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Effects of dietary lysozyme on immune response and fecal microflora in both sows and their offspring

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ABSTRACT - Two studies were conducted to investigate the effects of dietary lysozyme on immune response, fecal microflora in sows and their offspring fed lysozyme from late gestation to the onset of lactation, and growth performance in weaned piglets. Four antibiotic-based treatments (chlortetracycline, colistin, and lysozyme) were applied in experiment 1. Lysozyme addition significantly increased final body weight, average daily gain, and average daily feed intake, improved feed:gain ratio (F:G), and decreased diarrhea rate in weaned piglets. In experiment 2, postpartum sows were fed diets either with amoxicillin and cephalosporin (SC) or lysozyme (SE). Piglets from SC sows were administered enrofloxacin and those from SE sows were administered lysozyme. Lysozyme treatment decreased serum IL-1, IL-6, and IL-10, but did not influence IL-8, TNF-α, or IFN-γ in weaned piglets. Sequencing revealed that lysozyme significantly decreased Chao-1 index in sows and weaned piglets, increased *Bifidobacterium longum* in sows, and *Lactobacillus coleohominis*, *L. mucosae*, *L. amylovorus*, and *L. hamsteri* in weaned piglets. The results suggest that dietary supplementation of lysozyme improved the growth performance of weaned piglets, and dietary supplementation of lysozyme for sows increased immune function and modulated the intestinal flora structure in sows and their offspring.

Keywords: 16S high-throughput sequencing, feed additive, growth performance, immunological function, weaner

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1. Introduction

Antibiotics have been applied as growth promoters in swine production for more than half a century. However, problems surrounding the use of antibiotics, such as drug resistance, reduced safety of animal products for human consumption, and environmental pollution, have arisen and posed a risk to human health. The European Union banned the use of antibiotics in the livestock industry a decade ago (Wegener, 2003). In China, antibiotics will also be banned in animal feed from 2020. Hence, studies about potential antibiotic alternatives containing lysozyme, probiotics, acidifiers, or plant extracts are necessary.

A study found that lysozyme, a 1,4-b-N-acetylmuramidase, cleaves N-acetyl-glucosamine and N-acetylmu-raminic acid linkages in bacterial cell wall peptidoglycans (Wells et al., 2015). Lysozyme is secreted in tears, saliva, and the mucosa of the mammalian intestinal tract and can modulate anti-inflammatory (Goldman et al., 1986) and immune (Kawano et al., 1981) responses. Many studies have reported the beneficial effects of lysozyme on intestinal morphology and intestinal microflora

(Brundige et al., 2008; Maga et al., 2012) in piglets. Lysozyme from egg whites had a positive effect on the growth of young piglets (Oliver and Wells, 2013). In addition, lysozyme can directly or indirectly modulate the complement system and strengthen the function and multiplication of polymorphonuclear neutrophils and phagocytes (Torsteinsdóttir et al., 1999).

Recently, Xiong et al. (2019) reported that dietary lysozyme improved the development of intestinal structure and function and increased beneficial intestinal microbes in piglets. Xu et al. (2018) found that sows fed diets supplemented with lysozyme in late gestation had increased average daily feed intake (ADFI) and, subsequently, improved sow and piglet health status, which was evidenced by immunological characteristics and a lower incidence of diarrhea. Zhou et al. (2019) noted that supplementation of lysozyme provided to sows for 21 days effectively improved composition, metabolic functions, and phenotypes of gut microbiota of sows. However, limited studies have focused on the influence of lysozyme given to sows during late gestation on fecal microflora of sows and their offspring. Hence, this study was conducted to investigate the effects of lysozyme on immune response and fecal microflora in sows and their offspring. Meanwhile, growth performance was also studied in weaned piglets fed lysozyme.

2. Material and Methods

The experiments were conducted in Haining city, Zhejiang province, China (30°21'10" N and 120°23'23" E, and altitude of 7 m asl). All the following processes were performed according to the relevant institutional committee on animal use (No. 2017-82). All piglets were raised under standard conditions and had *ad libitum* access to feed and water.

In experiment 1, 240 healthy piglets, weaned on day 28 and with similar initial body weight, were randomly divided into four treatment groups with six replicates for each treatment (10 piglets/pen, five males and five females). The control group received a basal diet without any antibiotic additives, the antibiotic group (GB) received the basal diet with normal dose of antibiotic (75 mg chlortetracycline/kg and 20 mg colistin sulfate/kg), the high-antibiotic-dose group (IA) received the basal diet with a high dose of antibiotic (150 mg chlortetracycline/kg and 100 mg colistin sulfate/kg), and the lysozyme group (LSZ) received the basal diet with 0.15% lysozyme (Zhejiang Aegis Biotech Co., Ltd, Hangzhou, China).

