







DEVELOPMENT AND VALIDATION THE EFFICACY OF *BACILLUS*-BASED FERMENTED PRODUCTS AS AN ANTIBIOTICS ALTERNATIVE IN DOMESTIC ANIMALS

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ABSTRACT

Intestinal microflora plays a critical role in the process of nutrient digestion and immunomodulation. Normal gut microbiota is responsible for resistance to colonization by exogenous pathogenic microorganisms. Pathogen invasion alters the intestinal microbiota composition and causes intestinal disorder in domestic animals. Moreover, stress, infection, or dysbiosis during the feeding period also results in an imbalanced gut microbiota, which also leads to postweaning diarrhea of piglets and necrotic enteritis of chickens. It has been demonstrated that *B. licheniformis* and *B. subtilis* has antimicrobial activity against pathogens through the production of antibacterial cyclic lipopeptide surfactin. Therefore, in the present review a series research works on the *Bacillus* based-fermented products efficacy and validation by alleviating the diarrhea incidence, enteritis, coccidiosis, modified gut microbiota, and decrease postpartum dysgalactia syndrome in pigs and chickens.

Key words: validation, *Bacillus*-based fermented products, antibiotics alternative, domestic animals

INTRODUCTION

In the past decade, scientists have discovered that animal intestinal microbial disorders are related to many intestinal diseases. Therefore, how to choose and increase intestinal probiotics has become a topic of research. In fact, intestinal health is also very important for domestic animals. In order to reduce the use of antibiotics, our research team has recently discovered that the use of *Bacillus*-based fermented products can strengthen the resistance of livestock and poultry to intestinal diseases, prevent common intestinal viral bacteria and protozoan infections. These findings showed the potential in replacing intestinal antibiotics. As the digestive tract disease is a high-incidence disease of poultry and livestock, especially pigs and chickens, it is also an “invisible killer” for healthy animal farming. Traditional animals farming

was allowed pigs to use antibiotics or to add antibiotics to poultry drinking water or feed. However, this approach could lead to drug resistance, and drug residues to cause food safety concerns. Taiwan’s pig farming is developed and the breeding technology is also quite advanced, and it has achieved good results in breed improvement or disease resistance. However, due to the limited land resources, excessively intensive breeding, coupled with the island-type humid and hot climate condition and crowded stable environment is easy for bacteria to proliferate, and it is also easy for growing livestock and poultry to infect respiratory or intestinal bacteria. A secondary metabolite, surfactin showed in Fig. 1, was first found in *B. subtilis* and consists of multiple isoforms [Haddad et al. 2008, Sousa et al. 2014, Sumi et al. 2015]. The structure of surfactin is a seven amino acid peptide loop and a hydrophobic fatty acid chain [Carrillo et al. 2003]. It has

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been reported that surfactin has a broad spectrum of antimicrobial activity against pathogenic microbes [Chen et al. 2008, Sumi et al. 2015].

Therefore, our research mission and top priority are to focus on developing *Bacillus*-based-fermented products as a feed additives to be an antibiotics alternative in domestic animals.

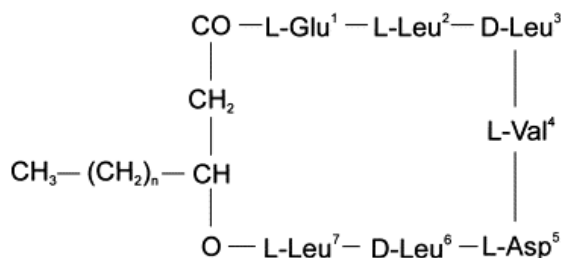


Fig. 1. Primary structure of surfactin ($n = 9-11$) [Carrillo et al. 2003]

Optimization of solid-state fermentation of *Bacillus* species and its antibacterial activity

Bacillus species are commonly used as probiotics in the poultry feed industry for preventing infectious diseases and improving productivity by altering gastrointestinal microbiota. We established a solid-state fermentation system to cultivate *Bacillus licheniformis*, during 4–6 days fermentation, the highest viable biomass was observed at 5% glucose, 10% soybean meal, 3% yeast, and 50% initial moisture content [Lin et al. 2019]. Furthermore, We evaluate the surfactin from *B. subtilis*-fermented products on *Clostridium perfringens*-induced necrotic enteritis and growth performance in broilers. Results showed the 4-day of *B. subtilis*-fermented products with the highest surfactin concentration (Fig. 2) and showed the maximal antimicrobial activity against pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhimurium*, and *C. perfringens*. The 4- and 6-day of *B. subtilis*-fermented products were heat-, acid- and bile-resistant. Furthermore, dietary *B. subtilis* fermented product supplementation in broilers significantly improved intestinal morphology and necrotic lesions under *C. perfringens* challenge [Cheng et al. 2018, Cheng et al. 2021].

Evaluation of the antiviral activity of *Bacillus licheniformis*-fermented products against porcine epidemic diarrhea virus

Our teamwork evaluate the antiviral activity of crude extracts from *B. licheniformis* against the porcine epidemic diarrhea virus (PEDV), a highly contagious enveloped porcine virus that has caused a huge economic loss in pigs. An animal trial were was performed as Control ($n = 5$), PEDV ($n = 5$); and PEDV + BLFP ($n = 5$). These

pigs were fed a commercial diet mixed with or without 5 kg/L BLFP as feed additives for 7 days prior to the viral challenged with or without 5×10^5 TCID₅₀ of the virulent PEDVPT-P6 at 5 weeks of age. Results showed milder clinical symptoms and decreased viral shedding (Fig. 3), In the PEDV group (the grey line), fecal viral shedding was first detected ($3.92 \pm 3.88 \log_{10}$ GE) at 2 DPI, found to gradually increase to peak viral load ($5.71 \pm 3.38 \log_{10}$ GE) at 5 DPI, and was continuously detected until 12 DPI. In the PEDV + BLFP group (the black line), the pattern of viral shedding was similar to but lower than that in the PEDV group during the study. Importantly, no significant systemic pathological lesions and no reduction in average daily gain were noted in pigs supplemented with the BLFP, which suggests that it is safe for use in pigs. In vitro experiments revealed that while *B. licheniformis* crude extracts exhibited no toxicity in Vero cells, co-cultivation of *B. licheniformis* crude extracts with PEDV significantly reduced viral infection and replication. In the present study, BLFP was investigated as an antiviral candidate against PEDV. In vivo by piglets challenge model, we first validated the safety and antiviral ability against of BLFP as a feed additive less than 5 kg/ton against PEDV after a long period of PEDV inoculation [Peng et al. 2019].

