

Effects of probiotic cell wall on growth performance and immune response against FMD, PRRS, and CSF viruses in growing pigs

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ABSTRACT— This study evaluated the effects of supplementing probiotic cell walls (PCW) containing *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* on growth performance, feed conversion, and immune responses of finishing pigs against Foot-and-Mouth Disease (FMD), Porcine Reproductive and Respiratory Syndrome (PRRS), and Classical Swine Fever (CSF) viruses. The experiment was conducted on 360 F1 crossbred pigs (Du × YL), divided into three treatments (three replicates of 30 pigs each): Control (C, no supplementation), FS (0.05% PCW in feed), and WS (0.05% PCW in drinking water). Results showed that PCW improved growth performance, nutrient absorption, and feed conversion efficiency, with an increase in weight gain of 3.23–4.52% and a reduction in feed intake of 2.09–4.72% compared to the control. PCW supplementation also enhanced antibody levels against FMD, PRRS, and CSF viruses, with statistically significant increases observed for FMD and PRRS, while the response to CSF was less pronounced and requires further investigation. Overall, PCW shows potential as an antibiotic alternative, contributing to improved economic efficiency, sustainability, and biosecurity in swine production.

KEYWORDS: Antibody, Growth performance, Immune response, Probiotic cell wall, Virus

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1. INTRODUCTION

Pig production significantly contributes to global food security and economic sustainability but is threatened by viral diseases such as Foot-and-Mouth Disease (FMD), Porcine Reproductive and Respiratory Syndrome (PRRS), and Classical Swine Fever (CSF). Although antibiotics and vaccines are widely used, concerns about resistance and limited efficacy have prompted interest in alternative approaches. Functional feed additives, particularly probiotic cell wall components, offer a promising strategy by enhancing immunity and gut health. These components— β -glucans, peptidoglycans, and lipoteichoic acids—stimulate innate and adaptive immune responses, improve antibody production, and support intestinal integrity. Peptidoglycans, in particular, are acid-stable immunostimulants that enhance mucosal and systemic immunity via cytokine pathways. However, research on their effects against multiple viral infections in pigs remains limited. This study investigates the impact of probiotic cell wall supplementation on growth performance and antibody responses to FMDV, PRRSV, and CSFV in weaned-to-market pigs, aiming to support the development of sustainable alternatives to antibiotics.

2. MATERIAL AND METHODS

2.1 Experimental materials

A total of 360 weaned F1 (Duroc × Yorkshire-Landrace) crossbred pigs were assigned to three treatments, with 30 pigs per treatment and four replicates. Treatment 1 served as the control, while Treatment 2 received 0.05% probiotic cell walls (PCW) in feed, and Treatment 3 received 0.05% PCW in drinking water. All pigs were raised under standard management conditions (Table 1) and vaccinated against FMD, PRRS, CSF, Donoban, atrophic rhinitis, hemorrhagic septicemia, infectious polyarthritis, and pleuropneumonia.

Table 1. Experimental design

Parameter	Control (C)	Feed suppl. (FS)	Water suppl. (WS)
Pigs per pen	30	30	30
Replicates	4	4	4
Initial weight (kg)	8.51	8.51	8.58
Experimental duration (days)	148	148	148
PCW - Immunevet (%)	0	0.05 (in feed)	0.05 (in water)
Feed composition	ME (Kcal/kg)	CP (%)	
10–30 kg phase	3200	20.0	
31–60 kg phase	3300	19.0	
61–90 kg phase	3250	18.5	
91 kg – market weight	3200	18.0	

The PCW supplement, a mixture of *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* cell walls (β -glucan $\geq 5\%$), was developed and produced by the Vietnam Institute of Functional Foods. *Lactobacillus rhamnosus* biomass was fermented, enzymatically lysed to break the bacterial cell structure, and purified to obtain peptidoglycan-based cell walls. *Saccharomyces cerevisiae* was enzymatically processed to yield yeast cell walls rich in β -Glucan 1/3-1/6. The final product was dried and formulated for appropriate supplementation in different livestock species.