The whole study lasted for 40 days. Days 29 to 42 were considered the creep stage, and days 43 to 70 were considered the nursery stage. The raw material formula and nutrient contents of creep and nursery diets were formulated according to recommendations of the NRC (2012) (Table 1).

In experiment 2, 24 healthy, pregnant sows were randomly divided into two groups. The postpartum sow control group (SC) received the basal diet supplemented with 1.5 kg amoxicillin/t for one week and a cephalosporin injection once a day for three days after farrowing. The postpartum sow experimental group (SE) received the basal diet with 0.3 kg lysozyme/t before farrowing for 30 days and 0.5 kg lysozyme/t after farrowing. Enrofloxacin was administered to piglets born from SC sows to prevent diarrhea (PC), while piglets born from SE sows were administered 50 mL of 250 U/mL lysozyme liquid to prevent diarrhea (PE) and were fed the basal diet without antibiotics. The PC piglets were fed a commercial diet with antibiotics (Shanghai New Type Feed Co., Ltd., Shanghai, China) and PE piglets were fed the basal diet with 1.5 kg lysozyme/t (Aegis, Hangzhou, China). The activity of lysozyme was tested at 500,000 U/g, which was derived by microbial fermentation.

On day 56, blood was collected from the portal vein from PC and PE piglets (12 per treatment) and then stored at −80 °C until samples were prepared for detection of immune response. Sterile cotton swabs were stretched into the anus to collect fresh feces from each pen in the morning and then stored at −80 °C for 16S high-throughput sequencing. The basal formula and nutritional levels of creep and nursery diets were formulated according to their commendations of the NRC (2012) (Table 1).

Residual feed and incidence of diarrhea in each pen were recorded daily. Final body weight was used to calculate average daily gain (ADG) and feed conversion ratio. Diarrhea incidence was evaluated as

Table 1 - Basal formula and nutritional level of diet[1] (air-dry basis)

Item (%)	Stage		
	Sow feed	Creep feed	Nursery feed
Corn	62	62	62
Expanded soybean		11	10
Common soybean meal	23		
Dehulled soybean meal		9	18
Fermented soybean meal	2		
Spray-dried plasma protein		2.7	
Whey powder		8	4
Fish meal	1	3	3
50% Fat powder	4		
Wheat bran	4		
Zinc oxide		0.3	
Premix[1]	4	4	3
Total	100	100	100
Nutrient level[2]			
Crude protein (%)	17.0	18.0	19.0
Digestible energy (MJ/kg)	14.6	14.4	14.6
Total lysine (%)	0.86	1.30	1.25
Ca (%)	0.80	0.65	0.70
Total P (%)	0.65	0.55	0.60

[1] Supplied per kilogram of diet: sow diet - vitamin A (retinyl acetate), 5,000 IU; cholecalciferol, 500 IU; vitamin E (DL-tocopheryl acetate), 44 IU; riboflavin, 3.75 mg; pantothenic acid, 12 mg; niacin, 10 mg; cobalamin, 0.02 mg; choline chloride, 1,000 mg; biotin, 0.2 mg; folic acid, 1.3 mg; thiamine, 1.0 mg; pyridoxine, 1.0 mg; Fe, 120 mg; Zn, 100 mg; Mn, 60 mg; I, 0.3 mg; Cu, 15 mg; Se, 0.3 mg.
Creep diet and nursery diet: vitamin A (retinyl acetate), 8,500 IU; cholecalciferol, 1000 IU; vitamin E (DL-tocopheryl acetate), 15 IU; riboflavin, 3.5 mg; pantothenic acid, 13 mg; niacin, 25 mg; cobalamin, 0.03 mg; choline chloride, 500 mg; biotin, 0.2 mg; folic acid, 0.7 mg; thiamine, 1.3 mg; pyridoxine, 1.2 mg; Fe, 130 mg; Zn, 120 mg; Mn, 30 mg; Cu, 15 mg; I, 0.3 mg; Se, 0.3 mg.
[2] Calculated values.

described by Liu et al. (2010). Briefly, the fecal consistency was classified at four levels: 0, normal; 1, pasty; 2, semiliquid; 3, liquid. Piglets were considered with diarrhea when the fecal consistency was at level 2 or 3, and the incidence of diarrhea was calculated by dividing the number of piglets with diarrhea in each pen by the total days of piglets during that interval.

