EISSN: 2231-0916 doi: 10.14202/vetworld.2026.5051-5061 OPEN ACCESS

Veterinary World

RESEARCH ARTICLE

Oregano oil emulsion as an alternative to enrofloxacin for multidrug-resistant *Escherichia coli*-associated diarrhea in piglets: Antibacterial activity and therapeutic efficacy

Kridda Chukiatsiri[1], Kittiphong Tippaya[1], Nattha Vigad[2], Sirichai Jaypong[3], Warintorn Jongpae[3], Wanna Chinawan[3], Nattanan Khamhom[3] and Ruttayaporn Ngasaman[4]

1. Faculty of Animal Science and Technology, Maejo University, Chiang Mai, 50290, Thailand.
2. School of Agriculture and Cooperatives, Sukhothai Thammathirat Open University, Pak Kret, Nonthaburi 11120, Thailand.
3. LIC Agrotech Company Limited, 432 Krisada Nakhon Village 20, Pinklao-Nakhon Chai Si Road, Sala Thammasop Subdistrict, Thawi Watthana District, Bangkok 10170, Thailand.
4. Faculty of Veterinary Science, Prince of Songkla University, Songkhla, 90110, Thailand.

ABSTRACT

Background and Aim: Post-weaning diarrhea associated with *Escherichia coli* is a major challenge in pig production, particularly with the increasing prevalence of multidrug-resistant (MDR) strains. Oregano oil has antimicrobial properties and may provide an alternative to conventional antibiotics. This study evaluated the *in vitro* antibacterial activity of oregano oil emulsion (OOE) against bacterial isolates, including MDR *E. coli* from diarrheic pigs, and compared its therapeutic efficacy with enrofloxacin in piglets with naturally occurring diarrhea.

Materials and Methods: *E. coli* was isolated from fecal samples of 11 diarrheic pigs and identified using culture and biochemical tests. Antimicrobial susceptibility to six antibiotics was determined using the Kirby–Bauer disk diffusion method. The minimum inhibitory concentration (MIC) of OOE was evaluated by agar dilution. For the therapeutic trial, 26 weaned piglets were allocated by litter into two groups (n = 13/group). Piglets received either 5% enrofloxacin orally once daily or OOE at 1 mL/2 kg body weight orally twice daily until fecal scores normalized. Fecal scores, bacterial counts, and weight gain were evaluated.

Results: All field *E. coli* isolates were resistant to at least two tested antibiotics. Resistance was highest to amoxicillin (100%), followed by amoxicillin–clavulanic acid and enrofloxacin (81.81% each). OOE inhibited field isolates at MICs of 2.5–10 mg/mL. Fecal scores decreased in both treatment groups, reaching 0 by day 7; however, enrofloxacin-treated piglets had greater odds of lower fecal scores (odds ratio = 6.11; 95% confidence interval = 1.23–30.44; p = 0.0274). Bacterial counts declined significantly over time ($p < 0.0001$), with no treatment effect (p = 0.727) or treatment × day interaction (p = 0.793). Weight gain did not differ between OOE (0.73 ± 0.14 kg) and enrofloxacin (0.71 ± 0.33 kg) groups (p = 0.884).

Conclusion: OOE exhibited antibacterial activity against antibiotic-resistant swine *E. coli* isolates and produced progressive clinical and bacterial count improvements in diarrheic piglets. Although enrofloxacin produced faster improvement in fecal consistency, OOE achieved fecal score resolution by day 7, with bacterial count reduction and weight gain comparable between treatments. These findings support further evaluation of OOE as a potential non-antibiotic approach for managing *E. coli*-associated diarrhea in pig production.

Keywords: antimicrobial resistance, antibiotic alternative, carvacrol, diarrheic piglets, *Escherichia coli*, multidrug resistance, oregano oil emulsion, post-weaning diarrhea.

INTRODUCTION

Diarrhea is a major cause of impaired growth performance, morbidity, and mortality in pigs, particularly in piglets and newly weaned pigs. Its etiology is multifactorial and involves interactions among infectious agents, environmental conditions, nutrition, sanitation, and physiological stress [1]. Infectious diarrhea may be caused by bacterial, viral, or parasitic pathogens. Among important bacterial agents, *Escherichia coli*, *Clostridium perfringens*, and *Salmonella* spp. can colonize the intestinal tract and cause anatomical or biochemical changes

Corresponding Author: Ruttayaporn Ngasaman
E-mail: ruttayaporn.n@gmail.com

Received: 11-05-2026, **Accepted:** 21-09-2026, **Published online:** 09-11-2026

Co-authors: KC: kridda003@hotmail.com; KT: kittipong@mju.ac.th; NV: Nattha.Vig@stou.ac.th; SJ: jaypong999@gmail.com; WJ: warintorn09@hotmail.com; WC: lic.puwanna@gmail.com; NK: lic.nattanan@gmail.com

How to cite: Chukiatsiri K, Tippaya K, Vigad N, Jaypong S, Jongpae W, Chinawan W, *et al.* Oregano oil emulsion as an alternative to enrofloxacin for multidrug-resistant *Escherichia coli*-associated diarrhea in piglets: Antibacterial activity and therapeutic efficacy. Vet World, 2026, 19(10): 5051–5061.

Copyright: Chukiatsiri *et al*. This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

that impair intestinal function and lead to the passage of liquid feces [2]. Effective control of diarrhea therefore requires appropriate sanitation, pathogen control, nutritional management, and therapeutic interventions.

The pig industry faces increasing challenges in preventing enteric diseases while improving animal welfare and reducing reliance on antimicrobial agents, particularly in the context of antimicrobial resistance. These concerns have stimulated interest in non-antibiotic strategies, including phytogenic products, essential oils, prebiotics, and minerals. Oregano and its essential oil have a long history of medicinal use [3]. The principal bioactive constituents of oregano essential oil (OEO), particularly thymol and carvacrol, have antimicrobial and anti-inflammatory properties that may help control diarrhea around weaning. Oregano oil has also been associated with improved intestinal health, feed utilization, and animal health and growth performance [4], suggesting its potential to reduce dependence on conventional antimicrobial therapy.

