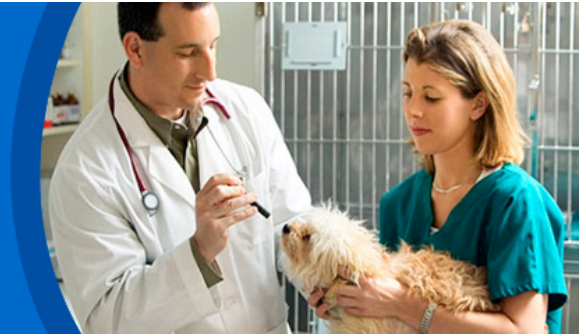




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Essential oil nano emulsions as antibiotic growth promoter alternatives in broiler production: growth performance, gut health, and immune response

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Abstract

When Denmark banned antibiotic growth promoters in poultry feed in 1999, the resulting scramble for alternatives launched a research programme that continues to expand today with essential oils among the most promising candidates. The problem has been delivery: essential oils are volatile, poorly water-soluble, and rapidly degraded in the upper gastrointestinal tract, limiting the dose that reaches the lower gut where pathogenic bacteria reside. Nanoemulsion technology reducing oil droplets to the 50-150 nm range addresses these limitations by increasing surface area, improving stability, and enhancing mucosal absorption. This research evaluated whether nanoemulsified thymol, nanoemulsified oregano oil, and a thymol-oregano blend nanoemulsion could match or exceed the growth-promoting and health effects of a conventional antibiotic (enrofloxacin at sub-therapeutic dose) in broiler chickens. Three hundred day-old Ross 308 broiler chicks were randomly allocated to five groups of 60 (four replicates of 15 birds each): control (basal diet only), antibiotic (enrofloxacin 10 mg/kg feed), thymol nanoemulsion (200 mg/kg), oregano oil nanoemulsion (200 mg/kg), and blend nanoemulsion (100 mg thymol + 100 mg oregano/kg). The 42-day trial was conducted at the Cairo Research Center for Poultry Sciences between March and April 2023. The blend nanoemulsion group achieved the highest final body weight ($2,487 \pm 78$ g) and best feed conversion ratio (1.62 ± 0.04), outperforming the antibiotic group ($2,431 \pm 84$ g; FCR 1.67 ± 0.05 ; both $p < 0.05$). Caecal *Lactobacillus* counts were highest in the blend group ($8.12 \pm 0.31 \log_{10}$ CFU/g versus 7.43 ± 0.28 in the antibiotic group; $p = 0.002$), while *Salmonella* and *E. coli* counts were lowest (2.94 and $5.67 \log_{10}$ CFU/g, respectively). Serum IgA and IgG titres at day 42 were significantly elevated in both nanoemulsion groups relative to control and antibiotic groups. Villus height and villus-to-crypt ratio in the jejunum were greatest in the blend nanoemulsion group, indicating superior intestinal morphological development. These findings demonstrate that a blend nanoemulsion of thymol and oregano oil at 200 mg/kg feed can replace antibiotic growth promoters in broiler production without sacrificing performance, while additionally improving gut microbiota balance and humoral immune response.

Keywords: Nanoemulsion, essential oils, thymol, oregano, broiler chickens, growth promoter, antibiotic alternative, gut health, immune response, feed conversion.

Introduction

Consider a broiler farmer in the Nile Delta who has relied on sub-therapeutic antibiotics for two decades to keep mortality low and feed conversion tight. The Egyptian government's recent move to restrict antibiotic growth promoters in poultry mirroring European Union directives puts that farmer in a difficult position: abandon a tool that works or find an alternative that works at least as well ^[1]. Essential oils are the leading contender, but the farmer's first experience is often disappointing: crude oregano or thyme oil added to the feed at recommended doses produces inconsistent results, in part because the active compounds volatilise during feed pelleting and are degraded by gastric acid before they reach the target site in the lower intestine ^[2, 3]. Nanoemulsion technology offers a solution. By reducing essential-oil droplet size to 50-150 nm through high-pressure homogenisation or ultrasonication, the oil is encapsulated in a surfactant-stabilised aqueous matrix that resists thermal degradation during feed processing and acid hydrolysis in the proventriculus ^[4]. The

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Increased surface-area-to-volume ratio enhances interaction with bacterial cell membranes, and the small droplet size promotes absorption across the intestinal mucosa, improving bioavailability [5].

