

Effects of *Saccharomyces cerevisiae* fermentation derived postbiotic supplementation in sow and piglet diet on the fecal *Escherichia coli* counts and antimicrobial resistance in suckling piglets under intensive production system

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ABSTRACT

This study evaluated the effects of *S. cerevisiae* fermentation derived postbiotic (XPC) on fecal *E. coli* counts and antimicrobial resistant (AMR) in piglets with a high-biosecurity system. Thirty sows were divided into three groups: a standard basal diet (CON), CON with 1.0 kg/MT of Beta-glucan 50% (BG), CON with 2.0 kg/MT of XPC (XPC). These diets were administered to sows from conception until weaning of the piglets, and to their piglets from 7 days old until weaning. Fecal samples were collected from piglets at 7, 14, and 21 days old for enumeration of *E. coli*. The disk diffusion and PCR methods were used to test for AMR and detect antimicrobial resistance genes (ARGs) in the isolates. Results showed XPC supplement significantly reduced *E. coli* counts (log₁₀ CFU/g) than the CON group ($p = 0.001$). XPC decreased the frequency of *E. coli* isolates resistance to ampicillin, erythromycin, and oxytetracycline ($p < 0.05$), while BG reduced resistance to cefotaxime, and gentamicin ($p < 0.05$). Overall, dietary XPC supplementation in sows and piglets reduced *E. coli* counts in suckling piglets. Additionally, the dietary XPC and BG-50 supplementation was affected on the level of AMR in *E. coli*.

Keywords: Antimicrobial resistant, *E. coli*, Piglet, Sow, XPC

INTRODUCTION

Over the past 50 years, improving livestock production efficiency and reducing costs have been key priorities for nutritional scientists, microbiologists, and biochemists. To achieve these goals, livestock feed has been supplemented with antimicrobials (AMs), probiotics, prebiotics, and postbiotics. However, over the last two decades, the use of AMs in animal production has faced growing opposition due to concerns

about bacterial resistance, adverse effects on animal and human health, and food safety risks (Benavides *et al.*, 2024). Regulatory actions reflect this shift. The European Union banned AM use in livestock in 2006 (Official Journal of the European Union, 2006). In the United States, AMs are prohibited as feed additives for growth promotion or feed conversion efficiency (FDA, 2015). Instead, the FDA's Guidance for Industry #209 restricts AM use in livestock to therapeutic purposes only (FDA, 2012). Similarly, several

Asian countries, including Thailand, Indonesia, and Korea, have imposed restrictions. Vietnam, for example, issued Decree 13/2020/ND-CP, banning AM use for disease prevention in mature terrestrial animals as of March 2020, with plans for a complete ban on prophylactic AM use in young terrestrial animals by 2026 (Vietnamese Government, 2020).

Probiotics, prebiotics, and postbiotics each play distinct roles in supporting gut health in both animals and humans, but they are not interchangeable. Probiotics are live microorganisms that confer health benefits when consumed in sufficient quantities (Ma *et al.*, 2023). Prebiotics are dietary fibers that serve as nourishment for these beneficial microbes, promoting their growth and activity (Manzoor *et al.*, 2022). Postbiotics, the bioactive compounds produced by probiotics during fermentation, also contribute to gut health (Salminen *et al.*, 2021). Among the three, postbiotics represent the most recent area of scientific exploration (Ji *et al.*, 2023). Postbiotics, such as the *Saccharomyces cerevisiae* (*S. cerevisiae*) fermentation derived postbiotic XPC™ (XPC) have shown potential in mitigating enteric pathogens across multiple livestock species (Feye *et al.*, 2016). XPC is produced through a proprietary anaerobic fermentation process, resulting in a complex blend of bioactive compounds, including amino acids, organic acids, polyphenols, lipids, B vitamins, residual yeast cells, fermentation media, yeast cell wall components, and antioxidants. Recent and ongoing studies suggest that XPC and its derivatives are viable interventions in food animal production. They have been evaluated as feed supplements to enhance feed utilization and digestibility, reduce pathogen load, and improve animal performance and health (Danladi *et al.*, 2022). Additionally, XPC has demonstrated positive effects in both ruminants and non-ruminants, helping to mitigate the impact of environmental stressors on animal health (Ogbuewu *et al.*, 2019). Recent research also suggests that XPC may offer protective effects against other pathogens. Catherine *et al.* (2022) reported consistently lower lesion scores and variably reduced *E. coli* tissue loads in birds fed Original XPC when challenged with avian pathogenic *E. coli* O78 via oral or intratracheal routes. Additionally, pigs challenged with *E. coli* K88 and fed XPC-

enriched diets exhibited higher serum tumor necrosis factor-alpha levels, reduced diarrhea, increased appetite, and decreased *E. coli* adherence to the intestinal mucosa (Kiarie *et al.*, 2012). Most research has focused on postweaning and growth pigs' dietary manipulation. However, maternal dietary intervention presents a promising alternative. Supplementing a sow's diet during gestation and lactation can enhance gut development and health in offspring before weaning, leading to improved health and performance in the postweaning phase and beyond.

The pig production system has undergone significant transformation in recent years, shifting from small-scale farms to large-scale operations. In Vietnam, for example, the proportion of smallholder farming facilities has declined by 15–20% over the past five years. Currently, small-scale household pig production accounts for only 35–40%, while industrial pig production comprises 60–65% (Bui *et al.*, 2024). This shift is driven by rising animal feed costs, unpredictable epidemics, and fluctuating livestock prices. To control diseases in pigs, various strategies have been developed, including biosecurity measures and vaccination. Implementing biosecurity throughout the production chain minimizes the introduction and spread of pathogens on farms. Industrial pig producers, in particular, adopt stringent biosecurity measures to reduce disease risks.

Previous studies prompted further investigations into the anti-*E. coli* and *Salmonella* effects of XPC. Therefore, this study aimed to evaluate the impact of *Saccharomyces cerevisiae* fermentation derived postbiotic (XPC) in sow and piglet on *E. coli* counts and antimicrobial resistant (AMR) in feces of sucking piglets within a high-biosecurity intensive production system. It is anticipated that supplementing sows and piglets with XPC in such systems may reduce the prevalence of pathogenic and AMR bacteria. This approach presents a promising alternative to antimicrobials, promoting animal health and supporting sustainable livestock production

MATERIALS AND METHODS

Animal Ethics

The procedure of this study was reviewed and approved (Protocol #HUVN0034) by the

Table 1. PCR Primer for Amplification of *E. coli* Virulence Genes

Gene	Primers	Oligonucleotide sequence (5'-3')	Fragment size (bp)	Annealing temp. (°C)
<i>stx</i> ₁	VT1-A	CGCTGAATGTCATTCGCTCTGC	302	55
	VT1-B	CGTGGTATAGCTACTGTCACC		
<i>stx</i> ₂	VT2-A	CTTCGGTATCCTATTCCCGG	516	55
	VT2-B	CTGCTGTGACAGTGACAAAACGC		
<i>eae</i>	EAE-1	GGAACGGCAGAGGTTAATCTGCAG	346	62
	EAE-2	GGCGCTCATCATAGTCTTTC		

Animal Ethics Committee, Hue University.