Antibacterial activity of *Bacillus* species-derived surfactin on *Brachyspira hyodysenteriae* and *Clostridium perfringens*

Swine dysentery and necrotic enteritis are a bane to animal husbandry worldwide. Some countries have already banned the use of antibiotics as growth promoters in animal production. Our development *Bacillus* produced surfactin is a potential alternative to antibiotics and antibacterial agents. Results showed that multiple surfactin isoforms were detected in *B. subtilis*, while only one surfactin isoform was detected in *B. licheniformis*-fermented products. The surfactin produced from *B. subtilis* exhibited significant antibacterial activity against *B. hyodysenteriae* compared with surfactin produced from *B. licheniformis* and *B. subtilis*-derived surfactin could inhibit bacterial growth and disrupt the morphology of *B. hyodysenteriae* (Shown as Fig. 4). Results of scanning electron microscopy showed that *B. subtilis*-derived surfactin could penetrate and disrupt the morphology of *B. hyodysenteriae* in a dose dependent manner after 1 h treatment compared with the untreated group. Furthermore, the surfactin produced from *B. subtilis* has the highest activity against *C. perfringens* growth. Due to Necrotic enteritis is an extremely common and important avian enteric disease caused mainly by *Clostridium perfringens* [Van Immerseel et al. 2004, Timbermont et al. 2011]. It leads to enormous economic losses in the poultry industry worldwide [Van der Sluis 2000, Timbermont et al.

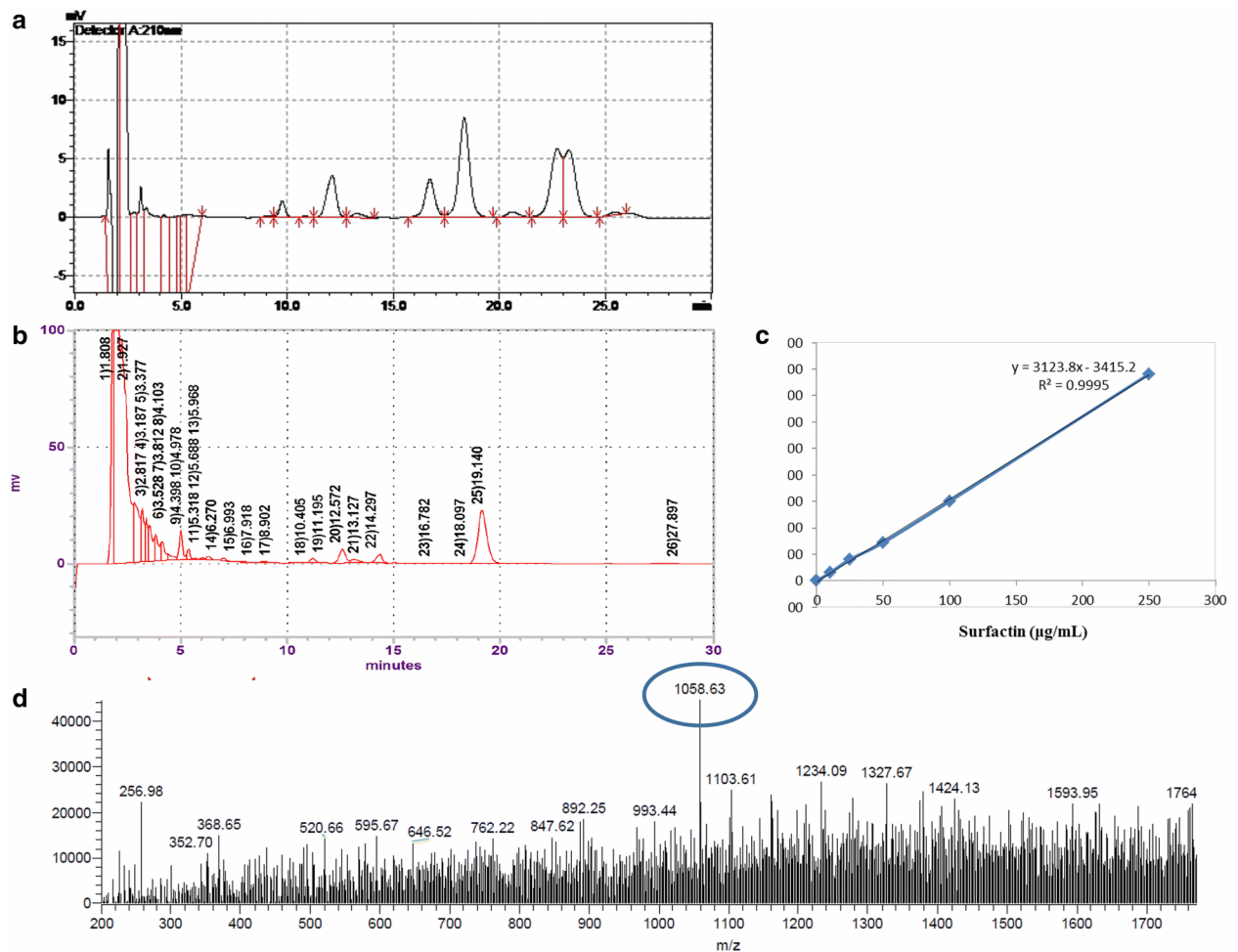


Fig. 2. Identification of the 2nd metabolites in standard substance surfactin derived from *B. subtilis* (a) and fermentative sample derived from *B. licheniformis*-fermented products (BLFP) (b) by liquid chromatography–mass spectrometry (LC–MS). Using the standard curve of surfactin derived from *B. subtilis* (c), the 2nd metabolite at [M+Na]⁺m/z 1058 in *B. licheniformis*-fermented products crude extract (d) was identified and quantified [Cheng et al. 2018]