Dietary OEO supplementation has been investigated for its effects on growth performance, intestinal health, immune function, stress responses, and resistance to infection in pigs. OEO supplementation can improve growth performance and nutrient digestibility by modulating intestinal bacterial populations, intestinal morphology, and antioxidant capacity [5]. Supplementation of sow diets with OEO has also been associated with increased fecal *Lactobacillus* abundance, reduced populations of potentially pathogenic bacteria, including *E. coli* and *Enterococcus* spp., and improved piglet performance, potentially through reduced oxidative stress [6, 7]. Marcin *et al.* [8] reported that dietary supplementation with oregano plant extract in weaned piglets reduced the abundance of Enterobacteriaceae by 40%. Furthermore, OEO supplementation during lactation increased the relative abundance of Lactobacillaceae and bacterial families associated with fiber digestion, including Fibrobacteriaceae and Akkermansiaceae, while reducing the incidence of therapeutic treatment and mortality among piglets [9].

Despite these promising findings, important gaps remain regarding the therapeutic application of oregano oil in pigs with clinically apparent diarrhea. Most previous studies have focused on OEO as a dietary feed additive administered for prophylactic purposes or over relatively prolonged periods, rather than as a targeted oral therapeutic intervention for acute post-weaning diarrhea (PWD). Conventional oregano oil formulations may also have limitations related to bioaccessibility and variable efficacy under field conditions. Emulsion-based formulations could provide a practical approach for administering oregano oil as an acute oral treatment; however, evidence regarding their antibacterial activity against field isolates of antibiotic-resistant *E. coli* and their therapeutic performance in naturally affected piglets remains limited. In particular, direct controlled comparisons between oregano oil emulsion (OOE) and conventional antimicrobial treatment, such as enrofloxacin, are lacking. This evidence gap is particularly relevant in the context of efforts to reduce antimicrobial use in food-producing animals and the increasing occurrence of antimicrobial-resistant enteric bacteria. Therefore, simultaneous evaluation of the *in vitro* antibacterial activity of OOE against field isolates and its *in vivo* therapeutic performance is needed to determine whether this formulation has potential as a non-antibiotic approach to the management of PWD.

This study aimed to evaluate the antibacterial activity and therapeutic efficacy of OOE in piglets with PWD and to compare its therapeutic effects with those of enrofloxacin. Specifically, the study aimed to isolate and identify *E. coli* from field cases of diarrhea in piglets and characterize the antimicrobial resistance profiles of the isolates; determine the *in vitro* antibacterial activity of OOE against the bacterial isolates; conduct a randomized comparative clinical trial to evaluate oral OOE and enrofloxacin treatment in weaned piglets with naturally occurring *E. coli*-associated diarrhea; and assess treatment-associated changes in fecal scores, bacterial counts, and short-term body weight gain. Through these complementary *in vitro* and *in vivo* evaluations, the study sought to determine the potential of OOE as an alternative approach for managing bacterial diarrhea while supporting efforts to reduce reliance on conventional antimicrobial treatment in pig production.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Maejo University, Thailand (Approval No. MACUC008A/2569). The study was conducted in accordance with the approved animal ethics protocol and applicable institutional animal welfare requirements. The experimental procedures and reporting were structured with consideration of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 guidelines, emphasizing transparency in experimental design, animal allocation, outcome assessment, and measures to minimize animal suffering.

Throughout the experiment, the piglets were maintained under controlled housing conditions with unrestricted access to feed and drinking water. Their general health, behavior, and clinical condition were monitored daily using a predefined clinical scoring system. Humane endpoints were established before the experiment. Piglets exhibiting more than 20% body weight loss, severe lethargy or unresponsiveness, or signs of acute distress, including labored breathing, abnormal posture, severe ulceration, or bleeding, were designated for immediate withdrawal from the study or humane euthanasia, as appropriate. These measures were intended to minimize pain, distress, and unnecessary suffering while maintaining the scientific validity of the experiment.

Study period and location

Sample collection and the *in vivo* study were conducted at Visut Farm, Chom Thong District, Chiang Mai, Thailand, from April to May 2026. The study was conducted at Maejo University, Thailand. Laboratory procedures, including bacterial isolation and identification, antimicrobial susceptibility testing, and evaluation of the antibacterial activity of OOE, were performed using fecal samples collected from diarrheic pigs. The therapeutic trial was conducted using weaned piglets originating from 10 contemporary sows housed at the same facility.

Study design

The study comprised laboratory and therapeutic components designed to evaluate the antibacterial activity and therapeutic efficacy of OOE. In the laboratory component, *E. coli* were isolated from fecal samples collected from 11 diarrheic pigs. A total of 11 isolates were recovered, with each isolate originating from an independent pig farm (one isolate per farm site across 11 farms) and subjected to antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) determination. In the therapeutic component, 26 piglets with diarrhea were allocated to two treatment groups (n = 13/group) using a randomized complete block design, with litter of origin as the primary blocking factor. Littermates were distributed between the two groups to minimize potential maternal and litter-associated effects. The therapeutic efficacy of orally administered OOE was compared with that of 5% enrofloxacin using fecal scores, bacterial counts, and body weight gain as outcome measures.

***E. coli* isolation and identification**

Fecal samples were collected from 11 diarrheic pigs. One gram of each fecal sample was mixed with Lauryl sulfate broth (BD BBL, MS, USA, catalog no. 224150) for pre-enrichment and incubated at 37°C for 24 h. Following incubation, the enriched samples were streaked onto MacConkey agar (BD BBL, catalog no. 211387) and eosin methylene blue agar (BD BBL, catalog no. 211215) to isolate presumptive *E. coli* [10]. Round, medium-sized colonies on MacConkey agar and colonies exhibiting a metallic sheen on eosin methylene blue agar were selected, subcultured on nutrient agar, and incubated at 37°C for 24 h.