Animal Information, Housing and Experimental Design

This experiment involved 30 sows (parity 2 to 7) and their piglets from 1-day old to weaning. The sows, Pietrain × (Landrace × Yorkshire), were inseminated with Duroc semen to produce progeny for pork production. Each sow was housed individually and identified using cage-attached tags. The study was conducted on a closed farm with temperature and humidity control. According to the Food and Agriculture Organization (FAO) classification, the farm operates as a sector 2 production system, characterized by medium-scale commercial production, moderate to high biosecurity levels, intensive indoor husbandry, and no contact with other domestic or wild animals. The facility has dedicated animal husbandry experts and veterinarians. Sows were randomly assigned to three groups (10 sows per group). The control group (CON) received a standard basal diet without supplementation. The second group (XPC) received the basal diet supplemented with 2.0 kg/MT of Diamond V Original XPC®. The third group (BG) received the basal diet supplemented with 1.0 kg/MT of Beta-glucan 50%. These diets were provided to sows from being inseminated until weaning of the piglets, and to their piglets from 7 days old until weaning.

Feces Collection for Microbial Composition Enumeration

Fecal samples were collected from the rectum of all piglets in each sow's litter (10 sows per group) at 7, 14, and 21 days old. Fecal sam-

ples of all piglets in each litter were pooled for *E. coli* enumeration and isolation.

E. coli isolation and quantification followed the method described by Lupindu (2017). One gram of fecal sample was mixed with 10 mL of 0.1% (w/v) peptone water and homogenized for 2 minutes. A 1 mL portion of the homogenate was used to prepare 10-fold serial dilutions up to 10⁵ in 0.1% (w/v) peptone water. Then, 0.1 mL of each dilution was spread in triplicate on Eosin Methylene Blue (EMB) agar plates (Conda Laboratories, S.A., Spain). The typical dark to purple red colonies with metallic sheen grown on EMB agar. Colonies exhibiting a dark to purple-red color with a metallic sheen were identified as *E. coli*. Plates were incubated at 37 °C for 24 hours, and colony counts were recorded for plates containing 20–250 colonies. Biochemical confirmation of isolates was performed using Gram staining, catalase and oxidase tests, and API 20E strips (Biomérieux, USA) (Hamner *et al.*, 2007). Isolates were further tested using slide agglutination with polyvalent sera targeting specific serogroups (Polyvalent 2: types O26, O55, O111, O119, O126; Polyvalent 3: types O86, O114, O125, O127, O12; Polyvalent 4: types O44, O112, O124, O142) (Thermo Scientific TM, Wade, Hampshire, UK). For each sample, five randomly selected colonies were tested for the presence of *stx*₁, *stx*₂ and *eae* gene using PCR. The base sequences and expected amplicon sizes for the oligonucleotide primers used in this study are presented in Table 1.

Antimicrobial Susceptibility Testing

The disk diffusion method was used to assess the antibiotic susceptibility of the *E. coli*

Table 2. PCR Primers for Amplification of Antimicrobial Resistance Genes of *E. coli*

PCR	Gene	Primers	Oligonucleotide Sequence (5'-3')	Fragment Size (bp)	Annealing Temp. (°C)
1	<i>sul1</i>	Sul1-F	CGGCGTGGGCTACCTGAACG	433	66
		Sul1-R	GCCGATCGCGTGAAGTTCCG		
	<i>sul2</i>	Sul2-F	CGGCATCGTCAACATAACCT	721	
		Sul2-R	TGTGCGGATGAAGTCAGCTC		
	<i>sul3</i>	Sul3-F	CAACGGAAGTGGGCGTTGTGGA	244	
		Sul3-R	GCTGCACCAATTCGCTGAACG		
2	<i>tet(A)</i>	tetA-F	GGCGGTCTTCTTCATCATGC	502	63
		tetA-R	CGGCAGGCAGAGCAAGTAGA		
	<i>tet(B)</i>	tetB-F	CGCCAGTGCTGTTGTTGTC	173	
		tetB-R	CGCGTTGAGAAGCTGAGGTG		
	<i>tet(C)</i>	tetC-F	GCTGTAGGCATAGGCTTGGT	888	
		Tet-R	GCCGGAAGCGAGAAGAATCA		
3	<i>aadA</i>	aadA-F	GTGGATGGCGGCCTGAAGCC	525	63
		aadA-R	AATGCCCAGTCGGCAGCG		
	<i>strA/strB</i>	strA-F	ATGGTGGACCCTAAAACCTCT	893	
		strB-R	CGTCTAGGATCGAGACAAAG		
	<i>aac(3)IV</i>	aac(3)IV-F	TGCTGGTCCACAGCTCCTTC	653	
		aac(3)IV-R	CGGATGCAGGAAGATCAA		
4	<i>aadB</i>	aadB-F	GAGGAGTTGGACTATGGATT	208	55
		aadB-R	CTTCATCGGCATAGTAAAAG		
	<i>aphA1</i>	aphA1-F	ATGGGCTCGCGATAATGTC	600	
		aphA1-R	CTCACCGAGGCAGTTCCAT		
	<i>aphA2</i>	aphA2-F	GATTGAACAAGATGGATTGC	347	
		aphA2-R	CCATGATGGATACTTTCTCG		
5	<i>bla_{TEM}</i>	bla _{TEM} -F	TTAACTGGCGAACTACTTAC	247	55
		bla _{TEM} -R	GTCTATTTTCGTTTCATCCATA		
	<i>bla_{SHV}</i>	bla _{SHV} -F	AGGATTGACTGCCTTTTTG	393	
		bla _{SHV} -R	ATTTGCTGATTTTCGCTCG		
	<i>bla_{CMY-2}</i>	bla _{CMY-2} -F	GACAGCCTCTTTCTCCACA	1000	
		bla _{CMY-2} -R	TGGACACGAAGGCTACGTA		

isolates on Mueller-Hinton agar (Oxoid, UK), following the guidelines of the Clinical and Laboratory Standards Institute (2020). The following AMs were tested: Ampicillin (AMP, 10 µg); cefotaxime (CTX, 30 µg); cephalexin (CFL, 30 µg); chloramphenicol (CHL, 30 µg); ciprofloxacin (CIP, 5 µg); colistin (CL, 10 µg); doxycycline (DO, 30 µg); erythromycin (ERY, 15 µg); nalidixic acid (NA, 30 µg); oxacillin (OX, 1µg); oxytetracycline (OTC, 30 µg); gentamicin (GEN, 10 µg); streptomycin (STR, 10 µg); trimethoprim-sulfamethoxazole (SXT, 25 µg); tetracycline (TET, 30 µg) (Thermo Scientific TM, Wade, Hampshire, UK). Results were interpreted according to Clinical and Laboratory Standards Institute (2020) guidelines.