Immune parameters in serum were measured by commercial kits designed for estimation of lysozyme, IL-1, IL-6, IL-8, IL-10, TNF-α, and IFN-γ (Cusibio Biotechnological Co. Ltd., Wuhan, China) following manufacturer's instructions.

A total of 40 fecal samples (10 per treatment) were selected for 16S high-throughput sequencing. DNA of fecal microflora was isolated using the QIAamp DNA Stool Mini Kit (Qiagen, CA, Germany) following manufacturer's protocol. The V3-V4 region was sequenced on the Illumina MiSeq platform (LC-Bio, Hangzhou, China) according to manufacturer's instructions. PCR was performed using the unique primers 319F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′).

Raw data were processed using Greengenes (DeSantis et al., 2006), and sequential analysis, comparison, and annotation were performed with Ribosomal Database Project (Version 11.3). Illumina MiSeq 2×300 bp paired-end was used for sequencing. Operational taxonomic units (OTU) with a similarity threshold of 97% were determined in R (version 3.1.0).

All statistical analyses were initially collated by Excel 2019 and performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Analyses of growth performance between groups were performed by one-way ANOVA with line segment detector (LSD). Results of immune response analyses were performed by t test. Analyses of diarrhea rate were performed by chi-square test. Alpha and beta

diversities were analyzed using Qiime 1.7.0. and displayed using R v.2.15.3 software. A level of P<0.05 was considered to indicate a significantly different response, and a tendency was revealed at 0.05<P<0.1.

3. Results

Supplementation of lysozyme and antibiotics significantly increased final body weight and ADG compared with the control treatment from days 30-70. Piglets from LSZ had a significantly higher ADG than those in GB treatment. Also, LSZ and IA piglets presented markedly increased ADFI and decreased diarrhea rate as compared with control piglets. Compared with control piglets, the application of lysozyme and antibiotics significantly improved F:G. Piglets of LSZ treatment also had significantly improved F:G compared with GB piglets (Table 2).

Piglets of PE treatment had higher (P<0.05) lysozyme content than PC piglets. The supplementation of lysozyme decreased the content of IL-1 and IL-6 compared with the control (P<0.05). Concentration of IL-10 tended to decrease in PE piglets. However, there was no significant difference in concentration of TNF-α and IFN-γ between PC and PE piglets (Table 3).

Effects of lysozyme on fecal microflora α-diversity of sows and piglets were consistent. Lysozyme markedly decreased Chao-1 index and tended to increase observed species in weaned piglets and sows (Figure 1).

The Venn diagram results showed that 4225 OTU were shared between SE and SC piglets, and 440 and 215 OTU were unique to SE and SC piglets, respectively (Figure 2A). Meanwhile, 3506 OTU were shared between PE and PC piglets, and 916 and 337 OTU were unique to PE and PC piglets, respectively (Figure 2B). Both the principal component analysis (PCA) and the PCoA and NMDS analyses showed that group swine were significantly different from group piglets (Figure 2C–E). Moreover, PC samples were mostly separated from PE group based on PCoA plot and NMDS analysis. In addition, samples in groups SC and SE were partly separated from each other based on the PCoA plot and NMDS analysis.

Table 2 - Effects of lysozyme on the growth performance of piglets from day 29 to 70[1]

