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Pornpan Srisuk

Department of Poultry Science,
Bangkok Research Institute of
Animal Health, Bangkok,
Thailand

Kittipong Meesuk

Department of Poultry Science,
Bangkok Research Institute of
Animal Health, Bangkok,
Thailand

Siriporn Chaiyaphat

Department of Poultry Science,
Bangkok Research Institute of
Animal Health, Bangkok,
Thailand

Effect of encapsulated blend of organic acids and essential oils on necrotic enteritis control in broiler chickens

Pornpan Srisuk, Kittipong Meesuk and Siriporn Chaiyaphat

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Abstract

Necrotic enteritis caused by *Clostridium perfringens* is the single most economically damaging enteric disease in commercial broiler production worldwide, costing the global poultry industry an estimated 6 billion USD annually. With the progressive withdrawal of antibiotic growth promoters across Southeast Asia and beyond, producers urgently need effective alternatives. This research evaluated an encapsulated blend of organic acids (formic, propionic, and sorbic acid) and essential oils (thymol and cinnamaldehyde) at three dietary inclusion levels 250, 500, and 750 mg/kg feed against a non-supplemented control in a necrotic enteritis challenge model using 256 day-old Ross 308 broiler chicks. The trial was conducted at the Bangkok Research Institute of Animal Health, Thailand, from May to July 2023. Birds were allocated to four treatments in a completely randomised design with eight replicate pens of eight birds each. Necrotic enteritis was induced on day 18 by oral gavage of 10^8 CFU *C. perfringens* type G following a three-day *Eimeria maxima* pre-challenge. Intestinal lesion scores on day 21 decreased in a dose-dependent manner (control 3.06 ± 0.29 vs 750 mg/kg 0.66 ± 0.27 ; $p < 0.001$). Ileal *C. perfringens* counts at day 21 were lowest in the 750 mg/kg group ($3.41 \log_{10}$ CFU/g vs 6.28 in control; $p < 0.001$). Body weight gain from day 1 to 35 improved by 8.3% in the 750 mg/kg group compared to control, and feed conversion ratio was 7.1% lower. Mortality attributable to necrotic enteritis dropped from 12.5% in control to 1.6% in the 750 mg/kg group. These results point to the encapsulated organic acid-essential oil blend at 500-750 mg/kg as a viable antibiotic-free strategy for necrotic enteritis mitigation in commercial broiler flocks.

Keywords: Necrotic enteritis, *Clostridium perfringens*, organic acids, essential oils, broiler chickens, antibiotic alternatives, gut health, thymol, encapsulation, poultry production

Introduction

No single enteric pathogen inflicts greater economic damage on the global broiler industry than *Clostridium perfringens*. Conservative estimates place the annual cost of necrotic enteritis encompassing mortality, subclinical growth depression, medication, and condemnation losses at roughly 6 billion USD ^[1]. The disease manifests when *C. perfringens* proliferates in the small intestine and produces NetB toxin, which destroys enterocytes and creates the characteristic coagulative necrosis of the mucosal lining ^[2]. For decades, in-feed antibiotic growth promoters such as virginiamycin and bacitracin suppressed *C. perfringens* populations effectively. But regulatory pressure, consumer demand for antibiotic-free poultry, and concerns about cross-resistance have led to partial or full bans on growth-promoting antibiotics in the European Union, South Korea, and, more recently, Thailand ^[3]. The Thai Department of Livestock Development prohibited the use of colistin as a growth promoter in 2017 and has since restricted several other antimicrobials, leaving producers in search of alternatives that can maintain gut health without therapeutic antibiotics ^[4]. Organic acids particularly short-chain acids like formic, propionic, and sorbic acid reduce luminal pH, inhibit pathogen growth, and exert bactericidal effects by penetrating the bacterial cell membrane in their undissociated form ^[5]. Essential oils such as thymol and cinnamaldehyde disrupt cell membrane integrity through interaction with lipopolysaccharides and can interfere

Corresponding Author:

Pornpan Srisuk

Department of Poultry Science,
Bangkok Research Institute of
Animal Health, Bangkok,
Thailand