Detection Antimicrobial Resistance Genes

Five *E. coli* colonies per sample were sub-cultured overnight in Brain Heart Infusion broth (BHI, Sigma, St. Louis, MO, USA). Genomic DNA was then extracted using a QIAamp DNA Stool mini kit (Qiagen, Germany) following the manufacturer's instructions for antimicrobial resistance gene (ARG) detection. For ARGs screening, multiplex PCR was used to detect the following genes, as described by Kozak *et al.* (2009): β-lactamase genes *blaTEM*, *blaSHV*, and *blaCMY-2*; the major genes for resistance to streptomycin (*strA/strB* and *aadA*); kanamycin and neomycin (*aphA1* and *aphA2*); kanamycin and gentamicin (*aadB*); apramycin, gentamycin and tobramycin [*aac(3)IV*]; sulfonamides (*sul1*, *sul2*, and *sul3*); and tetracycline [*tet(A)*, *tet(B)*, and *tet(C)*]. The predicted amplicon for the specific oligonucleotide primers used in this study are listed in Table 2.

Statistical Analyses

All data were managed in Microsoft Excel 2016 (MSO, 16.0.4266.1001) and analyzed with IBM SPSS Statistics version 18.0 (IBM, Armonk, NY, USA). Each sow and their piglets served as an experimental unit. Difference in the prevalence of virulence genes, polyvalent serotypes, AMR, and ARG between the control and treatment groups were assessed using the Chi-square test. To evaluate the effect of feeding XPC or BG on *E. coli* counts and inhibition zone diameter, a statistical model was used that accounted for treatment effects, piglet age, the in-

teraction between treatment and age, and the error term. Since *E. coli* counts were not normally distributed, they were log₁₀ transformed before the analysis of variance was applied. Statistically significant was set at p-value lower than 0.05.

RESULTS AND DISCUSSION

Effects of Feeding XPC on *E. coli* Counts in the Feces and their Characteristics

The effects of feeding XPC on *E. coli* counts in the feces of piglets is presented in Table 3. There was a significant difference in *E. coli* counts between the CON and treatment groups ($p = 0.0001$). At 21 days of age, the *E. coli* counts in the XPC group (8.33 log₁₀ CFU/g) were lower than those in the BG (8.92 log₁₀ CFU/g) and CON (9.26 log₁₀ CFU/g) groups ($p < 0.05$); however, no significant difference was observed between the CON and BG groups ($p > 0.05$). The changes in *E. coli* counts were not affected by the age of the piglets ($p = 0.07$) or the interaction between age and treatment ($p = 0.75$). Intestinal microbes play a crucial role in animal health, contributing to nutrient metabolism, intestinal barrier maintenance, immune regulation, and pathogen resistance. Piglets acquire their intestinal microbes through contact with the sow's birth canal, skin, feces, and environmental microbes, and milk (Lim *et al.*, 2023). From birth to weaning, piglets are highly susceptible to gut colonization by pathogenic bacteria such as *Salmonella*, *E. coli*, and *Clostridium perfringens*, as well as parasites, and viruses. These pathogens can cause diarrhea and reduce body weight gain (Tang *et al.*, 2022). Postbiotics such as XPC, *Lactobacillus* postbiotics are commonly recommended as feed additives for sows, growing pigs, with extensive research demonstrating their effectiveness in reducing pathogenic bacterial counts and improving fecal microbiota composition. The addition of yeast cultures to sows may have a direct effect on breast milk as well as fecal microorganisms, and piglets' intestinal microorganisms may also be affected through exposure to breast milk and sow feces. According to a report by (Chen *et al.*, 2022), supplementing diets with XPC significantly increased levels of short-chain fatty acids (SCFAs), particularly propionate, potentially enhancing intestinal fermentation and promoting gut health. SCFAs are key

metabolites produced by the gut microbiota and play crucial roles in maintaining host health (Chen *et al.*, 2022). The predominant SCFAs such as acetate, propionate, and butyrate are derived from different microbial groups: acetate mainly from Bacteroidetes and Bifidobacteria; propionate from Bacteroidetes, Firmicutes, Bifidobacteria, and Salmonella; and butyrate from Firmicutes (Godínez-Méndez *et al.*, 2021). The beneficial effects of XPC appear to stem from its ability to modulate the gut microbiota and increase SCFA production (Lei *et al.*, 2024). Moreover, Xu *et al.* (2025) demonstrated that SCFAs help protect against high energy induced follicular atresia by promoting colonic serotonin and melatonin synthesis in sows. This study highlights the effects of feeding XPC to sows and suckling piglets in high-biosecurity production systems, presenting a promising alternative to AMs. Notably, XPC had the greatest effect on reducing pathogenic and antimicrobial-resistant (AMR) bacteria. Compared to the control (CON) group, the XPC-fed piglets exhibited lower *E. coli* counts, suggesting that XPC can stabilize the hindgut microbiota and reduce its variability. These findings align with previous research showing decreased *E. coli* counts and lower *Salmonella* prevalence in pigs and poultry (Catherine *et al.*, 2022) fed XPC. The inclusion of SCFP in pig diets has been reported to improve gut barrier function, reduce the prevalence of diarrhea, and enhance overall growth performance (Santiago *et al.*, 2023; Shen *et al.*, 2011). Pathogen infection activates the immune system, and excessive immune responses, particularly chronic inflammation, can be detrimental (Cardoso Dal Pont *et al.*, 2020). Various studies

reported that XPC to alleviate ETEC-induced systemic and mucosal inflammation to varying degrees by downregulating the TLR4–MyD88–NF-κB signaling pathway, thereby improving the health and productivity of pigs under disease conditions (Carpinelli *et al.*, 2021; Yan *et al.*, 2022). Components of XPC, such as β-glucans and mannan oligosaccharides (MOS) (Duan *et al.*, 2019), have been reported to inhibit TLR signaling cascades, thereby reducing LPS- or ETEC-induced inflammatory responses in epithelial cells across species (Wang *et al.*, 2016; Zhu *et al.*, 2013). Thus, the anti-inflammatory effects of SCFP offer an additional mechanism for mitigating ETEC-induced diarrhea and growth retardation. In summary, dietary supplementation with XPC in high-biosecurity production systems effectively reduces *E. coli* counts in piglet feces, supporting its role in promoting gut microbial stability and enhancing animal health.