Single colonies were subsequently subjected to biochemical identification using indole, methyl red, Voges–Proskauer, and citrate utilization tests, together with catalase, nitrate reduction, and sugar fermentation tests. Isolates were considered consistent with *E. coli* when they were positive for indole, methyl red, catalase, and nitrate reduction; negative for Voges–Proskauer and citrate utilization; and demonstrated sugar fermentation with gas production [11].

Antimicrobial susceptibility testing

Phenotypic antimicrobial susceptibility was evaluated using the Kirby–Bauer disk diffusion method [12]. A pure culture of each *E. coli* isolate was prepared as a standardized bacterial suspension adjusted to a 0.5 McFarland standard. The standardized bacterial suspension was uniformly inoculated onto Mueller–Hinton agar (HiMedia, Mumbai, India), after which antimicrobial disks were placed on the agar surface.

The antimicrobial agents tested were ceftriaxone (30 µg/disk), gentamicin (10 µg/disk), amoxicillin (20 µg/disk), doxycycline (30 µg/disk), amoxicillin–clavulanic acid (20/10 µg/disk), and enrofloxacin (5 µg/disk).. The inoculated plates were incubated at 37°C for 18–24 h. Following incubation, inhibition-zone diameters were measured and compared with the referenced interpretive criteria to classify each isolate as susceptible, intermediate, or resistant [13, 14].

MIC assay

The oregano oil product (Essgano Emulsion, LIC Agrotech Company Limited, Bangkok, Thailand) was heated in a water bath and sonicated for 30 min. Twofold serial dilutions were prepared in Mueller–Hinton broth (HiMedia). For preparation of the agar dilution plates, 2 mL of each dilution was thoroughly mixed with 18 mL of molten Mueller–Hinton agar, poured into plates, and allowed to solidify.

A pure culture of *E. coli* was adjusted to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL, and then diluted to a final concentration of approximately 5×10^5 CFU/mL. Duplicate 2-µL aliquots of each bacterial suspension were inoculated at four points on the prepared agar plates with duplicated. Inoculated plates were incubated aerobically at 37°C for 24 h. Bacterial growth was evaluated visually by examining colony formation on agar plates, where MIC was defined as the lowest concentration that completely inhibited visible bacterial growth. Following incubation at 37°C for 24 h, bacterial growth was evaluated. The experiments were performed in triplicate to assess reproducibility. The MIC was defined as the lowest concentration of OOE that inhibited visible bacterial growth.

Treatment of diarrheic piglets with OOE

A total of 26 weaned piglets originating from 10 contemporary sows housed at the same facility were included in the therapeutic trial. A randomized complete block design was used, with litter of origin body weight, serving as the primary blocking factor and evaluated under single-blind conditions. Littermates were distributed between the two groups to minimize maternal clustering effects. Pretreatment bacterial isolation identified *E. coli* as the predominant organism in the diarrheic samples. All piglets had received anticoccidial treatment on day 3 of age.

Piglets were allocated to a control group with an enrofloxacin treatment (n = 13) and an OOE treatment group (n = 13). Piglets in the enrofloxacin group presenting with diarrhea (fecal score = 2–3) received 5% enrofloxacin orally once daily until clinical recovery, defined as a fecal score of 0. Piglets in the OOE group with comparable diarrheic signs received OOE orally at 1 mL/2 kg body weight (0.5 mL/kg) dose was based on target gut lumen concentration estimates exceeding the in vitro MIC, supported by preliminary pilot dosing for animal safety and palatability, twice daily until the fecal score reached 0. The OOE dosage was selected based on preliminary *in vitro* antimicrobial activity.

Fecal samples were collected from the same piglets repeatedly on Days 0, 3, and 5 post-treatments. Fecal samples (1 g) were collected from each piglet to enumerate total viable counts of total aerobic bacteria. Bacterial enumeration was performed using serial 10-fold dilutions in phosphate-buffered saline (PBS; pH 7.4) and spread-plating onto MacConkey agar. Plates were incubated aerobically at 37°C for 24 hours, and colonies were expressed as $\log_{10}$CFU/g of wet feces.

Statistical analysis

Bacterial counts and body weight gain were analyzed using analysis of variance, with paired t-tests for pairwise comparisons, and independent-samples t-tests assumed equal variances, as applicable. Fecal scores, recorded on an ordinal scale of 0–3, were analyzed using an ordinal generalized linear mixed model, with individual piglet identification included as a random effect to account for repeated measurements and between-animal variability [15]. A repeated-measures linear mixed model was used to evaluate the fixed effects of treatment, time, and the treatment × time interaction, with Tukey's adjustment for multiple comparisons [16, 17]. Data are presented as mean ± standard deviation, mean differences, and 95% confidence intervals (CI), as applicable. Statistical significance was evaluated using p-values [18, 19].

RESULTS

***E. coli* isolation and antimicrobial susceptibility**

All *E. coli* isolates (100%) were resistant to at least two of the six antimicrobial agents tested and were considered MDR. Isolates no. 1, 3, 4, and 11 showed the highest resistance, with each being resistant to five of the six antimicrobial agents (83.33%). Resistance was highest to amoxicillin (100%), followed by amoxicillin–clavulanic acid and enrofloxacin (90.91%. Resistance to gentamicin and doxycycline was 63.64% each, whereas 36.36% of the isolates showed intermediate susceptibility to doxycycline. All isolates (100%) were susceptible to ceftriaxone. The antimicrobial susceptibility profiles of the *E. coli* isolates are presented in Table 1.

