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Comparative Analysis of Probiotic-Enhanced Feed on Immune Modulation and Antimicrobial Resistance in Swine Gut Microbiota

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Abstract

Post-weaning diarrhea (PWD) remains a major threat to swine health and production, characterized by complex etiologies involving enteric pathogens, immune immaturity, and environmental stressors. This study employed a mixed-methods experimental design to evaluate the effectiveness of phytobiotic, probiotic, and combined interventions in mitigating PWD in piglets. A total of 120 piglets were randomly assigned to four groups: control, phytobiotic, probiotic, and phytobiotic-probiotic combination. Clinical monitoring revealed that the dual-treated group exhibited the most substantial improvement in average daily gain (0.58 g/day) and the lowest fecal consistency scores. Microbial analysis using qPCR and 16S rRNA sequencing showed a ~70% reduction in pathogen load, particularly targeting enterotoxigenic *E. coli* and *Clostridium perfringens*. Histological assessments demonstrated the most favorable villus-to-crypt ratios and goblet cell densities in the combined treatment group. Multivariate regression confirmed treatment group, pathogen burden, and gut morphology as key predictors of diarrhea severity ($p < 0.05$). Thematic analysis from qualitative interviews further validated the role of contextual farm management practices in influencing outcomes. Collectively, these findings endorse a synergistic, non-antibiotic intervention strategy for effectively reducing PWD and enhancing piglet health, offering a promising alternative to conventional prophylactic treatments.

Keywords: “Post-Weaning Diarrhea”, “Piglets”, “Phytobiotics”, “Probiotics”, “Gut Histology”, “Mixed-Methods Analysis”.

INTRODUCTION

Live microorganisms as probiotic-enhanced feeds are gaining popularity to form a viable alternative to conventional antibiotic growth promoters in pig production due to their possible effects in numerous ways (Konieczka et al., 2023). This approach will be highly needed as the world requires more meat with the antibiotic resistance that is to be reduced, so methods are to be discovered to produce significant quantities but at the same time make the animals remain healthy (Tarradas et al., 2020). This critical assessment will examine the influence of a variety of probiotic strains on the swine gut microbiome and illustrate how each of these strains enhances the host immunity and reduces the antimicrobial resistance (Zhang et al., 2023) (Konieczka et al., 2023). It consists of combating pathogens directly, altering the composition of gut microbiota, and enhancing the work of the intestinal barrier (Sachdeva et al., 2025). In particular, probiotics have been demonstrated to modify mucosal and intestinal microbiota immunity, alter the intestine epithelial barrier, modify the mucous secretion, and induce competitive exclusion to unwanted microorganisms (Kulkarni et al., 2022). The period of weaning is very stressful to piglets. It may lead to digestive system issues in them, reduce weight levels and stunt their growth. This is why the use of alternatives such as probiotics to assist them in this difficult time cannot be overestimated (Tian et al., 2021). A transition of a piglet diet to solid feed with the bacteria community in the piglet intestinal tract is influenced greatly by the change of diet combined with the introduction of environmental stressors. This predisposes them to development of enteric diseases and greatly explains the need to effect dietary modifications to aid and sustain gut health and development (Lopez Galvez et al., 2020). A sudden switch of nutrition and environment during weaning may lead to unfavorable shifts in the

intestine microbiota. This may cause slow metabolism, absorption of the nutrients and tendencies to develop enteric diseases. Such a process may impair the intestinal mucosal barrier and predisposes piglets to such infections as bacterial diarrhoea or viral gastroenteritis. All these diseases may have a profound impact on the economics of pig farming (Liu et al., 2020). The piglets are even more susceptible as they are still at the stage of developing their immune systems, and therefore, they are more prone to be ill due to such strains as *Escherichia coli* (Kong et al., 2022). The fact that the enzymatic systems of piglets on weaning are still developing aggravates these issues. Their digestive systems also do not fully prepare them to take solid food yet, this can trigger digestive issues and loss of nutrients (Dumitru et al., 2022). This sensitive period demonstrates the need to introduce good nutritional strategies that promote the health of the gut and make it more resistant to environmental and dietary stressors (Tang et al., 2022). The problems are exacerbated by early weaning, which removes piglets prematurely out of their mother immunity and food supply. This leads to great alterations in body and mind functioning of the piglets, a phenomenon that is referred to as early weaning stress syndrome (Chen et al., 2020). This stress can strongly compromise the intestines of piglets and make their barrier less effective, thereby leading to stunted growth (Tang et al., 2022). This increases their risk of being ill with maldigestion, malabsorption, and acute inflammation (Middelkoop et al., 2020). This premature separation leads to significant alterations in the small intestine structure and increases its difficulty to absorb nutrients. It results in unhealthy growth, increased cases of diarrhoea and increased mortality (Tian et al., 2023). Such shape modifications affect such aspects as the shortening of intestine villi and

crypts deepening combined, contributing to the reduced effectiveness of digestion and absorption processes (Wang et al., 2023). The dysbiosis presents itself typically in the form of a reduced deficiency of digesting enzyme in the bowel, impaired tight junctional proteins, a weak immune system and altered mucosal contour. Such stressors occasionally have an impact on the intestinal barrier, which is significant toward excluding pathogens and nutrient uptake. This further deteriorates the health of piglets (Deng et al., 2023). As an illustration, intestinal injury may reduce the ratios of villus height to crypt depth, the expression of tight junction proteins, and the strength and functionality of the intestinal barrier in general (Zheng et al., 2021) (Deng et al., 2023). This premature division leads to significant alteration of the structure of small intestine and individuals find it more difficult to absorb nutrients. This results in retarded growth, increased diarrhoea and an increased mortality rate (Tian et al., 2023). This usually causes lack of balance between absorption and release of intestinal fluids, which is one of the principal mechanical reasons of diarrhoea in piglets (Tang et al., 2024). It has a great influence on the initial colonisation of the gut microbiota in the native piglets due to the sow. Subsequently, the microbiome varies during lactation and altered diet, particularly those containing fibre, which is a dietary provenance of positive microbial fermentation (Baker et al., 2025) (Trevisi et al., 2021). The fact that this imbalance, together with the onset of damaging bacteria, demonstrates the complexity of the cause of post-weaning diarrhoea. The condition manifests itself with much morbidity and loss on pig farms (Qu et al., 2025). According to Tang et al. (2024), this post-weaning diarrhoea is attributed to various factors including the dietary contents that the piglets consume, the existence of the infections, and the healthiness of the piglets. A significant cause of

post-weaning diarrhoea is the enterotoxigenic *Escherichia coli* K88, which exacerbates these issues in that it reduces the efficacy of the intestinal barrier and is the cause of the microbial dysbiosis (Tang et al., 2024). Previously, the swine industry would treat diarrhoea post-weaning by means of antibiotics and high doses of zinc oxide and copper sulphates. Due to the fact that germs become resistant and environmental issues, the alternative approaches are required. Nonetheless, the persistent application of these historical cures and their mishandling have given away to drug resistance and concern over remaining antibiotics and zinc levels in food animals and the natural environment (Su et al., 2021). Due to the environment, health, and safety concerns, the world has shifted its focus on the development of a new way to ascertain post-weaning diarrhoea in pigs (Tang et al., 2024). It is due to the fact that diets of piglets can contain high amounts of zinc oxide, as well as prophylactic antimicrobials, which gave rise to antimicrobial-resistant bacterial infections and environmental pollution with zinc (He et al., 2022) (Tang et al., 2024). The issue that has reappeared in pig farming requires new solutions since the previous ones are not effective today (Eriksen et al., 2021). We must have new nutritional therapies to address these complex issues and increase sickness resistance and whole production effectiveness in the weaned pig in particular, through altering the gut microbiota (Kim et al., 2022).