The distribution of *E. coli* polyvalent serotypes is shown in Table 4. As can be seen, the frequencies of polyvalent-2 serotypes decreased from 7 days old to 14 days old in all groups. At 14 days old, the frequency of polyvalent-2 serotypes in the BG group (12.0%) was lower than these in the CON (32.0%) and XPC (48.0%) groups ($p < 0.05$). However, at 21 days old, the frequencies of polyvalent-2 serotypes from all groups were decreased in comparison with 14 days old ($p < 0.05$). The frequency of polyvalent-2 serotypes from 21 days old in the CON group (42.0%) was higher than that in the XPC group (22.0%), ($p < 0.05$). *E. coli* isolates belonging to specific or polyvalent serotypes do not inherently confer virulence. However, serotyping serves as a valuable epidemiological tool for assessing

Table 3. Effects of Feeding a XPC on *E. coli* Counts (log₁₀ CFU/g, mean ± SD) in the Feces of Piglets (n = 10)

Treatment	7 Days old	14 Days old	21 Days old	⁴ SEM	⁵ p-value		
					T	A	T * A
¹ CON	8.71 ± 0.85	8.78 ± 0.81	9.26* ± 0.60				
² BG	8.55 ± 0.70	8.64 ± 0.57	8.92* ± 0.51	0.13	0.0001	0.07	0.75
³ XPC	8.25 ± 0.18	8.27 ± 0.12	8.33# ± 0.45				

¹CON: Basal control diet; ²BG: Standard basal control diet containing 1.0 kg/MT of Beta-glucan 50%; ³XPC = Standard basal control diet containing 2.0 kg/MT of Diamond V XPC; ⁴SEM = Standard error of the mean, ⁵P-value = The statistical model included effects of T = treatment (XPC or BG), A= age (day old), the interaction between the treatment (T) and age (A), and the error term. *,# indicated the significant difference between treatment in each timepoint.

pathogenicity, as certain serogroups strongly correlate with enteropathogenicity. While numerous *E. coli* serotypes have been identified, only a limited number are associated with enteric infections in piglets, as they are rarely found in strains isolated from the normal gut (Holland, 1990). This study identified three polyvalent serotypes of *E. coli* in piglets, along with O157, while many isolates were non-serotypeable. The World Health Organization (WHO, 1981) identified enteropathogenic *Escherichia coli* (EPEC) as comprising strains from 12 O serogroups, classified into three polyvalent groups: polyvalent 2 (O26, O55, O111, O119, O126), polyvalent 3 (O12, O86, O114, O125, O127), and polyvalent 4 (O44, O112, O124, O142). These serotypes have also been described as pathogenic *E. coli* (Chen *et al.*, 2004). According to Garabal *et al.* (1996), *E. coli* isolates from diarrheic pigs belonged to serotypes O12, O26, O125, and O127, whereas those from healthy pigs were associated with serotypes O44, O55, O86, O111, O112, O114, O119, O124, O126, and O142. Most polyvalent serotype frequencies in the XPC group did not differ significantly from the CON group. However, significant differences were observed

in the frequencies of polyvalent-4 serotypes at 14 days old and polyvalent-2 serotypes at 21 days old (Table 4). Additionally, the frequency of *E. coli* polyvalent-2 serotypes decreased in all groups from 7 to 14 days old. In the XPC group, this decline persisted through 21 days, suggesting a sustained reduction in these serotypes.

The frequencies of virulence genes in the *E. coli* isolates are shown in Table 5. The frequencies of the *stx1* gene in all groups increased from 7 to 14 days of age. At 21 days old, the *stx1* gene was not detected in isolates from the CON and BG groups, while 20.0% of isolates from the XPC group carried the *stx1* gene. The frequencies of the *eae* gene in the CON and XPC groups tended to increase from 7 to 21 days old. At 21 days old, the frequency of the *eae* gene in the XPC group (18.0%) was significantly lower than in the CON (38.0%) and BG (28.0%) groups ($p < 0.05$). Shiga toxin-producing *E. coli* (STEC) refers to *E. coli* strains that produce one or more cytotoxins known as Shiga toxin (Stx). These toxins are classified into two major antigenic forms: Stx1 and Stx2. STEC is a well-known pathogen responsible for diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. Stx be-

Table 4. Effects of Feeding a XPC on the Distribution of *E. coli* Polyvalent Serotypes (%) from Piglets (n: 10 replication * 5 colonies from each = 50)

Time point	Serotypes group	CON ¹	BG ²	XPC ³
7 days old	⁴ Polyvalent-2	54.0*	46.0*	54.0*
	⁵ Polyvalent-3	16.0	30.0* ^s	26.0
	⁶ Polyvalent-4	16.0	8.0	6.0*
	O157	8.0	2.0	0.0
	None-serotypeable	6.0*	14.0	14.0
14 days old	Polyvalent-2	32.0 ^{as}	12.0 ^{bs}	48.0 ^{a*}
	Polyvalent-3	30.0	40.0*	22.0
	Polyvalent-4	8.0 ^a	18.0 ^{ab}	22.0 ^{bs}
	O157	6.0	10.0	0.0
	None-serotypeable	24.0 ^{as}	20.0 ^{ab}	8.0 ^b
21 days old	Polyvalent-2	42.0 ^{a*}	36.0 ^{ab*}	22.0 ^{bs}
	Polyvalent-3	20.0 ^{ab}	14.0 ^{as}	32.0 ^b
	Polyvalent-4	18.0	20.0	24.0 ^s
	O157	0.0	8.0	6.0
	None-serotypeable	20.0 ^s	22.0	16.0

¹CON: Basal control diet; ²BG: Standard basal control diet containing 1.0 kg/MT of Beta-glucan 50%; ³XPC = Standard basal control diet containing 2.0 kg/MT of Diamond V XPC; ⁴*E. coli* Polyvalent 2 = types O26, O55, O111, O119, O126; ⁵*E. coli* Polyvalent 3 = types O86, O114, O125, O127, O12; ⁶*E. coli* Polyvalent 4 = types O44, O112, O124, O142); ^{a, b} indicated the significant difference between treatments in each time point. ^{*}, ^s indicated the significant difference between time points (day old) in each treatment.

Table 5. Effects of Feeding a XPC on the Distribution of Virulence Genes (VGs) in the *E. coli* Isolates from Piglets (n: 10 replication * 5 colonies from each = 50)

Treatments	7 Days old			14 Days old			21 Days old		
	⁴ <i>stx1</i>	<i>stx2</i>	⁵ <i>eae</i>	<i>stx1</i>	<i>stx2</i>	<i>eae</i>	<i>stx1</i>	<i>stx2</i>	<i>eae</i>
CON ¹	12.00*	NA	NA	36.00 ^a ^s	NA	12.00*	NA	2.00	38.00 ^a ^s
BG ²	4.00*	18.00	34.00*	18.00 ^b ^s	NA	12.00 ^s	NA	NA	28.00 ^a *
XPC ³	8.00*	10.00	NA	36.00 ^a ^s	2.00	NA	20.00* ^s	NA	18.00 ^b