Antibacterial activity of OOE

OOE exhibited antibacterial activity against the tested bacterial strains, with MIC values ranging from 1.56 to 6.25 mg/mL. Among the swine *E. coli* isolates, isolates no. 2, 3, 4, 5, 10, and 11 showed the lowest MIC of 1.56 mg/mL. The chicken *E. coli* isolate β-Hemolytic *E. coli,* isolate no. 6, 8, 9, and the reference *E. coli* strain had MIC values of 3.125 mg/mL. *Staphylococcus aureus*, *Salmonella Typhimurium*, and swine *E. coli* isolate no. 7 showed the highest MIC of 6.25 mg/mL. Among the swine *E. coli* isolates with the highest antimicrobial resistance (isolate no. 7), OOE MIC values ranged from 1.56 to 6.25 mg/mL. The MIC values for all tested bacterial strains are

presented in Table 2.

Table 1: Antimicrobial susceptibility profiles of *Escherichia coli* isolates obtained from pig farms against the six standard antibiotics.

E. coli isolate	CTX	GEN	AMX	DOX	AMC	EN	Resistant (n/6)	Resistance (%)
Swine #1	S	R	R	R	R	R	5/6	83.33
Swine #2	S	R	R	I	R	R	4/6	66.67
Swine #3	S	R	R	R	R	R	5/6	83.33
Swine #4	S	R	R	R	R	R	5/6	83.33
Swine #5	S	R	R	I	R	R	4/6	66.67
Swine #6	S	S	R	R	R	R	4/6	66.67
Swine #7	S	S	R	I	R	I	2/6	33.33
Swine #8	S	S	R	R	R	R	4/6	66.67
Swine #9	S	R	R	I	I	R	3/6	50.00
Swine #10	S	S	R	R	R	R	4/6	66.67
Swine #11	S	R	R	R	R	R	5/6	83.33
Resistant (%)	0	63.64	100	63.64	90.91	81.82	—	—
Intermediate (%)	0	0	0	36.36	9.09	9.09	—	—
Susceptible (%)	100	36.36	0	0	0	9.09	—	—

CTX = Ceftriaxone; GEN = Gentamicin; AMX = Amoxicillin; DOX = Doxycycline; AMC = Amoxicillin–clavulanic acid; EN = Enrofloxacin; R = Resistant; I = Intermediate; S = Susceptible.

Table 2: Minimum inhibitory concentrations of oregano oil emulsion against reference bacterial strains and field isolates of *Escherichia coli.*

Bacterial strain/isolate	Resistance (%)	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.0975	Control (0)	MIC (mg/mL)
Staphylococcus aureus ATCC 25923	–	–	–	+	+	+	+	+	+	+	+	6.25
Salmonella Typhimurium ATCC 13076	–	–	–	+	+	+	+	+	+	+	+	6.25
E. coli ATCC 25922	–	–	–	–	+	+	+	+	+	+	+	3.125
β-Hemolytic *E. coli*	–	–	–	–	+	+	+	+	+	+	+	3.125
E. coli (chicken isolate)	–	–	–	–	+	+	+	+	+	+	+	3.125
E. coli (swine #1)	83.33	–	–	–	+	+	+	+	+	+	+	3.125
E. coli (swine #2)	66.67	–	–	–	–	+	+	+	+	+	+	1.56
E. coli (swine #3)	83.33	–	–	–	–	+	+	+	+	+	+	1.56
E. coli (swine #4)	83.33	–	–	–	–	+	+	+	+	+	+	1.56
E. coli (swine #5)	66.67	–	–	–	–	+	+	+	+	+	+	1.56
E. coli (swine #6)	66.67	–	–	–	+	+	+	+	+	+	+	3.125
E. coli (swine #7)	33.33	–	–	+	+	+	+	+	+	+	+	6.25
E. coli (swine #8)	66.67	–	–	–	+	+	+	+	+	+	+	3.125
E. coli (swine #9)	50.00	–	–	–	+	+	+	+	+	+	+	3.125
E. coli (swine #10)	66.67	–	–	–	–	+	+	+	+	+	+	1.56
E. coli (swine #11)	83.33	–	–	–	–	+	+	+	+	+	+	1.56

OOE = Oregano oil emulsion; MIC = Minimum inhibitory concentration; ATCC = American Type Culture Collection; – = No visible bacterial growth; + = Visible bacterial growth; Resistance (%) = Percentage of the six antibiotics tested to which the isolate was resistant. Concentrations are expressed in mg/mL.

Therapeutic efficacy of OOE in diarrheic piglets

Fecal scores differed significantly between the enrofloxacin and OOE treatment groups ($p = 0.0359$) and changed significantly over time ($p < 0.0001$). However, the treatment × day interaction was not significant ($p = 0.2488$), indicating that the pattern of change in fecal scores over the 7-day observation period did not differ significantly between the treatment groups. Overall, piglets receiving enrofloxacin had 6.11-fold greater odds of having a lower fecal score than those receiving OOE (odds ratio = 6.11; 95% CI = 1.23–30.44; $p = 0.0274$). Fecal scores decreased in both treatment groups during the experimental period, from a score of 2 on day 1 to 0 by day 7. Changes in fecal scores during the 7-day treatment period are presented in Figure 1.

Fecal bacterial counts did not differ significantly between the treatment groups ($p = 0.727$). On day 0, mean bacterial counts were 5.41 ± 0.68 and 5.34 ± 0.72 $\log_{10}$ CFU/g in the enrofloxacin and OOE groups, respectively. On day 3, the corresponding bacterial counts decreased to 4.49 ± 0.74 and 4.32 ± 0.61 $\log_{10}$ CFU/g and further decreased on day 5 to 3.97 ± 0.39 and 3.96 ± 0.83 $\log_{10}$ CFU/g, respectively. Bacterial counts changed significantly over time ($p < 0.0001$). However, the treatment × day interaction was not significant ($p = 0.793$), indicating that the temporal pattern of bacterial count reduction did not differ significantly between the enrofloxacin and OOE groups (Table 3).

