.2	4.5	Threonine (%)	0.88	0.78
Limestone	1.5	1.9	Calcium (%)	0.86	0.73
Fermented soybean meal	2.5	/	Total P (%)	0.70	0.71
Corn gluten meal	2.0	/	Available P (%)	0.43	0.44
CaHPO ₄ (2H ₂ O)	2.0	1.8			
NaCl	0.3	0.3			
Premix ^a	1.5	1.5			
Total	100.00	100.00			

^a The following substances were supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 2500 IU; vitamin E, 20 mg; vitamin B₁, 1.5 mg; vitamin B₂, 3.5 mg; pantothenic acid, 10 mg; vitamin B₁₂, 0.01 mg; folic acid, 1 mg; niacin 30 mg; Choline chloride, 1000 mg; Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 80 mg; Zn (MnSO₄·7H₂O), 60 mg; Se (NaSeO₃), 0.15 mg; I (KI), 0.2 mg.

2.2. Sample Collection

At 58 days old, blood was collected from the jugular vein after the chickens were starved overnight and weighted. It was centrifuged at $3500 \times g$ for 15 min at 4 °C, then stored at −20 °C for future analyses. The chickens were slaughtered by cervical dislocation.

The middle segments of jejunum were collected and fixed with 4% paraformaldehyde. The cecal contents were aseptically collected and assessed for volatile fatty acids (VFAs) and microflora composition.

2.3. Organ Index

The liver, spleen, thymus, and bursa of Fabricius of each sampling broiler were removed, the blood stains were wiped off the surface, then they were weighed. The relative organ weights were calculated as per the following equation:

$$\text{Organ weight indexes} = \text{Organ weight (g)} / \text{Body weight (g)} \times 100$$

2.4. Serum Immune Indicators

The concentrations of serum immunoglobulin A (IgA) (CAS:ANG-E32004C; 10–600 ng/mL), immunoglobulin M (IgM) (CAS:ANG-E32005C; 0.1405–11.25 µg/mL), immunoglobulin Y (IgY) (CAS:ANG-E32209C; 0.062.5–3.75 ng/mL), interleukin-1β (IL-1β) (CAS:ANG-E32031C; 1.875–112.5 ng/L), interleukin-6 (IL-6) (CAS:ANG-E32013C; 1–60 ng/L), and tumor necrosis factor-α (TNF-α) (CAS:ANG-E32030C; 1.25–75 ng/L) were measured using an enzymatic chromatometric method using the ELISA Kits that were purchased from Angle Gene Biotechnology Co., Ltd. (Nanjing, Jiangsu, China), according to the manufacturer's instructions.

2.5. Jejunum Morphology Analysis

The fixed and pruned jejunum was dehydrated with gradient ethanol, followed by cleaning with xylene, waxing, embedding, slicing, and staining with hematoxylin–eosin (HE). Finally, we took pictures using a microscope (Nikon, Tokyo, Japan). Ten intact villi for each sample were randomly selected to measure the villus height (V) and crypt depth (C) using the Image Pro Plus 6.0 software (Rockville, MD, USA), and the ratio of villus height to crypt depth (V/C) was calculated.

2.6. Volatile Fatty Acid (VFA) Analysis

The VFAs in cecum content were determined via gas chromatography according to the method of Yu et al. (2023) [18]. In brief, a sample containing about 0.5 g of cecum content was mixed with pre-cooled water at a mass volume ratio of 1:3 and centrifuged at 12,000× g for 10 min at 4 °C. The supernatant was mixed with 25% metaphosphoric acid in a 5:1 ratio and rested for 30 min, followed by centrifugation at 10,000× g for 10 min at 4 °C. Then, the supernatant was transferred into a sample bottle for testing using an Agilent Technologies 7890B GC System (column parameter: 30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). Pure acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid solutions were used as standards to calculate relevant concentrations in the sample.

2.7. Cecum Microflora

Total bacterial DNA of cecum microbiota was extracted using a DNA extraction kit, and the DNA concentration was determined using agarose gel electrophoresis. The V3–V4 regions of 16S rRNA were amplified with universal primers 515F/806R using the Applied Biosystems GENEAMP 9700 (Thermo Fischer Scientific, Waltham, MA, USA). The amplification products were recovered after purification using an AXYGEN DNA Gel Extraction Kit (Union City, CA, USA). The amplification products were sequenced on an Illumina miseq (PE300) platform provided by Majorbio Co., Ltd. (Shanghai, China), after quantitative analysis conducted using a quantifluor™ blue fluorescence quantitative system (Promega, WI, USA). In order to obtain the Amplicon Sequence Variant (ASV) variants and feature lists, the DADA2 variants in QIIME2 were used to optimize the data obtained through sequencing. The alpha diversity component, Shannon, Chao, and Simpson indices were adopted to indicate the diversity of microbiota. Principal component

analysis (PCA) and principal co-ordinates analysis (PCoA) were conducted to analyze the species indices, including the β -diversity component between different groups. Finally, linear discriminant analysis coupled with effect size (LEfSe) was used to identify microbial differences among all treatment groups.

2.8. Statistical Analysis

The data were tested for normality and homogeneity of variance through the Levene test. Then, two-factor analysis of variance and Tukey's HSD were carried out using the JMP Pro software 13.0 (SAS, Carrey, MS, USA). The model equation included the main effects (sodium butyrate addition and *C. perfringens* challenge) and their interactions. The differences among treatments were tested using Tukey's test when the main effects or interactions were significant. The statistical significance was set to $p < 0.05$. All values are shown as mean \pm SEM. The resulting data were plotted using the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Microencapsulated Sodium Butyrate Alleviated *C. perfringens* Infection

At 53–57 days old, the broilers were challenged with *C. perfringens* three times through gavage administration every other day. As shown in Table 2, the *C. perfringens* challenge significantly decreased the thymus index and significantly increased the spleen index ($p < 0.05$). The addition of SB significantly decreased the thymus index ($p < 0.05$), and MS-SB had the same effect as sodium butyrate. It is worth noting that the addition of SB and MS-SB decreased the thymus index under *C. perfringens* infection.

Table 2. Effect of microencapsulated sodium butyrate on the organ index of broiler chickens challenged with clostridium perfringens.

Diet	Challenge	Liver Index	Spleen Index	Bursa of Fabricius Index	Thymus Index
Control	CON	16.99	1.37	1.14	2.34 ^a
SB		18.04	1.49	1.23	1.84 ^{ab}
MS-SB		17.67	1.77	0.76	2.43 ^a
Control	CP	17.77	1.84	0.53	1.46 ^{bc}
SB		19.74	1.89	0.75	0.98 ^c
MS-SB		17.25	1.62	0.92	0.97 ^c
SEM		0.65	0.11	0.19	0.14
Main effect					
Diet	Control	17.38	1.60	0.83	1.90 ^a
	SB	18.86	1.70	0.99	1.41 ^b
	MS-SB	17.46	1.69	0.84	1.63 ^b
Challenge	CON	17.56	1.54 ^b	0.73	2.24 ^a
	CP	18.25	1.78 ^a	1.04	1.37 ^b
<i>p</i> -value					
Diet		0.057	0.743	0.694	0.005
Challenge		0.221	0.034	0.063	<0.001
Diet \times Challenge		0.304	0.051	0.128	0.023

Note. Number with different letters in the same column are statistically significant.