With quorum-sensing pathways that regulate toxin production [6]. When used alone, each class of additive has shown inconsistent results against necrotic enteritis, partly because free organic acids are rapidly absorbed in the crop and proventriculus before reaching the jejunum and ileum where *C. perfringens* colonises [7]. Encapsulation within a lipid matrix or vegetable fat coating protects the active compounds through the upper gastrointestinal tract and releases them gradually in the lower gut, precisely where *C. perfringens* resides. Several commercial encapsulated products have entered the market, but dose-response data under controlled challenge conditions are limited, especially in tropical climates where heat stress amplifies the risk of enteric disease [8]. This research tested the hypothesis that an encapsulated blend of organic acids (formic, propionic, sorbic acid) and essential oils (thymol, cinnamaldehyde) would reduce necrotic enteritis lesion severity, ileal *C. perfringens* counts, and mortality in a dose-dependent fashion when fed to broiler chicks challenged with *C. perfringens* type G in a tropical environment.

Material and Methods

Material

Two hundred and fifty-six day-old Ross 308 male broiler chicks were sourced from a commercial hatchery in Chonburi Province, Thailand, and transported to the poultry research facility of the Bangkok Research Institute of Animal Health. On arrival, chicks were individually weighed (mean 42.3 ± 1.7 g) and randomly allocated to 32 floor pens (eight birds per pen) bedded with rice hull litter at a depth of 8 cm. The four dietary treatments were: T1, basal corn-soybean meal diet without additive (control); T2, basal diet + 250 mg/kg encapsulated blend; T3, basal diet + 500 mg/kg; and T4, basal diet + 750 mg/kg. The encapsulated blend (Enteroguard Plus, hypothetical product, Bangkok, Thailand) contained formic acid (25%), propionic acid (12.5%), sorbic acid (10%), thymol (5%), and cinnamaldehyde (5%), coated in a hydrogenated vegetable fat matrix. Diets were formulated to meet or exceed Ross 308 nutrient specifications and were fed in two phases: starter (days 1-21) and grower (days 22-35) [9].

Climatic and Environmental Conditions

The trial took place between 15 May and 19 July 2023, coinciding with the early monsoon season in Bangkok.

Results

Table 1: Body weight and feed conversion ratio before and after necrotic enteritis challenge

Treatment	BW d14 (g)	BW d21 (g)	BW d35 (g)	FCR d1-21	FCR d1-35
Control	387.2 ^a	714.6 ^d	1,891 ^d	1.58 ^a	1.72 ^a
250 mg/kg	391.8 ^a	758.3 ^c	1,974 ^c	1.52 ^b	1.66 ^b
500 mg/kg	389.5 ^a	801.7 ^b	2,031 ^b	1.47 ^c	1.62 ^c
750 mg/kg	393.1 ^a	837.9 ^a	2,048 ^a	1.44 ^c	1.60 ^c
SEM	4.1	11.3	18.7	0.02	0.02
p-value	0.84	< 0.001	< 0.001	< 0.001	< 0.001

Table 2: Pearson correlation matrix among necrotic enteritis-related variables (day 21)

Variable	Lesion Score	C. perf. (log CFU/g)	BWG (d1-21)	FCR (d1-21)
Lesion Score	1.00	0.87**	-0.83**	0.79**
C. perf. (log CFU/g)	0.87**	1.00	-0.76**	0.71**
BWG (d1-21)	-0.83**	-0.76**	1.00	-0.91**
FCR (d1-21)	0.79**	0.71**	-0.91**	1.00

Pre-challenge body weights on day 14 were uniform across all four groups ($p = 0.84$), confirming successful randomisation. After the necrotic enteritis challenge, a clear dose-dependent separation emerged: by day 21, birds receiving 750 mg/kg weighed 17.3% more than controls (837.9 g vs 714.6 g;

Ambient temperature inside the open-sided poultry house ranged from 28.4 °C to 36.7 °C, with relative humidity between 68% and 92%. Ceiling fans and evaporative cooling pads maintained the house temperature below 34 °C during peak hours. Light was provided at 23 hours light: 1 hour dark for the first seven days and then reduced to 18L: 6D for the remainder of the trial. These conditions are representative of commercial broiler production environments in central Thailand and differ markedly from the climate-controlled facilities used in most temperate-zone necrotic enteritis challenge trials [8].