doi: 10.14202/vetworld.2026.5051-5061

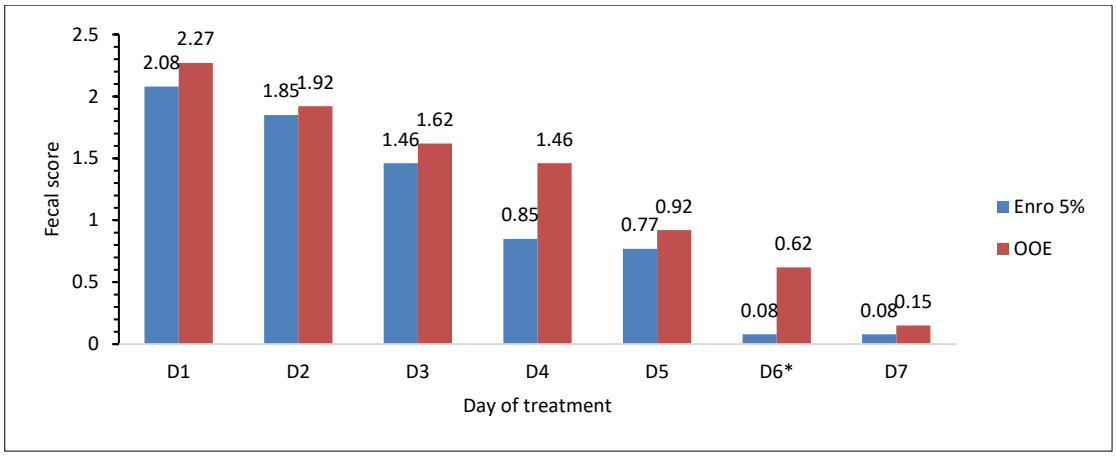


Figure 1: Comparison of fecal scores between treatment groups over a 7-day period. Data are expressed as mean values for the Enrofloxacin (Enro) 5% and Oregano oil emulsion (OOE) groups from Day 1 (D1) to Day 7 (D7). The asterisk (*) indicates a statistically significant difference between the treatment groups on D6 ($p < 0.05$).

Table 3: Comparison of bacterial counts between enrofloxacin treatment group and oregano oil emulsion treatment group over a 5-day period.

Day	Enrofloxacin	Oregano oil emulsion
0	5.41 ± 0.68	5.34 ± 0.72
3	4.49 ± 0.74	4.32 ± 0.61
5	3.97 ± 0.39	3.96 ± 0.83

Overall fixed effects

Effect	F-value	p-value
Treatment	0.13	0.727
Day	69.70	<0.0001
Treatment × day	0.23	0.793

Values are expressed as mean ± standard deviation (SD) of $\log_{10}$-transformed bacterial counts (CFU/g). Data were analyzed using a repeated-measures linear mixed model. CFU = Colony-forming unit.

Mean body weight gain was 0.71 ± 0.33 kg in the enrofloxacin group and 0.73 ± 0.14 kg in the OOE group. Body weight gain did not differ significantly between the treatment groups ($p = 0.884$). The reported mean difference between the groups was 0.015 kg (95% CI = –0.200–0.231 kg) (Table 4).

Table 4: Body weight gain of suckling piglets during the experimental period (5 days).

Item	Oregano oil emulsion (n = 13)	Enrofloxacin (n = 13)	Mean difference (95% CI)	p-value
Body weight gain (kg)	0.73 ± 0.14	0.71 ± 0.33	0.02 kg (–0.19 to 0.23kg)	0.840

Values are expressed as mean ± standard deviation (SD). Data were analyzed using an independent-samples t-test. The mean difference was calculated as the enrofloxacin group minus the oregano oil emulsion group. CI = Confidence interval.

DISCUSSION

Study design and methodological considerations

The experimental design aimed to reduce potential confounding by distributing piglets between treatment groups by litter. An untreated control group was not included because withholding treatment from clinically affected piglets could have resulted in progression of disease and compromised animal welfare. Therefore, enrofloxacin was used as the positive-control treatment. The number of animals available for the study was limited by field availability, with 11 diarrheic pigs included for bacterial isolation and 26 piglets included in the therapeutic trial. The sample size was also constrained by ethical considerations consistent with the principles of replacement, reduction, and refinement [20]. Consequently, the findings should be interpreted within the context of the relatively small sample size and field-based experimental conditions.

Antimicrobial resistance among *E. coli* isolates

The antimicrobial susceptibility findings demonstrated substantial resistance among the field *E. coli* isolates. All isolates were resistant to at least two of the six antimicrobial agents tested, and isolates no. 1, 3, 4, and 11 were resistant to five of six agents. Resistance was highest to amoxicillin (100%), followed by enrofloxacin and

amoxicillin–clavulanic acid (81.81% each). These findings suggest that several commonly used antimicrobial agents may be less effective against the *E. coli* isolates evaluated in this study.

A similarly high prevalence of antimicrobial resistance has previously been reported among *E. coli* isolated from diarrheic piglets in Thailand. In that study, 97.3% of isolates were classified as multidrug-resistant (MDR), with resistance reported to amoxicillin (100%), oxytetracycline (91.9%), enrofloxacin (89.2%), trimethoprim-sulfamethoxazole (86.5%), amoxicillin–clavulanic acid (81.1%), colistin and gentamicin (75.7% each), ceftriaxone and ceftiofur (64.9% each), and ceftazidime (35.1%) [21]. In Phayao Province, northern Thailand, antimicrobial-resistant *E. coli* was detected at a prevalence of 92.77%, with resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline. Ampicillin resistance was particularly high among isolates from piglets (95.7%), whereas 80% resistance was reported among isolates from adult and sick pigs [22]. Collectively, these reports and the present findings demonstrate substantial antimicrobial resistance among swine-associated *E. coli* in Thailand.