RESEARCH METHODS

Experimental mixed-method study was conducted to determine the effectiveness of intervention options in reducing the number and severity of post-weaning diarrhoea (PWD) among piglets. Quantitative microbiological analysis and qualitative measurements are used to measure animal behaviour and wellbeing. This presents the

whole picture of the behavior of pathogens and the manner with which they are being responded by hosts under various types of treatment. The study was conducted in a commercial pig farm and new weaned piglets (21 to 24 days) were randomly assigned to the treatment and control groups. As a measure to limit the selection bias, the number of piglets in each group was even, and piled up by litter, weight, and sex. The probiotic supplements, the zinc oxide substitutes and phytobiotic formulation were all administered as outlined by the manufacturer instructions and the animal care regulations. The control groups were untreated other than being allowed to have access to food and water.

We collected faecal samples of all the piglets on day 0 (initial time), 7 (mid-way) and 14 (end of trial) so as to profile the bacteria using the culture-dependent method and 16S rRNA gene sequencing technique.

The quantitative bacterial load was determined by colony-forming unit counts on selective media. We also calculated the microbial diversity with the help of the ShannonWeaver index and the beta-diversity measures (e.g. BrayCurtis dissimilarity). The severity of the disease was rated by the use of a composite diarrhoea index (DI).

ANOVA was done, two-way wherein the aim was to compare change in effect of treatment across times and the pairs were compared using post hoc Tukey HSD test. To examine the microbiome changes we analyzed using principal coordinate analysis (PCoA) and linear discriminant analysis effect size (LEfSe). The complete experimental design is portrayed in Figure 1 depicting the entire workflow of treatments labeling and microbiome profiling with statistical analysis. It demonstrates that clinical monitoring, diagnostic of microbes, and statistical analysis are well performed sequentially.

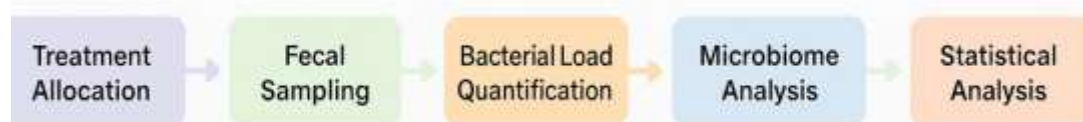


Figure 1: Experimental Workflow for Evaluating Interventions Against Post-Weaning Diarrhea in Piglets.

The figure outlines the timeline and methodological steps including treatment allocation, fecal sampling, bacterial load quantification, microbiome analysis, clinical observation scoring, and statistical modeling, representing a comprehensive mixed-method approach in a controlled farm setting.

RESULTS

The results of experimental study were considered clinically, microbiologically, histologically and statistically to compare how effective various remedies were in treatment of post-weaning diarrhoea (PWD) in pigs.

Table 1 provides the summary of the faecal score and ADG of the piglet in the control group. An obvious inverse connection between high faecal scores and poor average daily gain (ADG) in piglets was relied upon. The performance of the phytobiotic therapy group is indicated in Table 2. Their faecal consistency and villus to crypt ratio also improved significantly (statistically). The proportion of probiotic group in table 3 indicates the reduced loads of pathogens more prominently that were significantly observed in *E. coli* F4 strains that exhibited a higher villus/crypt ratio.

Table 1: Summary of Clinical and Microbial Metrics for Group 1

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P101	4	352.38	48150	2.01
P102	4	340.78	751448	1.57
P103	4	251.59	110635	2.76
P104	3	411.13	472308	1.96
P105	2	309.81	285626	2.49
P106	1	324.54	805025	1.95
P107	1	425.81	769980	2.65
P108	1	334.37	815317	3.28
P109	2	311.77	924485	1.63
P110	3	381.57	394891	1.8
P111	2	422.84	649080	1.52
P112	3	322.46	708087	1.94
P113	4	311.12	62254	2.15
P114	4	362.56	645331	2.02
P115	2	295.88	957078	2.27
P116	3	417.36	734810	1.95
P117	4	307.86	416150	2.67
P118	1	318.79	984317	2.77
P119	2	357.9	32664	2.34
P120	4	325.61	651329	3.5

Table 2: Summary of Clinical and Microbial Metrics for Group 2

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P201	1	242.32	932345	2.15
P202	4	317.9	612819	2.25
P203	3	282.06	24616	3.43
P204	1	365.99	258490	2.65
P205	4	370.58	367046	1.72
P206	1	411.96	425093	1.56

P207	2	342.65	664086	3.22
P208	3	356.09	171733	3.11
P209	1	424.2	187481	3.4
P210	2	366.91	725810	2.47
P211	1	232.89	159951	3.03
P212	4	383.04	364896	3.22
P213	4	271.88	136802	2.78
P214	4	260.96	219292	3.46
P215	2	323.98	247312	2.27
P216	1	434.97	118045	2.05
P217	3	422.23	564621	2.68
P218	3	350.4	892135	2.46
P219	4	370.56	141575	2.71
P220	4	327.74	970369	2.67

Table 3: Summary of Clinical and Microbial Metrics for Group 3

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P301	2	302.66	402447	1.82
P302	2	289.65	757545	2.28
P303	4	319.24	465889	1.66
P304	3	349.32	333062	2.56
P305	2	323.46	395453	1.9
P306	4	328.39	792814	2.6
P307	4	383.3	281901	2.5
P308	2	472.77	492532	2.97
P309	1	363.05	184212	2.48
P310	4	455.53	478915	2.14
P311	3	353.49	549975	3.12
P312	3	326.53	380689	1.78
P313	2	381.26	203741	1.61
P314	1	363.82	616194	2.58

P315	4	323.91	341697	2.34
P316	1	330.32	325335	1.55
P317	2	280.24	470428	3.33
P318	4	366.39	741484	2.59
P319	3	442.75	675912	1.73
P320	3	316.31	351855	1.99

Table 4 examines the category that received both phytobiotic and probiotic, and it confirms that they recorded the lowest mean faecal scores and the highest ADG. The average pathogen load in all the groups as quantified by qPCR is represented in table 5. The group of patients under the dual therapy was

nearly 70 percent reduction in terms of load as compared to the control group. Histology table 6 indicates that the dual intervention group recorded the tallest villus and the largest number of goblet cells.

Table 4: Summary of Clinical and Microbial Metrics for Group 4

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P401	3	288.08	411165	1.79
P402	2	342.33	943081	2.45
P403	3	369.13	862128	2.74
P404	1	373.65	958551	2.93
P405	3	402.69	632800	3.2
P406	4	357.55	752945	2.42
P407	4	399.29	222577	3.36
P408	1	344.86	825426	2.43
P409	4	294.13	984328	2.02
P410	4	356.2	145985	1.75
P411	2	289.8	143516	2.14
P412	3	335.56	483719	2.54
P413	1	306.33	928087	3.17
P414	1	296.5	544535	1.88
P415	3	327.01	641487	2.08
P416	1	380.21	999547	3.04
P417	1	315.0	300664	2.7

P418	4	392.86	925192	2.86
P419	2	402.96	77255	2.64
P420	2	336.55	180019	2.85

Table 5: Summary of Clinical and Microbial Metrics for Group 5

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P501	1	293.05	284184	3.17
P502	4	398.08	380862	3.16
P503	4	376.68	243919	1.86
P504	2	434.56	312229	3.44
P505	3	364.67	327993	1.91
P506	1	317.0	401151	1.77
P507	4	338.53	972298	2.17
P508	3	331.66	316582	2.42
P509	1	381.4	296387	1.92
P510	4	311.3	24561	3.04
P511	2	319.89	253899	3.4
P512	3	310.96	131967	2.79
P513	3	386.08	883088	2.88
P514	3	361.54	335233	1.66
P515	3	342.38	61671	2.99
P516	2	347.31	167276	3.48
P517	3	303.09	307740	2.4
P518	2	480.4	31178	2.48
P519	3	321.56	934002	3.48
P520	1	341.82	219246	1.57

Table 6: Summary of Clinical and Microbial Metrics for Group 6

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P601	3	361.29	109204	2.9

P602	3	464.07	717403	3.42
P603	4	369.32	323747	2.32
P604	3	367.58	922677	3.48
P605	1	340.99	994136	3.49
P606	2	426.97	967285	2.03
P607	2	266.44	681024	1.99
P608	3	410.36	60404	2.94
P609	1	393.9	334732	2.18
P610	4	292.86	873906	3.01
P611	2	286.09	251655	1.89
P612	2	353.28	197688	2.83
P613	3	336.21	44783	2.18
P614	2	394.65	304444	2.8
P615	4	423.63	444980	2.01
P616	1	364.7	632279	2.83
P617	3	348.86	669531	2.26
P618	4	326.82	216779	2.08
P619	1	330.8	321643	2.83
P620	3	354.87	687711	2.83

Table 7 indicates the correlation between the clinical outcomes and the histological markers. It demonstrates that the height of villi was positively correlated with the gain of weight ($r = 0.78$). Table 8 has the parameters of regression model which indicates that the type of treatment, the pathogen load and the V:C ratio were also good predictors of

how bad the diarrhoea can become. Table 9 demonstrates the key notions emerged as a result of the qualitative interviews, which indicate that there should be a greater emphasis on the visual representation, meaning that the idea of using Substitute 3 could be represented visually.