2.2 Research methods

Growth performance assessment

The growth performance of experimental pigs was evaluated by measuring individual body weight at the beginning and end of the trial. The feed conversion ratio (FCR) was determined by tracking daily feed intake over the entire experimental period and calculating feed efficiency based on weight gain across the three treatment groups

Antibody level assessment

Blood sampling method

Blood samples (5 mL) were randomly collected from the jugular vein of six pigs per treatment group before and after vaccination. The sampling timeline varied depending on the disease (Table 2). Blood samples were stored at 4–8°C and transported to the laboratory within 24 hours. A total of 108 blood samples were collected, including 54 pre-vaccination and 54 post-vaccination samples.

Table 2. Blood sampling schedule for antibody detection

Disease	Pre-vaccination		Post-vaccination	
	No. of samples	Age (days)	No. of samples	Age (days)

FMD	6	40	6	77
PRRS	6	38	6	60
CSF	6	60	6	87

Antibody detection methods

- **FMD antibodies:** Serum samples were diluted from 1/16 to 1/128 according to TCVN 8685-10:2014 guidelines [5]. Samples with PI < 50 were deemed negative. If PI > 50 at a 1/32 dilution, and all subsequent dilutions showed PI < 50, the antibody titer was recorded as 1/45 and considered positive.
- **CSF antibodies:** Measured using an ELISA assay with the Biochek kit. Samples were positive for CSFV antibodies if the S/P ratio was ≥ 0.500 and negative if ≤ 0.499 .
- **PRRS antibodies:** Assessed using the IDEXX ELISA kit, which employs an indirect ELISA method with HRP-conjugated recombinant PRRSV antigen. Samples were positive if the S/P ratio was ≥ 0.4 and negative if < 0.4.

2.3 Data analysis

Statistical analyses were performed using Minitab 16.0 software with one-way ANOVA for group comparisons.

3. RESULTS AND DISCUSSION

3.1 Growth performance and feed conversion ratio

Table 3. Growth performance of experimental pigs

Parameter	C	FS	WS	SEM	P-value
Initial weight (kg)	8.51	8.51	8.58	0.046	0.004
Final weight (kg)	116.88 ^b	119.98 ^a	121.62 ^a	0.723	<0.001
Weight gain (kg/pig)	108.37 ^b	111.47 ^a	113.04 ^a	0.707	<0.001
ADG (g/pig/day)	730.70 ^b	754.30 ^a	763.80 ^a	4.775	<0.001

Note: Different superscripts within the same row indicate statistically significant differences ($p < 0.05$).

At the start of the experiment, initial body weights across all treatments showed no significant differences. However, by the end of the trial, pigs in the control group (C) had a lower final weight (116.88 kg) compared to FS (119.98 kg) and WS (121.62 kg) ($P < 0.001$). Similarly, the highest average daily gain (ADG) was observed in WS (763.80 g/pig/day), followed by FS (754.30 g/pig/day), and the lowest in C (730.70 g/pig/day) ($P < 0.001$). Compared to the control, PCW supplementation increased growth performance by 3.23% to 4.52%. These findings align with previous studies. It was demonstrated that supplementing *Bacillus subtilis* in pig diets led to improved growth rates and nutrient absorption, consistent with the enhanced weight gain observed in the FS and WS groups [6]. Similarly, it was reported that *Lactobacillus plantarum* supplementation increased average daily gain (ADG) and reduced the incidence of diarrhea in pigs, further supporting the results of the current study [7]. It has also been highlighted that multi-strain probiotics significantly enhance gut health and growth performance in finishing pigs [8]. Similar outcomes have been reported in Vietnamese studies. In one study, supplementing 0.06% probiotic cell walls in feed did not affect feed intake but increased growth rates by 3.1% to 6.9%, with an ADG of 772.67 g/pig/day [9]. Additionally, β -glucan supplementation was shown to improve feed digestion and nutrient absorption, resulting in faster growth rates in pigs [10]. Overall, the findings of this study are in agreement with both international and domestic research, reinforcing the effectiveness of probiotic cell wall (PCW) supplementation in improving

growth performance in pigs. The highest total feed intake was observed in the WS group (270.0 kg/pig), followed by the control group (264.8 kg/pig), and the lowest was in the FS group (259.3 kg/pig) ($P < 0.001$). The FS group exhibited the best feed conversion ratio (FCR) (2.33), followed by the WS group (2.39), while the control group had the poorest FCR (2.45) ($P < 0.001$). This indicates that supplementing with probiotic cell walls reduced FCR by 2.09% to 4.72%. This effect may be attributed to the probiotic cell mixture stabilizing the gut microbiota, thereby enhancing digestion and nutrient absorption, leading to improved growth performance and feed utilization efficiency. Energy and protein consumption were positively correlated with FCR.