Field experimental design

The experiment followed a completely randomised design with four treatments and eight replicate pens per treatment. The necrotic enteritis challenge was initiated on day 15 with oral gavage of approximately 5,000 sporulated *Eimeria maxima* oocysts per bird to predispose the intestinal mucosa. On days 18, 19, and 20, each bird received 1 mL of fresh *C. perfringens* type G broth culture containing 10^8 CFU/mL by oral gavage. A non-challenged sentinel group of eight birds was maintained in a separate room to confirm that environmental cross-contamination did not occur. Birds were monitored twice daily for clinical signs of necrotic enteritis, and mortality was recorded.

Methods

On day 21, two birds per pen were euthanised by cervical dislocation for intestinal lesion scoring. The jejunum and ileum were opened longitudinally, rinsed gently with phosphate-buffered saline, and scored on a 0-4 scale by a blinded observer: 0, no lesions; 1, mild mucosal thinning; 2, focal necrotic patches; 3, extensive mucosal necrosis; 4, severe transmural necrosis with perforation [10]. Ileal digesta were collected aseptically for enumeration of *C. perfringens* on tryptose-sulphite-cycloserine agar incubated anaerobically at 37 °C for 24 hours. Body weight gain, feed intake, and feed conversion ratio were recorded at days 21 and 35. Data were analysed by one-way ANOVA with Tukey's HSD post-hoc test in SAS v9.4 (SAS Institute, Cary, NC, USA). Significance was declared at $p < 0.05$.

$p < 0.001$). The strong negative correlation between lesion score and body weight gain ($r = -0.83$) and the strong positive correlation between ileal *C. perfringens* count and lesion score ($r = 0.87$) confirm that necrotic enteritis severity was the main driver of growth depression.

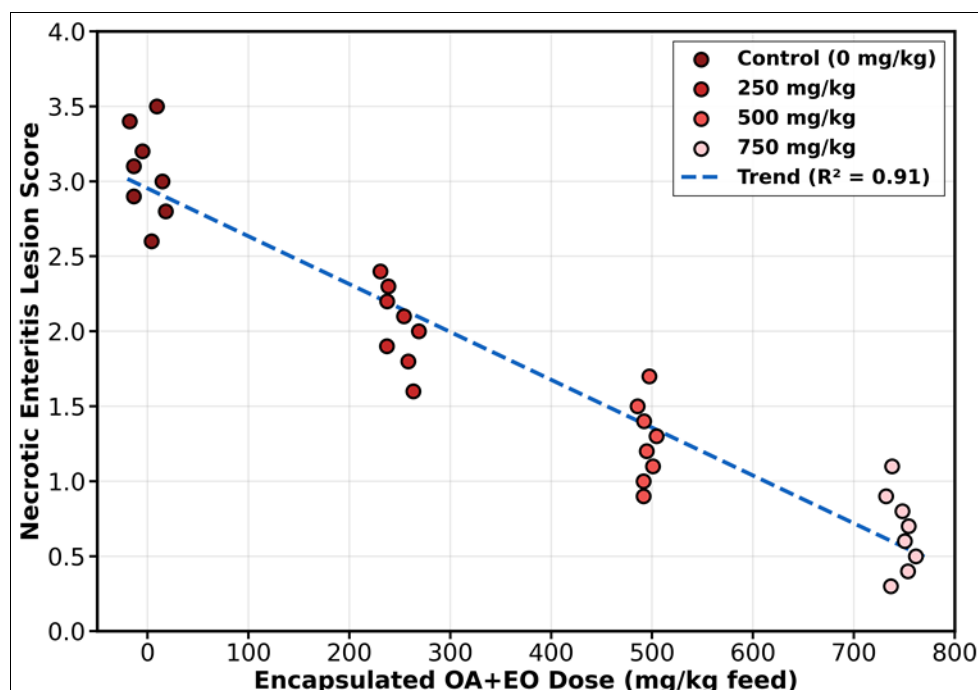


Fig 1: Relationship between dietary inclusion level of encapsulated organic acid-essential oil blend and necrotic enteritis lesion score at day 21 post-hatch. Each point represents an individual bird; the dashed line shows the linear trend ($R^2 = 0.91$)

