Journal of Animal and Feed Sciences, 34, 2, 2025, 189–199, https://doi.org/10.22358/jafs/193241/2025
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

ORIGINAL PAPER

Effects of glucose oxidase and probiotics (*Lactobacillus, subtilis*, *Bacillus licheniformis*) on growth performance, intestinal microvillus morphology and microfold cells in nursery pigs

S. Qin[1], X. Yuan[1], Z. Cheng[1], B. Zhang[2], Y. Ge[3], L.Yang[1,*] and W. Yang[1,*]

[1] Key Laboratory of Efficient Utilization of Non-grain Feed Resources (Co-construction by Ministry and Province), Ministry of Agriculture and Rural Affairs, Shandong Agricultural University, College of Animal Sciences and Technology, Tai'an 271018, Shandong, China
[2] Longkou City Animal Disease Prevention and Control Center, Long'kou 265700, Shandong, China
[3] Shandong University, SDU-ANU joint science college, Wei'hai 264209, Shandong, China

KEY WORDS: antibiotic substitutes, growth performance, microfold cell, microvilli morphology, nursery pigs

Received: 8 August 2024
Revised: 12 September 2024
Accepted: 12 September 2024

* Corresponding author:
e-mails: yanglijie@sdau.edu.cn;
wryang@sdau.edu.cn

ABSTRACT. Misuse of antibiotics in feed seriously affects pig intestinal health. Alternative feed additives are vital for maintaining pig health and productivity. This study investigated the effects of glucose oxidase (GOD) and probiotics on pig growth and the morphology of microfold cells in the small intestine. A total of 160 40-day-old (half males and half females) weaning piglets from the Duroc × Large White × Yorkshire (DLY) crossbreed was randomly assigned into 4 treatment groups with 4 replicates of 10 pigs per group. The dietary treatments included: (1) control (commercial basal diet without additive), (2) basal diet with antibiotics (50% gentamicin, 0.19 kg/t, 10% clomiphene, 0.42 kg/t, 10% bacitracin zinc, 0.44 kg/t), (3) basal diet with GOD (0.5 kg/t, 3000 U/g), and (4) basal diet with probiotics (*Lactobacillus* 3.0 × 10^9 CFU/g, *Bacillus subtilis* 1.4 × 10^{10} CFU/g, *B. licheniformis* 1.3 × 10^{10} CFU/g) and GOD. The trial included a 7-day preliminary period and a 35-day formal testing period. Results showed that the combination of probiotics and GOD significantly increased average daily gain (ADG) and reduced feed-to-gain ratio (F/G) of pigs ($P < 0.05$). The intestinal histology results indicated that antibiotics in feed severely damaged the morphology of intestinal microvilli and microfold cells, disrupting the intestinal microflora in nursery pigs. While GOD altered the morphology of microfold cells in the small intestine, enhancing phagocytosis, it also caused some damage to the intestinal mucosa. However, the combined application of probiotics and GOD helped repair the intestinal mucosa damage caused by GOD. In summary, the combination of probiotics and GOD as feed additives can serve as an effective alternative to antibiotics, improving both the growth and immune performance of nursery pigs.

Introduction

Antibiotics are widely utilised in human and veterinary medicine; their use in animal husbandry, including pigs, enhances growth rate and efficiency, reducing mortality and morbidity, and improving overall health and economic outcomes (Cromwell, 2002). In agriculture, antibiotics have been used for decades to prevent diseases and promote growth in livestock. However, their use can also lead to alterations in the composition and function of the microbiota, causing long-term detrimental effects in the host. The emergence of multidrug-resistant pathogens has raised serious concerns about

the widespread and often inappropriate use of antimicrobial agents (Becattini et al., 2016). The use of antibiotics as feed supplements has been banned in swine and livestock production in many countries around the world. On the other hand, alternative feed additives have been successfully employed to replace in-feed antibiotics, the most important being probiotics, prebiotics, bacteriocins, organic acids, enzymes, bioactive phytochemicals, and antimicrobial peptides (Thormar, 2012; Pearlin et al., 2022). The role of these additives and their potential in managing gut health and function in newly weaned pigs has been extensively reviewed (Heo et al., 2013; Thacker, 2013). Glucose oxidase (GOD) is an oxidoreductase that catalyses the conversion of glucose to gluconic acid and hydrogen peroxide in the presence of oxygen. It has been dubbed the 'Ferrari' of oxidative enzymes due to its rapid action, high stability and specificity (Wang et al., 2020; Bauer et al., 2022), GOD has been widely used in the feed production industry and has demonstrated strong antagonistic effects against various food-borne pathogens, such as *Salmonella infantis, Staphylococcus aureus, Clostridium perfringens, Bacillus cereus, Campylobacter jejuni*, or *Listeria monocytogenes* (Kapat, 1998). Supplementation of GOD in the diet has been shown to promote growth in weaning pigs (Dang et al., 2022). Wu et al. (2019) found that GOD improved growth performance and intestinal health in broilers by enhancing apparent nutrient digestibility and increasing the abundance of *Firmicutes* in the gut. Additionally, the inclusion of beneficial microorganisms in poultry diets has been found to enhance early growth, stimulate immune responses, and improve ileal morphology of broiler chickens (Salim et al., 2013). Combining prebiotics and probiotics in the diet of pigs from weaning to finishing has also demonstrated improvements in the feed conversion ratio (Méndez-Palacios et al., 2018). In addition to being an aerobic dehydrogenase, GOD is also a potential alternative to antibiotics and most importantly, can maintain the balance of intestinal flora and prevent oxidative stress (Wu et al., 2020; Cruz et al., 2012). Probiotics have also been demonstrated to enhance the immune system and improve the antioxidant capacity of animals (Xu et al., 2021). In recent years, studies on broiler production have demonstrated that the combined use of probiotics and GOD is more effective in improving overall health and production performance (Wang et al., 2022b). However, limited research has explored the impact of feed additive on the ultra-microstructure of the intestinal villi in pigs.