3.2. Microencapsulated Sodium Butyrate Alleviated Reduced Systemic Inflammation Caused by *C. perfringens*

The levels of inflammatory factors and immunoglobulin in sera are shown in Figure 1. The *C. perfringens* challenge had no influence on inflammatory factor and immunoglobulin content in sera. However, SB addition significantly increased the immunoglobulin content in sera, including IgA, IgM, and IgY ($p < 0.05$). In addition, SB significantly decreased the content of IL-1 β , IL-6, and TNF- α ($p < 0.05$). Compared with SB, MS-SB presented a stronger performance, with higher IgA content, as well as a lower IL-1 β level when normal or *C. perfringens*-challenged.

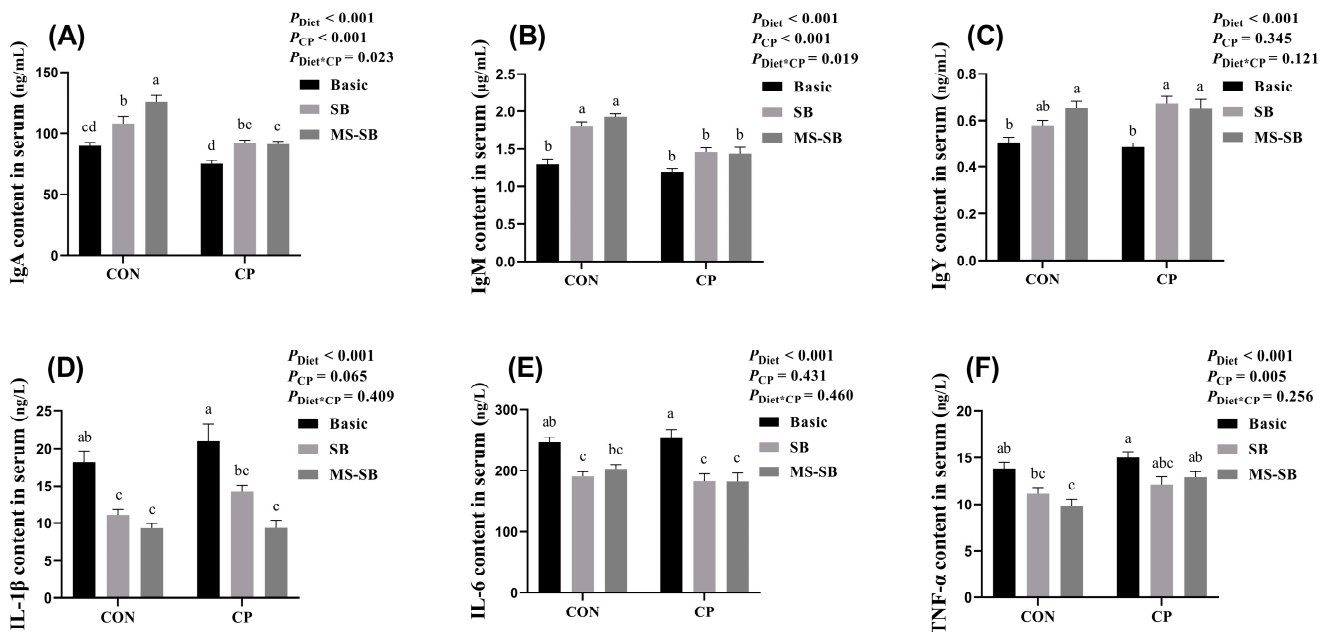


Figure 1. MS-SB modulates immunity through globulins and immune factor content in serum during *C. perfringens* infection. (A) IgA content; (B) IgM content; (C) IgY content; (D) IL-1 β content; (E) IL-6 content; (F) TNF- α content. Bars with different letters are statistically significant ($p < 0.05$) in different groups, $n = 6$.

3.3. Microencapsulated Sodium Butyrate Repaired Intestinal Morphology Damaged by *C. perfringens*

We also measured the parameters of jejunum villi through HE stains, and the results are shown in Figure 2. The results revealed that SB administration significantly increased the villus height and V/C ratio ($p < 0.05$), especially the MS-SB addition. However, *C. perfringens* challenge significantly decreased the villus height ($p < 0.05$) without affecting the V/C ratio.

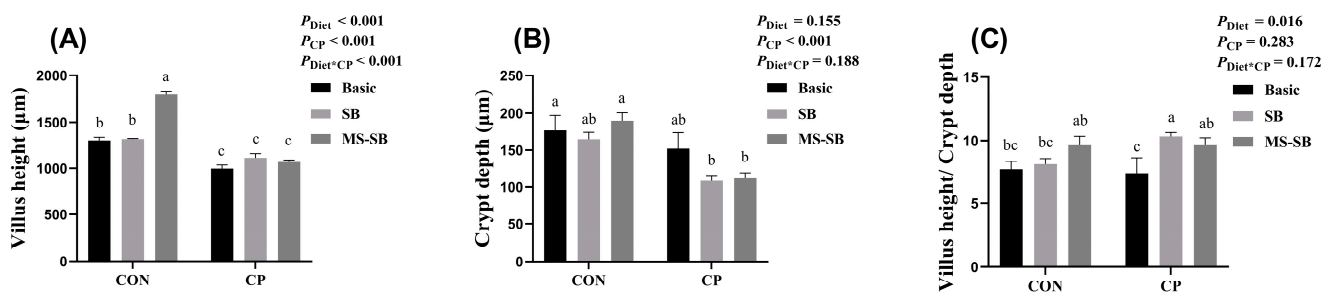


Figure 2. MS-SB repaired intestinal morphology damaged by *C. perfringens*. (A) Villus height of jejunum; (B) crypt depth of jejunum; (C) ratio of the villus height and crypt depth of jejunum. Bars with different letters are statistically significant ($p < 0.05$) in different groups, $n = 6$.

3.4. Microencapsulated Sodium Butyrate Ameliorated VFAs under *C. perfringens* Challenge

For this study, we also detected the VFA content in the broiler cecum, and the results are shown in Figure 3. The results indicated that the *C. perfringens* challenge had no effect on the VFA content. However, the addition of SB significantly decreased the content of acetic acid ($p < 0.05$) under *C. perfringens* non-challenge; at same time, SB or MS-SB presented varying degrees of increased fatty acid concentrations during *C. perfringens* challenge. It should be noted that MS-SB had a stronger performance, with a higher content of isobutyric acid, isovaleric acid, and valeric acid.