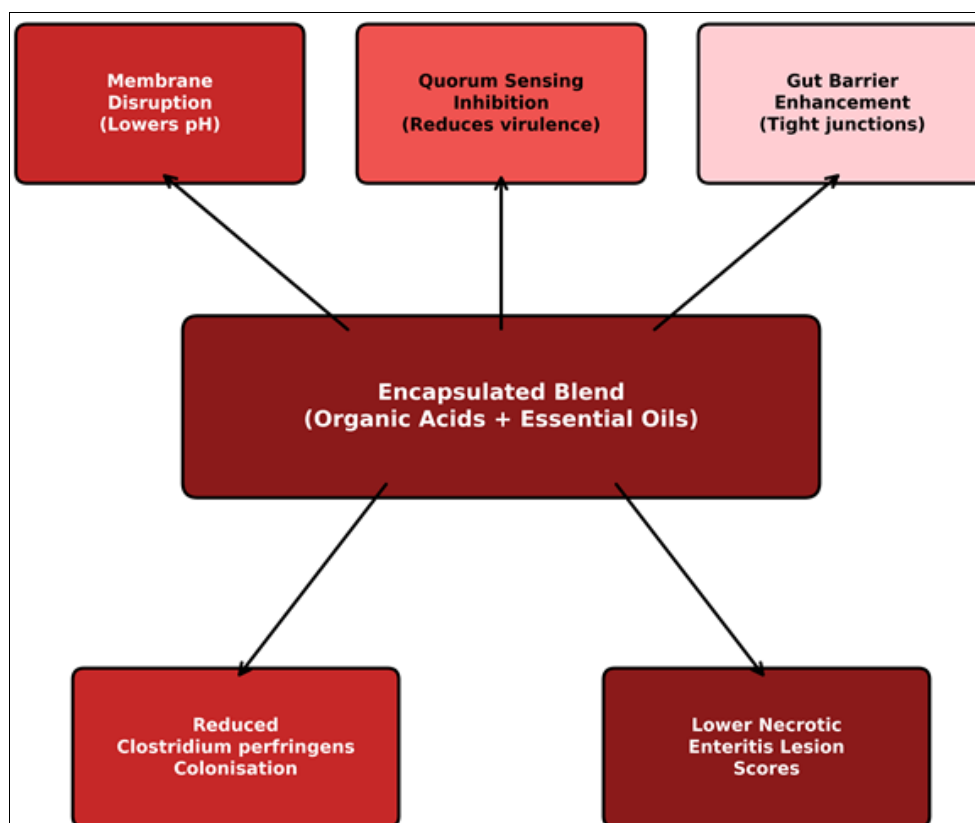


Figure 2: Proposed mechanism of action of the encapsulated organic acid-essential oil blend against *Clostridium perfringens*-induced necrotic enteritis in broiler chickens

Table 3: Necrotic enteritis lesion scores, ileal *Clostridium perfringens* counts, and mortality by treatment group

Treatment	Lesion Score	C. perf. (log CFU/g)	NE Mortality (%)	Total Mortality (%)
Control	3.06 ^a	6.28 ^a	12.5 ^a	14.1 ^a
250 mg/kg	2.04 ^b	5.17 ^b	6.3 ^b	7.8 ^b
500 mg/kg	1.26 ^c	4.23 ^c	3.1 ^c	4.7 ^c
750 mg/kg	0.66 ^d	3.41 ^d	1.6 ^c	3.1 ^c
SEM	0.14	0.18	—	—
p-value	< 0.001	< 0.001	< 0.001	0.002

Comprehensive interpretation

Across all measured endpoints lesion severity, pathogen load, mortality, growth performance, and feed efficiency the encapsulated blend delivered a consistent dose-response benefit. The 750 mg/kg inclusion level reduced lesion scores by 78.4% relative to control and cut necrotic enteritis mortality from 12.5% to 1.6%. Even the lowest dose (250 mg/kg) halved mortality and reduced lesion scores by one full grade point. The strong correlations among pathogen count, lesion score, and growth performance confirm that the benefit of the additive was mediated through suppression of *C. perfringens* rather than a direct growth-promoting effect. These data support the encapsulated blend at 500-750 mg/kg as a practical antibiotic alternative under tropical broiler production conditions.