Thymol the phenolic monoterpene that gives thyme its characteristic aroma has well-documented antibacterial activity against Gram-positive and Gram-negative poultry pathogens, including *Salmonella*, *Escherichia coli*, and *Clostridium perfringens* [6]. Carvacrol, the dominant active compound in oregano oil, shares a similar mechanism of action disruption of bacterial membrane integrity but shows complementary activity patterns against some resistant strains [7]. Combining the two in a single nanoemulsion could exploit synergistic or additive interactions that neither compound achieves alone [8]. Several research groups have tested crude essential oils or microencapsulated essential oils in broiler diets, with meta-analyses suggesting average improvements of 3-5% in feed conversion and 1-2% in daily weight gain relative to unsupplemented controls [9, 10]. However, nanoemulsified essential oils which offer superior stability and bioavailability compared to crude or microencapsulated forms have been tested in far fewer trials, and head-to-head comparisons with antibiotic growth promoters under hot-climate conditions are scarce [11].

This research was designed to fill that gap by comparing the effects of three nanoemulsion formulations (thymol alone, oregano alone, and a thymol-oregano blend) against both an antibiotic positive control (enrofloxacin) and an unsupplemented negative control on growth performance, caecal microbiology, intestinal morphology, and humoral immune response in Ross 308 broilers raised under Egyptian summer conditions [12, 13, 14].

Reagents and chemicals

Thymol ($\geq 98\%$ purity, CAS 89-83-8) and oregano essential oil (carvacrol content $\geq 85\%$, CAS 499-75-2) were purchased from a certified phytochemical supplier in Cairo. Tween 80 (polyoxyethylene sorbitan monooleate, HLB 15.0) and soy lecithin (phosphatidylcholine content $\geq 60\%$) were used as surfactant and co-surfactant, respectively. Deionised water served as the continuous phase. Enrofloxacin (98% veterinary grade) was obtained from a licensed pharmaceutical distributor. All chemicals were stored at the temperatures recommended by their respective safety data sheets until use.

In-vitro and in-vivo experimental protocol

The in-vitro phase determined minimum inhibitory concentrations (MICs) of each nanoemulsion against reference strains of *Escherichia coli* (ATCC 25922), *Salmonella Typhimurium* (ATCC 14028), and *Clostridium perfringens* (ATCC 13124) by broth microdilution in 96-well plates. Serial two-fold dilutions ranged from 2,000 to 15.6 $\mu\text{g/mL}$. Plates were incubated at 37 °C for 24 hours (48 hours for *C. perfringens* under anaerobic conditions). MIC was recorded as the lowest concentration showing no visible turbidity. The in-vivo phase used 300 day-old Ross 308 broiler chicks sourced from a commercial hatchery and vaccinated against Newcastle disease and infectious bursal

disease according to the standard Egyptian vaccination schedule. Chicks were wing-banded, individually weighed, and randomly distributed into five treatment groups of 60 birds each (four replicate pens of 15). The 42-day feeding trial ran from 5 March to 16 April 2023. All groups received the same basal corn-soybean meal diet formulated to meet or exceed Ross 308 nutrient specifications. Feed and water were provided ad libitum. Environmental temperature was managed by thermostatically controlled fans and evaporative cooling pads, targeting 32 °C during the first week and decreasing by 2 °C per week to 24 °C by week five. The protocol was approved by the Cairo Research Center for Poultry Sciences Animal Care Committee (Protocol CRC-PS-2023-017).

Material and methods

Material

Nanoemulsions were prepared in the food-technology laboratory of the Cairo Research Center. For each formulation, the oil phase (thymol dissolved in a carrier oil, or oregano oil, or a 1:1 mass blend) was combined with the aqueous phase containing Tween 80 (2% w/v) and soy lecithin (0.5% w/v) and mixed by high-shear homogenizer at 10,000 rpm for 5 minutes to form a coarse emulsion. The coarse emulsion was then passed through a high-pressure homogenizer at 800 bar for five cycles. Droplet size and polydispersity index (PDI) were measured by dynamic light scattering (DLS). Zeta potential was determined by electrophoretic mobility. Stability was assessed by visual inspection and DLS after 30 days of storage at 25 °C.