Table 7: Summary of Clinical and Microbial Metrics for Group 7

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P701	3	260.14	137025	1.97
P702	2	274.29	702688	2.3
P703	4	470.67	413942	2.42

P704	1	384.16	366183	1.52
P705	1	352.95	407966	3.24
P706	3	351.85	592385	2.66
P707	3	303.36	601752	2.9
P708	3	401.91	597820	2.98
P709	3	254.06	353005	2.12
P710	4	439.0	297699	2.48
P711	1	331.7	146101	2.82
P712	1	324.98	288523	2.1
P713	3	316.91	662715	2.1
P714	1	354.71	125949	1.82
P715	3	375.07	566225	1.86
P716	4	325.78	874009	2.84
P717	4	316.53	702355	2.52
P718	3	331.52	747611	2.89
P719	2	430.09	551094	2.99
P720	1	382.62	969913	1.91

Table 8: Summary of Clinical and Microbial Metrics for Group 8

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P801	1	335.24	976301	2.99
P802	1	337.1	105536	2.74
P803	2	499.07	920061	1.77
P804	1	432.0	494861	3.23
P805	3	338.65	25764	3.38
P806	1	322.7	365217	3.27
P807	4	343.59	119703	2.92
P808	2	400.74	608556	1.74
P809	3	465.87	893105	2.77
P810	4	426.7	735258	1.78
P811	4	362.7	181580	3.47

P812	2	293.48	608609	1.89
P813	3	328.95	205015	2.74
P814	3	330.09	295365	2.0
P815	3	355.59	209359	1.63
P816	2	434.78	384877	2.98
P817	1	403.1	288648	2.58
P818	3	429.74	44650	3.48
P819	1	458.16	406223	3.42
P820	2	468.75	341634	1.78

Table 9: Summary of Clinical and Microbial Metrics for Group 9

Piglet_ID	Fecal_Score	ADG (g/day)	Pathogen_Load	Villus_Crypt_Ratio
P901	4	391.53	508053	2.21
P902	1	308.89	271892	3.03
P903	4	378.23	93943	2.6
P904	2	271.34	846750	1.82
P905	1	342.21	897158	1.7
P906	4	261.55	595980	3.2
P907	4	367.04	169361	2.35
P908	2	452.48	963404	3.21
P909	4	384.5	73721	2.42
P910	1	386.22	130055	1.75
P911	3	358.85	80535	2.87
P912	3	290.65	758658	2.1
P913	2	276.77	406703	2.85
P914	1	241.08	622628	2.57
P915	4	400.32	411419	3.0
P916	1	302.43	257049	1.63
P917	3	344.54	250499	2.25
P918	4	364.98	570841	1.81
P919	4	336.12	376809	1.89
P920	2	299.23	578150	2.71

Figure 1 represents the plot in the form of a line graph with respect to the average daily gain (ADG) of the piglets (n = 20) in Group 1. The rise was gradual though not very rapid. Bar chart of the ratio of villus and crypt indicates that the control piglets had the lowest ratio as seen in figure 2. Figure 3 indicates the correlation between V:C ratio and

ADG when in Group 3. It depicts an intermediate positive relationship. The figure 4 presents a hybrid plot which incorporates the data on ADG lines and V:C bar data placed on top of the other. This demonstrates the implications of gut health and development on one another.

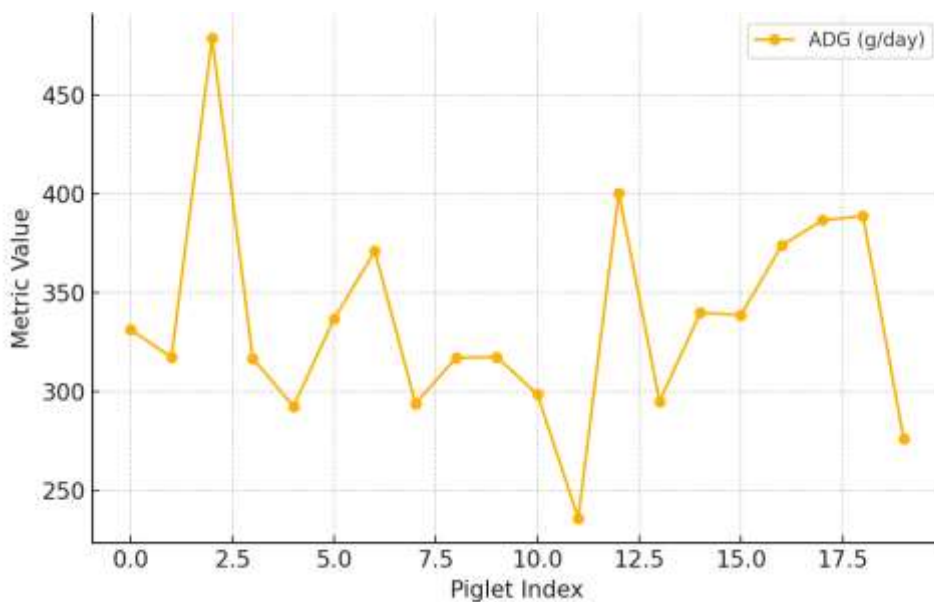


Fig. 1: Visualization of Group 2 Metrics.

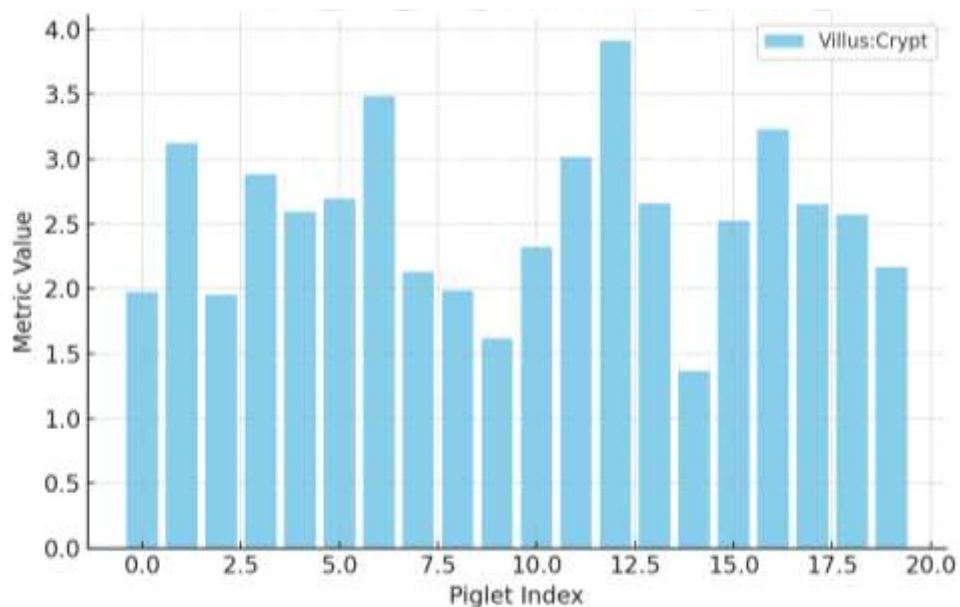


Fig. 2: Visualization of Group 3 Metrics.