Microfold cells (M cells) are antigen-sampling cells located in the epithelium of Peyer's patches, a component of the intestinal-associated lymphoid tissue in the small intestine (Honarpisheh et al., 2022). In newborn piglets, both continuous ileal and discrete jejunal Peyer's patches are presents (Pabst et al., 1988). They possess unique morphological characteristics, including irregular brush borders, reduced microvilli, and basolateral pockets containing mononuclear phagocytes and lymphocytes (Corr et al., 2008). These cells play a crucial role in initiating mucosal immune responses through the uptake and transcytosis of luminal microbial antigens, which are important for maintaining intestinal homeostasis (Kimura, 2018). It is well-established that antibiotics and probiotics can affect microbial populations in the intestinal tract. For instance, it was reported that GOD supplementation decreased the concentration of faecal *Salmonella* and improved the faecal microflora in growing piglets (Tang et al., 2016). However, whether feed additives such as antibiotics, GOD, and probiotics impact the morphology of M cells remains unclear.

We hypothesised that dietary antibiotic substitutes would affect morphological characteristics of intestinal microvilli and M cells, ultimately improving growth performance in nursery pigs. Therefore, the present study aimed to evaluate the effect of antibiotic alternatives on growth, intestinal microvillus morphology and M cell function in pigs.

Material and methods

Animals and management

All experimental procedures used in this study were approved by the Shandong Agricultural University Animal Care and Use Committee (Approval No.: SDAUA-2019-019). The pigs used in all experiments were cared for in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

A total of 160 healthy weaned piglets (Duroc × Landrace × Large White), with equal numbers of males and females of a similar weight (9.67 + 0.13) kg were selected as test animals. These piglets were sourced from Dongying Huayu Feed Co., Ltd., and the experiment was conducted at the pig house of the same company (Dongying, Shandong, CN, China). Preparations for cleaning and disinfection of the pig house were carried out before the start of the experiment. Once the trial began, the pigs were fed daily at 8:00 and had *ad libitum* access to feed

and water. At the conclusion of the experimental period, pigs were euthanised by intravenous injection of pentobarbital. Following euthanasia, the animals were processed in accordance with established animal research guidelines. Tissue samples from the jejunum and ileum were collected for analysis using transmission electron microscopy and scanning electron microscopy.

Experimental design

A single-factor design was applied to examine the effects of antibiotic substitutes on growth performance, intestinal microvillus and M cell morphology in pigs. There were no significant differences in starting body weight between treatments ($P > 0.05$). A total of 160 weaning piglets (Duroc × Large White × Yorkshire) aged 40 days were randomly divided into 4 treatment groups with 4 replicates of 10 pigs each. Dietary treatments were as follows: (1) control (commercial basal diet without additives; diet composition and nutrient content are shown in Table 1), (2) basal diet plus antibiotics (main ingredients: 50% gentamicin, 0.19 kg/t, 10% clomiphene, 0.42 kg/t, 10% bacitracin zinc, 0.44 kg/t), (3) basal diet plus GOD (0.5 kg/t, 3000 U/g, KRVAB Bio-tech group, Beijing, China), and (4) basal diet plus probiotics (*Lactobacillus,* 3.0 $\times 10^9$ CFU/g, *Bacillus subtilis* 1.4 × 10^{10} CFU/g, *B. licheniformis* 1.3 $\times 10^{10}$ CFU/g, commercial name: Kofulai, KRVAB Bio-tech group, Beijing, China) and GOD. The preliminary trial period lasted for 7 days, followed by a formal testing period of 35 days.

Table 1. Basic diet composition and nutrient content

Item	Content	Nutrient level	Content
Ingredients, %		Digestible energy, MJ/kg	13.86
maize	68.55	Crude protein	19.68
46% soybean	12.92	Ca	1.02
fermented soybean	3.15	P	1.89
full fat soybean	6.77	Lys	0.57
Peru fishmeal	3.15	Met	0.16
plasma protein	0.47	Thr	0.13
APC blood corpuscle	0.63		
$Ca(H_2PO_4)_2$	0.91		
limestone	0.79		
NaCl	0.47		
premix[1]	2.00		
Total	100.00		

[1] premix per kg of diet provided the following: IU: vit. A 8000, vit. D_3 3000; mg: vit. K_3 2.00, vit. B_1 1.50, vit. B_2 6.00, vit. B_6 2.20, vit. B12 0.04, pantothenic acid 14.00, niacin 45.00, biotin 0.15, folic acid 1.20, Mn 40.00, Fe 120, Cu 10.00, Zn 130, Se 0.30, I 0.50

Growth indicators

The quantity of feed and residue in the pig house were recorded weekly to calculate average daily feed intake (ADFI) of each pig. The weight of each pig was measured individually every two weeks to determine average daily gain (ADG) and feed to gain ratio.

Electron-microscopy analysis of intestinal villi

Epithelial samples measuring approximately 0.5 × 0.5 × 0.2 cm were collected from the middle sections of the ileum, washed with PBS, and fixed in a 3% glutaraldehyde fixing solution (pH 7.2) at 4 ℃ for 3 days. Subsequently, the samples were further fixed in 1% OsO_4 at 4 °C for 3 h. The prepared samples were sent to the electron microscopy centre at the State Key Laboratory of Crop Biology, Shandong Agricultural University for preparation of electron microscope slides. Observations and imaging were conducted using a JEM-1400Plus transmission electron microscope (JEOL, Tokyo, Japan) and a JSM-6610LV scanning electron microscope (JEOL, Tokyo, Japan).

Statistical analysis

All data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA implemented in SAS 9.1. Differences between treatments were compared using Duncan's test and considered statistically significant at $P < 0.05$.

Results

Effects of various antibiotic substitutes on growth performance of nursery pigs

Statistical analysis of growth performance of nursery pigs is presented in Table 2. The results showed significant differences in ADG, ADFI and F/G between treatments ($P < 0.01$). Compared

Table 2. Effects of various antibiotic substitutes on growth performance of nursery pigs

Items	ADG, kg/day	ADFI, kg/day	F/G
Control	0.38 ± 0.01^{d}	0.86 ± 0.01^{c}	2.29 ± 0.09^{a}
Antibiotic	0.43 ± 0.01^{c}	0.95 ± 0.08^{b}	2.19 ± 0.23^{ab}
GOD	0.53 ± 0.01^{a}	0.96 ± 0.04^{b}	1.82 ± 0.10^{c}
Microecological preparation + GOD	0.56 ± 0.03^{a}	1.02 ± 0.04^{a}	1.82 ± 0.06^{c}
P-value	<0.001	<0.001	0.009

ADG – average daily gain, ADFI – average daily feed intake, F/G – feed to gain ratio, GOD – glucose oxidase; feed was calculated based on dry weight; ^{abc} – values within a column with different superscripts differ significantly at $P < 0.05$

to the control group, the addition of antibiotics and antibiotic substitutes to the diet significantly increased ADG and ADFI ($P < 0.05$). Notably, ADG in the GOD and probiotics + GOD treatments increased by 23 and 30%, respectively, compared to the antibiotic treatment (0.43 kg/day), while the F/G ratio decreased significantly ($P < 0.05$). Overall, the probiotics + GOD group demonstrated superior growth performance during the nursery stage, achieving an ADG of 0.56 kg/day, an ADFI of 1.02 kg/day, and a F/G ratio of 1.82.