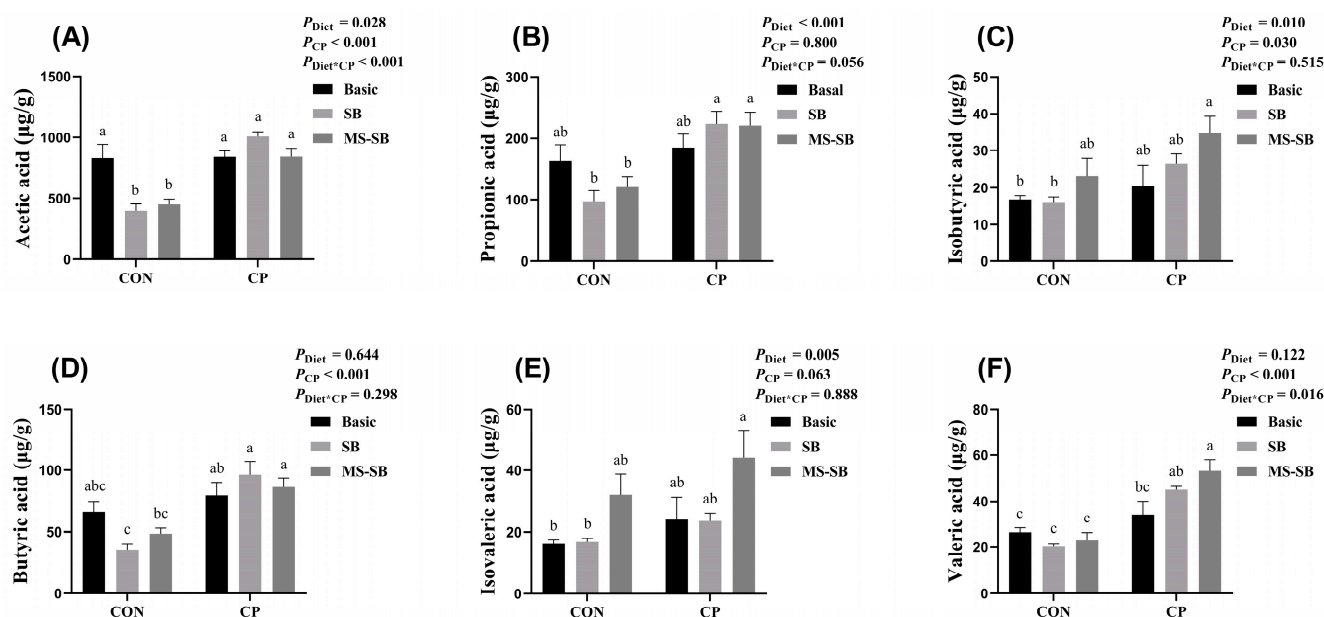


Figure 3. Microencapsulated sodium butyrate modulates the VFA content when challenged with *C. perfringens*. (A) Acetic acid; (B) propionic acid; (C) isobutyric acid; (D) butyric acid; (E) isovaleric acid; (F) valeric acid content in cecum of broiler chickens. Bars with different letters are statistically significant ($p < 0.05$) in different groups, $n = 6$.

3.5. Microencapsulated Sodium Butyrate Modulated Gut Microbiota Community Variation Caused by *C. perfringens*

The changes in the diversity of cecum microbiota are summarized in Figure 4. A total of 921 ASVs were shared among the five treatment groups based on the Venn diagram, with non-overlapping regions indicating unique OTUs in the CON group ($n = 569$), SB group ($n = 658$), MS-SB group ($n = 743$), CON-CP group ($n = 592$), and MS-SB-CP group ($n = 611$); see Figure 4A. We adopted the Chao index, Shannon index, and Simpson index to assess the microbiota community diversity. The results indicated that *C. perfringens* infection and SB addition individually presented more significant diversity in cecum microbiota when compared to the CON group, but there was no difference between them (Figure 4B–D). Principal component analysis (PCA) and principal coordinate analysis (PCoA) were performed to investigate the differences in species complexity and structural alterations in microbial communities. PCoA and PCA plots revealed the degree of diversity discrepancy in cecum microbiota between different treatments; in particular, the PCoA plot indicated that the CON group was separated from the other groups (Figure 4E,F).

3.6. Microencapsulated Sodium Butyrate Modulated Gut Microbiota Community Composition Caused by *C. perfringens*

In order to explore the change in cecum microbiota structure, we analyzed the relative abundance of cecum microbiota at phylum to species levels. At the phylum level, about 4 major phyla were detected, including *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, and *Actinobacteriota* (Figure 5A), and the relative abundance of *Proteobacteria* was higher in the *C. perfringens* infection groups than non-infection groups. The addition of MS-SB significantly increased the relative abundance of *Campilobacteria* (Figure 5D). At the genus level, about 13 major phyla were detected as dominant genera (Figure 5B). The relative abundances of *Bacteroides* in the *C. perfringens* infection groups were higher than in the non-infection groups, and the relative abundances of *Ruminococcus_torques* and *Alistipes* in the MS-SB-CP groups were higher than in other groups (Figure 5E). At the species level, about 11 major phyla were detected as dominant flora (Figure 5C). In addition, the relative abundances of 6 bacteria were higher with MS-SB addition than in the CON group, including *Bacteroides*, *Faecalibacterium*, *Clostridia_UCG-014*, *Ruminococcaceae*, *Alistipes_inops*,

and *Clostridia_vadinBB60*. The *C. perfringens* infection increased the abundance of *Bacteroides* and *Alistipes_inops* (Figure 5F). Finally, we analyzed the association between VFAs and microbiota composition. The results demonstrated that *Ruminococcaceae_torques* and *Alistipes* were positively correlated with isobutyric acid concentration, while *Lachnospiraceae*, *Ruminococcaceae_torques*, and *Alistipes* were positively correlated with valeric acid (Figure 6A). At the species level, *Ruminococcaceae_torques* and *Alistipes* were positively correlated with isobutyric acid, and *Lachnospiraceae*, *Ruminococcaceae_torques*, and *Alistipes* were positively correlated with valeric acid (Figure 6B).