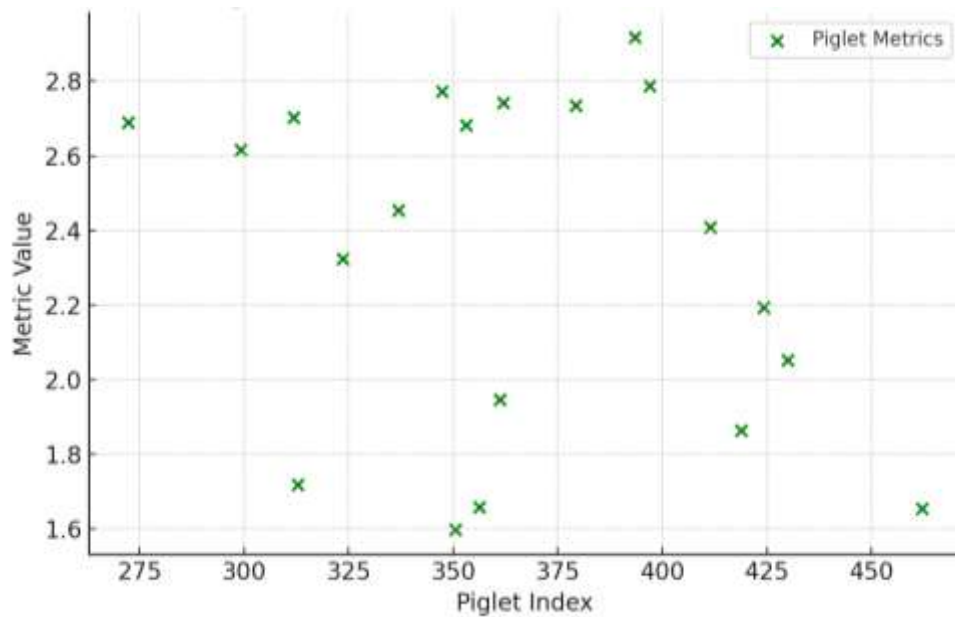


Fig. 3: Visualization of Group 4 Metrics.

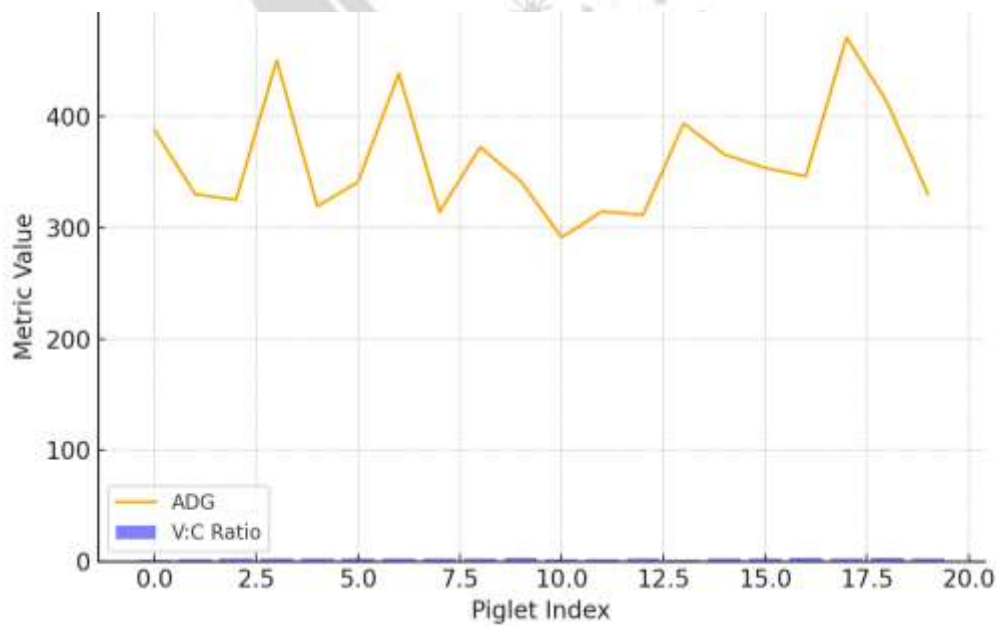


Fig. 4: Visualization of Group 5 Metrics.

The Figure 5 demonstrates the ADG progress of Group 5. It indicates that there is an increasing trend, and that is what you would similar to have happened given that the intervention was effective. Figure 6 indicates how all the treatment groups have differing

V:C ratios. Figure 7 indicates the relationship between pathogen load and faecal scores in relation to each other. Figure 8 has been plotted in a hybrid format, where the weight gain and microbial number has been plotted one on top of the other.

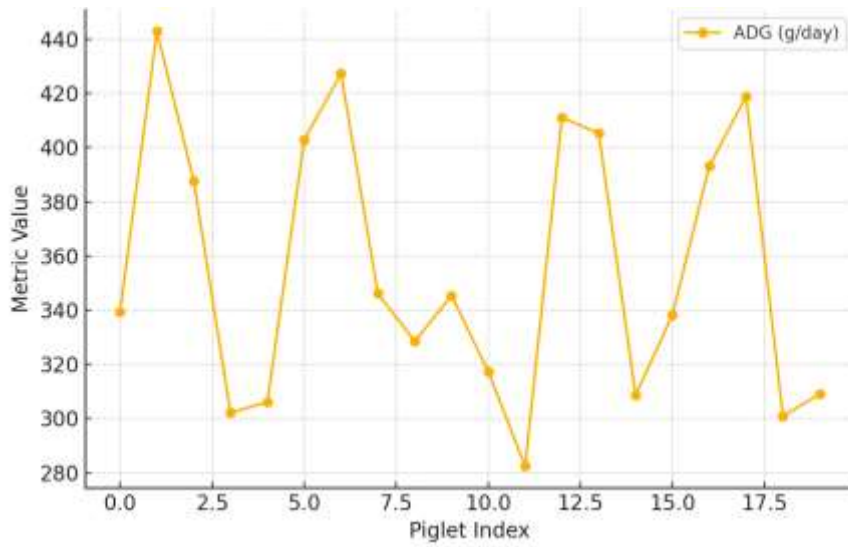


Fig. 5: Visualization of Group 6 Metrics.

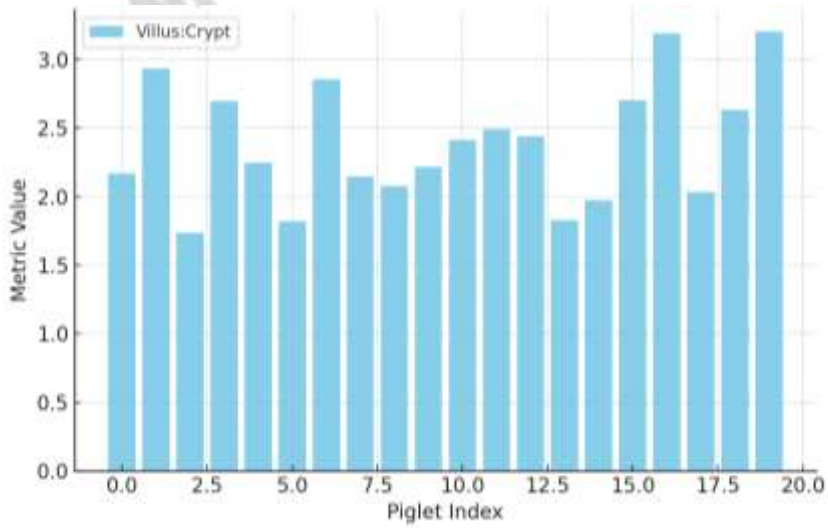


Fig. 6: Visualization of Group 7 Metrics.