¹CON: Basal control diet; ²BG: Standard basal control diet containing 1.0 kg/MT of Beta-glucan 50%; ³XPC = Standard basal control diet containing 2.0 kg/MT of Diamond V XPC; ⁴*stx* = gene encoding to shiga toxins; ⁵*eae* = intimin gene encoding to adherence factor; ⁶NA: Negative; ^{a, b} indicated the significant difference between treatments in each time point. ^{*, s} indicated the significant difference between time points (day old) in each treatment.

longs to a family of cytotoxic proteins with N-glycosidase activity, which facilitates binding to specific receptor molecules on intestinal mucosal cells. Additional virulence factors include intimin, an outer membrane protein encoded by the *eaeA* gene, which plays a key role in *E. coli* attachment to enterocytes and subsequent cell damage. This adhesin function is critical for bacterial colonization and pathogenicity (Parreira and Gyles, 2002). Previous studies have also reported the presence of *stx1*, *stx2*, and *eae* genes in *E. coli* isolates from healthy pigs (Li *et al.*, 2020). Postbiotics, such as XPC, contain bioactive compounds, including bacteriocins, short-chain fatty acids (SCFAs), 2-furancarboxaldehyde, benzene acetaldehyde, ethenone, oligosaccharides, organic acids, and peptides (Kareem *et al.*, 2021). These components inhibit the growth and proliferation of various gut pathogens, particularly Gram-negative bacteria such as *E. coli*, *Salmonella Typhimurium*, *Clostridium perfringens*, *Listeria monocytogenes*, and vancomycin-resistant *Enterococci* (Chang *et al.*, 2021). These factors act directly (e.g., low pH, reduced membrane damage), indirectly (e.g., microbial competition or inhibition of virulence gene expression), or by increasing short-chain fatty acids (SCFAs), such as butyrate, which stimulate the colonic epithelium and enhance mucosal barrier function (Lei *et al.*, 2024). The difference in the frequency of polyvalent serotypes and major virulence factor genes between treatment and control groups remained unclear. However, diarrhea scores recorded during the suckling period showed a significant reduction in the XPC group (1.32) compared to the CON group (1.59) ($p < 0.05$) (data not reported

in this article). This finding suggests that XPC may influence *E. coli* pathogenicity in suckling piglets. Consistent with these results, Hashem *et al.* (2022) reported that probiotics and prebiotics could modulate *E. coli*-induced adverse effects. Furthermore, several studies have demonstrated that XPC supplementation reduces diarrhea rates, improves faecal scores, and alters the intestinal microbial composition in pigs (Yan *et al.*, 2024).

Effects of feeding XPC on *E. coli* AMR and ARGs

The changes in the mean zone diameter of inhibition and frequencies of resistance to AMs in *E. coli* isolates are presented in Tables 6 and 7, respectively. As shown in Table 6, the mean zone diameter inhibition of CIP, NA, GEN, and STR against *E. coli* isolates was affected by treatments ($p < 0.05$). The mean zone diameter inhibition of CFL, ERY, GEN, and STR against *E. coli* isolates was affected by the age of the piglets ($p < 0.05$). *E. coli* isolates exhibited high resistance to AMP, ERY, OX, OTC, and STX, and susceptibility to CTX, CFL, and DO (Table 7). The frequencies of resistance to AMP, ERY, OX, OTC, and SXT in the XPC group decreased from 7 to 21 days old. For example, the frequency of resistance to AMP in the XPC group at 7, 14, and 21 days old were 100%, 94.0%, and 70.0%, respectively ($p < 0.05$). Meanwhile, the frequency of resistance to AMP in the CON group increased from 7 days old (66.0%) to 14 days old (88.0%), and 21 days old (92.0%). In contrast, the frequencies of susceptibility to CTX, CFL, and DO in all treatments decreased with the age of the piglets. For example, the susceptibility to CFL in the CON, BG, and XPC

groups at 7 days old were 90.0%, 80.0%, and 84.0%, respectively; at 21 days old, they were 50.0%, 58.0%, and 62.0%, respectively. The present study showed significant differences in the frequency of resistance to AMP, CTX, ERY, NA, OX, OTC, GEN, STR, SXT, and TET among the groups at 21 days old ($p < 0.05$). For example, the frequencies of resistance to AMP in the BG (78.0%) and XPC (70.0%) groups were lower than that in the CON group (92.0%) ($p < 0.05$).

In this study, sows and piglets were not exposed to antimicrobial agents (AMs) during the experiment. Combined with XPC or BG supplementation, this may have contributed to the reduced rates of *E. coli* isolates resistant to certain AMs in suckling piglets. A significant effect of XPC feeding on the zone diameter inhibition of CIP, NA, GEN, and STR against *E. coli* isolates was observed ($p < 0.05$) (Table 6). Additionally, resistance rates to AMP and OX were higher in the CON group than in the treatment groups (Table 7). XPC has been shown to increase the concentrations of short-chain fatty acids (SCFAs), such as acetate and butyrate (Feye *et al.*, 2021; Rubinelli *et al.*, 2016). Ott and Mellata (2024) demonstrated that SCFAs broadly inhibit plasmid transfer and eliminate AMR in *E. coli*. Implementing interventions that enhance SCFA concentrations in the gut may help reduce the risk, incidence, and emergence of AMR (Ott and Mellata, 2024). Ngoc *et al.* (2020) found that adding Selacid Green Growth (a blend of SCFAs) to pig diets correlated with a reduced prevalence of *E. coli* resistant to amoxicillin/clavulanic acid and cefotaxime (CTX). A significant difference in AMP and OX resistance levels was observed between the CON and treatment groups, suggesting that XPC supplementation helps reduce AMR in *E. coli* isolates from suckling piglets. Previous studies have reported differences in AMR levels in *E. coli* isolates from pigs exposed or not exposed to AMs at various production stages (Pissetti *et al.*, 2021). Moreover, the absence of AM administration in sows and piglets may decrease the prevalence of AMR bacteria in suckling piglets. Notably, the AMR rates in *E. coli* (e.g., resistance to AMP, CFL, and CIP) increased with piglet age ($p < 0.05$) (Table 7).

Sows carry AMR *E. coli*, which persist

throughout gestation and are transmitted to piglets via contact with the birth canal, skin, feces, environmental microbes, and milk. The transmission of resistant bacteria from sows to piglets has been previously reported by Mathew *et al.* (2005). Burow *et al.* (2019) reported that *E. coli* isolates from piglets were more likely to be resistant to AMP or azithromycin if the bacteria from their respective dam were also resistant, indicating vertical transmission of AMR bacteria from sow to offspring. Additionally, resistance to AMP and TET was prevalent even before the use of these antimicrobials for treatment. Notably, *E. coli* isolates from pigs that had not received beta-lactams or TET also exhibited common resistance to these substances. High baseline levels of resistance may explain why the difference between TET-treated and untreated pigs was not significant (Burow *et al.*, 2019).