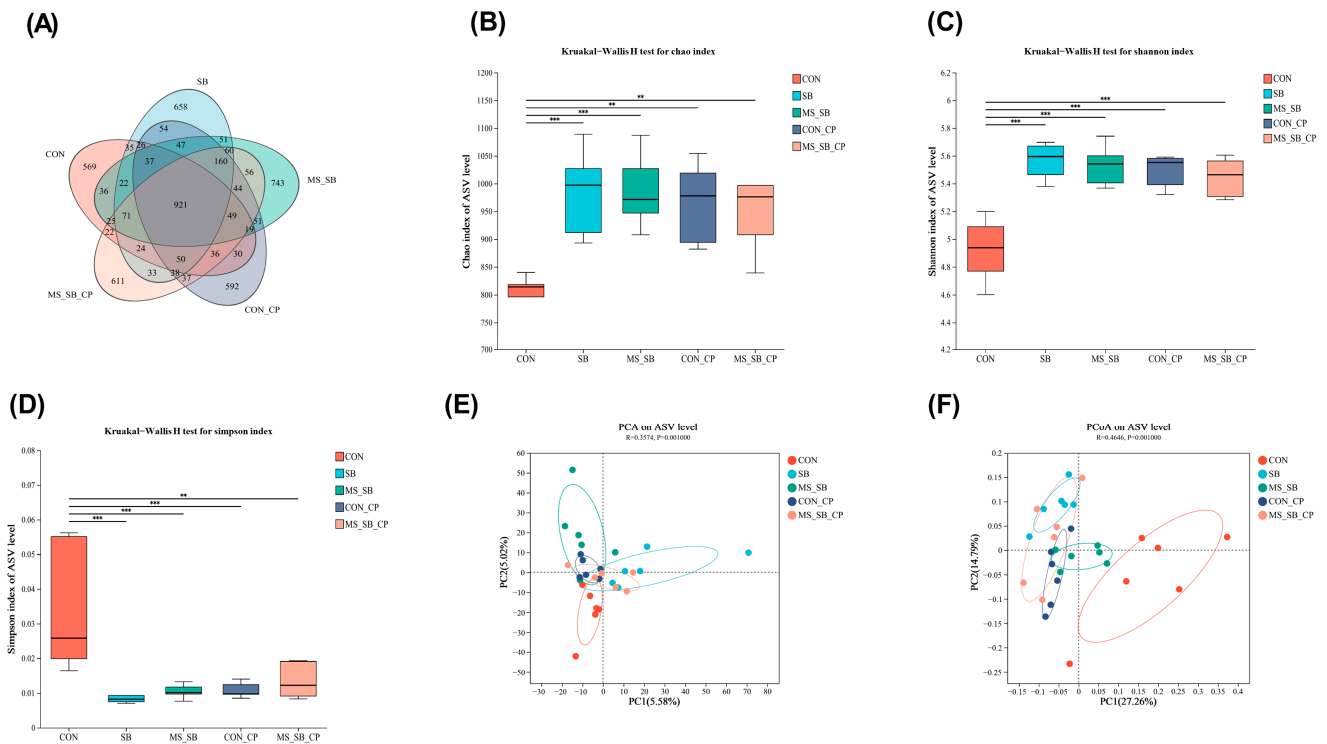


Figure 4. Analysis of the diversity of gut microbiota. (A) The Venn diagram summarizing the numbers of common and unique OTUs in cecum microflora community. (B–D) The Chao index, Shannon index, and Simpson index reflecting species alpha diversity between groups. (E,F) The principal component analysis (PCA) and principal co-ordinates analysis (PCoA) reflecting beta diversity within and between groups at the species level. The column with * are statistically significant, ** means $p < 0.01$, *** means $p < 0.001$, $n = 6$.

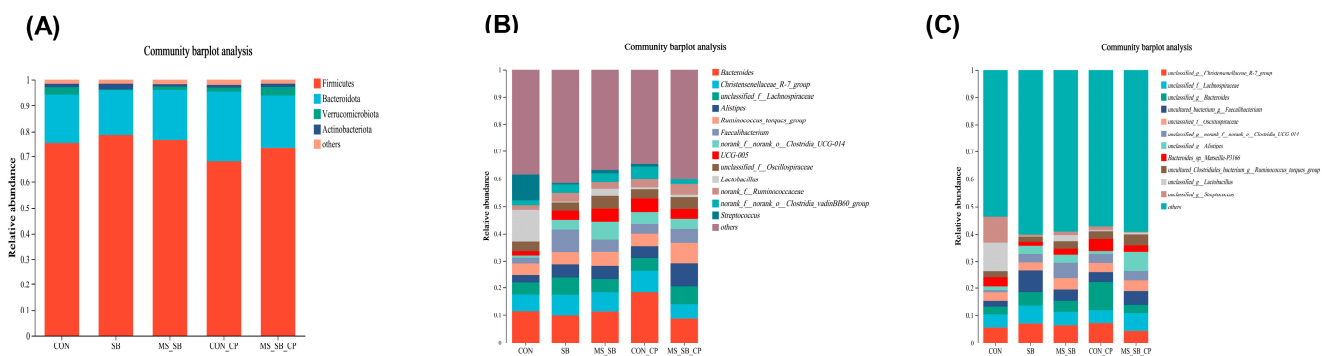


Figure 5. Cont.

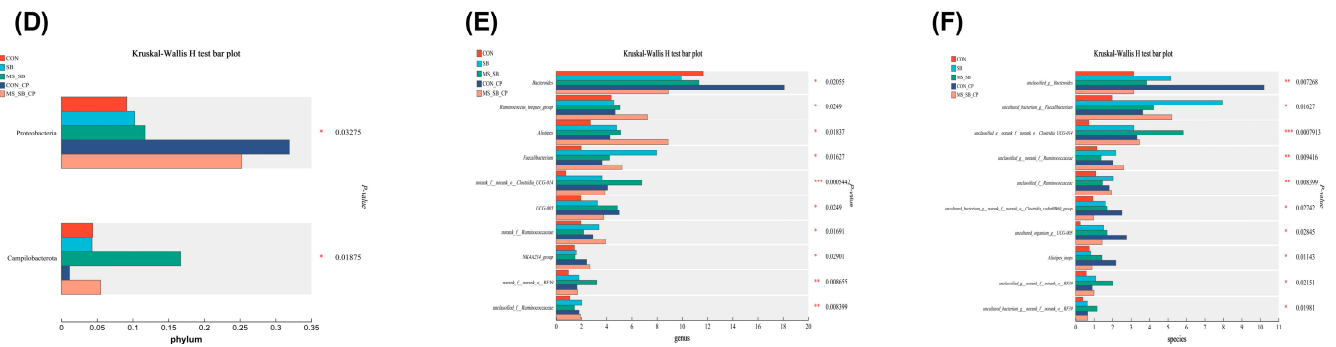


Figure 5. The abundance of the microbial community in cecum content. (A–C) The top relative abundance of the microflora community between groups (phylum level, genus level, and species level). (D–F) The bacteria with significant differences between groups (phylum level, genus level, and species level). The column with * are statistically significant, * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$, $n = 6$.

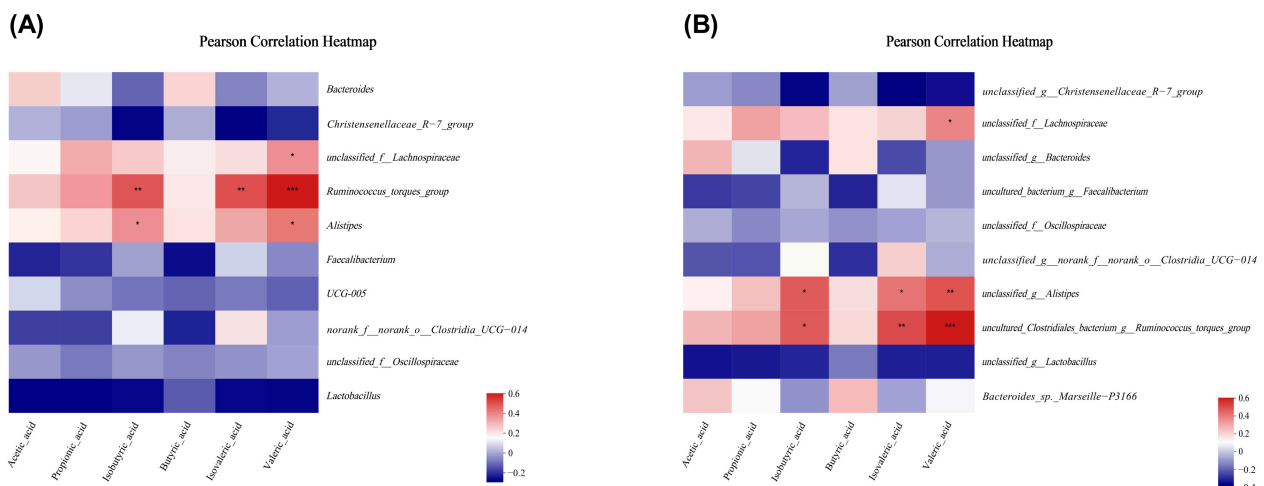


Figure 6. Correlation analysis between gut microbiota and VFAs. (A) Genus level and VFAs. (B) Species level and VFAs. The column with * are statistically significant, * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$, $n = 6$.