Effects of various antibiotic substitutes on the morphology of intestinal microvilli of nursery pigs

Representative microvillus morphology from the ileum and jejunum is illustrated in Figure 1 and 4. Scanning and transmission electron microscopy results revealed that the inclusion of antibiotics to the feed led to extensive microvillus shedding in the jejunum and ileum (Figure 2A), as well as structural damage to epithelial cells (Figure 2D), especially in the ileum compared to the control group. The epithelial cells on the surface of the intestinal villi showed signs of histological lesions, including hollow areas (Figure 1B). Transmission electron microscopy further revealed that epithelial cells maintained lateral junctions with adjacent cells (Figure 4A, D). However, antibiotic treatment resulted in considerable degradation of the microvilli (Figure 4B, C, G, H). Supplementation with GOD also caused some damage to the microvilli of the intestinal mucosa (Figure 2B). In contrast, the inclusion of probiotics in the diet significantly alleviated the damage to the intestinal villus barriers caused by antibiotics. The combined administration of probiotics and GOD resulted in denser (Figure 1D) and longer (Figure 4J) intestinal microvilli.

Intestinal microflora was also observed in the folds and on the surface of the villi. A small number of cocci were present among the villous folds, noticeably fewer than in the other treatments (Figure 3), suggesting that only a small number of drug-resistant cocci remained in the intestinal tract. Interestingly, under all treatments, including the basal diet, bacilli were rarely found on the surface of the intestinal microvilli. Following microbial treatment, a small number of brevibacteria was observed between the villous folds. The combination treatment involving probiotics and GOD significantly increased both the number and richness of microorganisms between the microvilli of the jejunum

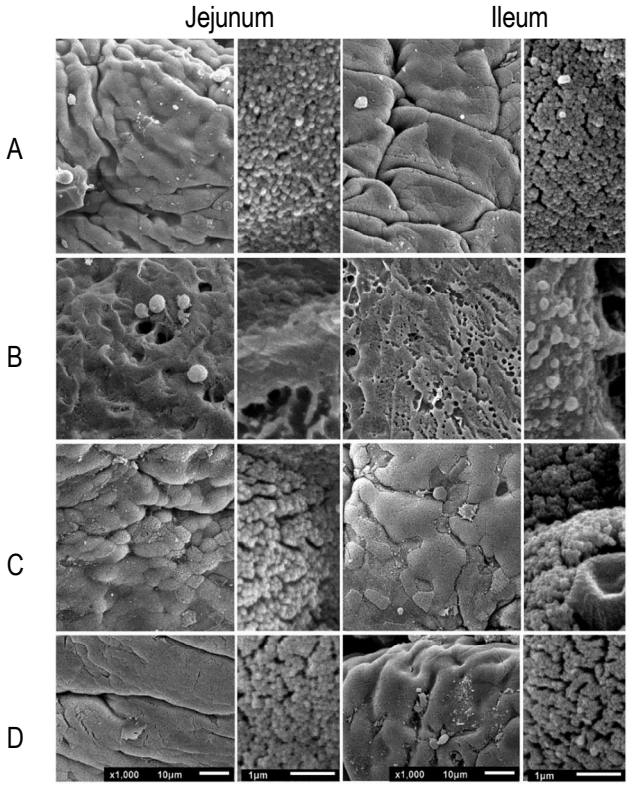

Figure 1. Effects of antibiotics and their substitutes on microvilli in the intestinal epithelium of nursery pigs under scanning electron microscopy. Row A – control; B – antibiotics (50% gentamicin, 0.19 kg/t; 10% clomiphene, 0.42 kg/t; 10% bacitracin zinc, 0.44 kg/t); C – glucose oxidase (GOD); D – probiotics (*Lactobacillus*, 3.0 × 109 CFU/g, *Bacillus subtilis* 1.4 × 1010 CFU/g, *B. licheniformis* 1.3 × 1010 CFU/g) + GOD. Scale bar: 10 μm for left 1st and 3rd columns, 1 μm for right 1st and 3rd column