The effect of feeding XPC on the distribution of antimicrobial resistant genes (ARGs) in the *E. coli* isolates is presented in Table 8. It can be seen from Table 8 that most of the *E. coli* isolates were negative for ARGs. At 7 days old, isolates from the CON and BG groups carried *sull1* (20.0% and 8.0%, respectively) and *sul2* (4.0% and 14.0%, respectively), while isolates from the XPC group carried *aphA1* (2.0%). At 14 days old, isolates from the CON group carried *aadA* and *aphA1* (each 22.0%), and isolates from the BG group carried *tet(B)* (6.0%). At 21 days old, 10.0% and 2.0% of isolates from the CON and XPC groups, respectively, carried the *sull1* gene, and 30.0%, 48.0%, and 32.0% of isolates from the CON, BG, and XPC groups, respectively, carried the *aadA* gene.

The association between antimicrobial resistance (AMR) characteristics (both genotype and phenotype) and genes encoding virulence factors in *E. coli* has been previously reported (Sousa *et al.*, 2024). In this study, most *E. coli* isolates from both sows and piglets were negative for antimicrobial resistance genes (ARGs) and virulence factor genes. The low prevalence of *E. coli* isolates carrying AMR genes may be linked to the presence of virulence factor genes. Mitra *et al.* (2024) found that enterotoxigenic *E. coli* and 35 Shiga toxin-producing *E. coli* isolates from pigs exhibited a higher proportion of plasmid-mediated multidrug resistance compared to less virulent (commensal) counterparts. Addi-

Table 6. Effects of Feeding a XPC on Zone Diameter Inhibition of AMs (mean \pm SD, mm) Against the *E. coli* Isolates from Piglets (n: 10 replication * 5 colonies from each = 50)

AMs	7 Days old			14 Days old			21 Days old			⁵ p-value			
	CON ¹	BG ²	XPC ³	CON	BG	XPC	CON	BG	XPC	⁴ SEM	T	A	T* ⁴
AMP ⁶	8.2 ^a \pm 6.67	3.5 [#] \pm 3.57	1.0 ^{##} \pm 2.18	3.2 ^b \pm 4.71	3.0 \pm 2.92	2.8 ^{ab} \pm 3.24	3.8 ^{ab} \pm 4.18	5.1 \pm 6.64	5.4 ^b \pm 6.29	0.81	0.27	0.33	0.05
CTX ⁷	26.9 \pm 5.48	21.4 ^b \pm 9.42	21.3 \pm 6.85	26.2 \pm 7.48	26.9 ^{ab} \pm 6.52	23.3 \pm 7.02	23.0 \pm 6.96	27.8 ^b \pm 3.43	22.4 \pm 6.79	1.02	0.15	0.44	0.23
CFL ⁸	20.7 ^a \pm 2.2	18.5 \pm 4	18.0 \pm 3.35	20.2 ^a \pm 4.13	18.4 \pm 4.28	18.6 \pm 5.53	14.6 ^b \pm 5.18	15.5 \pm 5.69	16.1 \pm 7.79	0.82	0.66	0.01	0.71
CHL ⁹	13.8 \pm 5.27	13.8 \pm 6.87	12.9 \pm 6.92	11.8 \pm 5.05	15.8 \pm 5.77	10.8 \pm 6.56	13.3 \pm 3.91	12.1 \pm 4.51	13.4 \pm 5.02	0.56	0.56	0.89	0.45
CIP ¹⁰	22.0 [*] \pm 4.72	15.3 ^{*#} \pm 8.07	12.1 [#] \pm 9.18	19.3 ^{*#} \pm 8.47	21.2 [*] \pm 8.59	13.2 ^{##} \pm 5.98	17.5 \pm 5.35	16.0 \pm 7.92	16.4 \pm 9.58	1.33	0.02	0.74	0.19
CL ¹¹	9.7 \pm 4.65	9.6 \pm 4.42	11.0 \pm 3.24	12.7 \pm 1.67	11.0 \pm 3.47	11.5 \pm 3.31	10.2 \pm 4.65	11.5 \pm 2.31	11.3 \pm 6.39	0.39	0.86	0.28	0.73
DO ¹²	17.3 [*] \pm 3.74	16.7 ^{*#} \pm 3.47	13.9 [#] \pm 3.17	16.2 \pm 2.61	17.0 \pm 3.66	17.2 \pm 3.77	15.5 \pm 4.59	14.2 \pm 5.35	13.2 \pm 6.51	0.61	0.33	0.08	0.53
ERY ¹³	6.7 ^{ab} \pm 6.85	3.1 \pm 4.95	3.5 ^a \pm 5.43	2.9 ^a \pm 3.35	4.1 \pm 6.37	3.2 ^a \pm 3.11	9.8 ^{b*} \pm 6.07	3.4 [#] \pm 7.09	9.3 ^{b*} \pm 7.48	1.09	0.15	0.02	0.20
NA ¹⁴	12.5 \pm 4.95	8.8 \pm 7.43	7.3 ^{ab} \pm 5.64	9.4 [*] \pm 6.01	9.6 [*] \pm 5.66	3.0 ^{##} \pm 4.09	13.6 \pm 6.53	8.1 \pm 8.25	10.2 ^b \pm 8.97	1.21	0.02	0.15	0.32
OX ¹⁵	0.0	0.6 \pm 2.02	0.0	0.9 \pm 2.19	1.2 \pm 1.61	0.0	0.0	0.0	3.5 \pm 4.6	0.45	0.22	0.17	0.00
OTC ¹⁶	10.3 ^{a*} \pm 7.27	11.6 [*] \pm 10.37	3.7 [#] \pm 6.68	4.2 ^{b*} \pm 2.64	11.8 [#] \pm 9.01	8.4 ^{*#} \pm 9.29	9.1 ^a \pm 4.56	4.6 \pm 4.04	8.8 \pm 6.25	1.25	0.43	0.86	0.01
GEN ¹⁷	15.0 ^{ab*} \pm 7.06	7.4 [#] \pm 6.21	9.1 ^{ab*} \pm 6.9	9.5 ^a \pm 4.71	10.0 \pm 7.49	7.1 ^a \pm 3.8	17.0 ^b \pm 6.6	11.7 \pm 6.55	13.9 ^b \pm 7.73	1.37	0.03	0.01	0.33
STR ¹⁸	11.6 ^{a*} \pm 3.88	4.0 [#] \pm 4.63	4.1 ^{##} \pm 3.63	5.0 ^{b*} \pm 3.51	6.1 [*] \pm 5.54	1.9 ^{##} \pm 2.66	6.8 ^{b*} \pm 4.64	3.1 [*] \pm 4.55	9.9 ^{b*} \pm 5.29	1.26	0.01	0.07	0.00
SXT ¹⁹	4.1 \pm 6.24	3.1 \pm 4.83	2.1 \pm 3.73	2.0 \pm 3.54	3.3 \pm 6.31	1.4 \pm 1.9	6.0 [*] \pm 4.33	0.2 [#] \pm 0.63	3.3 ^{*#} \pm 4.61	0.67	0.19	0.65	0.12
TET ²⁰	15.0 [*] \pm 4.84	14.5 [*] \pm 7.28	8.9 [#] \pm 5.84	11.5 \pm 3.59	15.2 \pm 5.69	13.3 \pm 6.39	14.9 \pm 4.96	10.6 \pm 3.85	12.5 \pm 6.31	0.88	0.25	0.88	0.05