Table 4. Feed conversion efficiency of experimental pigs

Parameter	C	FS	WS	SEM	P-value
Total feed intake (kg/pig)	264.8 ^a	259.3 ^b	270.0 ^a	1.622	<0.001
Total weight gain (kg/pig)	108.4 ^b	111.5 ^a	113.0 ^a	0.707	<0.001
FCR (kg feed/kg gain)	2.44 ^a	2.33 ^b	2.39 ^c	0.007	<0.001
Energy consumption (kcal/kg gain)	7907.30 ^a	7520.10 ^c	7720.3 ^b	21.63	<0.001
Protein consumption (g/kg gain)	455.7 ^a	432.9 ^c	444.4 ^b	1.195	<0.001

Note: Different superscripts within the same row indicate statistically significant differences ($p < 0.05$).

The supplementing probiotic cell walls in pig diets resulted in a feed conversion ratio (FCR) of 2.44–2.46 [8]. In comparison, better results were demonstrated in the present study, with FCR values ranging from 2.33 to 2.39. In another study, 0.03%–0.04% probiotics were supplemented in finishing pigs, leading to a significantly lower FCR of 2.13 kg/kg weight gain [11]. Similarly, it was indicated that probiotics significantly reduced FCR, which aligned with the FS group in the current study, where the lowest FCR was recorded [12]. It has been suggested that probiotic supplementation optimizes the digestion of protein and energy [13]. Further confirmation has been provided that probiotics improve feed conversion efficiency and reduce waste production in pig farming [14]. Significant improvements in growth performance, nutrient digestibility, blood parameters, fecal consistency, and gut microbiota composition—along with reductions in gas emissions—were demonstrated when complex probiotics were supplemented, compared to single-strain supplementation or no supplementation [15]. However, research on the supplementation of complex probiotics remains limited, highlighting the need for further studies. It was also reported that supplementing probiotic cell wall mixtures increased villus height in the duodenum (292.9 μm), jejunum (302.63 μm), and ileum (235.2 μm), and improved the villus height/crypt depth ratio in the duodenum (5.2), jejunum (2.76), and ileum (3.2), thereby enhancing digestive and absorptive capacity [9]. Additionally, this supplementation was found to reduce harmful gut microorganisms while tending to increase the total aerobic microbial population and *Lactobacillus* spp., ultimately lowering the incidence of common diseases.

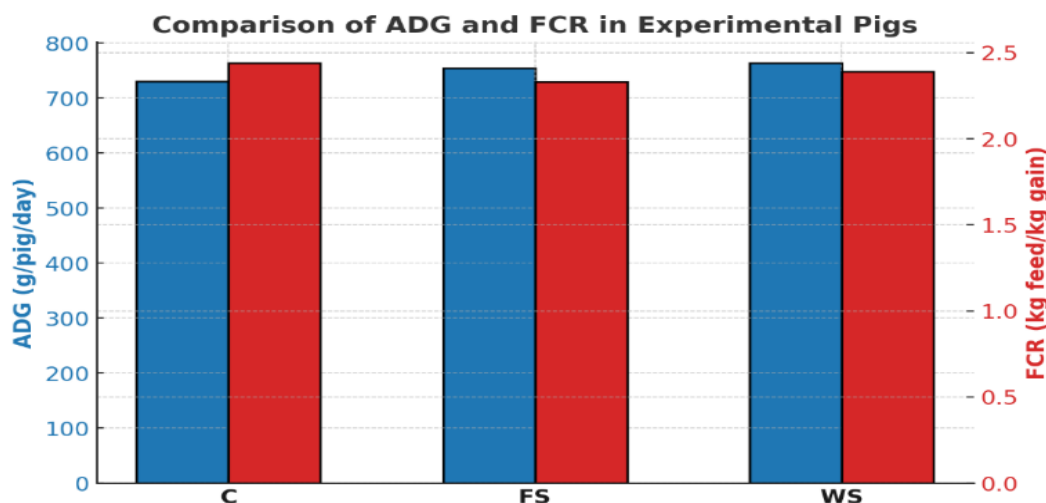


Figure. Comparison of ADG and FCR of experimental pigs

3.2 Immune response against Foot-and-Mouth, Porcine Reproductive and Respiratory Syndrome, and Classical Swine Fever virus