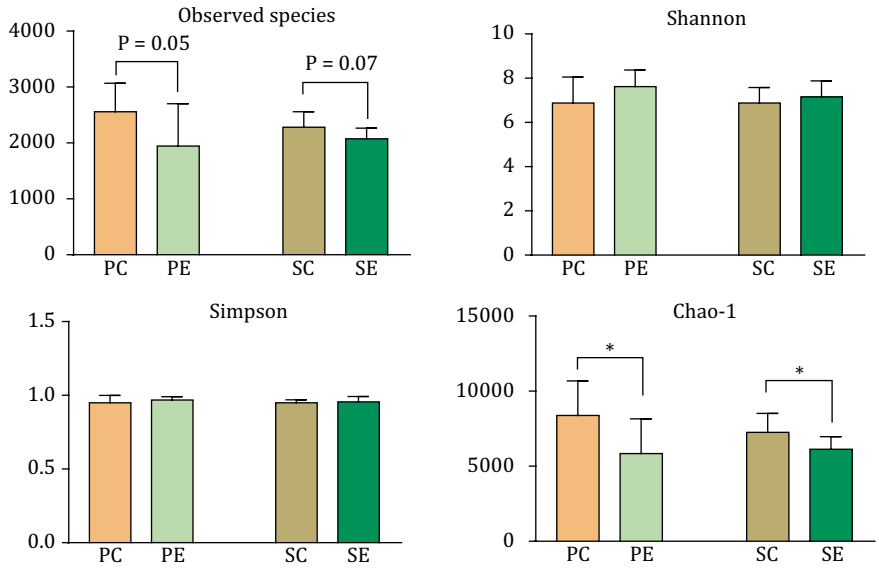
Item	Treatment				P-value
	Control	GB	IA	LSZ	
Initial body weight	8.33±0.32	8.30±0.43	8.30±0.43	8.30±0.42	0.99
Final body weight	18.38±1.12c	20.43±0.96b	22.04±0.51a	21.94±1.49a	0.01
ADG (g)	251±31.25c	303±26.28b	344±22.62a	341±40.30a	0.02
ADFI (g)	521±59.73b	565±52.09ab	609±34.93a	599±47.63a	0.03
F:G	2.07±0.07a	1.86±0.04b	1.77±0.09c	1.76±0.07c	0.01
Diarrhea rate (%)	8.70±3.52a	4.60±1.42a	2.90±1.58b	1.80±0.97b	0.01

ADG - average daily gain; ADFI - average daily feed intake; F:G - feed to gain ratio.
[1] Control: piglets fed a basal diet; GB: piglets fed a basal diet supplemented with standard