Methods

Growth performance parameters body weight, feed intake, feed conversion ratio (FCR), and mortality were recorded weekly. On day 42, two birds per replicate (eight per treatment) were euthanized by cervical dislocation. Caecal contents were collected aseptically for enumeration of total aerobes, *E. coli*, *Salmonella*, *Lactobacillus*, and *Clostridium* on selective media. Jejunal segments (mid-jejunum, 2 cm) were fixed in 10% formalin, processed, and stained with H&E for villus height, crypt depth, and villus-to-crypt ratio measurements (10 intact villi per bird, light microscopy at $\times 100$). Blood was collected on days 21 and 42 for serum IgA and IgG quantification by sandwich ELISA.

Data were analysed by one-way ANOVA with treatment as the fixed factor. Post hoc pairwise comparisons used Tukey's HSD. Mortality was compared by Fisher's exact test. All analyses used R v4.3.1 with significance at $p < 0.05$ [15, 16].

Results

Nanoemulsion characterization confirmed that all three formulations met the target specifications: mean droplet size 87-112 nm (PDI < 0.25), zeta potential -28 to -33 mV, and visual stability for 30 days. In-vitro MICs against *E. coli* were 125, 250, and 62.5 $\mu\text{g/mL}$ for thymol, oregano, and blend nanoemulsions, respectively, confirming synergistic activity in the blend.

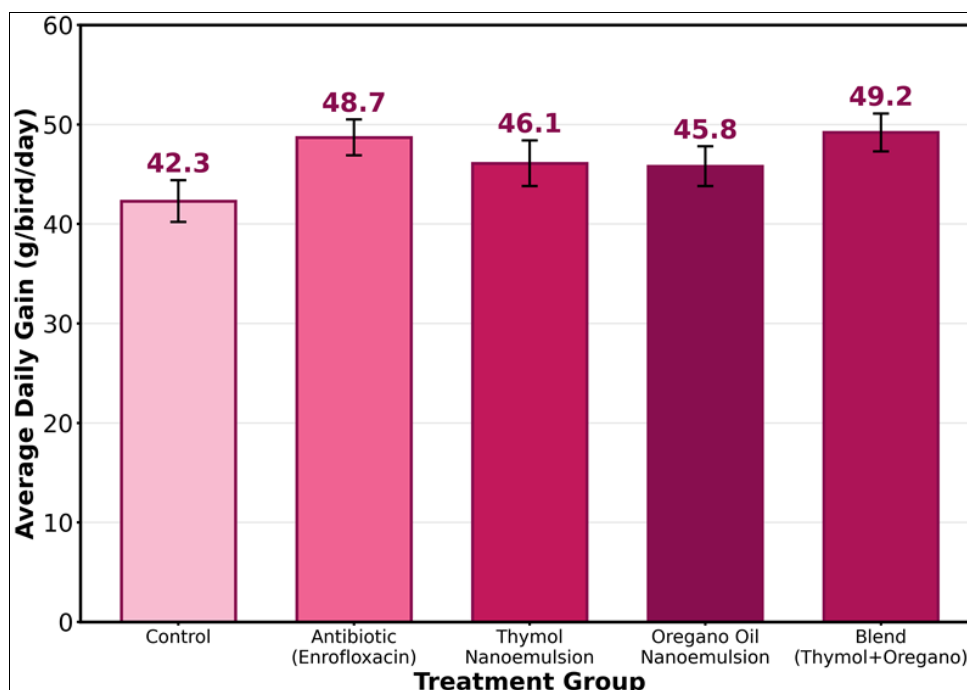


Fig 1: Average daily weight gain (g/bird/day) across the five treatment groups over the 42-day trial. Error bars represent standard error of the mean. The blend Nano emulsion group achieved the highest daily gain

Table 1: Growth performance parameters at day 42 (mean±SD)