Pretreatment bacterial isolation in the present study identified *E. coli* as the predominant organism in the diarrheic samples. However, we did not molecularly characterize virulence factors associated with enterotoxigenic *E. coli, and* we did not investigate concurrent viral pathogens. Consequently, bacterial isolation alone cannot identify the specific pathotype responsible for the clinical signs or rule out the contribution of other enteric pathogens. Future studies incorporating molecular detection of relevant virulence determinants and simultaneous screening for other enteric pathogens would provide more comprehensive etiological characterization.

The high resistance to amoxicillin and enrofloxacin observed in the present study is also relevant to broader antimicrobial stewardship efforts in food-producing animals [23]. Excessive antimicrobial use in livestock can increase selection pressure and drive the persistence and dissemination of antimicrobial resistance determinants through manure, agricultural environments, the food chain, and other pathways connecting animal and human populations [24]. From a One Health perspective, these concerns reinforce the importance of investigating effective non-antibiotic approaches for controlling bacterial enteric diseases in livestock.

Antibacterial activity of OOE

OOE demonstrated *in vitro* antibacterial activity against the bacterial strains evaluated in this study, with MIC values ranging from 2.5 to 10 mg/mL. Among the swine *E. coli* isolates, isolates no. 2, 3, 4, 5, 10, and 11 had an MIC of 2.5 mg/mL, whereas isolates no. 1, 6, 8, and 9 had an MIC of 5 mg/mL. Isolate no. 7 required 10 mg/mL for growth inhibition (Table 2). Importantly, isolates no. 1, 3, 4, and 11, which exhibited resistance to five of the six antimicrobial agents tested (Table 1), remained susceptible to inhibition by OOE at concentrations of 2.5–5 mg/mL. These findings demonstrate that OOE retained *in vitro* antibacterial activity against isolates displaying extensive phenotypic antimicrobial resistance.

The MIC values observed in the present study were higher than the 0.25–1.05 mg/mL values previously reported for OEO [26]. Differences among studies may reflect variation in oil composition, formulation, bacterial strains, and experimental conditions. Oregano oil contains bioactive constituents, including carvacrol and thymol, and its antibacterial effects have been associated with disruption of bacterial cell membranes and increased membrane permeability [27]. Previous studies have also demonstrated antibacterial activity of oregano oil-based nanoemulsions against *E. coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* [29, 30]. In addition, oregano oil formulations have been investigated for controlling microbial spoilage and foodborne pathogens in food preservation applications [31-33], and oregano oil delivery systems have demonstrated potential for improving its application as an antimicrobial agent [34]. These observations provide context for the antibacterial activity of OOE observed in the present study.

Therapeutic effects on diarrhea and bacterial counts

The *in vivo* findings showed improved fecal scores in both treatment groups over the 7-day observation period. Fecal scores differed significantly between treatments ($p = 0.0359$), and piglets receiving enrofloxacin had 6.11-fold greater odds of having a lower fecal score than those receiving OOE ($p = 0.0274$). However, the treatment × day interaction was not significant ($p = 0.2488$), indicating that the overall temporal pattern of fecal score change did not differ significantly between treatments. Both groups reached a fecal score of 0 by day 7. Thus, although enrofloxacin was associated with lower fecal scores overall, both treatments showed progressive improvement during the observation period.

Fecal bacterial counts also decreased significantly over time ($p < 0.0001$), but there was no significant treatment effect ($p = 0.727$) or treatment × day interaction ($p = 0.793$). By day 5, bacterial counts had decreased to 3.97 ± 0.39 $\log_{10}$ CFU/g in the enrofloxacin group and 3.96 ± 0.83 $\log_{10}$ CFU/g in the OOE group. Therefore, the data do not show a statistically significant difference between OOE and enrofloxacin in reducing fecal bacterial counts during the study period.

Previous research has demonstrated potential effects of OEO on intestinal health. OEO has been associated with antioxidant capacity, intestinal defense, and intestinal structural characteristics However, OEO supplementation increased sow feed intake during certain weeks of lactation but did not improve growth performance or the health status of suckling piglets. [36]. Dietary supplementation of sows during lactation has also been associated with changes in intestinal bacterial communities, including increased relative abundance of *Lactobacillaceae* and bacterial families involved in fiber utilization [9]. These findings indicate that responses to OEO may vary according to formulation, administration strategy, animal population, and measured outcomes.

Effects on body weight gain

Body weight gain did not differ significantly between the treatment groups. Piglets receiving OOE gained 0.73 ± 0.14 kg compared with 0.71 ± 0.33 kg in the enrofloxacin group ($p = 0.884$). Although the numerical mean was marginally higher in the OOE group, the absence of a statistically significant difference does not support a treatment-related advantage in short-term body weight gain.

This finding is consistent with the variable effects of oregano oil on productive performance reported previously. Henn *et al.* [28] reported antimicrobial and antioxidant properties of oregano oil but did not observe improvements in zootechnical performance or effective prevention of diarrhea in weanling pigs. The short duration of the present experiment may also have limited the ability to detect treatment-associated differences in growth. Longer-term studies are needed to determine whether OOE administration has measurable effects on subsequent growth performance.

Implications and study limitations

The combined *in vitro* and *in vivo* findings provide preliminary evidence regarding OOE as a non-antibiotic intervention for diarrhea associated with *E. coli* in piglets. OOE inhibited the tested swine *E. coli* isolates, including isolates exhibiting resistance to several antimicrobial agents, and OOE-treated piglets showed progressive reductions in fecal scores and bacterial counts. Nevertheless, enrofloxacin was associated with significantly lower fecal scores overall, and the present results do not demonstrate superiority of OOE over enrofloxacin. Rather, the absence of significant between-treatment differences in bacterial count reduction and body weight gain indicates that OOE warrants further investigation as a potential alternative or complementary management strategy.