level antibiotic; IA: piglets fed a basal diet supplemented with extensive standard level antibiotic; LSZ: piglets fed lysozyme; N = 12.
a-c - Means in the same row with different superscript letters differ significantly (P<0.05).

Table 3 - Effects of lysozyme on the immune responses of weaned piglets on d 56[1]

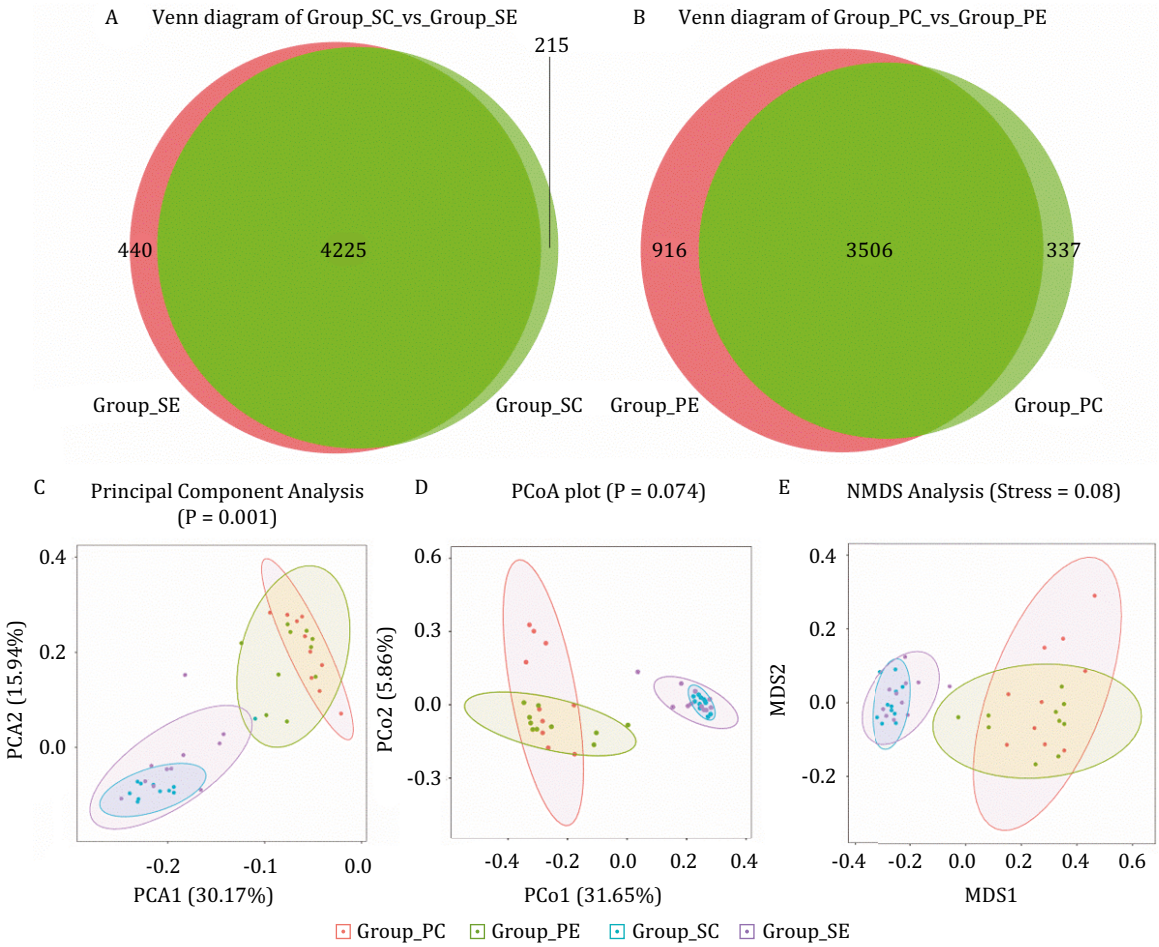
Item	Lysozyme (U/mL)	IL-1 (pg/mL)	IL-6 (ng/L)	IL-8 (ng/L)	IL-10 (pg/mL)	TNF-α (pg/mL)	IFN-γ (pg/mL)
PC	152.3±26.8	153.4±18.6	71.8±4.7	34.6±3.8	85.7±5.8	92.6±7.6	228.5±29.8
PE	177.1±35.1	138.89±13.2	66.9±6.0	33.8±3.4	82.1±4.7	90.3±4.3	222.1±16.7
P-value	0.04	0.02	0.02	0.51	0.09	0.36	0.49