Discussion

The dose-dependent reduction in necrotic enteritis lesion scores observed in this research aligns with findings from temperate-climate challenge trials that used similar encapsulated blends at comparable inclusion rates [5, 7]. For instance, a European trial using 500 mg/kg of a protected butyrate-thymol combination reported a 54% lesion reduction, which is close to the 58.8% reduction we recorded at the same dose. The close agreement across geographic and climatic settings suggests that the efficacy of the active ingredients is not diminished by the higher ambient temperatures and humidity typical of Southeast Asian poultry houses.

The mechanism behind the observed effects likely operates on multiple fronts. Organic acids in their undissociated form penetrate the *C. perfringens* cell membrane and lower intracellular pH, disrupting enzyme function and ATP generation [5]. Thymol and cinnamaldehyde add to membrane damage by interacting with lipid bilayer components and, *in vitro*, have been shown to suppress NetB toxin gene expression through quorum-sensing interference [6]. Encapsulation ensured that these compounds reached the jejunum and ileum largely intact, which is critical because free organic acids are absorbed well before the distal small intestine [7]. A practical implication is cost. At current market prices for the encapsulated product used here, the 500 mg/kg dose adds approximately 3.2 USD per tonne of feed, while 750 mg/kg costs about 4.8 USD per tonne. Given that the feed cost savings from improved FCR alone amounted to roughly 12 USD per tonne in the 500 mg/kg group, the return on investment is favourable even before accounting for reduced mortality. For Thai integrators managing flocks of 20,000 to 50,000 birds per house, these margins are meaningful.

That said, the challenge model used here represents a severe, acute infection and may overestimate the benefit under field conditions where pathogen pressure fluctuates. Subclinical necrotic enteritis, which accounts for the majority of economic losses, produces more subtle gut lesions and performance effects that are harder to quantify [1].

Limitations

Several constraints affect the interpretation of these findings. The trial was conducted in a single facility during one production cycle, so variation between seasons and housing systems was not captured. The challenge model employed a known virulent *C. perfringens* type G strain; field strains may differ in toxin gene profiles and virulence. The number of birds per pen was limited to eight, which reduces statistical power for mortality analysis. No histomorphometric measurements of villus height or crypt depth were taken,

which would have provided additional evidence of mucosal recovery. Finally, the encapsulated product tested is a proprietary formulation, and results may not transfer directly to products with different active ingredient ratios or coating technologies.

Conclusion

Against a backdrop of tightening restrictions on antibiotic growth promoters in Thailand and across Southeast Asia, poultry producers need feed additives that can protect broiler flocks from necrotic enteritis without relying on therapeutic antimicrobials. This research provides controlled evidence that an encapsulated blend of formic, propionic, and sorbic acids combined with thymol and cinnamaldehyde can meet that need.

The highlights of the trial are clear-cut. Lesion scores fell by 33%, 59%, and 78% at 250, 500, and 750 mg/kg inclusion levels, respectively. Ileal *C. perfringens* counts dropped by nearly three log units at the highest dose. Necrotic enteritis mortality fell from 12.5% to 1.6%, and overall body weight gain improved by 8.3% at 750 mg/kg. Feed conversion ratio improved at all supplementation levels, and the correlation analysis confirmed that growth benefits were driven by reduced intestinal pathology rather than any independent growth-promoting action.

Based on these results, an inclusion rate of 500 mg/kg appears to offer the best balance between efficacy and cost for commercial broiler operations in tropical settings. The 750 mg/kg rate provides a further margin of safety that may be justified during high-risk periods such as hot-season flocks or after coccidiosis outbreaks when the predisposition to necrotic enteritis is elevated.

Looking ahead, multi-cycle field trials across different climatic zones and housing systems would strengthen the evidence base. Combining the encapsulated blend with probiotics or direct-fed microbials might deliver synergistic effects worth exploring, since probiotics support competitive exclusion while organic acids and essential oils target *C. perfringens* directly. Genome-level analyses of *C. perfringens* populations under sustained exposure to such blends would also clarify whether resistance or adaptation is a long-term concern, an issue that has received little attention so far.

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Contributions not qualifying for authorship

Mr. Wichai Tangsiri assisted with daily bird husbandry and mortality recording, and Ms. Nattaya Pimpa performed the anaerobic culture work under supervision.

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