Several limitations should be considered when interpreting these findings. The study included a relatively small number of animals, lacked an untreated negative-control group, had a short follow-up period, and included animals originating from a single facility. In addition, the *E. coli* isolates were not molecularly characterized for specific virulence determinants, and the study did not systematically investigate concurrent enteric pathogens. These factors restrict causal attribution of the observed diarrhea specifically to pathogenic *E. coli* and limit the generalizability of the therapeutic findings. Larger, adequately powered, multi-farm studies incorporating comprehensive pathogen characterization, longer follow-up periods, and standardized OOE formulations are needed to confirm therapeutic efficacy and determine whether the observed effects are reproducible under different production conditions.

CONCLUSION

The present study demonstrated substantial antimicrobial resistance among swine *E. coli* isolates, with particularly high resistance to amoxicillin, enrofloxacin, and amoxicillin–clavulanic acid. OOE showed *in vitro* antibacterial activity against the tested *E. coli* isolates, with MIC values ranging from 2.5 to 10 mg/mL, including activity against isolates resistant to several conventional antimicrobial agents. In the therapeutic trial, fecal scores progressively decreased in both treatment groups and reached 0 by day 7. Although enrofloxacin was associated with significantly lower fecal scores overall, fecal bacterial count reduction and body weight gain did not differ significantly between the OOE and enrofloxacin groups.

A key strength of this study was the combined evaluation of OOE under *in vitro* and *in vivo* conditions. The laboratory component characterized the antimicrobial susceptibility of field *E. coli* isolates and determined their susceptibility to OOE, whereas the therapeutic component directly compared OOE with enrofloxacin in diarrheic

piglets. The randomized complete block design, with litter of origin used as the primary blocking factor, helped reduce potential litter-associated variation. In addition, the simultaneous assessment of fecal scores, quantitative bacterial counts, and short-term body weight gain provided complementary measures of therapeutic response.

Future studies should evaluate OOE in larger, adequately powered, multi-farm trials involving animals from diverse production systems. Future studies should incorporate molecular characterization of *E. coli* virulence determinants and concurrent screening for other bacterial, viral, and parasitic enteric pathogens to strengthen etiological attribution. Further research should also standardize the composition and physicochemical characteristics of OOE and determine the optimal dose, administration frequency, treatment duration, and safety. Longer follow-up periods are required to evaluate sustained clinical recovery, recurrence of diarrhea, bacterial shedding, and subsequent growth performance.

OOE demonstrated *in vitro* antibacterial activity against swine *E. coli* isolates with substantial antimicrobial resistance and was associated with progressive improvement in fecal scores and reductions in bacterial counts in treated piglets. Although enrofloxacin produced a greater overall improvement in fecal scores, no significant differences between treatments were detected in bacterial count reduction or body weight gain. Collectively, these findings support further investigation of OOE as a potential non-antibiotic intervention for *E. coli*-associated diarrhea in pig production, particularly within strategies intended to reduce dependence on conventional antimicrobials.

DATA AVAILABILITY

The data generated or analyzed during the study are included within the manuscript.

GENERATIVE AI DECLARATION

The authors declare that ChatGPT-5.2 (OpenAI) was used solely to improve language, grammar, and readability during manuscript preparation. All scientific content, data analysis, interpretation of results, and conclusions were developed and verified by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

AUTHORS' CONTRIBUTIONS

KC: Conceptualization, Methodology, and writing – original draft. KT, NV, and SJ: Formal analysis and investigation, WJ and WC: Data curation. NK: Supervision, RN: Conceptualization, and writing – review and editing. All authors have read and approved the final version of the manuscript.

ACKNOWOLEDGMENTS

We would like to express our sincere gratitude to Visut Farm for granting us permission to access their facilities and conduct our research. We also extend our thanks to LIC Agrotech Co., Ltd. for their support in providing Essgano Emulsion®. The authors did not receive any funds for this study.

COMPETING INTERESTS

WJ, WC, and NK are employees of LIC Agrotech Co., Ltd. Other authors declare that they have no competing interests.

PUBLISHER'S NOTE

Veterinary World remains neutral with regard to jurisdictional claims in the published institutional affiliations.

REFERENCES

1. Biovet SA. Main causes of diarrhea in pigs [Cited 6 July 2025]. Available from: https://www.veterinariadigital.com/en/articulos.
2. Bergeland ME, Henry SC. Infectious diarrheas of young pigs. Vet Clin North Am Large Anim Pract. 2017;4(2):389–399.
3. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013;6:1451–1474.
4. Nurzyńska-Wierdak R, Walasek-Janusz M. Chemical composition, biological activity, and potential uses of