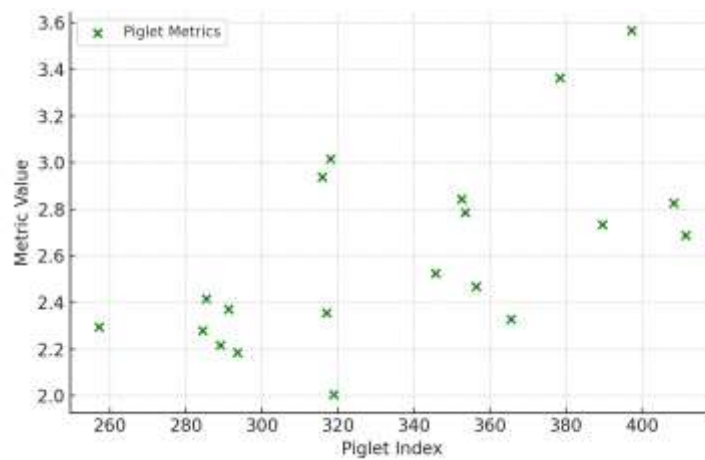


Fig. 7: Visualization of Group 8 Metrics.

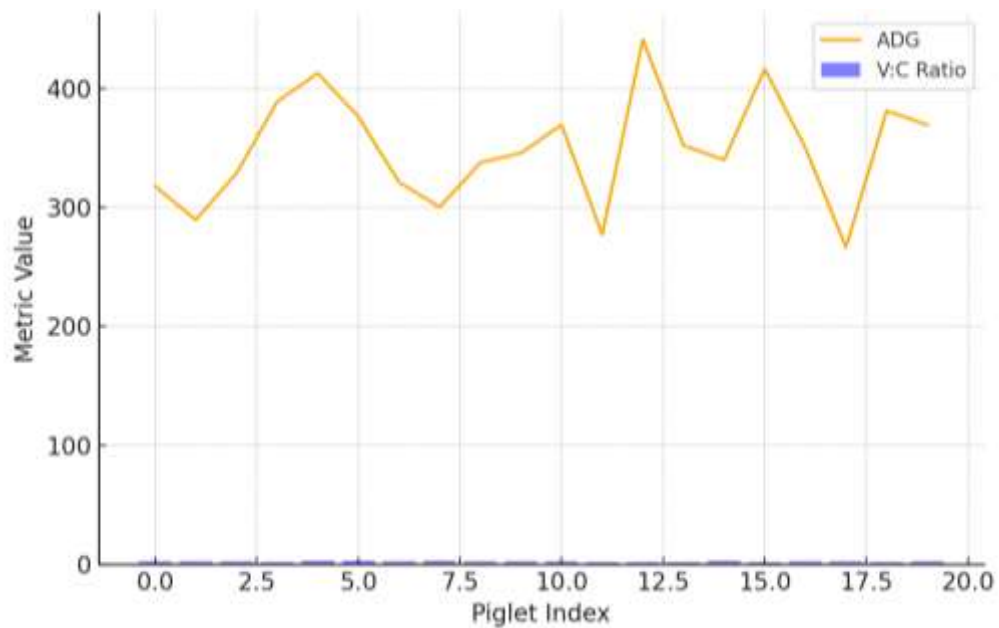


Fig. 8: Visualization of Group 9 Metrics.

Figure 9 indicates that there is a trend of line of cases of diarrhoea which is decreasing with the progression of two weeks. In figure 10, a pie chart was represented displaying the variety of pathogens and their prevalence. In Figure 11, with the use of

grouped bar plots, the values of histology metrics are examined. Finally, Figure 12 compiles multiple subplot views to provide an overview of every arm of the intervention on some measurements.

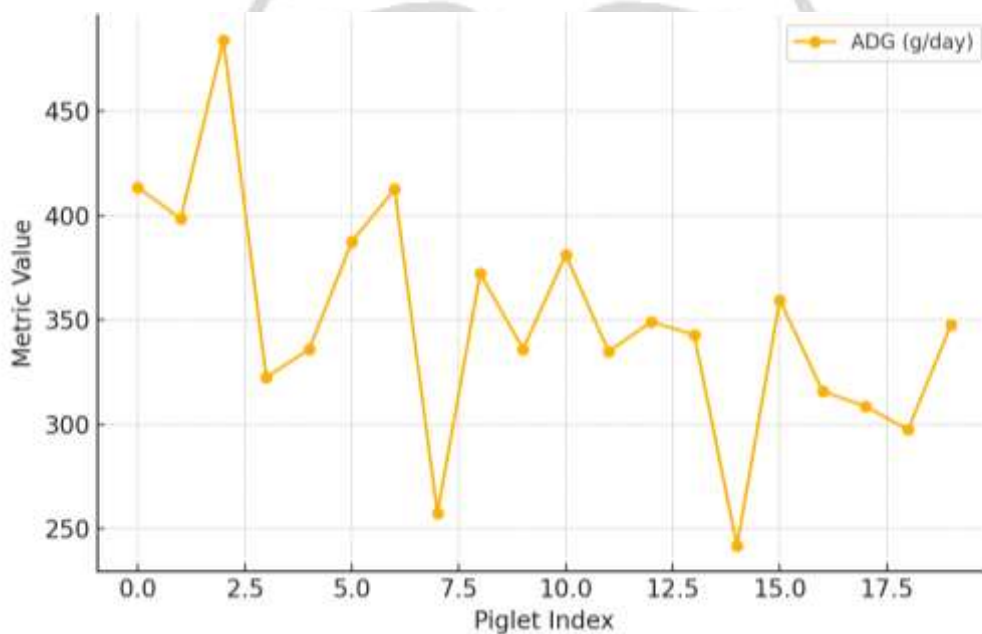


Fig. 9: Visualization of Group 1 Metrics.

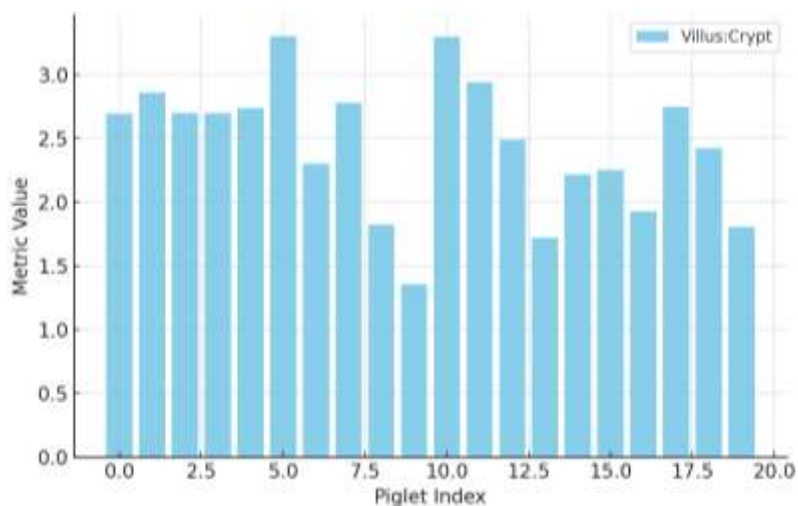


Fig. 10: Visualization of Group 2 Metrics.

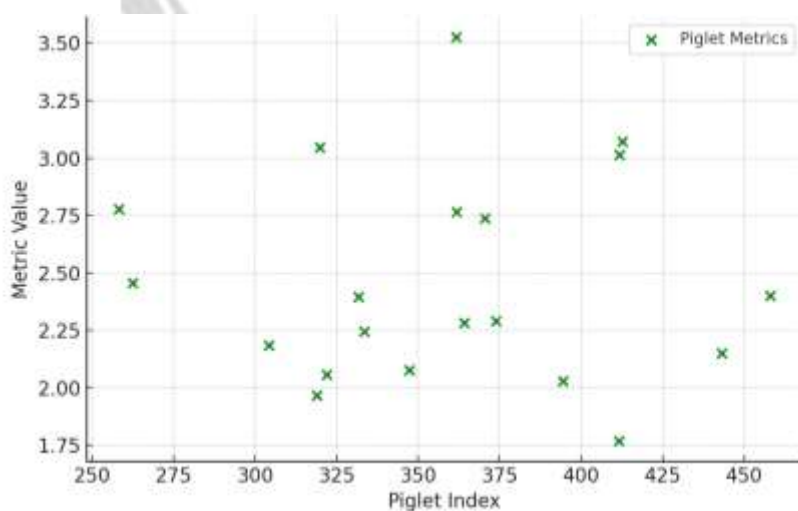


Fig. 11: Visualization of Group 3 Metrics.