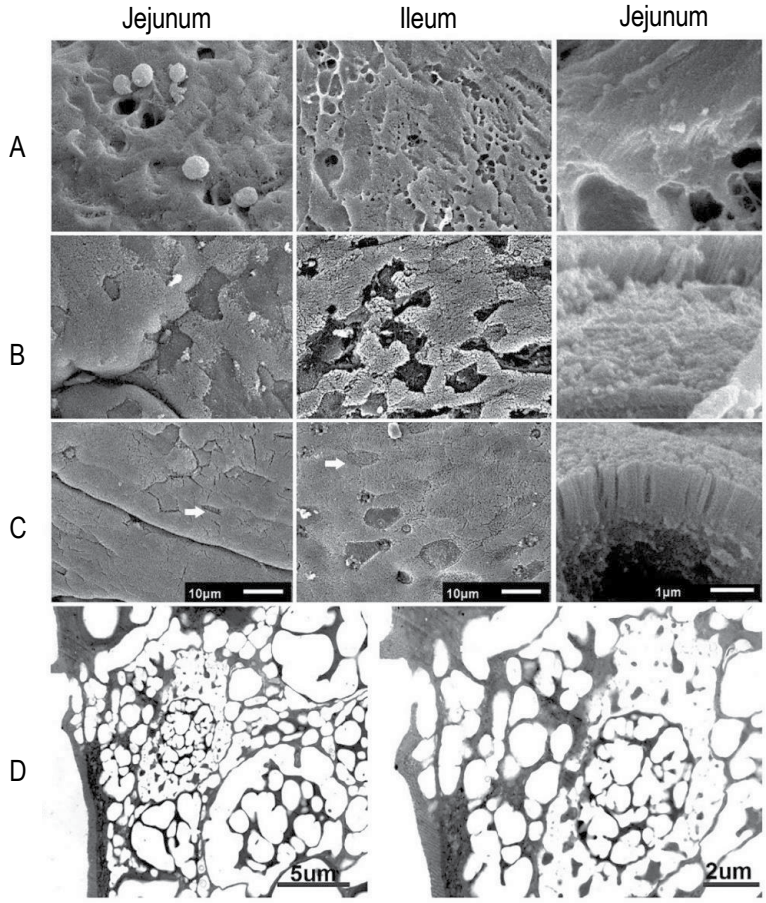

Figure 2. Effects of antibiotics and their substitutes on the development of intestinal villi in nursery pigs. Rows A–C – scanning electron micrographs, D – transmission electron micrographs; A – antibiotics (50% gentamicin, 0.19 kg/t; 10% clomiphene, 0.42 kg/t; 10% bacitracin zinc, 0.44 kg/t); B – glucose oxidase (GOD); C – probiotics (*Lactobacillus*, 3.0 × 10^9 CFU/g, *Bacillus subtilis* 1.4 × 10^{10} CFU/g, *B. licheniformis* 1.3 × 10^{10} CFU/g) + GOD; D – antibiotics. Arrows indicate lesions of microvilli. Scale bar: for scanning electron micrographs, 10 μm for the left two columns, 1 μm for the right column, for row D, 5 μm for the left, 2 μm for the right

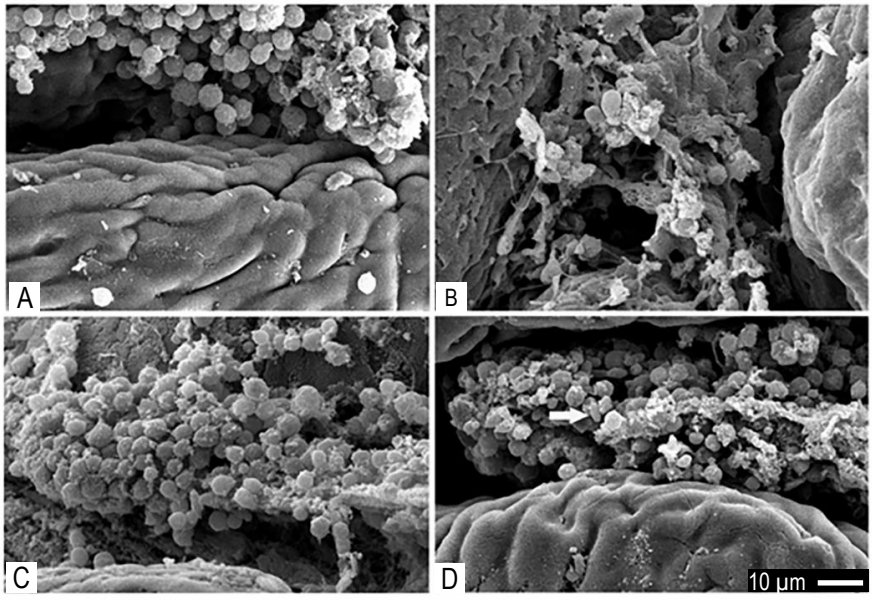

Figure 3. Effects of antibiotics and their substitutes on the number of bacteria in the intestine of nursery pigs. Row A – control; B – antibiotics (50% gentamicin, 0.19 kg/t; 10% clomiphene, 0.42 kg/t; 10% bacitracin zinc, 0.44 kg/t); C – glucose oxidase (GOD); D – probiotics (*Lactobacillus*, 3.0 × 10^9 CFU/g, *Bacillus subtilis* 1.4 × 10^{10} CFU/g, *B. licheniformis* 1.3 × 1010 CFU/g) + GOD. Arrows indicate B. brevis. Scale bar: 10 μm

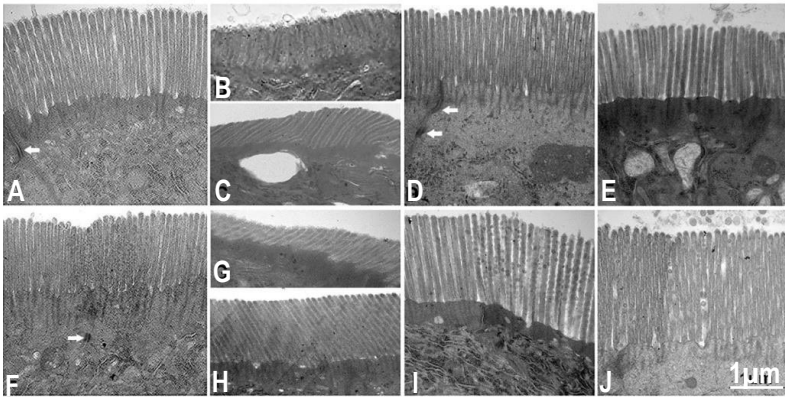

Figure 4. Effects of antibiotics and their substitutes on the morphology of microvilli in the intestinal epithelium of nursery pigs in transmission electron microscopy. Row A–E – jejunum; F–J – ileum; A and F – control; B, C, G and H – antibiotics (50% gentamicin, 0.19 kg/t; 10% clomiphene, 0.42 kg/t; 10% bacitracin zinc, 0.44 kg/t); D and I – glucose oxidase (GOD); E and J – probiotics (*Lactobacillus*, 3.0 × 10^9 CFU/g, *Bacillus subtilis* 1.4 × 10^{10} CFU/g, *B. licheniformis* 1.3 × 10^{10} CFU/g) + GOD. White arrow indicates gap junction between cells. Scale bar: 1 µm

(Figure 3). These findings indicated that excessive use of antibiotics and additives disrupted the normal intestinal flora of pigs.