oregano (*Origanum vulgare* L.) and oregano essential oil. Pharmaceuticals. 2025;18(2):267.
5. Cheng C, Xia M, Zhang X, Wang C, Jiang S, Peng J. Supplementing oregano essential oil in a reduced-protein diet improves growth performance and nutrient digestibility by modulating intestinal bacteria, intestinal morphology, and antioxidative capacity of growing-finishing pigs. Animals (Basel). 2018;8(9):159.
6. Zhang Y, Deng Y, Hao Y, Fang J, Feng J. Effects of supplementation with oregano essential oil during late gestation and lactation on serum metabolites, antioxidant capacity and fecal microbiota of sows. Animals. 2024;14:753.
7. Tan C, Wei H, Sun H, *et al*. Effects of dietary supplementation of oregano essential oil to sows on oxidative stress status, lactation feed intake of sows, and piglet performance. Biomed Res Int. 2015:525218.
8. Marcin A, Sústriková A, Mati R. The effects of aromatic oils on growth performance and physiological parameters in the intestine of weaned pigs. Slovak J Anim Sci. 2006;39:103–107.
9. Hall HN, Wilkinson DJ, Le Bon M. Oregano essential oil improves piglet health and performance through maternal feeding and is associated with changes in the gut microbiota. Anim Microbiome. 2021;3(1):2.
10. Lupindu A. Isolation and characterization of *Escherichia coli* from animals, humans, and environment. London: InTech; 2017.
11. Biochemical test of *Escherichia coli* [Cited 6 July 2025]. Available from: https://microbenotes.com/biochemical-test-of-escherichia-coli-e-coli/. Accessed July 6, 2025.
12. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology; 2016. p. 1–23.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Wayne, PA: CLSI; 2021.
14. Andrews JM, Howe R. BSAC standardized disc susceptibility testing method (version 11). J Antimicrob Chemother. 2011;66:2726–2757.
15. Kyriazakis I, Alameer A, Bučková K, Muns R. Toward the automated detection of behavioral changes associated with the post-weaning transition in pigs. Front Vet Sci. 2023;9:1087570.
16. Gaire TN, Scott HM, Noyes NR, Ericsson AC, Tokach MD, Menegat MB, *et al*. Age influences the temporal dynamics of microbiome and antimicrobial resistance genes among fecal bacteria in a cohort of production pigs. Anim Microbiome. 2023;5:2.
17. Jenkins TP, Ács N, Arendrup EW, Swift A, Duzs Á, Chatzigiannidou I, *et al*. Protecting the piglet gut microbiota against ETEC-mediated post-weaning diarrhoea using specific binding proteins. NPJ Biofilms Microbiomes. 2024;10:42.
18. Pu J, Chen D, Tian G, He J, Zheng P, Mao X, *et al*. Protective effects of benzoic acid, *Bacillus coagulans*, and oregano oil on intestinal injury caused by enterotoxigenic *Escherichia coli* in weaned piglets. Biomed Res Int. 2018:1829632.
19. Song D, Lee J, Yoo Y, Oh H, Chang S, An J, *et al*. Effects of probiotics on growth performance, intestinal morphology, intestinal microbiota weaning pig challenged with *Escherichia coli* and *Salmonella enterica*. J Anim Sci Technol. 2025;67(1):106–136.
20. Russell WMS, Burch RL. The principles of humane experimental technique. London: Methuen & Co Ltd; 1959.
21. Nguyet LTY, Keeratikunakorn K, Kaeoket K, Ngamwongsatit N. Antibiotic resistant *Escherichia coli* from diarrheic piglets from pig farms in Thailand that harbor colistin-resistant *mcr* genes. Sci Rep. 2022;12(1):9083.
22. Boonkerd N, Chaikhiandee S. Antibiotic resistance and genetic variation of *Escherichia coli* isolated from swine feces in Phayao Province. BUU Sci J. 2026;27:1530–1543.
23. Lekagul A, Kirivan S, Tansakul N, Krisanaphan C, Srinha J, Laoprasert T, *et al*. Antimicrobial consumption in food-producing animals in Thailand between 2017 and 2019: the analysis of national importation and production data. PLoS One. 2023;18(4):e0283819.
24. Trinchera M, De Gaetano S, Sole E, Midiri A, Silvestro S, Mancuso G, *et al*. Antimicrobials in livestock farming and resistance: public health implications. Antibiotics. 2025;14(6):606.
25. Placha I, Bacova K, Plachy L. Current knowledge on the bioavailability of thymol as a feed additive in humans and animals with a focus on rabbit metabolic processes. Animals. 2022;12(9):1131.
26. Walasek-Janusz M, Grzegorczyk A, Malm A, Nurzyńska-Wierdak R, Zalewski D. Chemical composition, and antioxidant and antimicrobial activity of oregano essential oil. Molecules. 2024;29(2):435.
27. Fan K, Li L, Wu J. Antibacterial mechanism of ultrasound combined with oregano essential oil nano-emulsion on *Escherichia coli* O157:H7 and application in cherry tomatoes storage. Int J Food Microbiol.

2025;442:111348.
28. Henn J, Bertol T, Fernandes de Moura N, Coldebella A, Brum P, Casagrande M. Oregano essential oil as food additive for piglets: antimicrobial and antioxidant potential. Rev Bras Zootec. 2010;39:1761–1767.
29. Manaa AO, Baghdadi HH, El-Nikhely NA, Heikal LA, El-Hosseiny LS. Oregano oil-nanoemulsions: formulation and evaluation of antibacterial and anticancer potentials. J Drug Deliv Sci Technol. 2022;78:103978.
30. Zhou H, Xu S, Wang J. Oregano nanoemulsion antibacterial effects in *Enterococcus faecalis* root canals. Int Dent J. 2025;75:104841.
31. Ghosh V, Chandwani S, Lonhare A, Balan N. Antibacterial nanoemulsion of oregano oil for food preservation: *in vitro* and *in situ* evaluation against *Escherichia coli*. BioNanoScience. 2024;14:1–11.
32. Hamouda MA, El-saharawy TMHA, El-Saadany AAE-kA, El-diasty EM, El-Ashaal ES. Comparative study of oregano essential oil nanoemulsion and encapsulated in chitosan nanoparticles for enhancing the shelf life of Karish cheese. Innov Food Sci Emerg Technol. 2025;106:104267.
33. Yoncheva K, Benbassat N, Zaharieva MM, Dimitrova L, Kroumov A, Spassova I, *et al*. Improvement of the antimicrobial activity of oregano oil by encapsulation in chitosan-alginate nanoparticles. Molecules. 2021;26(22):7017.
34. Zou Y, Xiang Q, Wang J, Peng J, Wei H. Oregano essential oil improves intestinal morphology and expression of tight junction proteins associated with modulation of selected intestinal bacteria and immune status in a pig model. Biomed Res Int. 2016:5436738.
35. Ding X, Wu X, Zhang K, Bai S, Wang J, Peng H, *et al*. Dietary supplement of essential oil from oregano affects growth performance, nutrient utilization, intestinal morphology and antioxidant ability in Pekin ducks. J Anim Physiol Anim Nutr. 2020;104(4):1067–1074.
36. Ivanova S, Dimitrova A, Nikolova T, Pirgozliev V. Effect of oregano essential oil on the performance and microbiological status of sucking piglet. Sci Pap Ser D Anim Sci. 2023;66(1):93–100.

****