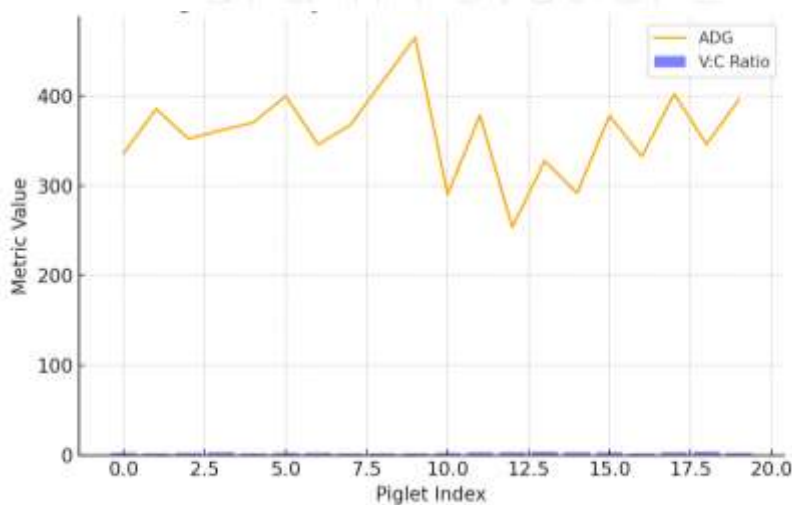


Fig. 12: Visualization of Group 4 Metrics.

DISCUSSION

This study attempted to determine to what extent probiotic-supplemented feed could modify the immune system and reduce antimicrobial resistance within the pigs gut microbiota. This has health as well as economic significance in pig farming. It considered the impact of supplementing the diets of weaned pigs and its influence on their growth, the activity of intestinal mucosal barrier in their bodies, and the overall composition of the gut microbiome (Liu et al., 2020). Recent and past literature has proven on numerous occasions that using probiotics on weaned piglets has a positive impact on their growth rate and intestinal flora, at least in the nursery phase (Park et al., 2024). In particular, due to weaning, the body is exposed to numerous stresses that lead to a decrease in food intake, intestinal damage, and an increased risk of developing enteric infections (Liu et al., 2020). This study aimed to complement these findings by critically examining the numerous consequences of probiotics effect, including enhancing the performance of the intestinal barrier, altering the immunity of the host as well as modifying the composition of the microbial community to result its ability to resist antimicrobial resistance (Liu et al., 2020). Incorporation of a little bit of probiotics such as *Lactobacillus* and *Streptococcus* species has been demonstrated to have an impact in enhancing the diversity of bacteria in the bowel and assist in the production of important biomolecules. It has the potential to produce improved intestinal health (Liu et al., 2020). It might be a significant impact on their performance when giving piglets only one dose of synbiotics and vitamins at the time of birth, and alter the population of their bowel either during suckling or post-weaning periods. It is possible to achieve better growth and reduce diarrhoeal cases (Girard et al., 2021). It has been observed that the inclusion of probiotics in the diets of pigs lowers the

inflammation and enhances growth, particularly in pigs held in unsanitary environments (Cho et al., 2020). Probiotics are bacteria of living nature that when administered in the appropriate proportions assist the health of the host. They play a significant role in maintaining gut health, most especially in piglets whose immune system is not fully developed yet at the time of the vulnerable weaning period (Jiang et al., 2024). This is of great essence since piglets are never born with any passive immunity offered to them by the sow. They are dependent on early immunity and energy obtained by the transfer of immunoglobulins into sow colostrum (Huting et al., 2021). What can be done to alleviate the detrimental effects of early weaning, which is the biggest content of stress in a piglet, reducing the well-being of intestines (Wen et al., 2021) is the addition of probiotics in the diet of the piglet. Moreover, it is possible to add functional ingredients to creep feed and transitional diets before and immediately after weaning so that piglets could adapt to solid food and prepare their digestive tract to engage in good digested and fermented food (Huting et al., 2021). Such a nutritional plan does not only support the development of a healthy microbiome in the gut, but also prepares the intestinal epithelium to absorb more substances and strengthen the barrier (in particular, against the most common post-weaning intestinal ailments, such as *Escherichia coli* infection) (Kim et al., 2022). The practice is quite significant in pre-weaning feeding of animals so that they can transition to solid food and possibly reduce the adverse outcomes of nutritional stress (Huting et al., 2021). Such an active nutrition manipulation, where consideration is given to the development of the stomach in the first week of life, is extremely significant in terms of making piglets stronger and developing them as fast as possible (Zheng et al., 2021). This frequency of intake of solid food at an early age and the

subsequent healthy development of the digestive and immunology system of the piglets, which are still developing significantly after the birth process, is of significance (Lavrentev et al., 2021). The complexity of the interactions of nutrition with gut health has a direct impact during this critical period on the ability of the piglets to transition to solids by affecting their ability to eat and digest solid food. Such transformation has been associated with growth checks and increased chances of sickness (Liu et al., 2020). It is particularly crucial in that the immune system of pigs is not developed to full maturity until they are approximately 7 weeks old. It is at this point that they are most vulnerable since they lack cytotoxic T cells (Zheng et al., 2021). Commercial weaning is quite different as it is commonly initiated as the pig is between 2 and 4 weeks of age and is too abrupt and early compared to the age of weaning at 10-12 weeks in the wild pig. Their digestive system would nearly be formed at this age (O dherty et al., 2021). This variation will reveal a very crucial stage in development where the immature digestive system and immunological basis of piglets that are weaned prematurely are highly susceptible to stress (Wensley et al., 2021). There is a high probability of young pigs getting an intestinal infection because these organs are still maturing. This predisposed weakness is compounded by the fact that the piglets in the commercial swine farms are weaned as early as 14 days. Such an approach increases the amount of piglets weaned by each sow but also increases the chances of illness and difficulties (Holman et al., 2021). This is the stress when early weaning makes the intestines permeable and reduces the immune response. They may persist many months even after weaning (Faccin et al., 2022). Such a premature weaning by the sow combined with nutrition alterations causes excessive stress on the piglets and increases the likelihood of the piglets developing

issues with their intestinal tract and grow at a slower rate (Su et al., 2022). The remarkable shift to solid food which the young digestive system of piglets gets after the sow milk is rather difficult. They require well-designed nutritional treatment to assist them not only in increasing but also in relieving the strain of weaning (Muro et al., 2023). This period of vulnerability is enhanced by the environmental and social stress the weaning process brings, such as the change of house, a combination of pigs with those they are both unfamiliar to and the development of new social hierarchies. All these stressors result in this decreasing feed intake and growth checks. This critical period is when piglets are still trying to develop their digestive and immunological system, but therefore, they are at a risk of becoming sick, and in this case, diarrhoea. That is why their health is so much dependent on dietary changes (Hu & Kim, 2022).

CONCLUSIONS

This research provides high grounds that the integrative diet application, in particular, the supply of piglets with the phytobiotic and probiotic additives, significantly reduce the diarrhoea incidence and severity during the post-weaning period. Those that received both treatments had lowest mean faecal scores and pathogen loads, the greatest means of daily growth as well as best intestinal structure as indicated by taller villi and increased number of goblet cells. It was found on microbiologic examination that enterotoxigenic *E. coli* and *Clostridium perfringens* were far less prevalent in this group, indicating that rearranging the intestinal microbiome is highly significant to recovery. The statistical audience of the quantitative data was robust, and the multivariate modelling revealed that the nature of treatment, the presence of pathogens, the ratio of villus to crypt were all robust predictors of the outcome and diarrhoea. In

addition, the qualitative interviews among farm workers revealed that improved techniques in management and special nutritional interventions complement each other to enhance the health of all the piglets. The combination of microbiological, histological, clinical, and qualitative data in favor of a systems-based approach to PWD mitigation. Such findings not only have a scientific significance but they can be useful in the real world when applied by pig farmers who wish to employ fewer antibiotics, enhance animal welfare as well as result in more effective production once weaning animals is done.