4. Discussion

In intensive animal production, the animals are faced with a variety of external factors at any time, such as production environment stress and physiological stress. In healthy animals, bacteria and health stand at either end of a pair of scales; once this balance is broken, harmful bacteria in the environment and body can cause damage to animal health, including intestinal inflammation, immune stress, and so on [19]. *C. perfringens* is consumed by chickens from environmental sources during rearing, including contaminated feed, water, and the farm environment [20]. As previously reported, the immune organ changes in response to infection with *C. perfringens*, shown in terms of the morphology and weight of the bursa of Fabricius, spleen, and thymus [21]. In this study, the results indicated that the spleen index increased, which is consistent with previous findings, as well as showing expected changes in the thymus index. This may be attributed to the age and species, as broilers near the end of growth were used in this study. Thus, these results indicate the validity of the *C. perfringens* infection model.

Moreover, we demonstrated that the addition of SB or MS-SB had no promotive or protective effects on the immune organs in this study. The results are not consistent with the results of a previous study, in which the thymus, spleen, and bursa weighed more in the SB addition group compared with control group [22]. Moreover, in a study in quails,

dietary supplementation with 1000 mg/kg SB significantly increased the thymus and bursa of Fabricius [23]. As a matter of fact, we have also confirmed that SB and MS-SB could enhance immune organ development throughout the growth period in a previous study (unpublished). Thus, according to the results of this study, SB or MS-SB have no protective effect against the immune organ changes caused by *C. perfringens* infection, evidenced by the fact that SB or MS-SB were unable to reverse the organ changes induced by *C. perfringens*.

The immune organs, immune cells, and immune molecules make up the immune system of animals, and can be categorized into two parts—the innate immune system and the adaptive immune system—according to the manner in which they act against invading pathogens [24]. As our results indicated that SB and MS-SB could enhance immune organ development during the growth period (unpublished), we further determined the levels of immunoglobulins and immunomodulatory cytokines in sera. Immunoglobulins are synthesized and secreted by B-cells after immune cells are activated by antigens, which can bind to specific antigens in order to defend against invading pathogens [25]. In this study, the results showed that *C. perfringens* infection decreased the immunoglobulin levels in sera, while SB and MS-SB improved the secretion of immunoglobulins, consistent with the results of previous studies [26]. However, it should be noted that the enhancement brought by SB and MS-SB was diminished under *C. perfringens* infection, when compared to normal conditions. Immunomodulatory cytokines are produced by immune cells and act on other immune cells, which can be classified as pro-inflammatory or anti-inflammatory according to their function [27]. Pro-inflammatory cytokines (e.g., IL-1 α / β , TNF- α / β , and IL-6) up-regulate inflammatory reactions, while anti-inflammatory cytokines (e.g., IL-10) down-regulate inflammatory responses and promote tissue healing [28]. In rat models, necrotic enteritis causes enteric inflammation accompanied by serum proinflammatory cytokine production [29]. Thus, we proceeded to determine the content of inflammatory factors in sera. The results demonstrated that SB or MS-SB exhibited a potent anti-inflammatory effect, evidenced by reductions in IL-1 β , IL-6, and TNF- α , especially in the case of the slight increase in inflammatory factors caused by *C. perfringens*. Similar to the results of our study, the study published by Sun et al. (2021) reported that sodium butyrate inhibited intestinal inflammation through the HMGB1-TLR4/NF- κ B pathway [3]. Therefore, we speculate that SB or MS-SB may enhance immune function by regulating serum inflammatory cytokines in broiler chickens. Moreover, compared with SB, MS-SB presented a better anti-inflammatory effect.

As an organ for nutrient digestion and absorption, the intestinal tract is also an important barrier to maintain the homeostasis of the internal environment, and is the first barrier to deal with foreign harmful bacteria [30]. Normal intestinal permeability prevents water and electrolyte loss, promotes the absorption of dietary nutrients, and prevents the entry of antigens and micro-organisms into the body, which are dependent on the integrity of the intestinal villi [31]. In a previous study, necrotic enteritis caused by *C. perfringens* was mostly observed in the jejunum, manifesting as intestinal morphological damage and inflammation [9]. In addition, SCFA can improve the proliferation of gut epithelial cells and increase their villi height, which subsequently helps to improve the capacity of the intestine for nutrient absorption [32]. Thus, we detected the morphology of the jejunum using HE staining, and the results indicated that *C. perfringens* negatively affected the intestinal villi morphology, decreasing both the villus height and crypt depth. These results are similar to those previously reported in broilers [33,34] and mice [35]. We cannot ignore that the addition of SB or MS-SB alleviated the morphological damage to the intestinal villi, and the performance of MS-SB was particularly prominent compared with that of SB. Similar results have also been confirmed in a study using a mouse model [5].

In previous research, VFAs have been shown to inhibit pathogenic micro-organisms and increase the absorption of nutrients, which may contribute to a reduction in the luminal pH [36]. Acetic acid is the shortest fatty acid with a carbon chain, which has been shown to be an intermediary involved in *bifidobacteria* inhibiting the proliferation of intestinal

pathogens [37]. Propionate and butyrate have been reported to assist in controlling intestinal inflammation by inducing the differentiation of T-regulatory cells, and the inhibition of histone deacetylation may be involved in the relevant regulatory mechanism [2,38]. In addition, recent studies have shown that valerate inhibits the proliferation of *Clostridium difficile* in the intestinal tract, thereby protecting or treating intestinal diseases [39]. Moreover, butyric acid is usually produced in the large intestine by intestinal bacteria and plays important roles, such as fueling intestinal epithelial cells and increasing mucin production, which may result in changes in bacterial adhesion and improved tight-junction integrity [40,41]. Thus, VFAs seem to play an important role in the maintenance of gut barrier function [42]. We also measured the concentrations of VFAs in the cecum, such as acetic acid, butyric acid (isobutyric acid), and valeric acid (isovaleric acid). The results indicated that *C. perfringens* infection has no effect on the VFA content in the cecum. However, SB or MS-SB increased the concentrations of fatty acids to varying degrees under the *C. perfringens* challenge. Our results are partially consistent with previous findings in broilers [43], and the dietary composition and butyric acid originating from foregut may be the cause of this result, as a result of butyric acid and other VFAs being produced through the bacterial fermentation of unabsorbed carbohydrates or food scraps [44]. Meanwhile, MS-SB was used in our study, which can significantly delay the enteric release of butyric acid, thus reducing small intestinal absorption and enhancing colonic delivery [44–46]. This may explain the difference between our results and those reported in previous studies.