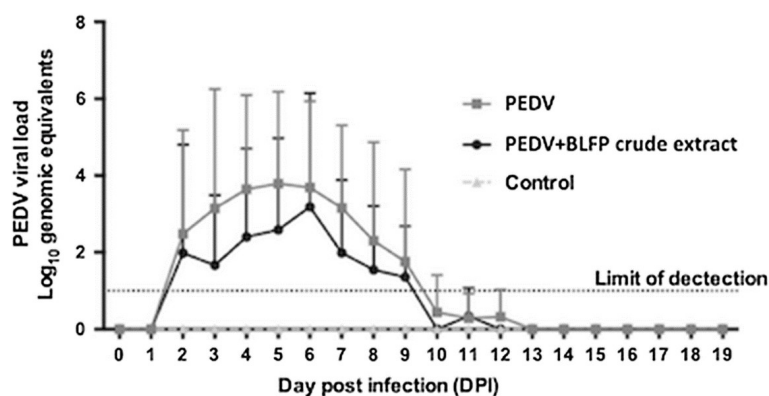


Fig. 3. Fecal shedding of PEDVPT-P6 detected in piglets fed <5 kg/ton BLFP in each group (n=5). The pattern of viral shedding in the PEDV + BLFP group was similar to but lower than that of the PEDV group during the study, with no significant difference detected. Changes in the mean values of genomic equivalents (GE)/mL are presented as log₁₀ values ±SD [Peng et al. 2019]

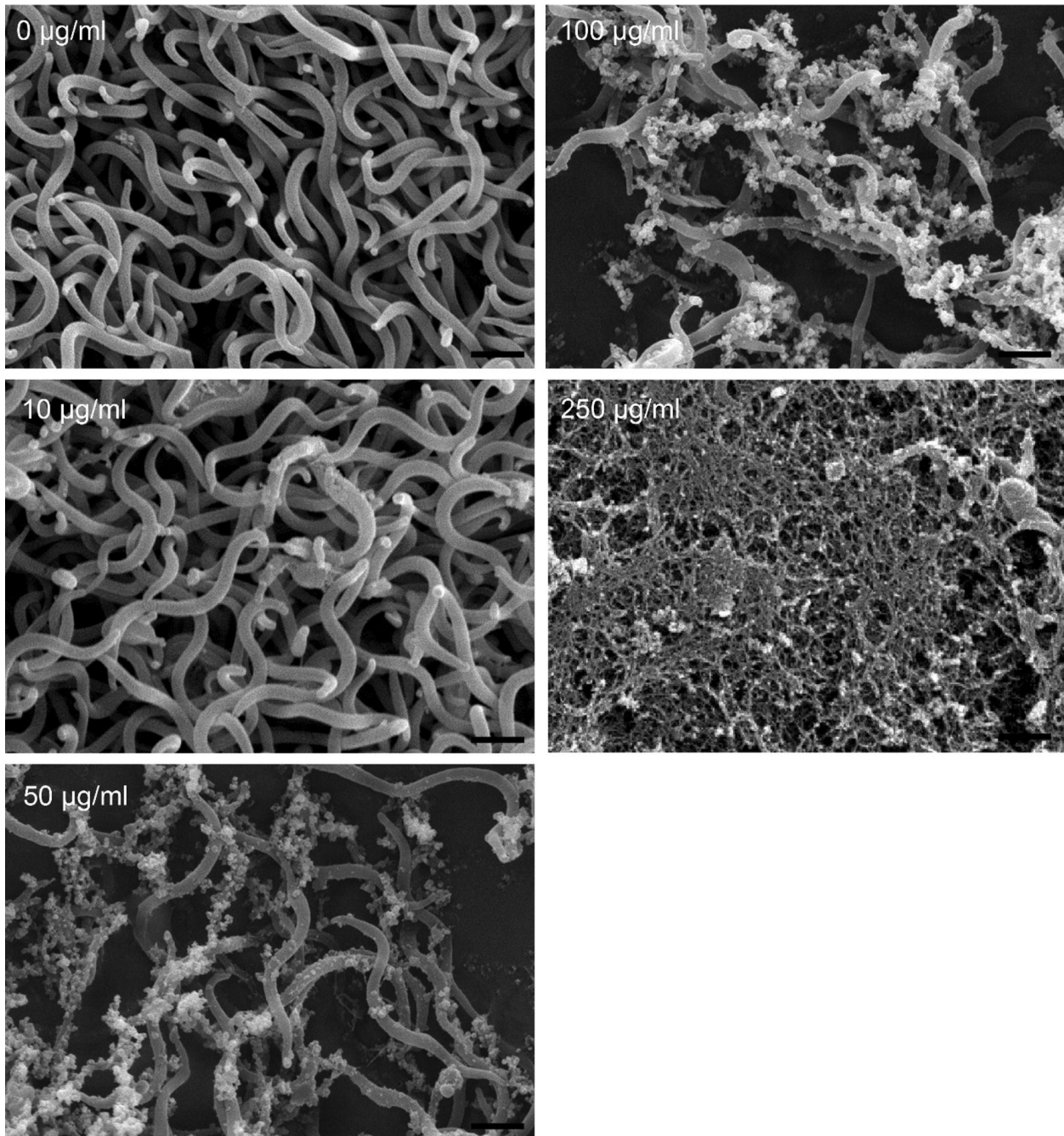


Fig. 4. Scanning electron microscope images of *B. hyodysenteriae* illustrating the Surfactin could penetrate and disrupt the morphology of *B. hyodysenteriae* in a dose dependent manner after 1 h treatment compared with the untreated group [Horng et al. 2019]