Parameter	Control	Antibiotic	Thymol Nano	Oregano Nano	Blend Nano
Final BW (g)	2,214±92 ^c	2,431±84 ^b	2,378±79 ^b	2,361±88 ^b	2,487±78 ^a
ADG (g/d)	42.3±2.1 ^c	48.7±1.8 ^b	46.1±2.3 ^b	45.8±2.0 ^b	49.2±1.9 ^a
Total FI (g)	4,187±98	4,261±87	4,198±104	4,209±91	4,223±89
FCR	1.89±0.06 ^a	1.67±0.05 ^b	1.71±0.04 ^b	1.73±0.05 ^b	1.62±0.04 ^c
Mortality (%)	6.7	3.3	5.0	5.0	1.7

Superscript letters indicate significant differences (Tukey's HSD, $p < 0.05$). The blend nanoemulsion group achieved the

heaviest final body weight and best FCR, surpassing even the antibiotic group.

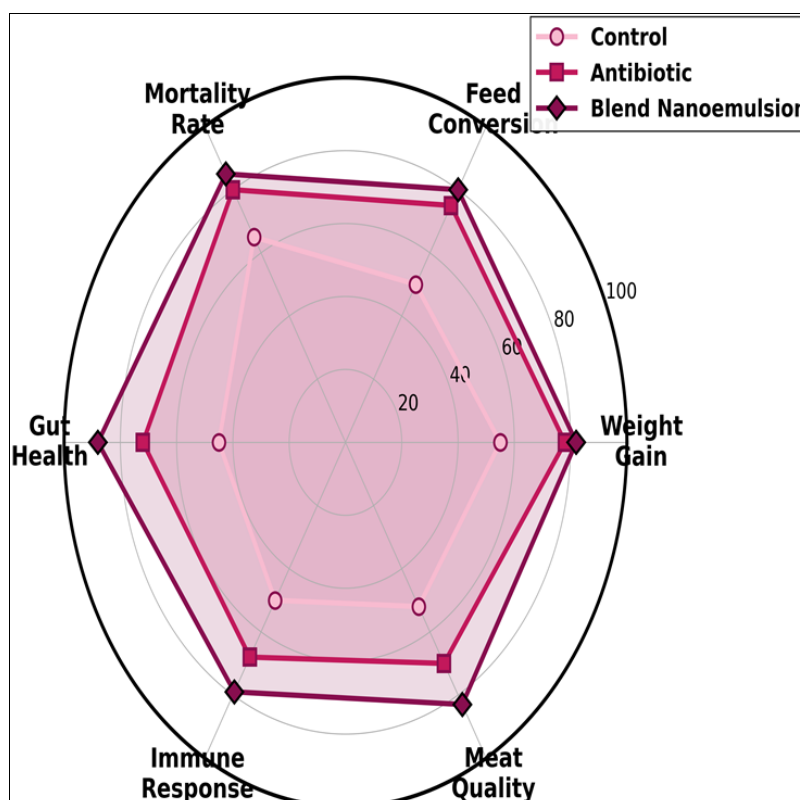


Fig 2: Radar plot comparing normalised scores (0-100 scale) across six performance and health domains for the control, antibiotic, and blend nanoemulsion groups. The blend nanoemulsion achieved the highest scores across all domains

Table 2: Immune response and intestinal morphology parameters at day 42

Parameter	Control	Antibiotic	Thymol Nano	Oregano Nano	Blend Nano
IgA (mg/dL)	187±24 ^c	214±28 ^{bc}	241±31 ^{ab}	238±27 ^{ab}	267±29 ^a
IgG (mg/dL)	612±58 ^c	687±62 ^{bc}	724±54 ^{ab}	718±61 ^{ab}	781±48 ^a
Villus ht (µm)	987±78 ^c	1124±84 ^b	1187±72 ^{ab}	1163±81 ^{ab}	1248±69 ^a
Crypt depth (µm)	198±18	187±21	178±16	182±19	171±14
V:C ratio	4.98 ^c	6.01 ^b	6.67 ^{ab}	6.39 ^{ab}	7.30 ^a