Numerous studies have revealed that the gut microbiota that improve growth and metabolism promote host nutrient absorption and modulate the immune system, which are all behaviors providing irreplaceable functionality [47,48]. Furthermore, the diversity of the microbial community helps to maintain the homeostasis of the intestinal microbiome and improve resistance to pathogens in the host [49]. Our results on alpha diversity and beta diversity in the cecum content revealed a degree of diversity discrepancy in the cecum microbiota. Both the *C. perfringens* challenge and addition of SB improved the diversity of bacterial flora. This result is not consistent with previous studies, such as that of Pammi et al. (2017), in which the intestinal flora diversity in necrotizing enterocolitis patients was lower than that in unaffected patients, such as the lower relative abundance of *Firmicutes* and *Bacteroides* and higher relative abundance of *Proteobacteria* [50]. Meanwhile, our results were consistent with those of Zhang et al. (2018), who revealed the α -diversity index of broiler gut microbial community after *C. perfringens* infection [51]. This may be explained by the fact that *C. perfringens* infection can destroy the ecological balance of intestinal flora, resulting in intestinal ecological imbalance [52]. In addition, *C. perfringens* strains and dietary components, as well as the timing and duration of the *C. perfringens* challenge, may have contributed to the observed discrepancy [53]. In this study, the results indicated that the proportions of *Bacteroides*, *Faecalibacterium*, *Clostridia*, *Ruminococcaceae*, *Alistipes*, and *Clostridia* were significantly higher in the MS-SB addition group; at the same time, *C. perfringens* infection increased the abundance of *Bacteroides* and *Alistipes*. In previous studies, the genera *Alistipes* and *Bacteroides* have been identified as butyrate producers in the gut and have demonstrated good anti-inflammatory effects through butyrate [54]. The results of the correlation analysis considering VFAs and microbiota indicated that the isobutyric acid content increased and was positively correlated with the abundance of *Alistipes* and *Clostridia*. Thus, our findings demonstrate that SB and MS-SB exhibit a protective role to suppress *C. perfringens*-induced intestinal damage and microbiota disturbances.

5. Conclusions

In summary, sodium butyrate ameliorated *C. perfringens* infection by reducing inflammation, repairing intestinal damage, and modulating the cecum microbiota. Compared to sodium butyrate, microencapsulated sodium butyrate presented a better effect. This study highlights the effectiveness of microencapsulated sodium butyrate and provides a novel strategy for protection against *C. perfringens* infection through the effective utilization of gastrointestinal-sensitive biological agents.

Author Contributions: B.D. designed the experiments. S.X., Y.L. and X.W. performed the experiments. C.Y., J.L., S.X. and Z.D. analyzed the data. T.Y. and Y.S. wrote the main manuscript. Z.D., S.Y. and R.Z. revised the main manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Leading Innovation and Entrepreneurship Team Project of Zhejiang Province (2020R01015), Zhejiang Provincial Key R&D Program of China (2021C02008), the Natural Science Foundation of China (32260847, 32202687), Jiangxi Modern Agricultural Research Collaborative Innovation Project (JXXTCXBSJJ202208), and Hangzhou Key Projects for Agricultural and Social Development (202203A08).

Institutional Review Board Statement: The animal study protocol was approved by the Animal Health and Care Committee of Zhejiang Agricultural and Forestry University (No. ZAFUAC2022005, 15 October 2022).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study were not deposited in an official repository, but they are available from the authors upon request.

Conflicts of Interest: Jinsong Liu, Shiping Xiao, Yulan Liu, and Caimei Yang are employees of Zhejiang Vegamax Biotechnology. This paper reflects the views of the scientists, and not the company. We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript, and the authors declare no conflict of interest.

References

1. Li, Z.; Wang, W.; Liu, D.; Guo, Y. Effects of *Lactobacillus acidophilus* on the growth performance and intestinal health of broilers challenged with *Clostridium perfringens*. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 25. [\[CrossRef\]](#)
2. Salvi, P.S.; Cowles, R.A. Butyrate and the Intestinal Epithelium: Modulation of Proliferation and Inflammation in Homeostasis and Disease. *Cells* **2021**, *10*, 1775. [\[CrossRef\]](#)
3. Sun, Q.; Ji, Y.-C.; Wang, Z.-L.; She, X.; He, Y.; Ai, Q.; Li, L.-Q. Sodium Butyrate Alleviates Intestinal Inflammation in Mice with Necrotizing Enterocolitis. *Mediat. Inflamm.* **2021**, *2021*, 6259381. [\[CrossRef\]](#)
4. Manrique Vergara, D.; González Sánchez, M.E. Short chain fatty acids (butyric acid) and intestinal diseases. *Nutr. Hosp.* **2017**, *34*, 58–61. [\[CrossRef\]](#)
5. Chen, G.; Ran, X.; Li, B.; Li, Y.; He, D.; Huang, B.; Fu, S.; Liu, J.; Wang, W. Sodium Butyrate Inhibits Inflammation and Maintains Epithelium Barrier Integrity in a TNBS-induced Inflammatory Bowel Disease Mice Model. *EBioMedicine* **2018**, *30*, 317–325. [\[CrossRef\]](#)
6. Zhou, D.; Pan, Q.; Xin, F.Z.; Zhang, R.N.; He, C.X.; Chen, G.Y.; Liu, C.; Chen, Y.W.; Fan, J.G. Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J. Gastroenterol.* **2017**, *23*, 60–75. [\[CrossRef\]](#)
7. Posthaus, H.; Kittl, S.; Tarek, B.; Bruggisser, J. *Clostridium perfringens* type C necrotic enteritis in pigs: Diagnosis, pathogenesis, and prevention. *J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn. Inc.* **2020**, *32*, 203–212. [\[CrossRef\]](#)
8. Wade, B.; Keyburn, A. The true cost of necrotic enteritis. *World Poult.* **2015**, *31*, 16–17.
9. Hustá, M.; Tretiak, S.; Ducatelle, R.; Van Immerseel, F.; Goossens, E. *Clostridium perfringens* strains proliferate to high counts in the broiler small intestinal tract, in accordance with necrotic lesion severity, and sporulate in the distal intestine. *Vet. Microbiol.* **2023**, *280*, 109705. [\[CrossRef\]](#)
10. Daneshmand, A.; Kermanshahi, H.; Mohammed, J.; Sekhavati, M.H.; Javadmanesh, A.; Ahmadian, M.; Alizadeh, M.; Razmyar, J.; Kulkarni, R.R. Intestinal changes and immune responses during *Clostridium perfringens*-induced necrotic enteritis in broiler chickens. *Poult. Sci.* **2022**, *101*, 101652. [\[CrossRef\]](#)
11. Marcano, V.; Gamble, T.; Maschek, K.; Stabler, L.; Fletcher, O.; Davis, J.; Troan, B.V.; Villegas, A.M.; Tsai, Y.Y.; Barbieri, N.L.; et al. Necrotizing Hepatitis Associated with *Clostridium perfringens* in Broiler Chicks. *Avian Dis.* **2022**, *66*, 337–344. [\[CrossRef\]](#)
12. Tian, M.; Li, L.; Tian, Z.; Zhao, H.; Chen, F.; Guan, W.; Zhang, S. Glyceryl butyrate attenuates enterotoxigenic *Escherichia coli*-induced intestinal inflammation in piglets by inhibiting the NF- κ B/MAPK pathways and modulating the gut microbiota. *Food Funct.* **2022**, *13*, 6282–6292. [\[CrossRef\]](#)
13. Pace, F.; Rudolph, S.E.; Chen, Y.; Bao, B.; Kaplan, D.L.; Watnick, P.I. The Short-Chain Fatty Acids Propionate and Butyrate Augment Adherent-Invasive *Escherichia coli* Virulence but Repress Inflammation in a Human Intestinal Enteroid Model of Infection. *Microbiol. Spectr.* **2021**, *9*, e0136921. [\[CrossRef\]](#)
14. Kaczmarek, S.A.; Barri, A.; Hejdysz, M.; Rutkowski, A. Effect of different doses of coated butyric acid on growth performance and energy utilization in broilers. *Poult. Sci.* **2016**, *95*, 851–859. [\[CrossRef\]](#)
15. Pourjafar, H.; Noori, N.; Gandomi, H.; Basti, A.A.; Ansari, F. Viability of microencapsulated and non-microencapsulated *Lactobacilli* in a commercial beverage. *Biotechnol. Rep.* **2020**, *25*, e00432. [\[CrossRef\]](#)