Effects of various antibiotic substitutes on M cell morphology in the intestinal epithelium of nursery pigs

Scanning electron microscopy revealed that M cells in the antibiotic treatment group had fewer or completely lacked normal microvilli on their apical plasma membrane, instead showing short, fold-like structures, with a concave shape compared to the normal villous epithelial cells (Figure 5A). The addition of antibiotics damaged the microvilli of intestinal epithelial cells, making it difficult to distinguish M cells from other cells; only 'M-like' cells were recorded in these cases (Figure 5B). GOD treatment stimulated the development of short, fold-like structures, resulting in the formation of papillary, elevated structures on the apical surface of M cells. This promoted

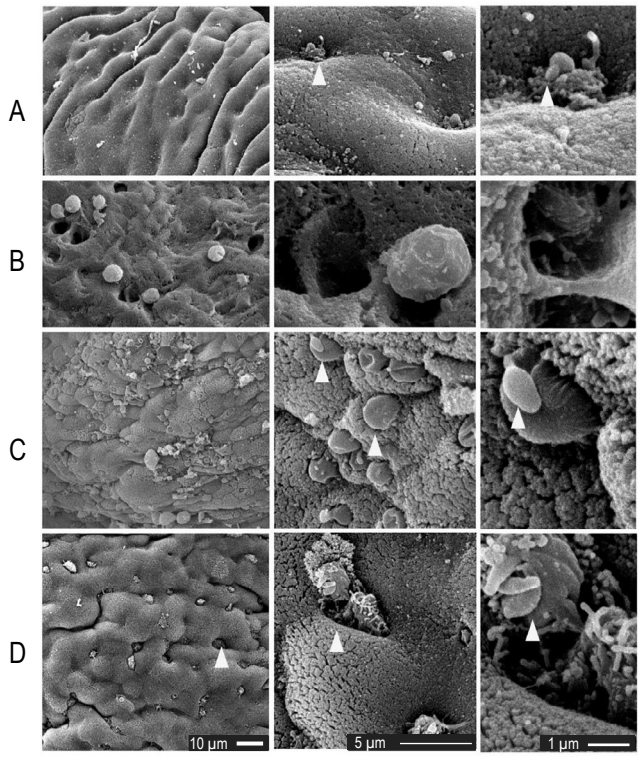

Figure 5. Effects of antibiotics and their substitutes on M cells in the intestinal epithelium of nursery pigs in scanning electron microscopy. Row A – control, B – antibiotics (50% gentamicin, 0.19 kg/t; 10% clomiphene, 0.42 kg/t, 10% bacitracin zinc, 0.44 kg/t); C – glucose oxidase (GOD); D – probiotics (*Lactobacillus*, 3.0 × 10^9 CFU/g, *Bacillus subtilis* 1.4 × 10^{10} CFU/g, *B. licheniformis* 1.3 × 10^{10} CFU/g) + GOD. Triangle indicates M cells. Scale bar: 10 µm for the left 1st column, 5 µm for the left 2nd column, 1 µm for the right columns

enhanced phagocytosis, with bacterial-like particles observed attached to the protrusions of M cells (Figure 5C). Similarly, the combined treatment with GOD and probiotics promoted the development of these fold-like structures on M cells compared to the morphology of these cells in the group fed the normal diet. Additionally, particles captured on the protrusion of M cells were also observed.

Transmission electron microscopy showed that normal M cells completely lacked microvilli or had sparse, disorganized microvilli (Figure 6A, C,E). The basal plasma membrane was deeply invaginated, forming a large sac-like structure, known as the M-cell pocket, where dendritic cells and/or lymphocytes could enter and reside. M cells possessed tight junctions and desmosomes that contacted adjacent columnar cells and lymphocytes (Figure 6D, 7E,F, 8B, G, white arrow). In the cytoplasm, phagocytic endosomes and secondary lysosomes were observed (Figure 6E,F). Treatment with antibiotics caused vacuolisation of the organelles and degradation of the microvilli, as well as fragmentation rendering the subcellular structure unrecognisable (Figure 6G, H).

GOD supplementation altered the morphology of M cells in the small intestine, causing irregular elevation of microvilli on the apical surface of M cells, enhancing their capacity for capture and phagocytosis (Figure 7C,D). In addition, cell fragments were found in the deeper cytoplasm (Figure 7G), while more phagocytic endosomes and secondary lysosomes were visible in the superficial cytoplasm (Figure 6E,F). Many neighbouring M cells were observed to have gap junctions between them (Figure 7E, F). Interestingly, the combined application of GOD and probiotics stimulated lipid mobilisation, resulting in the accumulation of lipid droplets in M cells and adjacent epithelial cells (Figure 8). Large phagocytic fusion vesicles were found in the superficial cytoplasm of M cells (Figure 8F, H), and endosomes were present in the deeper cytoplasm (Figure 8D).

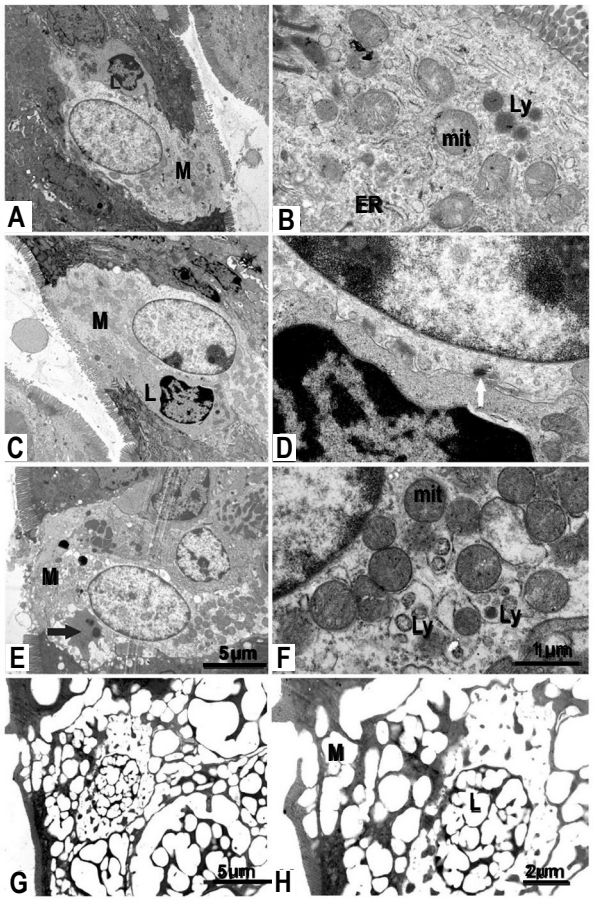

Figure 6. Ultramicrostructure of M cells in the intestinal epithelium of nursery pigs in transmission electron microscopy. Row A–F – control; G–H – antibiotic (50% gentamicin, 0.19 kg/t; 10% clomiphene, 0.42 kg/t; 10% bacitracin zinc, 0.44 kg/t). White arrow indicates desmosome between lymphocytes and M cells, and black arrow indicates endocytosed cell fragment. Scale bar: 5 μm for the left column, 1 μm for the right columns beside 7-H (2 μm).