[1] PC: piglets fed a basal diet; PE: piglets fed a basal diet supplemented with lysozyme; N = 12.
P<0.05 was considered a significant difference and 0.05<P<0.1 was considered a significant tendency.



PC: piglets fed a basal diet; PE: piglets fed a basal diet supplemented with lysozyme; SC: sows fed a basal diet; SE: sows fed a basal diet supplemented with lysozyme.
N = 10.
* Significant when P<0.05.

Figure 1 - Effects of lysozyme on fecal microbial community diversity of swine and piglets.

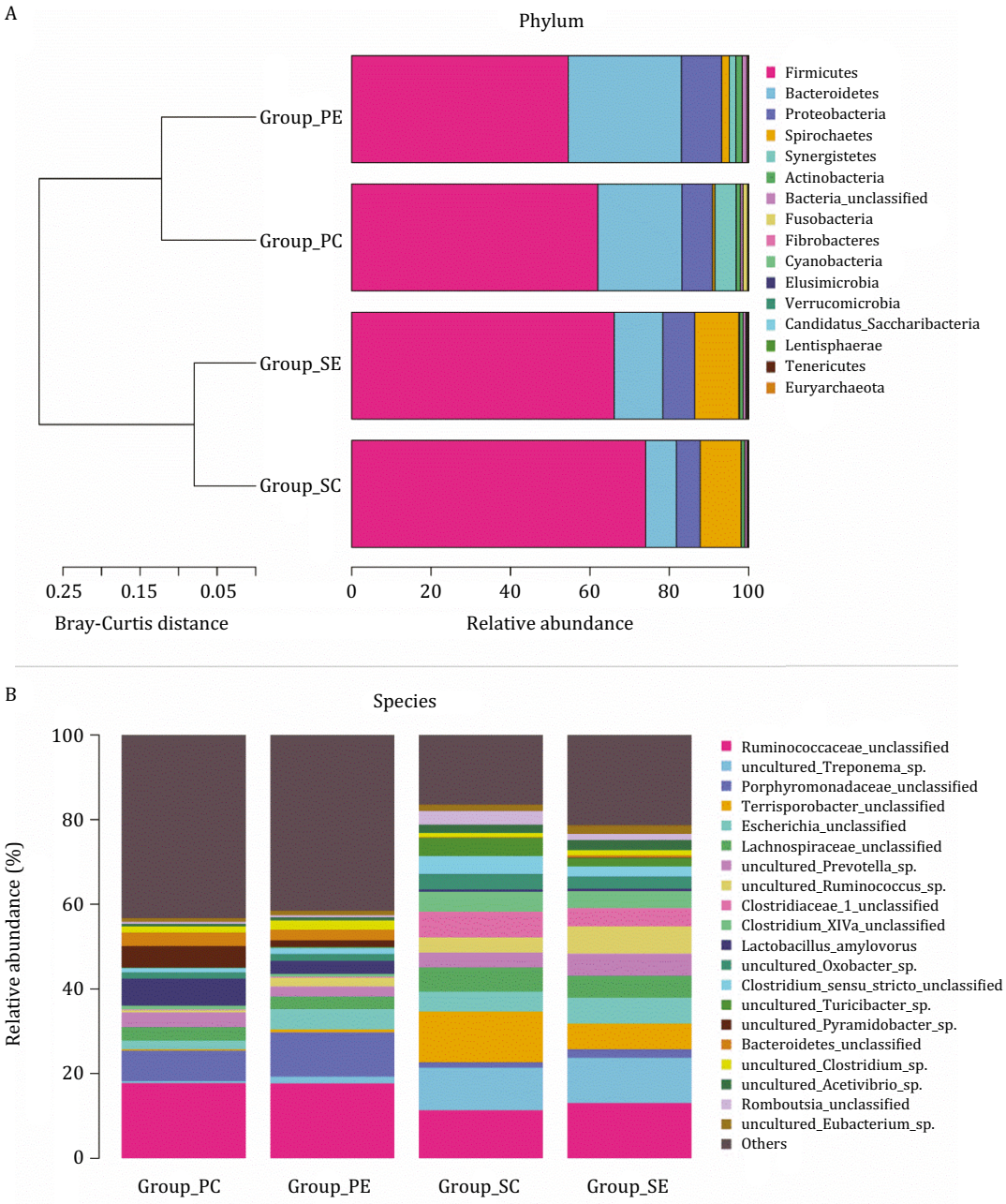


PC: piglets fed a basal diet; PE: piglets fed a basal diet supplemented with lysozyme; SC: sows fed a basal diet; SE: sows fed a basal diet supplemented with lysozyme.
A - Venn diagram of SC and SE; B - Venn diagram of PC and PE; C, D, and E - PCA, PCoA, and NMDS analysis, respectively.
N = 10.

Figure 2 - Effects of treatments on the colonic microflora of swine and piglets.

From the unweighted pair-group method with arithmetic mean based on the Bray-Curtis distance at the phylum level (Figure 3A), the microfloral composition of weaned piglets was clustered. In terms of relative abundance, the top six phyla were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Spirochaetes*, *Synergistetes*, and *Actinobacteria* in all groups. Lysozyme treatment decreased the relative abundance of *Firmicutes* and increased *Bacteroidetes* and *Proteobacteria* in both sows and weaned piglets.

From the relative abundance of the major bacterial strains at species level, the following were the dominant groups in weaned piglets on day 28 (Figure 3B): *Ruminococcaceae*, *Porphyromonadaceae*, *Lachnospiraceae*, *Prevotella* spp., *Lactobacillus amylovorus*, *Oxobacter* sp., *Clostridium sensu stricto*, and *Bacteroidetes* predominated in groups of swine. Moreover, *Ruminococcaceae*, *Treponema* sp., *Porphyromonadaceae*, *Terrisporobacter*, *Lachnospiraceae*, *Prevotella* sp., *Ruminococcus* sp., *Clostridiaceae*,



PC: piglets fed a basal diet; PE: piglets fed a basal diet supplemented with lysozyme; SC: sows fed a basal diet; SE: sows fed a basal diet supplemented with lysozyme.
N = 10.

Figure 3 - Effects of lysozyme on the colonic microflora of swine and piglets on phylum and species levels.

and *Clostridium*. Supplementation with lysozyme significantly increased the relative abundance of *Bifidobacterium longum* and *Citrobacter freundii* and tended to increase *Parabacteroides distasonis*, *Enterococcus avium*, *Sphingomonas yabuuchiae*, *Bacteroides ovatus*, and *Bacteroides coprophilus* in the feces of sows (Table 4). Supplementation with lysozyme tended to increase the relative abundance of *Lactobacillus coleohominis*, *L. hamsteri*, *L. mucosae*, *L. plantarum*, *Weissella paramesenteroides*, and *Ruminococcus flavefaciens* in the feces of weaned piglets (Table 5).