2011]. Our research findings that the *B. licheniformis*-fermented product-derived surfactin had a strong bacterial killing activity against *C. perfringens* compared with surfactin produced from *B. subtilis*. These results together suggest that *Bacillus* species-derived surfactin has potential for development as a possible substitute for antibiotics to prevent swine dysentery and necrotic enteritis in

domestic animals [Horng et al. 2019]. Due to the chemical structure of surfactin which is an acidic lipopeptide, because of its amphiphilic nature, with a polar amino acid head and a hydrocarbon chain, it is presumed that the interaction of surfactin with its target bacterial membrane and the alteration of the bilayer properties [Carrillo et al. 2003]. Surfactin have been proposed and characterized,

including insertion into lipid bilayers, membrane solubilization, destabilization of membrane permeability by channel formation and chelating of mono- and divalent cations [Seydlová and Svobodová 2008]. However, the precise mechanisms through which surfactin exerts antibacterial activity on *B. hyodysenteriae* need further investigation.

Effect of *Bacillus licheniformis*-fermented products on postpartum dysgalactia syndrome of sows

Postpartum dysgalactia syndrome (PDS) is a multifactor disease in sows with a recently reported prevalence varying from 6.0 to 48.2%. Sows with PDS exhibit clinical signs of mastitis, dysgalactia, and a fever above 39.5°C within 12 to 48 h postpartum. In addition to sows, the increased mortality rate and growth retardation are also observed in her offspring due to dysgalactia. PDS leads to enormous economic losses in the swine industry worldwide. Pathogens can invade the mammary gland of sows and cause a local inflammation via the endogenous or the galactogenous route, thereby inducing PDS. Therefore, alteration of the gut microbiota of sows may prevent PDS by inhibiting pathogen growth and protecting the mammary glands against fecal contamination. We organized a total of fifty multiparous cross-bred pregnant sows were randomly assigned to two groups in a completely randomized design. The dietary treatments comprised a basal diet (pregnancy and nursery diet) as control and a basal diet supplemented with 1.5 g · kg⁻¹ of *B. licheniformis*-fermented products. Sows with PDS in the two groups were further verified 12 h post-partum. Results show that the piglet body weight at weaning was increased in sows fed the *B. licheniformis*-fermented products compared to those fed the control diet (Table 1). The milk fat content of prepartum sows was

reduced in sows fed the *B. licheniformis*-fermented products. Postpartum sows with PDS had increased milk solid content compared with healthy sows. Microbial composition and species relative abundance analysis indicated distinct bacterial clusters between the groups (Fig. 5). The abundance of the family Prevotellaceae in the feces decreased in sows with PDS. *B. licheniformis*-fermented products increased the average abundance of the genus *Eubacterium coprostanoligenes* group in the feces of sows. These findings demonstrate that *B. licheniformis*-fermented products in the diet of sows can improve the piglet body weight at weaning and modulate the fecal microbiota of sows. PDS also has an impact on milk composition and fecal microbiota in sows [Yu et al. 2020].

Bacillus licheniformis-fermented products reduce diarrhea incidence and alter the fecal microbiota community in weaning piglets

The weaning period is an important time in the management of piglets. Postweaning diarrhea is the most frequent cause of heavy economic losses in pig herds. The use of antibiotic growth promoters (AGP) has been effective in decreasing the incidence of diarrhea in weaning piglets. However, the overuse of AGP in animal feed led to the evolution of drug-resistant bacteria and antibiotic residues in pigs. AGP has been banned in animal production in the European Union since 2006 and this policy is expected to expand to other countries. Hence, finding alternative solutions for preventing diarrhea in weaning piglets is urgent. Intestinal microflora plays a critical role in the process of nutrient digestion and immunomodulation. Normal gut microbiota is responsible for resistance to colonization by exogenous pathogenic microorganisms. Pathogen invasion alters the intestinal microbiota composition and causes diarrhea in weaning piglets.

Table 1. Effect of *Bacillus licheniformis*-fermented products on the litter performance traits of sows [Yu et al. 2020]

| Item | C ¹ | F ² | SEM ³ | P value ⁴ |
|--|--------------------|----------------|------------------|----------------------|
| Number of piglets totally born/litter | 12.47 ⁵ | 13.48 | 0.60 | 0.408 |
| Number of piglets born alive/litter | 10.65 | 11.91 | 0.57 | 0.274 |
| Number of weaned piglets/litter | 9.00 | 8.37 | 0.41 | 0.478 |
| Number of dead piglets during suckling period/litter | 5.20 | 6.33 | 0.66 | 0.378 |
| Pre-weaning mortality (%)/litter | 38.37 | 40.68 | 4.05 | 0.784 |
| Piglet body weight (kg) at birth/litter | 1.55 | 1.47 | 0.04 | 0.338 |
| Piglet body weight (kg) at weaning/litter | 6.61 | 7.22 | 0.15 | 0.041 |
| Fever rate of sow (%) | 45.45 | 39.29 | | |

¹C = Pregnancy and nursery diet

²F = Pregnancy and nursery diet supplemented with 1.5 g · kg⁻¹ BLFP

³SEM = Standard error of mean

⁴Analyzed using Student's *t*-test (two-tailed)