16. Pesti, G.M. *Nutrient Requirements of Poultry*; National Academy Press: Washington, DC, USA, 1994.
17. Sciences, G.A.o.A. *Nutrient Requirements of Yellow Chickens*; Ministry of Agriculture and Rural Affairs, PRC: Beijing, China, 2020.
18. Yu, X.; Dai, Z.; Cao, G.; Cui, Z.; Zhang, R.; Xu, Y.; Wu, Y.; Yang, C. Protective effects of *Bacillus licheniformis* on growth performance, gut barrier functions, immunity and serum metabolome in lipopolysaccharide-challenged weaned piglets. *Front. Immunol.* **2023**, *14*, 1140564. [[CrossRef](#)]
19. Cong, J.; Zhou, P.; Zhang, R. Intestinal Microbiota-Derived Short Chain Fatty Acids in Host Health and Disease. *Nutrients* **2022**, *14*, 1977. [[CrossRef](#)]
20. He, W.; Goes, E.C.; Wakaruk, J.; Barreda, D.R.; Korver, D.R. A Poultry Subclinical Necrotic Enteritis Disease Model Based on Natural *Clostridium perfringens* Uptake. *Front. Physiol.* **2022**, *13*, 788592. [[CrossRef](#)]
21. Villagrán-de la Mora, Z.; Vázquez-Paulino, O.; Avalos, H.; Ascencio, F.; Nuño, K.; Villarruel-López, A. Effect of a Synbiotic Mix on Lymphoid Organs of Broilers Infected with *Salmonella typhimurium* and *Clostridium perfringens*. *Animals* **2020**, *10*, 886. [[CrossRef](#)]
22. Sikandar, A.; Zaneb, H.; Younus, M.; Masood, S.; Aslam, A.; Khattak, F.; Ashraf, S.; Yousaf, M.S.; Rehman, H. Effect of sodium butyrate on performance, immune status, microarchitecture of small intestinal mucosa and lymphoid organs in broiler chickens. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 690–699. [[CrossRef](#)]
23. Elnesr, S.; Ropy, A.; Abdel-Razik, A. Effect of dietary sodium butyrate supplementation on growth, blood biochemistry, haematology and histomorphometry of intestine and immune organs of Japanese quail. *Anim. Int. J. Anim. Biosci.* **2019**, *13*, 1234–1244. [[CrossRef](#)]
24. Ferreira, N.S.; Tostes, R.C.; Paradis, P.; Schiffrin, E.L. Aldosterone, inflammation, immune system, and hypertension. *Am. J. Hypertens.* **2021**, *34*, 15–27. [[CrossRef](#)]
25. Hand, T.W.; Reboldi, A. Production and function of immunoglobulin A. *Annu. Rev. Immunol.* **2021**, *39*, 695–718. [[CrossRef](#)]
26. Zhang, R.; Qin, S.; Yang, C.; Niu, Y.; Feng, J. The protective effects of *Bacillus licheniformis* against inflammatory responses and intestinal barrier damage in broilers with necrotic enteritis induced by *Clostridium perfringens*. *J. Sci. Food Agric.* **2023**, *13*, 6958–6965. [[CrossRef](#)]
27. Azad, M.A.K.; Sarker, M.; Wan, D. Immunomodulatory effects of probiotics on cytokine profiles. *BioMed Res. Int.* **2018**, *2018*, 8063647. [[CrossRef](#)]
28. Crawford, C.K.; Lopez Cervantes, V.; Quilici, M.L.; Armién, A.G.; Questa, M.; Matloob, M.S.; Huynh, L.D.; Beltran, A.; Karchemskiy, S.J.; Crakes, K.R. Inflammatory cytokines directly disrupt the bovine intestinal epithelial barrier. *Sci. Rep.* **2022**, *12*, 14578. [[CrossRef](#)]
29. Yu, R.; Jiang, S.; Tao, Y.; Li, P.; Yin, J.; Zhou, Q. Inhibition of HMGB1 improves necrotizing enterocolitis by inhibiting NLRP3 via TLR4 and NF- κ B signaling pathways. *J. Cell. Physiol.* **2019**, *234*, 13431–13438. [[CrossRef](#)]
30. Di Tommaso, N.; Gasbarrini, A.; Ponziani, F.R. Intestinal barrier in human health and disease. *Int. J. Environ. Res. Public Health* **2021**, *18*, 12836. [[CrossRef](#)]
31. Bischoff, S.C.; Barbara, G.; Buurman, W.; Ockhuizen, T.; Schulzke, J.-D.; Serino, M.; Tilg, H.; Watson, A.; Wells, J.M. Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol.* **2014**, *14*, 189. [[CrossRef](#)]
32. Pérez-Reytor, D.; Puebla, C.; Karahanian, E.; García, K. Use of short-chain fatty acids for the recovery of the intestinal epithelial barrier affected by bacterial toxins. *Front. Physiol.* **2021**, *12*, 650313. [[CrossRef](#)]
33. Fasina, Y.O.; Lillehoj, H.S. Characterization of intestinal immune response to *Clostridium perfringens* infection in broiler chickens. *Poult. Sci.* **2019**, *98*, 188–198. [[CrossRef](#)]
34. Ibrahim, D.; Ismail, T.A.; Khalifa, E.; Abd El-Kader, S.A.; Mohamed, D.I.; Mohamed, D.T.; Shahin, S.E.; Abd El-Hamid, M.I. Supplementing Garlic Nanohydrogel Optimized Growth, Gastrointestinal Integrity and Economics and Ameliorated Necrotic Enteritis in Broiler Chickens Using a *Clostridium perfringens* Challenge Model. *Animal* **2021**, *11*, 2027. [[CrossRef](#)]
35. Navarro, M.A.; Li, J.; McClane, B.A.; Morrell, E.; Beingesser, J.; Uzal, F.A. NanI sialidase is an important contributor to *Clostridium perfringens* type F strain F4969 intestinal colonization in mice. *Infect. Immun.* **2018**, *86*, 10–1128. [[CrossRef](#)]
36. Dąbek-Drobny, A.; Kaczmarczyk, O.; Woźniakiewicz, M.; Paśko, P.; Dobrowolska-Iwanek, J.; Woźniakiewicz, A.; Piątek-Guziewicz, A.; Zagrodzki, P.; Zwolińska-Wcisło, M. Association between Fecal Short-Chain Fatty Acid Levels, Diet, and Body Mass Index in Patients with Inflammatory Bowel Disease. *Biology* **2022**, *11*, 108. [[CrossRef](#)]
37. Fukuda, S.; Toh, H.; Hase, K.; Oshima, K.; Nakanishi, Y.; Yoshimura, K.; Tobe, T.; Clarke, J.M.; Topping, D.L.; Suzuki, T.; et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* **2011**, *469*, 543–547. [[CrossRef](#)]
38. Yang, J.; Wei, H.; Zhou, Y.; Szeto, C.H.; Li, C.; Lin, Y.; Coker, O.O.; Lau, H.C.H.; Chan, A.W.H.; Sung, J.J.Y.; et al. High-Fat Diet Promotes Colorectal Tumorigenesis Through Modulating Gut Microbiota and Metabolites. *Gastroenterology* **2022**, *162*, 135–149.e132. [[CrossRef](#)]
39. McDonald, J.A.; Mullish, B.H.; Pechlivanis, A.; Liu, Z.; Brignardello, J.; Kao, D.; Holmes, E.; Li, J.V.; Clarke, T.B.; Thursz, M.R. Inhibiting growth of *Clostridioides difficile* by restoring valerate, produced by the intestinal microbiota. *Gastroenterology* **2018**, *155*, 1495–1507.e1415. [[CrossRef](#)]
40. Pietrzak, A.; Banasiuk, M.; Szczepanik, M.; Borys-Iwanicka, A.; Pytrus, T.; Walkowiak, J.; Banaszkiwicz, A. Sodium Butyrate Effectiveness in Children and Adolescents with Newly Diagnosed Inflammatory Bowel Diseases-Randomized Placebo-Controlled Multicenter Trial. *Nutrients* **2022**, *14*, 3283. [[CrossRef](#)]