Table 4 - Change of the relative abundance of fecal microflora in lysozyme-supplemented sows at the species level

Item	Mean (SC)	SEM (SC)	Mean (SE)	SEM (SE)	P-value
Citrobacter freundii	0.000002	0.000007	0.000019	0.000031	0.008
Bifidobacterium longum	0.000001	0.000004	0.000016	0.000021	0.015
Petrimonas sulfuriphila	0.000018	0.000031	0.000000	0.000000	0.033
Lactobacillus paralimentarius	0.000010	0.000015	0.000000	0.000000	0.033
Peptostreptococcus anaerobius	0.000000	0.000000	0.000011	0.000028	0.047
Victivallis vadensis	0.000000	0.000000	0.000011	0.000021	0.047
Sphingomonas echinoides	0.000000	0.000000	0.000008	0.000014	0.047
Helicobacter winghamensis	0.000000	0.000000	0.000007	0.000023	0.047
Bacteroides coprophilus	0.000038	0.000068	0.001408	0.002975	0.091
Bacteroides eggerthi	0.000000	0.000000	0.000104	0.000337	0.091
Bacteroides ovatus	0.000007	0.000012	0.000069	0.000095	0.091
Bacteroides plebeius	0.000041	0.000100	0.000000	0.000000	0.091
Parabacteroides distasonis	0.000002	0.000008	0.000234	0.000689	0.091
Enterococcus avium	0.000002	0.000008	0.000047	0.000056	0.091
Lactobacillus brevis	0.000021	0.000040	0.000000	0.000000	0.091
Sphingomonas yabuuchiae	0.000002	0.000008	0.000035	0.000042	0.091
Helicobacter rappini	0.000081	0.000264	0.000000	0.000000	0.091

SEM - standard error of the mean.
SC: sows fed a basal diet; SE: sows fed a basal diet supplemented with lysozyme; N = 10.
P<0.05 was considered a significant difference and 0.05<P<0.1 was considered a significant tendency.

Table 5 - Change of the relative abundance of fecal microflora in lysozyme-supplemented weaned piglets at the species level

Item	Mean (PC)	SEM (PC)	Mean (PE)	SEM (PE)	P-value
Sphingomonas yabuuchiae (G−)	0.000020	0.000034	0.000000	0.000000	0.009
Streptococcus minor (G+)	0.000015	0.000021	0.000000	0.000000	0.020
Zea luxurians	0.000012	0.000038	0.000000	0.000000	0.044
Lactobacillus plantarum	0.000000	0.000000	0.000016	0.000033	0.067
Aeromonas simiae	0.000022	0.000040	0.000007	0.000014	0.089
Parabacteroides gordonii	0.000044	0.000103	0.000000	0.000000	0.091
Myroides odoratimimus	0.000322	0.000790	0.000020	0.000044	0.091
Lactobacillus coleohominis	0.000228	0.000282	0.001173	0.001819	0.091
Lactobacillus hamsteri	0.000035	0.000051	0.000131	0.000095	0.091
Lactobacillus mucosae	0.000052	0.000075	0.000357	0.000646	0.091
Weissella paramesenteroides	0.000003	0.000009	0.000040	0.000076	0.091
Ruminococcus callidus	0.000096	0.000304	0.000000	0.000000	0.091
Ruminococcus flavefaciens	0.001443	0.003141	0.015734	0.034198	0.091
Helicobacter trogontum	0.000004	0.000014	0.000085	0.000130	0.091
Pseudoxanthomonas mexicana	0.000073	0.000216	0.000000	0.000000	0.091
Staphylococcus equorum	0.000010	0.000017	0.000000	0.000000	0.095

SEM - standard error of the mean.
PC: piglets fed a basal diet; PE: piglets fed a basal diet supplemented with lysozyme; N = 10.
P<0.05 was considered a significant difference and 0.05<P<0.1 was considered a significant tendency.

4. Discussion

Lysozyme is a natural antimicrobial substance and has been widely used as a feed additive to improve growth and feed efficiency in livestock production (Oliver and Wells, 2015). Previous studies have reported contradictory effects of lysozyme in weaned piglets. A higher ADG and feed efficiency were observed at weaning in piglets fed lysozyme from days 0-28 (Oliver and Wells, 2013). Oliver et al. (2014) also noted that lysozyme positively influenced weight gain and feed efficiency. However, several studies using human lysozyme from transgenic goat milk failed to observe an increase in growth performance of pigs consuming the transgenic goat milk (Brundige et al., 2008; Maga et al., 2006).