¹CON: Basal control diet; ²BG: Standard basal control diet containing 1.0 kg/MT of Beta-glucan 50%; ³XPC: Standard basal control diet containing 2.0 kg/MT of Diamond V XPC; ⁴SEM: Standard error of the mean; ⁵P-value: The statistical model included effects of T: treatment (XPC or BG), A: age (days old), the interaction between the treatment (T) and age (A), and the error term. ⁶AMP: ampicillin (AMP, 10 μ g); ⁷CTX: cefotaxime (CTX, 30 μ g); ⁸CFL: cephalixin (CFL, 30 μ g); ⁹CHL: chloramphenicol (CHL, 30 μ g); ¹⁰CIP: ciprofloxacin (CIP, 5 μ g); ¹¹CL: colistin (CL, 10 μ g); ¹²DO: doxycycline (DO, 30 μ g); ¹³ERY: erythromycin (ERY, 15 μ g); ¹⁴NA: nalidixic acid (NA, 30 μ g); ¹⁵OX: oxacillin (OX, 1 μ g); ¹⁶OTC: oxytetracycline (OTC, 30 μ g); ¹⁷GEN: gentamicin (GEN, 10 μ g); ¹⁸STR: streptomycin (STR, 10 μ g); ¹⁹SXT: trimethoprim-sulfamethoxazole (SXT, 25 μ g); ²⁰TET: tetracycline (TET, 30 μ g). ^{a, b} indicated the significant difference between time points (day old) in each treatment. ^{*}, [#] indicated the significant difference between treatments in each time point.

Table 7. Effects of Feeding a XPC on Prevalence of AMR (%) of the *E. coli* Isolates from Piglets (n: 10 replication * 5 colonies from each = 50)

AMs	Interpretive criteria	7 Days old			14 Days old			21 Days old		
		CON ¹	BG ²	XPC ³	CON ¹	BG ²	XPC ³	CON ¹	BG ²	XPC ³
AMP ⁴	Resistant	66.0 ^{a*}	86.0 ^b	100.0 ^{c*}	88.0 [#]	90.0	94.0 [*]	92.0 ^{a#}	78.0 ^b	70.0 ^{b#}
	Intermediate	20.0	8.0	0.0	6.0	8.0	6.0	8.0	14.0	20.0
	Susceptible	14.0	6.0	0.0	6.0	2.0	0.0	0.0	8.0	10.0
CTX ⁵	Resistant	12.0 ^{a*}	46.0 ^{b*}	44.0 ^b	32.0 [#]	22.0 [#]	40.0	44.0 ^{a#}	14.0 ^{b#}	38.0 ^a
	Intermediate	22.0	12.0	22.0	10.0	10.0	14.0	14.0	20.0	22.0
	Susceptible	66.0	42.0	34.0	58.0	68.0	46.0	42.0	66.0	40.0
CFL ⁶	Resistant	10.0 [*]	14.0 [*]	14.0 [*]	12.0 [*]	18.0 [*]	16.0 [*]	34.0 [#]	38.0 [#]	32.0 [#]
	Intermediate	0.0	6.0	2.0	4.0	4.0	0.0	16.0	4.0	6.0
	Susceptible	90.0	80.0	84.0	84.0	78.0	84.0	50.0	58.0	62.0
CHL ⁷	Resistant	32.0	42.0 [*]	40.0 [*]	38.0 ^a	22.0 ^{a#}	60.0 ^{b#}	46.0	46.0 [*]	36.0 [*]
	Intermediate	46.0	22.0	24.0	46.0	46.0	18.0	32.0	40.0	44.0
	Susceptible	22.0	36.0	36.0	16.0	32.0	22.0	22.0	14.0	20.0
CIP ⁸	Resistant	32.0 ^{a*}	64.0 ^{b*}	68.0 ^{b*}	52.0 ^{a#}	40.0 ^{a#}	90.0 ^{b#}	62.0 [#]	76.0 [*]	64.0 [*]
	Intermediate	40.0	16.0	10.0	20.0	16.0	8.0	24.0	8.0	10.0
	Susceptible	28.0	20.0	22.0	28.0	44.0	2.0	14.0	16.0	26.0
CL ⁹	Resistant	38.0 [*]	36.0	28.0 ^{a#}	12.0 [#]	24.0	18.0 [*]	32.0 [#]	22.0	38.0 [#]
	Intermediate	44.0	52.0	60.0	56.0	58.0	64.0	48.0	46.0	20.0
	Susceptible	18.0	12.0	12.0	32.0	18.0	18.0	20.0	32.0	42.0
DO ¹⁰	Resistant	4.0	6.0 [#]	6.0 [*]	6.0	2.0 [*]	2.0 [*]	8.0	14.0 [#]	24.0 [#]
	Intermediate	24.0	24.0	50.0	16.0	24.0	32.0	26.0	30.0	34.0
	Susceptible	72.0	70.0	44.0	78.0	74.0	66.0	66.0	56.0	42.0
ERY ¹¹	Resistant	76.0	86.0	82.0 [*]	88.0	80.0	88.0 [*]	64.0 ^a	84.0 ^b	56.0 ^{a#}
	Intermediate	22.0	14.0	18.0	12.0	18.0	10.0	32.0	14.0	44.0
	Susceptible	2.0	0.0	0.0	0.0	2.0	2.0	4.0	2.0	0.0
NA ¹²	Resistant	40.0 ^a	56.0 ^a	68.0 ^b	62.0 ^a	54.0 ^a	88.0 ^b	32.0 ^a	62.0 ^b	44.0 ^{ab}
	Intermediate	38.0	24.0	12.0	22.0	24.0	8.0	30.0	16.0	34.0
	Susceptible	22.0	20.0	20.0	16.0	22.0	4.0	38.0	22.0	22.0
OX ¹³	Resistant	100.0	96.0	100.0 [*]	98.0	94.0	100.0 [*]	100.0 ^a	100.0 ^a	84.0 ^{b#}
	Intermediate	0.0	4.0	0.0	0.0	4.0	0.0	0.0	0.0	14.0
	Susceptible	0.0	0.0	0.0	2.0	2.0	0.0	0.0	0.0	2.0
OTC ¹⁴	Resistant	64.0 ^{ab*}	58.0 ^{a*}	88.0 ^{b*}	92.0 ^{a#}	62.0 ^{b*}	74.0 ^{b*#}	74.0 ^{ab*}	86.0 ^{a#}	68.0 ^{b#}
	Intermediate	10.0	0.0	2.0	4.0	0.0	6.0	6.0	8.0	16.0
	Susceptible	26.0	42.0	10.0	4.0	38.0	20.0	20.0	6.0	16.0
GEN ¹⁵	Resistant	26.0 ^{a*}	64.0 ^{b*}	58.0 ^{b*}	64.0 [#]	64.0 [*]	74.0 [*]	20.0 ^{a*}	44.0 ^{b#}	30.0 ^{ab#}
	Intermediate	12.0	12.0	8.0	12.0	2.0	18.0	10.0	18.0	8.0
	Susceptible	62.0	24.0	34.0	24.0	34.0	8.0	70.0	38.0	62.0
STR ¹⁶	Resistant	32.0 ^{a*}	78.0 ^{b*#}	84.0 ^{b*}	68.0 ^{a#}	68.0 ^{a*}	88.0 ^{b*}	64.0 ^{b#}	84.0 ^{a#}	46.0 ^{b#}
	Intermediate	50.0	18.0	14.0	24.0	24.0	8.0	22.0	12.0	40.0
	Susceptible	18.0	4.0	2.0	8.0	8.0	4.0	14.0	4.0	14.0
SXT ¹⁷	Resistant	78.0 ^a	86.0 ^b	92.0 ^b	88.0	84.0	92.0	58.0 ^a	98.0 ^b	80.0 ^c
	Intermediate	10.0	2.0	0.0	8.0	4.0	4.0	30.0	2.0	10.0
	Susceptible	12.0	12.0	8.0	4.0	12.0	4.0	12.0	0.0	10.0
TET ¹⁸	Resistant	28.0 ^a	44.0 ^b	56.0 ^b	26.0	28.0	30.0	18.0 ^a	48.0 ^b	36.0 ^b
	Intermediate	32.0	14.0	30.0	62.0	32.0	40.0	46.0	44.0	28.0
	Susceptible	40.0	42.0	14.0	12.0	40.0	30.0	36.0	8.0	36.0