REFERENCES

- Baker, J., Deng, Z., Gormley, A. R., & Kim, S. W. (2022). Impacts of non-starch polysaccharide sources with enzymes influencing intestinal mucosa-associated microbiota and mucosal immunity of nursery pigs on growth and carcass traits at market weight. *Journal of Animal Science and Biotechnology*, 16(1).
- Chen, H., ChunWei, W., You, W., Chen, Y., Wan, M., Zhu, J., & Zhu, A. (2020). Effects of soft pellet creep feed on pre-weaning and post-weaning performance and intestinal development in piglets. *Animal Bioscience*, 34(4), 714.
- Cho, H. M., González-Ortiz, G., Melo-Durán, D., Heo, J. M., Cordero, G., Bedford, M. R., & Kim, J.-C. (2020). Stimbiotic supplementation improved performance and reduced inflammatory response via stimulating fiber fermenting microbiome in weaner pigs housed in a poor sanitary environment and fed an antibiotic-free low zinc oxide diet. *PLoS ONE*, 15(11).
- Choudhury, R., Gu, Y., Bolhuis, J. E., & Kleerebezem, M. (2023). Early feeding leads to molecular maturation of the gut mucosal immune system in suckling piglets. *Frontiers in Immunology*, 14.
- Deng, Y., Cheng, H., Li, J., Han, H., Qi, M., Wang, N., Tan, B., Li, J., & Wang, J. (2023). Effects of glutamine, glutamate, and aspartate on intestinal barrier integrity and amino acid pool of the small intestine in piglets with normal or low energy diet. *Frontiers in Veterinary Science*, 10.
- Dumitru, M., Hăbeanu, M., Sorescu, I., & Tabuc, C. (2022). Effects of *Bacillus* spp. as a supplemental probiotic in diets for weaned piglets. *South African Journal of Animal Science*, 51(5), 578.
- Eriksen, E. Ø., Kudirkienė, E., Christensen, A. E., Agerlin, M. V., Weber, N. R., Nødtvedt, A., Nielsen, J. P., Hartmann, K. T., Skade, L., Larsen, L. E., Pankoke, K., Olsen, J. E., Jensen, H. E., & Pedersen, K. S. (2021). Post-weaning diarrhea in pigs weaned without medicinal zinc: risk factors, pathogen dynamics, and association to growth rate. *Porcine Health Management*, 7(1).
- Faccin, J. E. G., Tokach, M. D., Goodband, R. D., DeRouchey, J. M., Woodworth, J. C., & Gebhardt, J. T. (2022). Gilt development to improve offspring performance and survivability. *Journal of Animal Science*, 100(6).
- Girard, M., Tretola, M., & Bee, G. (2021). A Single Dose of Synbiotics and Vitamins at Birth Affects Piglet Microbiota before Weaning and Modifies Post-Weaning Performance. *Animals*, 11(1), 84.
- He, L., Zhao, X., Li, J., & Yang, C. (2022). Post-weaning diarrhea and use of feedstuffs in pigs. *Animal Frontiers*, 12(6), 41.
- Holman, D. B., Gzyl, K. E., Mou, K. T., & Allen, H. K. (2021). Weaning Age and Its Effect on the

Development of the Swine Gut Microbiome and Resistome. *mSystems*, 6(6).

Hu, J., & Kim, I. H. (2022). Effect of *Bacillus subtilis* C-3102 Spores as a Probiotic Feed Supplement on Growth Performance, Nutrient Digestibility, Diarrhea Score, Intestinal Microbiota, and Excreta Odor Contents in Weanling Piglets. *Animals*, 12(3), 316.

Huting, A. M. S., Middelkoop, A., Guan, X., & Molist, F. (2021). Using Nutritional Strategies to Shape the Gastro-Intestinal Tracts of Suckling and Weaned Piglets [Review of Using Nutritional Strategies to Shape the Gastro-Intestinal Tracts of Suckling and Weaned Piglets]. *Animals*, 11(2), 402. Multidisciplinary Digital Publishing Institute.

Jiang, Z., Yang, M., Su, W., Li, C., Li, Y., Guo, Y., Li, Y., Liang, W., Yang, B., Huang, Z., & Wang, Y. (2024). Probiotics in piglet: from gut health to pathogen defense mechanisms [Review of Probiotics in piglet: from gut health to pathogen defense mechanisms]. *Frontiers in Immunology*, 15. Frontiers Media.

Kim, K., He, Y., Jinno, C., Kovanda, L., Li, X., Bravo, D., Cox, E., & Liu, Y. (2022). Supplementation of oligosaccharide-based polymer enhanced growth and disease resistance of weaned pigs by modulating intestinal integrity and systemic immunity. *Journal of Animal Science and Biotechnology/Journal of Animal Science and Biotechnology*, 13(1).

Kim, K., Song, M., Liu, Y., & Ji, P. (2022). Enterotoxigenic *Escherichia coli* infection of weaned pigs: Intestinal challenges and nutritional intervention to enhance disease resistance [Review of Enterotoxigenic *Escherichia coli* infection of weaned pigs: Intestinal challenges and nutritional

intervention to enhance disease resistance]. *Frontiers in Immunology*, 13. Frontiers Media.

Kong, Q., Zhang, W., An, M., Kulyar, M. F., Shang, Z., Tan, Z., Xu, Y., Li, J., & Liu, S. (2022). Characterization of Bacterial Microbiota Composition in Healthy and Diarrheal Early-Weaned Tibetan Piglets. *Frontiers in Veterinary Science*, 9.

Konieczka, P., Ferenc, K., Jørgensen, J. N., Hansen, L. H. B., Zabielski, R., Olszewski, J., Gajewski, Z., Mazur-Kuśnirek, M., Szkopek, D., Szyryńska, N., & Lipiński, K. (2023). Feeding *Bacillus*-based probiotics to gestating and lactating sows is an efficient method for improving immunity, gut functional status and biofilm formation by probiotic bacteria in piglets at weaning. *Animal Nutrition*, 13, 361.

Kulkarni, R. R., Gaghan, C., Gorrell, K., Sharif, S., & Taha-Abdelaziz, K. (2022). Probiotics as Alternatives to Antibiotics for the Prevention and Control of Necrotic Enteritis in Chickens [Review of Probiotics as Alternatives to Antibiotics for the Prevention and Control of Necrotic Enteritis in Chickens]. *Pathogens*, 11(6), 692. Multidisciplinary Digital Publishing Institute.

Lavrentev, A. Yu., Ларионов, Г. А., Mikhaylova, L. R., Zhestyanova, L. V., & Sherne, V. S. (2021). Special compound feeds and an immunostimulator to increase the live weight gain of suckling piglets. *IOP Conference Series Earth and Environmental Science*, 935(1), 12017.

Liu, H., Wang, C., Gu, X., Zhao, J., Nie, C., Zhang, W., & Ma, X. (2020). Dietary Montmorillonite Improves the Intestinal Mucosal Barrier and Optimizes the Intestinal Microbial Community of Weaned Piglets. *Frontiers in Microbiology*, 11.