⁵Data are mean values of 12–14 litters per group

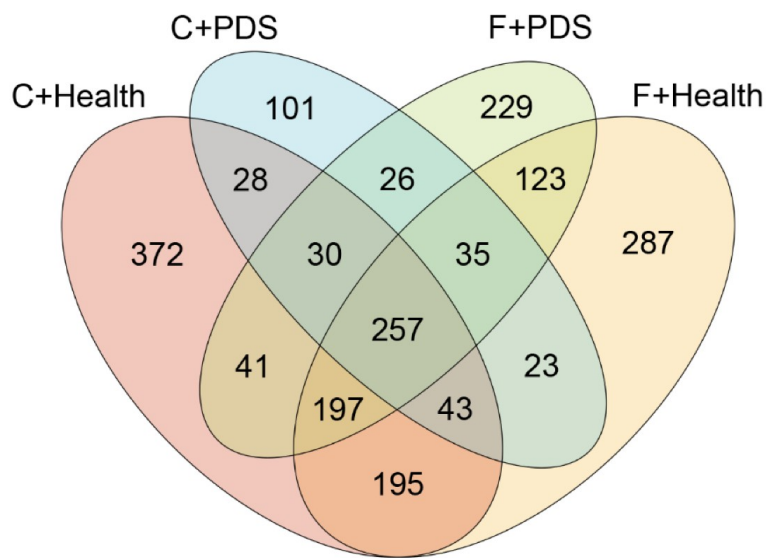


Fig. 5. Operational taxonomic unit distribution across the analyzed fecal content. Venn diagram representing the number of unique and shared operational taxonomic units among the four groups: healthy sows plus control diet group (C + Health), sows with postpartum dysgalactia syndrome (PDS) plus control diet group (C + PDS), healthy sows plus *B. licheniformis*-fermented product group (F + Health), and sows with PDS plus *B. licheniformis*-fermented product group (F + PDS) [Yu et al. 2020]

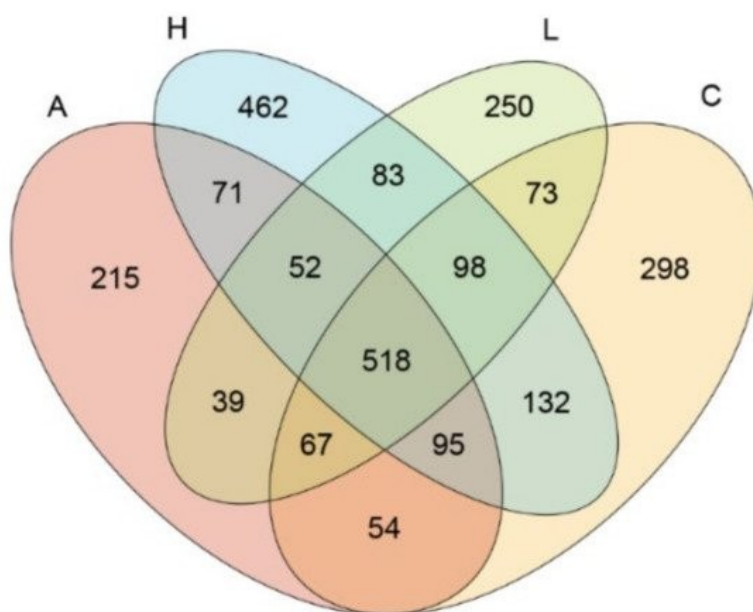


Fig. 6. Operational taxonomic unit distribution and composition analysis of fecal content. A Venn diagram of the operational taxonomic unit (OTU) distribution of the fecal contents. Each ellipse represents one group. The overlapping regions between the ellipses represent the OTU that is shared between the following: basal diet as the control (C), basal diet plus 1 g · kg⁻¹ of *B. licheniformis*-fermented products (L), basal diet plus 4.5 g · kg⁻¹ of *B. licheniformis*-fermented products (H), and basal diet plus 30 mg/kg antibiotics (bacitracin methylene disalicylate) (A). The value of each region represents the number of OTUs corresponding to the region [Hung et al. 2019]