The results in Table 5. indicate that before vaccination, all treatment groups (C, FW, WS) had 100% seronegative samples, with a neutralizing antibody titer of $\leq 1/16$. This confirms that before vaccination, none of the samples had antibody levels high enough to be considered positive. After vaccination, 100% of the tested samples in all three groups became seropositive. However, the neutralizing antibody titers in the FS and WS groups ($>1/45$) were significantly higher than those in the control group ($1/45$) (P -value < 0.05). This demonstrates that supplementing PCW in feed and drinking water enhanced antibody production against the FMD virus compared to the non-supplemented group, with a statistically reliable difference.

Table 5. FMD antibody levels before and after vaccination

Treatment	Number of Samples	Before vaccination				After vaccination		P-value
		Negative samples	(%)	Neutralizing antibody titer	Positive samples	(%)	Neutralizing antibody titer	
C	6	6	100	$\leq 1/16$	6	100	1/45	P< 0.05
FS	6	6	100	$< 1/16$	6	100	$>1/45$	
WS	6	6	100	$< 1/16$	6	100	$>1/45$	

Negative sample: Antibody titer $\leq 1/16$; Positive sample: Antibody titer $\geq 1/45$; Suspicious sample: Antibody titer from 1/16 to 1/32)

The use of *Lactobacillus plantarum* to enhance immunity in pigs following FMD vaccination was investigated, and it was found that higher antibody titers were exhibited by the probiotic-supplemented group compared to the control group, with these elevated levels being maintained for up to six weeks post-vaccination [1]. Similarly, a significant increase in antibody titers in the probiotic-supplemented group compared to the non-supplemented group was demonstrated when *Lactobacillus acidophilus* was used as a natural adjuvant in FMD vaccines [16]. The effects of *Bacillus subtilis* on the immune response of FMD-infected pigs were also assessed, showing that cellular immune responses were improved and tissue damage was reduced by probiotic supplementation in comparison to the control group [17]. In another study, increased levels of IgG, IgA, IgM, secretory IgA (sIgA), and neutralizing antibodies were observed in pigs administered recombinant *Lactobacillus plantarum* expressing VP1. Stronger cell-mediated immune responses and enhanced protection against the FMD virus were also exhibited by these pigs compared to the control group [18]. Based on these findings, it can be concluded that immune protection against FMD can be effectively

enhanced by recombinant *Lactobacillus plantarum* expressing VP1.

Table 6. PRRS antibody levels before and after vaccination

Treatment	Number of samples	Before vaccination		After vaccination		P-value
		Negative samples (%)	S/P (Mean $\pm$ SD)	Positive samples (%)	S/P (Mean $\pm$ SD)	
C	6	100	0.0067 $\pm$ 0.0008	100	1.6523 ^b $\pm$ 0.1712	0.001
FS	6	100	0.2268 $\pm$ 0.0652	100	2.5060 ^a $\pm$ 0.0773	
WS	6	100	0.1073 $\pm$ 0.0453	100	2.1763 ^a $\pm$ 0.0985	

(Note: Means within the same column with different superscripts indicate statistically significant differences ($P < 0.05$). Positive sample: $S/P \geq 0.4$; Negative sample: $S/P < 0.4$.)