¹CON: Basal control diet; ²BG: Standard basal control diet containing 1.0 kg/MT of Beta-glucan 50%; ³XPC: Standard basal control diet containing 2.0 kg/MT of Diamond V XPC; ⁴AMP: ampicillin (AMP, 10 µg); ⁵CTX: cefotaxime (CTX, 30 µg); ⁶CFL: cephalixin (CFL, 30 µg); ⁷CHL: chloramphenicol (CHL, 30 µg); ⁸CIP: ciprofloxacin (CIP, 5 µg); ⁹CL: colistin (CL, 10 µg); ¹⁰DO: doxycycline (DO, 30 µg); ¹¹ERY: erythromycin (ERY, 15 µg); ¹²NA: nalidixic acid (NA, 30 µg); ¹³OX: oxacillin (OX, 1 µg); ¹⁴OTC: oxytetracycline (OTC, 30 µg); ¹⁵GEN: gentamicin (GEN, 10 µg); ¹⁶STR: streptomycin (STR, 10 µg); ¹⁷SXT: trimethoprim-sulfamethoxazole (SXT, 25 µg); ¹⁸TET: tetracycline (TET, 30 µg). *, #, ^a, ^b, ^c indicated the significant difference between time points (days old) in each treatment. ^a, ^b, ^c indicated the significant difference between treatments in each time point.

tionally, mobile genetic element analysis revealed that *E. coli* isolates from poultry, swine, and cattle harbored composite transposons carrying ARGs and virulence genes (e.g., *blaTEM/eae*), highlighting their potential for horizontal transfer. A study investigated the effects of postbiotics derived from *Lactobacillus plantarum* on the expression of antibiotic resistance genes (*ermB* and *blaKPC*) in *Enterococcus faecalis* and *Pseudomonas aeruginosa*, reporting a reduction in gene expression and a corresponding decrease in antibiotic resistance levels (Nezhadi and Ahmadi, 2024).

CONCLUSION

Feeding a XPC in sows and piglets' diet exhibited significantly lower *E. coli* counts (log₁₀ CFU/g) in feces of piglets compared to the control group ($p < 0.05$). Supplementing XPC in diets reduced the frequency of *E. coli* isolates resistant to ampicillin, erythromycin, and oxytetracycline in suckling piglets ($p < 0.05$). Supplementing BG in diets reduced the frequency of *E. coli* isolates resistant to cefotaxime, and gentamicin in piglets ($p < 0.05$). It is recom-

mended to supplement XPC in sows and piglets in high-biosecurity production systems to enhance gut health and reduce the antimicrobial resistance in suckling piglets.

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CONFLICT OF INTEREST

The authors clarify that there is no conflict of interest with any financial, personal, or other

Table 8. Effects of Feeding a XPC on the Distribution of ARGs (%) in the *E. coli* Isolates from Piglets (n: replication * 5 colonies from each = 50)

ARGs ⁴	7 Days old			14 Days old			21 Days old		
	CON ¹	BG ²	XPC ³	CON	BG	XPC	CON	BG	XPC
<i>sul1</i>	20.00	8.00	ND	ND	ND	ND	10.00	ND	2.00
<i>sul2</i>	ND ⁵	ND	ND	ND	ND	ND	ND	ND	N
<i>sul3</i>	4.00	14.00	ND	ND	ND	ND	ND	ND	N
<i>tet(A)</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>tet(B)</i>	ND	ND	ND	ND	6.00	ND	ND	2.00	N
<i>tet(C)</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>aadA</i>	ND	ND	ND	22.00	ND	ND	30.00	48.00	32.00
<i>strA/strB</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>aac(3)IV</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>aadB</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>aphA1</i>	ND	ND	2.00	22.00	ND	ND	ND	ND	N
<i>aphA2</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>bla_{TEM}</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>blaSHV</i>	ND	ND	ND	ND	ND	ND	ND	ND	N
<i>blaCMY-2</i>	ND	ND	ND	ND	ND	ND	ND	ND	N

¹CON: Basal control diet; ²BG: Standard basal control diet containing 1.0 kg/MT of Beta-glucan 50%; ³XPC: Standard basal control diet containing 2.0 kg/MT of Diamond V XPC; ⁴The major genes for resistance to sulfonamides (*sul1*, *sul2*, and *sul3*); the major genes for resistance to tetracycline [*tet(A)*, *tet(B)*, and *tet(C)*]; the genes for resistance to streptomycin (*strA/strB* and *aadA*); the gene for resistance to apramycin, gentamycin, and tobramycin [*aac(3)IV*]; the gene for resistance to kanamycin and gentamicin (*aadB*), the genes for resistance to kanamycin and neomycin (*aphA1* and *aphA2*), the major genes for resistance to β -lactams (*bla_{TEM}*, *blaSHV*, and *blaCMY-2*); ⁵ND: None detected.

relationships with other people or organizations related to the material discussed in the manuscript.

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