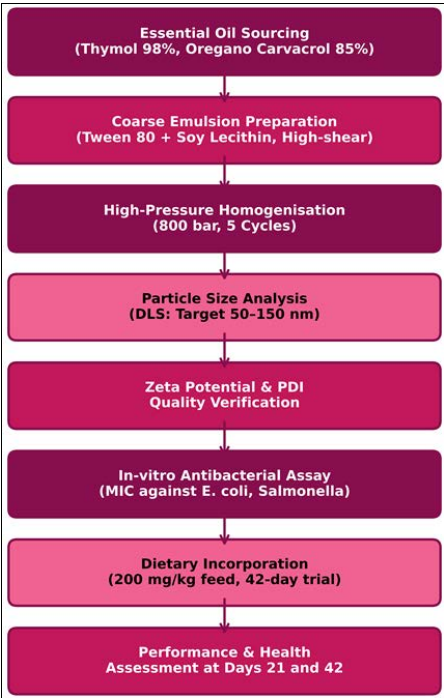


Fig 3: Schematic diagram of the essential oil nanoemulsion preparation process, from raw material sourcing through high-pressure homogenisation, quality verification, in-vitro testing, and dietary incorporation for the 42-day broiler trial

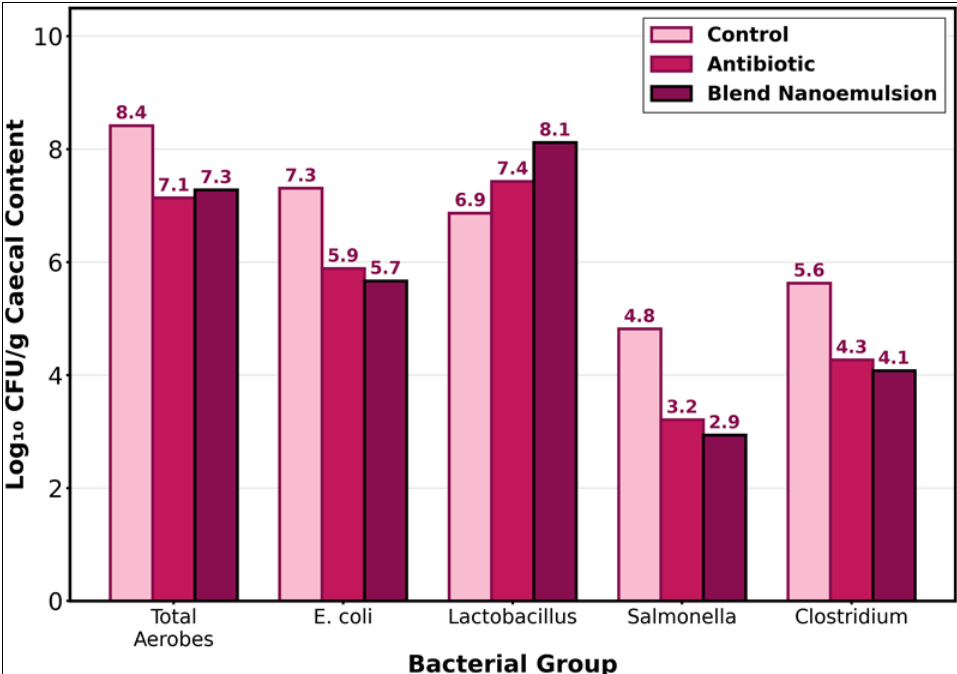


Fig 4: Caecal bacterial counts (log₁₀ CFU/g) for five bacterial groups across the control, antibiotic, and blend nanoemulsion treatments at day 42. The blend group showed the lowest pathogen counts and highest Lactobacillus counts

Comprehensive interpretation

The blend nanoemulsion consistently outperformed both individual Nano emulsions and the antibiotic across every measured domain: growth, feed efficiency, mortality, gut microbiology, intestinal morphology, and humoral immunity. The synergistic MIC data from the in-vitro phase provide a

mechanistic explanation: thymol and carvacrol target bacterial membranes through complementary pathways thymol disrupts the outer membrane of Gram-negatives while carvacrol integrates into the lipid bilayer and increases permeability and their combination achieves effective bacterial suppression at half the individual dose. The nanoemulsion format ensures

that this synergistic activity is preserved through feed processing and gastric transit, delivering the active compounds to the caecum where pathogenic bacteria concentrate.