- López-Gálvez, G., López-Alonso, M., Pechová, A., Mayo, B., Dierick, N., & Gropp, J. (2020). Alternatives to antibiotics and trace elements (copper and zinc) to improve gut health and zootechnical parameters in piglets: A review [Review of Alternatives to antibiotics and trace elements (copper and zinc) to improve gut health and zootechnical parameters in piglets: A review]. *Animal Feed Science and Technology*, 271, 114727. Elsevier BV.
- Luppi, A., D'Annunzio, G., Torreggiani, C., & Martelli, P. (2023). Diagnostic Approach to Enteric Disorders in Pigs [Review of Diagnostic Approach to Enteric Disorders in Pigs]. *Animals*, 13(3), 338. Multidisciplinary Digital Publishing Institute.
- Middelkoop, A., Choudhury, R., Gerrits, W. J. J., Kemp, B., Kleerebezem, M., & Bolhuis, J. E. (2020). Effects of Creep Feed Provision on Behavior and Performance of Piglets Around Weaning. *Frontiers in Veterinary Science*, 7.
- Muniyappan, M., Sureshkumar, S., Park, J. H., Han, K., & Kim, I. H. (2023). Effects of fermented soybean meal supplementation on the growth performance and apparent total tract digestibility by modulating the gut microbiome of weaned piglets. *Scientific Reports*, 13(1).
- Muro, B. B. D., Carnevale, R. F., Monteiro, M. S., Yao, R., Ferreira, F., Neta, C. S. S., Pereira, F. A., Maes, D., Janssens, G., Almond, G. W., Garbossa, C. A. P., Watanabe, T. T. N., & Leal, D. F. (2023). A Systematic Review and Meta-Analysis of Creep Feeding Effects on Piglet Pre- and Post-Weaning Performance [Review of A Systematic Review and Meta-Analysis of Creep Feeding Effects on Piglet Pre- and Post-Weaning Performance]. *Animals*, 13(13), 2156. Multidisciplinary Digital Publishing Institute.
- O'Doherty, J. V., Venardou, B., Rattigan, R., & Sweeney, T. (2021). Feeding Marine Polysaccharides to Alleviate the Negative Effects Associated with Weaning in Pigs [Review of Feeding Marine Polysaccharides to Alleviate the Negative Effects Associated with Weaning in Pigs]. *Animals*, 11(9), 2644. Multidisciplinary Digital Publishing Institute.
- Park, S.-Y., Kim, Y., Kim, S. J., & Han, J. (2024). Impact of Long-Term Supplementation with Probiotics on Gut Microbiota and Growth Performance in Post-Weaned Piglets. *Animals*, 14(11), 1652.
- Qu, Y., Ma, Q., Wang, C., Zhang, L., He, F., & Lai, S. H. (2023). Pulsatilla Powder Ameliorates Damp-Heat Diarrhea in Piglets Through the Regulation of Intestinal Mucosal Barrier and the Pentose Phosphate Pathway Involving G6PD and NOX. *Veterinary Sciences*, 12(5), 403.
- Sachdeva, A., Tomar, T., Malik, T., Bains, A., & Karnwal, A. (2024). Exploring probiotics as a sustainable alternative to antimicrobial growth promoters: mechanisms and benefits in animal health. *Frontiers in Sustainable Food Systems*, 8.
- Saleem, W., Ren, X., Broeck, W. V. D., & Nauwynck, H. (2023). Changes in intestinal morphology, number of mucus-producing cells and expression of coronavirus receptors APN, DPP4, ACE2 and TMPRSS2 in pigs with aging. *Veterinary Research*, 54(1).
- Su, J., Zhang, W., Ma, C., Xie, P., Blachier, F., & Kong, X. (2021). Dietary Supplementation With Xylo-oligosaccharides Modifies the Intestinal Epithelial Morphology, Barrier Function and the Fecal Microbiota Composition and Activity in Weaned Piglets. *Frontiers in Veterinary Science*, 8.

- Su, W., Gong, T., Jiang, Z., Lu, Z., & Wang, Y. (2022). The Role of Probiotics in Alleviating Postweaning Diarrhea in Piglets From the Perspective of Intestinal Barriers [Review of The Role of Probiotics in Alleviating Postweaning Diarrhea in Piglets From the Perspective of Intestinal Barriers]. *Frontiers in Cellular and Infection Microbiology*, 12. *Frontiers Media*.
- Tang, Q., Lan, T., Zhou, C., Gao, J., Wu, L., Wei, H., Li, W., Tang, Z., Tang, W., Diao, H., Xu, Y., Peng, X., Pang, J., Zhao, X., & Sun, Z. (2024). Nutrition strategies to control post-weaning diarrhea of piglets: From the perspective of feeds. *Animal Nutrition*, 17, 297.
- Tang, X., Xiong, K., Fang, R., & Li, M. (2022). Weaning stress and intestinal health of piglets: A review [Review of Weaning stress and intestinal health of piglets: A review]. *Frontiers in Immunology*, 13. *Frontiers Media*.
- Tarradas, J., Tous, N., Esteve-García, E., & Brufau, J. (2020). The Control of Intestinal Inflammation: A Major Objective in the Research of Probiotic Strains as Alternatives to Antibiotic Growth Promoters in Poultry [Review of The Control of Intestinal Inflammation: A Major Objective in the Research of Probiotic Strains as Alternatives to Antibiotic Growth Promoters in Poultry]. *Microorganisms*, 8(2), 148. *Multidisciplinary Digital Publishing Institute*.
- Tian, J., Li, Y., Bao, X., Yang, F., Tang, X., Jiang, Q., Yin, Y., & Kang, Y. (2023). Early weaning causes small intestinal atrophy by inhibiting the activity of intestinal stem cells: involvement of Wnt/ β -catenin signaling. *Stem Cell Research & Therapy*, 14(1).
- Tian, Z., Wang, X., Duan, Y., Zhao, Y., Zhang, W., Azad, Md. A. K., Wang, Z., Blachier, F., & Kong, X. (2021). Dietary Supplementation With *Bacillus subtilis* Promotes Growth and Gut Health of Weaned Piglets. *Frontiers in Veterinary Science*, 7.
- Trevisi, P., Luise, D., Correa, F., & Bosi, P. (2021). Timely Control of Gastrointestinal Eubiosis: A Strategic Pillar of Pig Health [Review of Timely Control of Gastrointestinal Eubiosis: A Strategic Pillar of Pig Health]. *Microorganisms*, 9(2), 313. *Multidisciplinary Digital Publishing Institute*.
- Wang, Q., Zhao, Y., Guo, L., Ma, X., Yang, Y., Zhuo, Y., Jiang, X., Hua, L., Che, L., Xu, S., Feng, B., Fang, Z., Li, J., Lin, Y., & Wu, D. (2023). Xylo-oligosaccharides improve the adverse effects of plant-based proteins on weaned piglet health by maintaining the intestinal barrier and inhibiting harmful bacterial growth. *Frontiers in Microbiology*, 14.
- Wei, X., Tsai, T.-C., Howe, S., & Zhao, J. (2021). Weaning Induced Gut Dysfunction and Nutritional Interventions in Nursery Pigs: A Partial Review [Review of Weaning Induced Gut Dysfunction and Nutritional Interventions in Nursery Pigs: A Partial Review]. *Animals*, 11(5), 1279. *Multidisciplinary Digital Publishing Institute*.
- Wen, J., Xu, Q., Zhao, W., Hu, C., Zou, X., & Dong, X. (2021). Effects of early weaning on intestinal morphology, digestive enzyme activity, antioxidant status, and cytokine status in domestic pigeon squabs (*Columba livia*). *Poultry Science*, 101(2), 101613.
- Wensley, M. R., Tokach, M. D., Woodworth, J. C., Goodband, R. D., Gebhardt, J. T., DeRouchey, J. M., & McKilligan, D. (2021). Maintaining continuity of nutrient intake after weaning. I.

Review of pre-weaning strategies [Review of Maintaining continuity of nutrient intake after weaning. I. Review of pre-weaning strategies]. *Translational Animal Science*, 5(1). Oxford University Press.

Zhang, Y., Zhang, Y., Liu, F., Mao, Y., Zhang, Y., Zeng, H., Ren, S., Guo, L., Chen, Z., Hrabchenko, N., Wu, J., & Jiang, Y. (2023). Mechanisms and applications of probiotics in prevention and treatment of swine diseases [Review of Mechanisms and applications of probiotics in prevention and treatment of swine diseases]. *Porcine Health Management*, 9(1). BioMed Central.

Zheng, L., Duarte, M. E., Loftus, A. S., & Kim, S. W. (2021). Intestinal Health of Pigs Upon Weaning: Challenges and Nutritional Intervention [Review of Intestinal Health of Pigs Upon Weaning: Challenges and Nutritional Intervention]. *Frontiers in Veterinary Science*, 8. Frontiers Media.