Before vaccination, all three groups had 100% negative samples with $S/P < 0.4$. After vaccination, all treatment groups showed 100% seropositive samples, with S/P values of 1.6523, 2.5060, and 2.1763 for the C, FS, and WS groups, respectively. The differences in S/P values between the FS and WS groups compared to the C group were statistically significant ($P < 0.001$), indicating that PCW supplementation enhanced antibody production against PRRSV compared to the non-supplemented group. It has been demonstrated that the abundance of *Lactobacillus* in the gut microbiota of pigs is closely linked to immune responses following PRRSV vaccination, suggesting that the efficacy of PRRS vaccines may be enhanced by probiotic supplementation [19], [20]. The role of *Lactobacillus plantarum* JDFM LP11 in modulating gut microbiota and PRRS immune responses in weaned pigs has been investigated, and it was shown that the growth of beneficial bacteria was promoted and intestinal structure was improved by *L. plantarum* JDFM LP11, thereby contributing to enhanced immunity [21]. The effects of supplementing *Bacillus subtilis* DSM 32540 and *Bacillus pumilus* DSM 32539 in the diets of weaned pigs have also been evaluated, with significant improvements in growth performance and disease resistance being demonstrated through gut microbiota modulation and enhanced systemic immunity [22]. In addition, the mechanisms by which probiotics influence gut health and defend against pathogens in piglets have been analyzed, including microbiota modulation, pathogen competition, antimicrobial compound production, and immune system regulation [4]. A stronger immune response in weaned pigs compared to non-supplemented pigs has also been reported as a result of β -glucan supplementation [10]. Together, these findings provide strong evidence that the immune response of pigs against PRRS can be effectively enhanced through probiotic supplementation.

Table 7. CSF antibody levels before and after vaccination

Treatment	Number of samples	Before vaccination		After vaccination		P-value
		Negative samples (%)	S/P (Mean $\pm$ SD)	Positive samples (%)	S/P (Mean $\pm$ SD)	
C	6	100	0.2260 $\pm$ 0.0629	100	1.330 $\pm$ 0.141	0.125
FS	6	100	0.2237 $\pm$ 0.0434	100	1.255 $\pm$ 0.335	
WS	6	100	0.1667 $\pm$ 0.0469	100	1.254 $\pm$ 0.120	

(Note: Means within the same column with different superscripts indicate statistically significant differences ($P < 0.05$). Positive sample: $S/P \geq 0.5$; Negative sample: $S/P < 0.5$.)

Before vaccination, all groups had 100% negative samples, with S/P values ranging from 0.1667 to 0.2260.

After vaccination, all groups showed 100% seropositive samples, with S/P values significantly increasing to a range of 1.254–1.330. While all groups transitioned from negative to positive samples after vaccination, indicating an immune response, the P-value (0.125) across the three groups suggests that the difference in S/P values before and after vaccination was not statistically significant. This may be attributed to the small sample size ($n=6$), highlighting the need for further studies with larger sample sizes. Several researchers have reported similar findings. A study evaluated the immunogenicity of orally administered recombinant *Lactobacillus plantarum* expressing the E2 protein of classical swine fever virus (CSFV), combined with Thymosin α -1 as an adjuvant. The results indicated that the recombinant *Lactobacillus plantarum* could stimulate an immune response against CSFV [23]. Another study investigated the immune response of pigs to oral administration of recombinant *Lactobacillus* expressing antigens of African swine fever virus (ASFV). The findings suggested that recombinant *Lactobacillus* can stimulate immune responses against ASFV [24]. Additionally, research demonstrated that β -glucan influences lymphocyte proliferation, thereby enhancing immunity in weaned pigs. β -glucan also activates immune responses related to exogenous antigens, such as vaccination [25]. Another study described glucan as a biological immunomodulator with various immunological properties, capable of stimulating both innate and adaptive immunity [26]. Similarly, β -glucan supplementation in weaned pigs improved the immune response to CSF compared to non-supplemented pigs [27].

4. CONCLUSIONS AND RECOMMENDATION

Supplementing *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* cell walls in feed and drinking water improved growth performance, nutrient absorption, and feed conversion efficiency in finishing pigs. This supplementation increased growth performance by 3.23–4.52% and reduced the feed conversion ratio (FCR) by 2.09–4.72% compared to the control group.

The study on the effects of PCW supplementation on immune responses against three viral diseases (FMD, PRRS, and CSF) demonstrated that both supplementation methods (via feed or drinking water) enhanced antibody production against all three viruses. Notably, antibody levels against FMD and PRRS viruses showed a statistically significant increase compared to the control group, whereas the difference for CSF was less conclusive and requires further investigation.

These findings suggest that probiotic cell wall (PCW) supplementation could serve as a promising alternative to antibiotics, contributing to improved economic efficiency, sustainability, and biosecurity in pig production.

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