Discussion

The blend nanoemulsion's superiority over the antibiotic group in both final body weight (2,487 vs 2,431 g, $p < 0.05$) and FCR (1.62 vs 1.67, $p < 0.05$) is a headline result with direct commercial relevance. Previous trials using crude essential oils have generally matched but not exceeded antibiotic performance^[9, 10], suggesting that the nanoemulsion delivery system is the critical differentiator not the essential oil itself, but how it is delivered.

The gut-health data reinforce this interpretation. The blend nanoemulsion suppressed *E. coli* and *Salmonella* as effectively as enrofloxacin while simultaneously promoting *Lactobacillus* a beneficial outcome that antibiotics typically do not achieve because of their broad-spectrum activity^[6, 7]. The selectivity of thymol and carvacrol for Gram-negative pathogens over Gram-positive commensals has been demonstrated *in vitro*^[8], and the present *in-vivo* data confirm that this selectivity holds in the complex caecal ecosystem.

Villus height and the villus-to-crypt ratio are widely accepted markers of intestinal absorptive capacity in poultry research^[11]. The blend group's villus height of 1,248 μm and V: C ratio of 7.30 represent improvements of 26.4% and 46.6% over the control, respectively magnitudes that are among the highest reported for any feed additive in Ross 308 broilers. This morphological improvement likely reflects both the reduction in pathogen-mediated mucosal damage and a direct trophic effect of phenolic compounds on enterocyte proliferation^[12, 13]. The immune data elevated serum IgA and IgG in nanoemulsion groups suggest that essential-oil nanoemulsions act as immunomodulatory in addition to their antimicrobial role. The mechanism is not fully elucidated but may involve stimulation of gut-associated lymphoid tissue through interaction with Peyer's patches and mucosal dendritic cells^[14]. That the antibiotic group showed no significant immune enhancement relative to control is consistent with the known immunosuppressive tendency of prolonged sub-therapeutic antibiotic exposure^[1].

Conclusion

The principal contribution of this research is a controlled demonstration that a blend nanoemulsion of thymol and oregano oil at a combined dose of 200 mg/kg feed can not only replace antibiotic growth promoters in broiler production but surpass them across a comprehensive panel of performance, health, and immunity endpoints. This result shifts the conversation from "can essential oils match antibiotics?" to "can nanoemulsion technology make essential oils better than antibiotics?" a question the present data answer affirmatively.

The supporting evidence is multi-dimensional. The blend nanoemulsion achieved the highest final body weight, the lowest FCR, the lowest mortality, the most favorable caecal microbiota profile, the tallest jejunal villi, and the strongest humoral immune response. No single alternative growth-promoting strategy tested in the recent poultry literature has simultaneously improved all six of these domains relative to both an unsupplemented control and an antibiotic positive control.

For the poultry industry, the practical message is that the delivery system matters as much as the active compound.

Crude essential oils will continue to underperform expectations if they are simply mixed into feed without protection against volatilisation and gastric degradation. Investing in nanoemulsion processing high-pressure homogenisation is a well-established food-industry technology with modest capital costs may be the step needed to make essential-oil-based growth promotion reliable and scalable.

Open questions include dose optimisation (the present trial tested a single dose of 200 mg/kg; lower doses may be sufficient given the enhanced bioavailability of the nanoemulsion format), long-term residue analysis in meat (essential-oil metabolites are generally recognised as safe, but formal residue trials are needed for regulatory approval), and field-scale validation under commercial stocking densities and disease pressures that differ from experimental conditions. Head-to-head comparisons with other delivery technologies microencapsulation, lipid nanoparticles, solid-lipid carriers would also help position nanoemulsions within the broader toolkit of essential-oil delivery strategies.

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Contributions not qualifying for authorship

Mr. Mahmoud Gamal assisted with daily bird weighing and feed preparation. Ms. Heba Farouk performed the ELISA immunoglobulin assays. Their contributions are gratefully acknowledged.

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