41. Jung, T.-H.; Park, J.H.; Jeon, W.-M.; Han, K.-S. Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway. *Nutr. Res. Pract.* **2015**, *9*, 343–349. [[CrossRef](#)]
42. Liu, P.; Wang, Y.; Yang, G.; Zhang, Q.; Meng, L.; Xin, Y.; Jiang, X. The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacol. Res.* **2021**, *165*, 105420. [[CrossRef](#)]
43. Mallo, J.J.; Sol, C.; Puyalto, M.; Bortoluzzi, C.; Applegate, T.J.; Villamide, M.J. Evaluation of sodium butyrate and nutrient concentration for broiler chickens. *Poult. Sci.* **2021**, *100*, 101456. [[CrossRef](#)]
44. Krix-Jachym, K.; Onyszkiewicz, M.; Swiatkiewicz, M.; Fiedorowicz, M.; Sapierzynski, R.; Grieb, P.; Rekas, M. Evaluation of butyric acid as a potential supportive treatment in anterior uveitis. *Ophthalmol. J.* **2022**, *7*, 117–126. [[CrossRef](#)]
45. Pham, V.T.; Fehlbaum, S.; Seifert, N.; Richard, N.; Bruins, M.J.; Sybesma, W.; Rehman, A.; Steinert, R.E. Effects of colon-targeted vitamins on the composition and metabolic activity of the human gut microbiome—A pilot study. *Gut Microbes* **2021**, *13*, 1875774. [[CrossRef](#)]
46. Makowski, Z.; Lipiński, K.; Mazur-Kuśnirek, M. The Effects of sodium butyrate, coated sodium butyrate, and butyric acid glycerides on nutrient digestibility, gastrointestinal function, and fecal microbiota in turkeys. *Animals* **2022**, *12*, 1836. [[CrossRef](#)]
47. Xu, Y.; Yu, Y.; Shen, Y.; Li, Q.; Lan, J.; Wu, Y.; Zhang, R.; Cao, G.; Yang, C. Effects of *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, immunity, short chain fatty acid production, antioxidant capacity, and cecal microflora in broilers. *Poult. Sci.* **2021**, *100*, 101358. [[CrossRef](#)]
48. Yao, H.; Zhang, D.; Yu, H.; Yuan, H.; Shen, H.; Lan, X.; Liu, H.; Chen, X.; Meng, F.; Wu, X. Gut microbiota regulates chronic ethanol exposure-induced depressive-like behavior through hippocampal NLRP3-mediated neuroinflammation. *Mol. Psychiatry* **2023**, *28*, 919–930. [[CrossRef](#)]
49. Li, C.; Wang, S.; Chen, S.; Wang, X.; Deng, X.; Liu, G.; Chang, W.; Beckers, Y.; Cai, H. Screening and characterization of *Pediococcus acidilactici* LC-9-1 toward selection as a potential probiotic for poultry with antibacterial and antioxidative properties. *Antioxidants* **2023**, *12*, 215. [[CrossRef](#)]
50. Pammi, M.; Cope, J.; Tarr, P.I.; Warner, B.B.; Morrow, A.L.; Mai, V.; Gregory, K.E.; Kroll, J.S.; McMurtry, V.; Ferris, M.J. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: A systematic review and meta-analysis. *Microbiome* **2017**, *5*, 31. [[CrossRef](#)]
51. Zhang, B.; Lv, Z.; Li, Z.; Wang, W.; Li, G.; Guo, Y. Dietary l-arginine Supplementation Alleviates the Intestinal Injury and Modulates the Gut Microbiota in Broiler Chickens Challenged by *Clostridium perfringens*. *Front. Microbiol.* **2018**, *9*, 1716. [[CrossRef](#)]
52. Gong, L.; Wang, B.; Zhou, Y.; Tang, L.; Zeng, Z.; Zhang, H.; Li, W. Protective effects of *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 against *Clostridium perfringens* infection in broilers. *Front. Immunol.* **2021**, *11*, 628374. [[CrossRef](#)]
53. Liu, Y.; Zhang, S.; Luo, Z.; Liu, D. Supplemental *Bacillus subtilis* PB6 improves growth performance and gut health in broilers challenged with *Clostridium perfringens*. *J. Immunol. Res.* **2021**, *2021*, 2549541. [[CrossRef](#)] [[PubMed](#)]
54. Wu, M.; Cao, W.; Chen, X.; She, J. Intelligent Optimization and Control for Reheating Furnaces. In *Intelligent Optimization and Control of Complex Metallurgical Processes*; Springer: Singapore, 2020; pp. 223–271. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.