M – M cell, Ly – lysosome, mit – mitochondria, ER – endoplasmic reticulum, L – lymphocyte

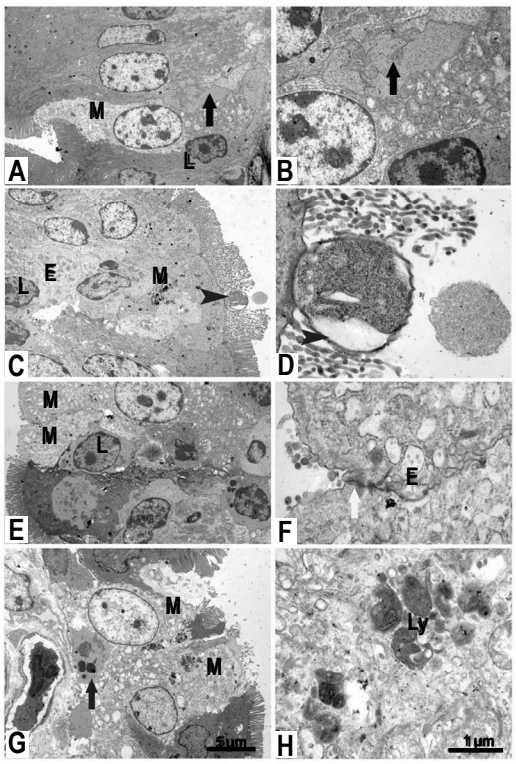

Figure 7. Effects of glucose oxidase (GOD) on M cells in the intestinal epithelial cells of nursery pigs in transmission electron microscopy. Row A–H – GOD. The white arrow indicates the gap junction between M cells; black arrow indicates endocytosed cell fragment, and arrowhead indicates particle to be phagocytosed. Scale bar: 5 µm for the left column, 1 µm for the right columns

M – M cells, Ly – lysosome, E – endosome, L – lymphocyte

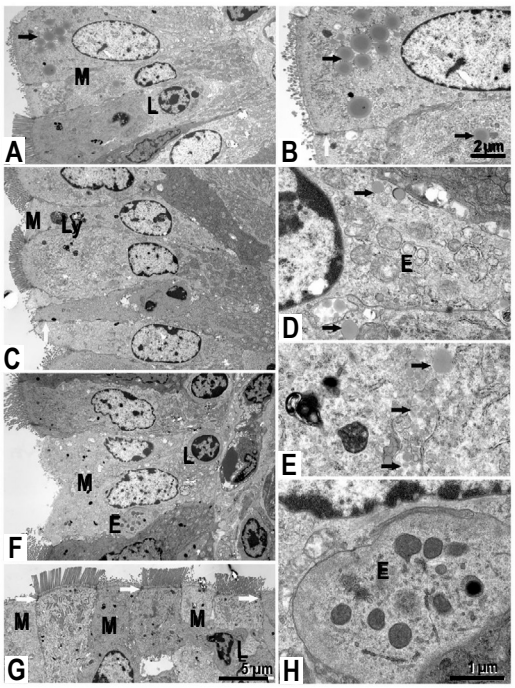

Figure 8. Effects of the combination of probiotics and glucose oxidase (GOD) on M cells in the intestinal epithelial cells of nursery pigs in transmission electron microscopy. Row A–H – probiotics (*Lactobacillus*, 3.0×10^9 CFU/g, *Bacillus subtilis* 1.4×10^{10} CFU/g, *B. licheniformis* 1.3×10^{10} CFU/g) + GOD. White arrow indicates gap junction; black arrow indicates lipid droplet. Scale bar: 5 µm for the left column, 1 µm for the right columns beside part B (2 µm)

M – M cells, Ly – lysosome, E – endosome, L – lymphocyte

Discussion

The prohibition of antibiotics in animal feed has prompted the need for alternative feeding strategies to support gut health and development in weaned pigs, ensuring the long-term sustainability of the pig industry. This study carried out a comprehensive examination of the ultra-microstructure of intestinal villi and M cells using electron microscopy. The findings showed that antibiotics in feed severely impacted the morphology of these structures, while probiotics and GOD, used as antibiotic substitutes, improved the microflora and the condition of microvilli in nursery pigs.

It is well known that antibiotics in feed improve health and economic benefits while disrupting the gastrointestinal microbiota (Becattini et al., 2016). This work provides visual ultrastructural evidence of the effects of antibiotics on intestinal microvilli and microflora using electron microscopy. Earlier research has shown that feed additives affect the morphology of intestinal villi at the level of optical microscopy, affecting parameters such as the length and width of the villi and crypt depth (Chen et al., 2019; González et al., 2024; Yu et al., 2024). In the present experiment, the administration of antibiotics clearly resulted in damage to microvilli and markedly reduced the number of microorganisms in the gastrointestinal tract. The observed damage to microvilli was likely associated with mucosal inflammation induced by antibiotics (Knoop et al., 2016; 2017). Interestingly, even in the control group, bacilli were infrequently found on the folds and surfaces of the intestinal villi. The addition of probiotics significantly increased the abundance of microflora in the small intestine. This suggests that the prolonged use of antibiotics in feed on pig farms adversely affects the microbiota in the gastrointestinal tract, which deserves further attention. The current findings also show that probiotics could be applied as viable alternatives to antibiotics in feed.