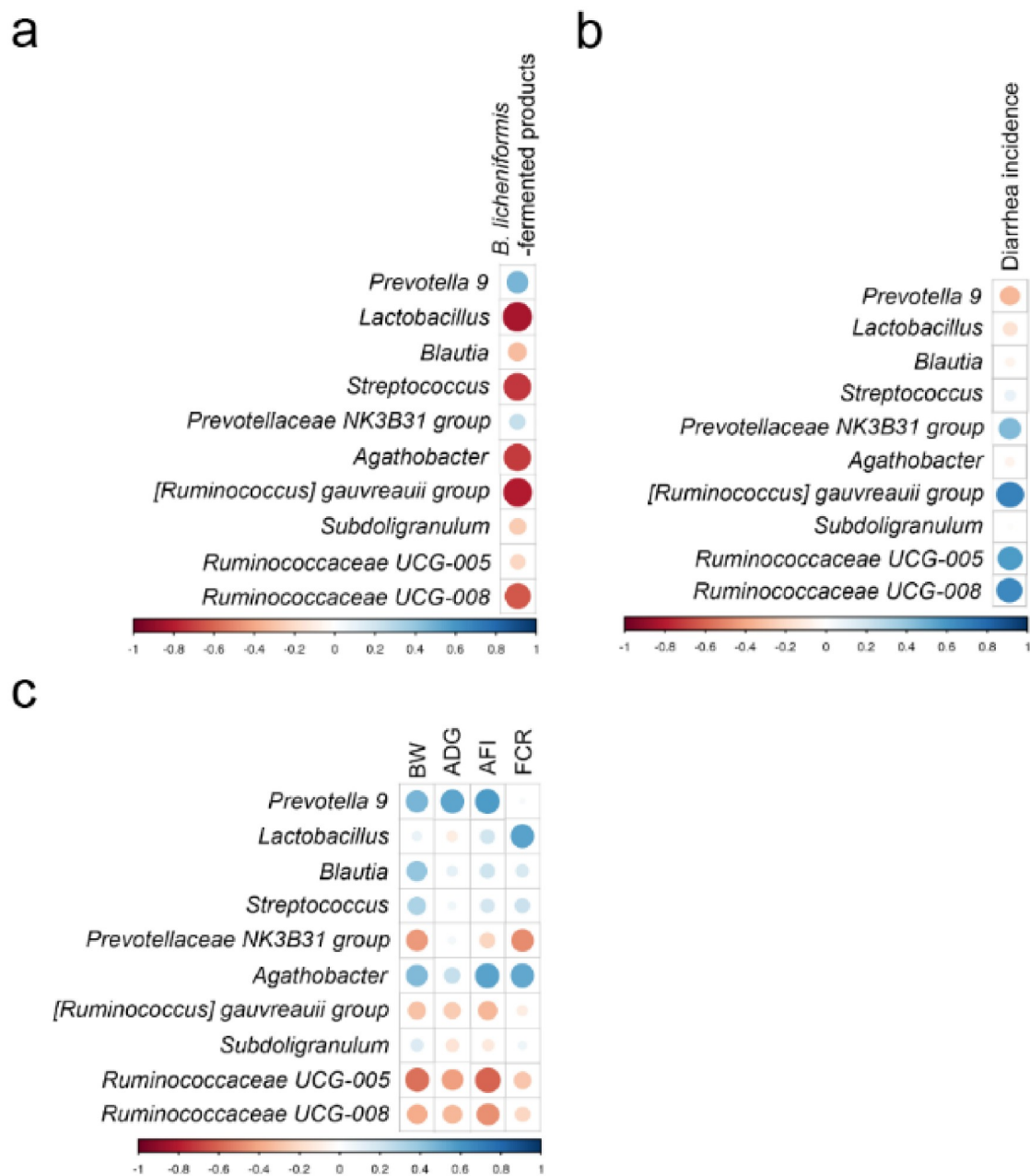


Fig. 7. Correlation analysis of fecal microbiota. (a) Correlation analysis between concentration of *B. licheniformis*-fermented products and abundant genera in piglets of different groups. (b) Correlation analysis between diarrhea incidence and abundant genera in piglets of different groups. (c) Correlation analysis between growth performance and abundant genera in piglets of different groups. Circle sizes and color intensity represent the magnitude of correlation. Blue circle represents positive correlations; red circle represents negative correlations. BW = body weight, ADG = average daily gain, AFI = average daily feed intake, FCR = feed conversion ratio [Hung et al. 2019]

In addition, stress or dysbiosis during the weaning period also results in an imbalanced gut microbiota, which also leads to postweaning diarrhea.

Recently, we study the effects of *B. licheniformis*-fermented products on diarrhea incidence and the fecal microbial community in weaning piglets. A total of 120 crossbred piglets with an average initial

body weight of 9.87 ± 1.43 kg were randomly allotted to four dietary treatments consisting of three replicate stalls with 10 piglets in each. The dietary treatments comprised a basal diet as control, control plus $1 \text{ g} \cdot \text{kg}^{-1}$ or $4.5 \text{ g} \cdot \text{kg}^{-1}$ of *B. licheniformis*-fermented products, and control plus 30 mg/kg antibiotics (bacitracin methylene disalicylate). Results showed

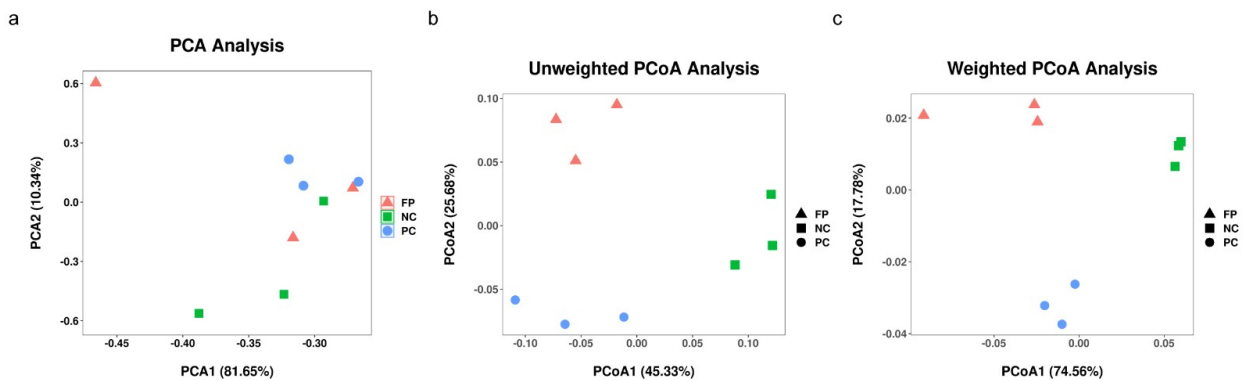


Fig. 8. Advanced analysis of the bacterial communities of cecal digesta. (a) Principal component analysis of the cecal digesta of basal diet without treatment (NC), basal diet plus coccidial challenge (PC), and basal diet plus the coccidial challenge and 1 g/kg of *B. licheniformis*-fermented products (FP) (n = 3). Principal coordinate analysis of quantitative traits (unweighted UniFrac distances) (b) and qualitative traits (weighted UniFrac distances) (c) of the cecal bacterial communities from NC, PC, and FP (n = 3) [Cheng et al. 2021]