In the present study, lysozyme supplementation significantly increased body weight, ADG, and ADFI and improved F:G in weaned piglets. The difference might due to the feed formula and source of lysozyme (May et al., 2012; Oliver and Wells, 2013). In addition, lysozyme supplementation significantly decreased diarrhea incidence in their piglets. Huang et al. (2018) also found that the intake of milk supplemented with lysozyme played a beneficial role in recovery from infection with lower mortality in ETEC-challenged piglets. Compared with piglets fed a basal diet, those fed lysozyme had a higher villus length:crypt depth ratio in the jejunum (Oliver and Wells, 2013) and ileum (Huang et al., 2018), and increased ileal villus height (Nyachoti et al., 2012). These results indicate that lysozyme is beneficial for nutrient absorption and growth performance. Small intestine morphology of piglets was not measured in the current study, and future studies should focus on this.

Cytokines are important indicators of immune status and inflammation response. Studies have reported that lysozyme can modulate innate immunity and prevent intestinal inflammation (Lee et al., 2015; Patel and Kuyucak, 2017; Ragland and Criss, 2017; Huang et al., 2018). Xu et al. (2018) found that piglets from sows fed lysozyme had significantly higher serum concentrations of IgA, IgG, IgM, and IL-10. A recent study by Xiong et al. (2019) reported that the content of colonic mucosal IL-4 was significantly increased. In DSS-induced colitis porcine model, dietary lysozyme upregulated the mRNA abundance of IL-4 and TGF-β (Lee et al., 2009). The current study was similar to above studies, which found that the lysozyme supplementation to sows significantly decreased the concentrations of IL-1 and IL-6 and tended to decrease the concentration of IL-10 in their offspring. These results indicate a better immune status and inflammation resistance of piglets.

Intestinal microbes are closely related to animal growth performance, especially in terms of intestinal barrier functions and immune response, as well as digestion and absorption of nutrients (Cox et al., 2014). Maternal diet during pregnancy is associated with intestinal development and microfloral composition of the offspring (Chu et al., 2016; Cheng et al., 2018). In the present study, we noted that dietary lysozyme decreased microbial diversity in both sows and weaned piglets evidenced by Chao-1 index. The results were similar to those of Zhou (2019), who found a reduction of gut microbial diversity in sows' diet with 1.0 kg/t lysozyme for 24 days. The reduced microbial diversity might due to the decreased gastrointestinal pathogens (Zhang et al., 2016; Zhou et al., 2019). In the current study, the OTU, PCA, PCoA, and NMDS analyses revealed that fecal microfloral composition was changed by lysozyme supplementation in sows and weaned piglets. A recent study by Xiong et al. (2019) revealed that lysozyme treatment significantly modulated the gut microfloral composition by decreasing the relative abundance of *Firmicutes*, increasing the relative abundance of *Bacteroidetes*, *Proteobacteria*, and *Fibrobacteres* and increasing *Lactobacillus*, *Treponema*, and the *Prevotellaceae* NK3B31group in piglets.

At species level, supplementation of lysozyme modulated the fecal microflora by increasing *B. longum* in sows and tended to increase *L. coleohominis*, *L. mucosae*, *L. amylovorus*, and *L. hamsteri* in weaned piglets. These bacteria are kinds of conventional probiotics that were thought to be associated with intestinal function and mucosal immunity (Yang et al., 2014). Our results are consistent with study of Xu et al. (2020), which found that *Lactobacillus* was significantly increased in feces by supplementation of 150 mg/kg lysozyme in diet of sows on day 21 of lactation. Zhou et al. (2019) also noted that dietary lysozyme significantly increased the relative abundance of sow fecal microbiota, such as *Lactobacillus amylovorus* and *Treponema bryantii*. Huang et al. (2018) revealed that lysozyme intake had a protective effect by improving gut health of piglets with ETEC-induced diarrhea, including the enrichment of intestinal bacteria (*Lactobacillus*).

5. Conclusions

Lysozyme have positive effects on growth performance of weaned piglets. Supplementation of lysozyme for sows could increase immune function and modulate microbiota by enriching beneficial microbes in sows and their offspring.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: B. Deng and Z.W. Xu. Data curation: B. Deng. Formal analysis: W.D. Hua and Y.M. Li. Funding acquisition: B. Deng, H.T. Pan and H.L. Pan. Investigation: W.D. Hua, H.L. Pan and Z.W. Xu. Project administration: B. Deng, H.T. Pan, J. Wu, W.D. Hua and Y.M. Li. Resources: H.T. Pan, Y.M. Li and H.L. Pan. Software: Y.M. Li. Supervision: H.L. Pan and Z.W. Xu. Visualization: J. Wu. Writing-original draft: J. Wu. Writing-review & editing: J. Wu.

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