In this study, GOD treatment enhanced the growth of pigs, which was consistent with previous reports in broiler chickens (Meng et al., 2021; Hoque et al., 2022). Although treatment with GOD promoted pig growth, it is important to note that the by-product of GOD, hydrogen peroxide, may also induce injury to intestinal microvilli. GOD specifically catalyses the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide, the latter of which has been shown to cause intestinal mucosal damage in a closed circulating intestinal loop (Kohen et al., 1992; Wang et al., 2022a). This underscores the significance of selecting appropriate additive dosage in promoting pig health. The combined use of probiotics and GOD effectively improved the repair of microvillus damage, indicating that probiotics are advantageous for maintaining intestinal health in pigs. Supporting this hypothesis, *B. subtilis*-based probiotic supplementation has been shown to enhance gut barrier integrity through increased tight junction gene expression in broilers (Gadde et al., 2017). It is worth noting that directly administered GOD altered the microfold morphology of M cells in the intestinal tract, resulting in elevated and elongated papillary structures on the apical membrane and the presence of gap junctions between adjacent M cells. Moreover, Wang et al. (2018) also demonstrated that dietary treatment with GOD positively affected the expression of intestinal tight junction genes in broilers. This result indicates that direct feeding of GOD can enhance the phagocytic function of M cells, though the underlying mechanisms need to be further identified.

Interestingly, numerous lipid droplets were observed in both M and intestinal epithelial cells in the probiotic-fed group. This raises intriguing questions about why this many lipid droplets formed in these cells after probiotic treatment and what roles they play in cellular function. Currently, these questions remain unresolved. Existing data indicate that lipid droplets are organelles involved in lipid metabolism and cell signalling (Cruz et al., 2020). We speculate that they may be involved in the mobilisation of lipids or in immunomodulation. Supporting this idea, Aghaei et al. (2023) found that exercise training and probiotic supplementation in rats with hepatic steatosis increased the content of lipids and lipid droplets in rat hepatocytes, which in turn increased the body's antioxidant capacity and alleviated liver damage.

Conclusions

In summary, the present results show that excessive use of antibiotics has a detrimental impact on the development and health of the small intestine. However, the application of probiotics and glucose oxidase (GOD) can serve as effective alternatives to antibiotics, improving the growth and resistance of nursery pigs. Moreover, probiotics significantly contribute to intestinal health.

Acknowledgements

This work was supported by the Key Research and Development Plan of Shandong Province (2023TZXD041), and the Shandong Province Pig Industry Technology System (SDAIT-08-04).

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Aghaei F., Arabzadeh E., Mahmoodzadeh Hosseini H., Shirvani H., 2023. Exercise training and probiotic *Lacticaseibacillus rhamnosus* GG reduce tetracycline-induced liver oxidative stress and inflammation in rats with hepatic steatosis. Probiotics Antimicrob. Proteins 15, 1393–1405, https://doi.org/10.1007/s12602-022-09994-6

Bauer J.A., Zámocká M., Majtán J., Bauerová-Hlinková V., 2022. Glucose oxidase, an enzyme "Ferrari": Its structure, function, production and properties in the light of various industrial and biotechnological applications. Biomolecules 12, 472, https://doi.org/10.3390/biom12030472

Becattini S., Taur Y., Pamer E.G., 2016. Antibiotic-induced changes in the intestinal microbiota and disease. Trends Mol. Med. 22, 458–478, https://doi.org/10.1016/j.molmed.2016.04.003

Chen J., Kang B., Yao K., Fu C., Zhao Y., 2019. Effects of dietary *Macleaya cordata* extract on growth performance, immune responses, antioxidant capacity, and intestinal development in weaned piglets. J. Appl. Anim. Res. 47, 349–356, https://doi.org/10.1080/09712119.2019.1636800

Corr S.C., Gahan C.C., Hill C., 2008. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol. Med. Microbiol. 52, 2–12, https://doi.org/10.1111/j.1574-695X.2007.00359.x

Cromwell G.L., 2002. Why and how antibiotics are used in swine production. Anim. Biotechnol. 13, 7–27, https://doi.org/10.1081/ABIO-120005767

Cruz A.G., Castro W.F., Faria J.A., Bogusz Jr S., Granato D., Celeguini R.M., Lima-Pallone J., Godoy H.T., 2012. Glucose oxidase: A potential option to decrease the oxidative stress in stirred probiotic yogurt. LWT-Food Sci. Technol. 47, 512–515, https://doi.org/10.1016/j.lwt.2012.01.037

Cruz A.L.S., Barreto E.A., Fazolini N.P.B., Viola J.P.B., Bozza P.T., 2020. Lipid droplets: platforms with multiple functions in cancer hallmarks. Cell Death Dis. 6, 105, https://doi.org/10.1038/s41419-020-2297-3

Dang X., Liu Y., Chen N., Kim I.H., 2022. Dietary supplementation of A*spergillus niger*-expressed glucose oxidase ameliorates weaning stress and improves growth performance in weaning pigs. J. Anim. Physiol. Anim. Nutr. 106, 258–265, https://doi.org/10.1111/jpn.13576

Gadde U., Oh S.T., Lee Y.S., Davis E., Zimmerman N., Rehberger T., Lillehoj H.S., 2017. The effects of direct-fed microbial supplementation, as an alternative to antibiotics, on growth performance, intestinal immune status, and epithelial barrier gene expression in broiler chickens. Probiotics Antimicrob. Proteins 9, 397–405, https://doi.org/10.1007/s12602-017-9275-9

González F., Cervantes M., Morales A., Valle J.A., Camacho R.L., Morales-Becerra A., Bernal H., Mendoza S.M., Htoo J.K., 2024. Effect of supplementing a *Bacillus subtili*s-based probiotic on performance, intestinal integrity, and serum antioxidant capacity and metabolites concentrations of heat-stressed growing pigs. J. Anim. Sci. 102, skae012, https://doi.org/10.1093/jas/skae012

Heo J.M., Opapeju F.O., Pluske J.R., Kim J.C., Hampson D.J., Nyachoti C.M., 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J. Anim. Physiol. Anim. Nutr. 97, 207–237, https://doi.org/10.1111/j.1439-0396.2012.01284.x

Honarpisheh P., Banerjee A., Ko K.A., Honarpisheh P., Venna V.R., McCullough L., Lee J., 2022. Stroke-induced gut microbiota dysbiosis regulates microfold cells in Peyer's patches. Stroke 53, A83–A83, https://doi.org/10.1161/str.53.suppl_1.83

Hoque M.R., Chen N., Liu Y., Kim I.H., 2021. Possibility of using glucose oxidase in the diet to improve selected indicators of blood antioxidant defense, digestibility and growth performance of broiler chicken. Ital. J. Anim. Sci. 21, 455–462, https://doi.org/10.1080/1828051X.2021.2024457