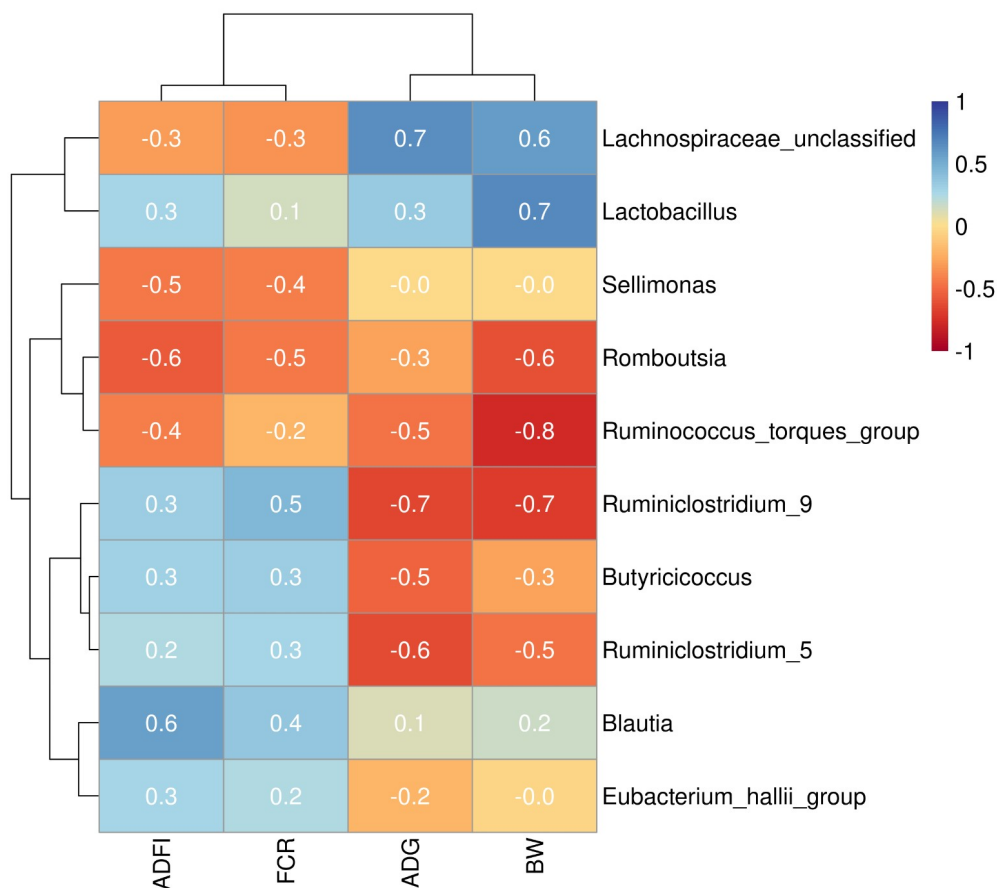


Fig. 9. The correlation coefficient between the dominant 10 genera and growth performance of broilers. ADFI: average daily feed intake, FCR: feed conversion ratio, ADG: average daily gain, BW: body weight. The positive correlations are shown in blue, while the negative correlations are shown in red. The color intensity and the size of the circle are proportional to the correlation coefficients. The values from +1 to -1 indicate the strength of the association [Cheng et al. 2021]

Table 2. Effect of *B. licheniformis*-fermented products on the growth performance and anti-coccidial index of broilers under coccidial challenge [Cheng et al. 2021]

| Item | NC ¹ | PC | FP | SEM | P value | | |
|--------------------------------------|-----------------|--------|--------|-------|----------|----------|----------|
| | | | | | NC vs PC | PC vs FP | FP vs NC |
| Body weight (g/bird) | | | | | | | |
| 1 d | 43.8 | 43.8 | 44.1 | 0.07 | 0.889 | 0.133 | 0.101 |
| 20 d | 823.1 | 795.3 | 792.3 | 14.37 | 0.380 | 0.952 | 0.068 |
| 35 d | 2065.8 | 1880.8 | 2006.7 | 32.78 | 0.031 | 0.144 | 0.310 |
| Average daily gain (g/d/bird) | | | | | | | |
| 1–20 d | 39.0 | 37.6 | 35.8 | 0.68 | 0.380 | 0.319 | 0.067 |
| 21–35 d | 88.8 | 77.5 | 89.0 | 2.17 | 0.037 | 0.050 | 0.939 |
| 1–35 d | 57.8 | 52.5 | 56.1 | 0.94 | 0.031 | 0.145 | 0.307 |
| Average daily feed intake (g/d/bird) | | | | | | | |
| 1–20 d | 66.0 | 67.0 | 69.6 | 0.99 | 0.490 | 0.386 | 0.208 |
| 21–35 d | 137.3 | 165.7 | 172.8 | 5.38 | 0.012 | 0.566 | 0.003 |
| 1–35 d | 95.5 | 106.5 | 110.9 | 2.49 | < 0.001 | 0.464 | 0.006 |
| Feed conversion ratio | | | | | | | |
| 1–20 d | 1.7 | 1.8 | 2.0 | 0.06 | 0.326 | 0.280 | 0.074 |
| 21–35 d | 1.6 | 2.2 | 2.0 | 0.10 | 0.014 | 0.384 | 0.023 |
| 1–35 d | 1.6 | 2.0 | 2.0 | 0.07 | 0.011 | 0.745 | 0.020 |
| Anti-coccidial index | 172.5 | 112.0 | 169.9 | 7.07 | < 0.001 | < 0.001 | 0.581 |

¹NC = Basal diet; PC = Basal diet in combination with the coccidial challenge; FP = Basal diet plus 1 g · kg⁻¹ of *B. licheniformis*-fermented products in combination with the coccidial challenge