Kapat A., Jung J., Park Y., 1998. Improvement of extracellular recombinant glucose oxidase production in fed-batch culture of Saccharomyces cerevisiae: Effect of different feeding strategies. Biotechnol. Lett. 20, 319–323, https://doi.org/10.1023/A:1005354608653

Kimura S., 2018. Molecular insights into the mechanisms of M-cell differentiation and transcytosis in the mucosa-associated lymphoid tissues. Anat. Sci. Int. 93, 23–34, https://doi.org/10.1007/s12565-017-0418-6

Knoop K., Gustafsson J.K., McDonald K.G., Kulkarni D.H., Kassel R., Newberry R.D., 2017. Antibiotics promote the sampling of luminal antigens and bacteria via colonic goblet cell associated antigen passages. Gut Microbes 8, 400–411, https://doi.org/10.1080/19490976.2017.1299846

Knoop K.A., McDonald K.G., Kulkarni D.H., Newberry R.D., 2016. Antibiotics promote inflammation through the translocation of native commensal colonic bacteria. Gut. 65, 1100–1109, https://doi.org/10.1136/gutjnl-2014-309059

Kohen R., Kakunda A., Rubinstein A., 1992. The role of cationized catalase and cationized glucose oxidase in mucosal oxidative damage induced in the rat jejunum. J. Biol. Chem. 267, 21349–21354, https://doi.org/10.1016/S00219258(19)36616-5

Méndez-Palacios N., Méndez-Mendoza M., Vázquez-Flores F., Castro-Colombres J.G., Ramírez-Bribiesca J.E., 2018. Productive and economic parameters of pigs supplemented from weaning to finishing with prebiotic and probiotic feed additives. Anim. Sci. J. 89, 994–1001, https://doi.org/10.1111/asj.13008

Meng Y., Huo H., Zhang Y., Bai S., Wang R., Zhang K., Ding X., Wang J., Zeng Q., Peng H., Xuan Y., 2021. Effects of dietary glucose oxidase supplementation on the performance, apparent ileal amino acids digestibility, and ileal microbiota of broiler chickens. Animals 11, 2909, https://doi.org/10.3390/ani11102909

Pabst R., Geist M., Rothkötter H.J., Fritz F.J., 1988. Postnatal development and lymphocyte production of jejunal and ileal Peyer's patches in normal and gnotobiotic pigs. Immunology 64, 539–544

Pearlin B.V., Muthuvel S., Govidasamy P., Villavan M., Alagawany M., Ragab Farag M., Dhama K., Gopi M., 2020. Role of acidifiers in livestock nutrition and health: A review. J. Anim. Physiol. Anim. Nutr. 104, https://doi.org/10.1111/jpn.13282

Salim H.M., Kang H.K., Akter N., Kim D.W., Kim J.H., Kim M.J., Na J.C., Jong H.B., Choi H.C., Suh O.S., Kim W.K., 2013. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poult. Sci. 92, 2084–2090, https://doi.org/10.3382/ps.2012-02947

Tang H., Yao B., Gao X., Yang P., Wang Z., Zhang G., 2016. Effects of glucose oxidase on the growth performance, serum parameters and faecal microflora of piglets. South Afr. J. Anim. Sci. 46, 14–20, https://doi.org/10.4314/sajas.v46i1.2

Thacker P.A., 2013. Alternatives to antibiotics as growth promoters for use in swine production: A review. J. Anim. Sci. Biotechnol. 14, 35, https://doi.org/10.1186/2049-1891-4-35

Thormar H., 2012. Patented non-antibiotic agents as animal feed additives. Recent Pat. Food Nutr. Agric. 4, 155–168, https://doi.org/10.2174/2212798411204020155

Wang C., Yang J., Dong C., Shi S., 2020. Glucose oxidase-related cancer therapies. Adv. Therap. 3, 2000110, https://doi.org/10.1002/adtp.202000110

Wang F., Chen X., Wang Y., Li X., Wan M., Zhang G., Leng F., Zhang H., 2022a. Insights into the structures, inhibitors, and improvement strategies of glucose oxidase. Int. J. Mol. Sci. 23, 9841, https://doi.org/10.3390/ijms23179841

Wang Y., Wang B., Zhan X., Wang Y., Li W., 2022b. Effects of glucose oxidase and its combination with B. amyloliquefaciens SC06 on intestinal microbiota, immune response and antioxidative capacity in broilers. Animal 16, 100473, https://doi.org/10.1016/j.animal.2022.100473

Wang Y., Wang Y., Xu H., Mei X., Gong L., Wang B., Li W., Jiang S., 2018. Direct-fed glucose oxidase and its combination with *B. amyloliquefaciens* SC06 on growth performance, meat quality, intestinal barrier, antioxidative status, and immunity of yellow-feathered broilers. Poult. Sci. 97, 3540–3549, https://doi.org/10.3382/ps/pey216

Wu S., Chen X., Li T., Ren H., Zheng L., Yang X., 2020. Changes in the gut microbiota mediate the differential regulatory effects of two glucose oxidases produced by *Aspergillus niger* and *Penicillium amagasakiense* on the meat quality and growth performance of broilers. J. Anim. Sci. Biotechnol. 11, 73, https://doi.org/10.1186/s40104-020-00480-z

Wu S., Li T., Niu H., Zhu Y., Liu Y., Duan Y., Sun Q., Yang X., 2019. Effects of glucose oxidase on growth performance, gut function, and cecal microbiota of broiler chickens. Poult. Sci. 98, 828–841, https://doi.org/10.3382/ps/pey393

Xu Y., Yu Y., Shen Y., Li Q., Lan J., Wu Y., Zhang R., Cao G., Yang C., 2021. Effects of *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, immunity, short chain fatty acid production, antioxidant capacity, and cecal microflora in broilers. Poult. Sci. 100, 101358, https://doi.org/10.1016/j.psj.2021.101358

Yu S.J., Morris A., Kayal A., Milošević I., Van T.T.H., Bajagai Y.S., Stanley D., 2024. Pioneering gut health improvements in piglets with phytogenic feed additives. Appl. Microbiol. Biotechnol. 108, 142, https://doi.org/10.1007/s00253-023-12925-2