Table 3. Bacterial taxonomic assignment and ranking within the cecal digesta of broilers [Cheng et al. 2021]

| Genus | Relative abundance (%) | | | SEM | P value | | |
|-------------------------------------|------------------------|------|------|------|----------|----------|----------|
| | NC ¹ | PC | FP | | NC vs PC | PC vs FP | FP vs NC |
| <i>Lachnospiraceae_unclassified</i> | 53.3 | 41.5 | 41.5 | 2.26 | < 0.001 | 1.000 | 0.032 |
| <i>Ruminococcus_torques_group</i> | 21.5 | 27.0 | 17.2 | 1.51 | 0.022 | 0.003 | 0.019 |
| <i>Lactobacillus</i> | 2.2 | 1.4 | 8.6 | 1.30 | 0.026 | 0.031 | 0.045 |
| <i>Ruminiclostridium_9</i> | 2.6 | 4.2 | 3.7 | 0.24 | 0.001 | 0.052 | < 0.001 |
| <i>Butyricoccus</i> | 2.0 | 3.9 | 3.8 | 0.32 | < 0.001 | 0.780 | < 0.001 |
| <i>Blautia</i> | 0.5 | 2.5 | 4.8 | 0.64 | < 0.001 | 0.009 | < 0.001 |
| <i>Eubacterium_hallii_group</i> | 0.3 | 1.1 | 1.6 | 0.20 | 0.016 | 0.095 | 0.001 |
| <i>Ruminiclostridium_5</i> | 1.1 | 2.1 | 1.9 | 0.15 | 0.004 | 0.510 | 0.005 |
| <i>Sellimonas</i> | 2.7 | 1.4 | 1.3 | 0.25 | 0.001 | 0.680 | 0.015 |
| <i>Romboutsia</i> | 1.6 | 1.8 | 0.5 | 0.20 | 0.382 | 0.003 | 0.001 |

¹NC = Basal diet; PC = Basal diet in combination with the coccidial challenge; FP = Basal diet plus 1 g · kg⁻¹ of *B. licheniformis*-fermented products in combination with the coccidial challenge

that 4.5 g · kg⁻¹ of *B. licheniformis*-fermented product supplementation significantly reduced diarrhea incidence in weaning piglets. Principal coordinate analysis and a

heatmap of species abundance indicated distinct clusters between the groups treated with antibiotics and *B. licheniformis*-fermented products (Fig. 6). The bacterial

richness and evenness in the feces decreased in weaning piglets fed $1 \text{ g} \cdot \text{kg}^{-1}$ of *B. licheniformis*-fermented products and antibiotics. The abundance of the genera [*Ruminococcus*] *gavreaii* group, *Ruminococcaceae* UCG-005, and *Ruminococcaceae* UCG-008 in the feces decreased in weaning piglets fed *B. licheniformis*-fermented products or antibiotics. The average abundance of the genus *Prevotella 9* in the feces was positively correlated with the concentration of *B. licheniformis*-fermented products and negatively correlated with the diarrhea incidence in weaning piglets (Fig. 7). Furthermore, the average abundance of the genus *Prevotella 9* in the feces was positively correlated with the growth performance of weaning piglets [Hung et al. 2019].

***Bacillus licheniformis*-fermented products prevent coccidiosis and regulate cecal microbial community of broilers**

Coccidiosis is a severe enteric disease in poultry caused mainly by protozoa from the *Eimeria* genus. Coccidiosis costs the poultry industry about 3 billion US dollars annually worldwide due to high mortality, impaired growth, and high medical costs. Anti-coccidial drugs have been widely used to control coccidiosis, but drug resistance of *Eimeria* species in chickens has become prevalent worldwide. Since the disadvantages of current anti-coccidial drugs and vaccines, alternative strategies to prevent coccidiosis in broilers are an urgent unmet need in the poultry industry. We demonstrated that *B. licheniformis*-fermented products could increase the average daily gain of broilers at 21 to 35 days of age compared with the coccidial challenge-only group (Table 2). The anti-coccidia index in the *B. licheniformis*-fermented product-treated group was elevated compared with the coccidial challenge-only group (Table 2). Principal coordinate analysis showed that there was significant segregation in bacterial community composition in the cecal digesta among the groups (Fig. 8). The genus *Lactobacillus* was more abundant in the cecal digesta of the *B. licheniformis*-fermented product-treated group compared with the coccidial challenge-only group (Table 3). There was a positive correlation between the abundance of the genus *Lactobacillus* in the cecal digesta and growth performance (Fig. 9) [Cheng et al. 2021].

CONCLUSION

Bacillus species are spore-forming probiotics and are heat-, acid-, and bile-resistant. *Bacillus* species show antimicrobial activity against pathogens through the production of antibacterial cyclic lipopeptide. *Bacillus* species-based fermented products produced from solid-state fermentation containing probiotics and antibacterial cyclic lipopeptide, surfactin, can improve growth perfor-

mance, prevent disease, and modulate gut microbiota in domestic animals. Summarized, *Bacillus* species-based fermented products might provide an alternative source for preventing pathogens or a substitute for antibiotics.

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OPRACOWANIE I WALIDACJA SKUTECZNOŚCI PRODUKTÓW FERMENTOWANYCH NA BAZIE *BACILLUS* JAKO ALTERNATYWY DLA ANTYBIOTYKÓW U ZWIERZĄT DOMOWYCH

STRESZCZENIE

Mikroflora jelitowa odgrywa kluczową rolę w procesie trawienia składników odżywczych i immunomodulacji. Prawidłowa mikroflora jelitowa jest odpowiedzialna za odporność na kolonizację przez egzogenne mikroorganizmy chorobotwórcze. Inwazja patogenów zmienia skład mikroflory jelitowej i powoduje zaburzenia jelitowe u zwierząt domowych. Co więcej, stres, infekcja lub dysbioza w okresie karmienia również powodują nie zrównoważoną mikroflorę jelitową, co prowadzi do biegunki poodsadzeniowej prosiąt i martwiczego zapalenia jelit u kurcząt. Wykazano, że *B. licheniformis* i *B. subtilis* wykazują aktywność przeciwdrobnoustrojową wobec patogenów poprzez wytwarzanie przeciwbakteryjnej cyklicznej surfaktyny lipopeptydowej. Dlatego w niniejszym artykule przytoczono szereg badań nad skutecznością i walidacją produktów fermentowanych opartych na *Bacillus* poprzez łagodzenie objawów biegunki, zapalenia jelit, kokcydiozy, zmodyfikowanej mikroflory jelitowej i zespołu bezmleczności poporodowej u świń i kur.

Słowa kluczowe: walidacja, produkty fermentowane na bazie *Bacillus*, alternatywa dla antybiotyków, zwierzęta domowe

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