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Co-encapsulation of formic acid and *Satureja hortensis* essential oil enhances antibacterial and anti-inflammatory activities against *Salmonella enterica*

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ABSTRACT

The emergence of multidrug-resistant *Salmonella enterica* has increased the need for alternative antibacterial strategies based on natural bioactive compounds with multifunctional properties. This study developed spray-dried co-encapsulated formic acid (FA) and *Satureja hortensis* essential oil (SEO) nanocapsules and evaluated their physicochemical properties, antibacterial activity, and anti-inflammatory potential. GC–MS analysis showed SEO was rich in carvacrol (41.26%), thymol (18.74%), p-cymene (14.32%), and γ-terpinene (11.48%). SEM and DLS analyses indicated spherical nanoparticles with a mean dry diameter of 442.91 nm and a hydrodynamic diameter of 346.93 nm (PDI 0.246), indicating uniform distribution. Encapsulation efficiency was 81.47 ± 2.16%. The formulation showed strong antibacterial activity against *Salmonella enterica*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Klebsiella pneumoniae* in disk diffusion, MIC, and MBC assays. In vivo, Salmonella-infected BALB/c mice receiving FA-SEO nanocapsules showed improved body weight, feed intake, antioxidant enzymes, and immunoglobulin levels, with reduced liver enzymes and intestinal inflammation. Histopathology confirmed protective effects on intestinal and hepatic tissues, while iNOS expression in the jejunum was significantly reduced, indicating anti-inflammatory action. Overall, FA-SEO co-encapsulation enhanced stability and biological activity, offering a promising supportive strategy against Salmonella infection and inflammation, though further mechanistic and safety studies are needed. These findings suggest that combining organic acid and essential oil components through nano-encapsulation may improve antimicrobial stability, enhance bioavailability, and enable synergistic interactions between active compounds, thereby increasing efficacy compared with non-encapsulated forms. Such multifunctional nanoformulations may also reduce required dosages, minimize potential side effects, and provide a natural alternative to conventional antibiotics in food-producing animals, contributing to antimicrobial resistance mitigation strategies. Moreover, the observed modulation of inflammatory markers such as iNOS highlights the potential immunomodulatory role of the formulation beyond direct antibacterial effects, supporting tissue protection during infection. Further long-term in vivo studies are required to confirm safety, dosage optimization, and mechanistic pathways and clinical applicability in the future.

1. Introduction

The accelerating emergence of antimicrobial-resistant bacterial pathogens represents one of the most critical threats to global public health, food safety, and sustainable livestock production (Elbehiry et al., 2025). Among foodborne pathogens, *Salmonella enterica* remains a leading cause of gastrointestinal infection worldwide and is associated with substantial morbidity, economic losses, and increased healthcare burden in both humans and animals (Mkangara, 2023). The widespread and often indiscriminate use of antibiotics in clinical practice and animal agriculture has significantly contributed to the evolution of multidrug-resistant Salmonella strains, thereby compromising the efficacy of conventional antimicrobial therapies and increasing the likelihood of treatment failure (Gajic et al., 2025). These concerns have intensified

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the search for alternative antimicrobial strategies based on natural bioactive compounds capable of reducing pathogen load while minimizing the selective pressure associated with antibiotic resistance development.

In recent years, organic acids and plant-derived essential oils have received considerable scientific attention as promising alternatives to conventional antimicrobial agents due to their broad-spectrum biological activities and favorable safety profiles. Formic acid (FA), a short-chain organic acid extensively used in food preservation and animal nutrition, has demonstrated substantial antimicrobial activity against a wide range of pathogenic microorganisms, particularly Gram-negative bacteria such as Salmonella spp. (Ricke et al., 2020). The antimicrobial efficacy of FA is primarily attributed to its ability to penetrate bacterial cell membranes in the undissociated form, reduce intracellular pH, disrupt membrane integrity, and interfere with essential metabolic pathways (Wang et al., 2024). In addition to its direct antibacterial effects, FA has also been reported to improve intestinal health and modulate gastrointestinal microbial balance under infection-associated conditions (Abd El-Hack et al., 2025).

Satureja hortensis L. (summer savory), a medicinal and aromatic plant belonging to the *Lamiaceae* family, is recognized as a rich source of biologically active essential oils with pronounced pharmacological potential (Ejaz et al., 2023). The biological properties of *Satureja hortensis* essential oil (SEO) are mainly attributed to its high content of phenolic monoterpenes, particularly carvacrol and thymol, along with other bioactive constituents such as p-cymene and γ-terpinene (Tumbarski et al., 2025). These compounds exhibit potent antibacterial, antioxidant, and anti-inflammatory activities through multiple mechanisms, including disruption of bacterial membrane structure, increased membrane permeability, leakage of intracellular components, and modulation of oxidative and inflammatory signaling pathways (Ejaz et al., 2023; Sivamaruthi et al., 2024). Moreover, increasing evidence suggests that SEO and its phenolic constituents may attenuate inflammation and oxidative stress associated with bacterial infections (Ejaz et al., 2023).

Despite their promising biological activities, the practical application of organic acids and essential oils is frequently constrained by several physicochemical limitations, including volatility, low aqueous solubility, chemical instability, sensitivity to environmental conditions, and rapid degradation during processing and gastrointestinal transit (Dima et al., 2021). To overcome these limitations, encapsulation technologies have emerged as effective approaches for improving the stability, controlled release behavior, bioavailability, and biological performance of sensitive bioactive compounds (Kaushalya and Rupasinghe, 2024). Among available encapsulation techniques, spray drying is widely regarded as one of the most practical and industrially scalable methods due to its operational simplicity, cost-effectiveness, reproducibility, and ability to efficiently entrap volatile compounds within protective carrier matrices (Rezvankhah et al., 2020). Furthermore, encapsulation may reduce undesirable sensory properties and improve the dispersibility of hydrophobic essential oils in aqueous systems (Kandasamy and Naveen, 2022).

The combination of formic acid and *Satureja hortensis* essential oil may provide complementary and potentially synergistic biological effects through simultaneous targeting of multiple bacterial and inflammatory pathways. Formic acid primarily exerts antimicrobial activity through intracellular acidification and metabolic disruption, whereas phenolic constituents of SEO such as carvacrol and thymol destabilize bacterial membranes and modulate oxidative and inflammatory responses. Therefore, co-delivery of FA and SEO through encapsulation may enhance antibacterial efficacy while simultaneously providing antioxidant and anti-inflammatory protection during Salmonella-associated infection. In addition, co-encapsulation may improve the physicochemical stability and retention of volatile bioactive compounds during storage and gastrointestinal exposure.

Although the antimicrobial properties of formic acid and essential oils have been extensively investigated individually, limited information is available regarding the biological activity of co-encapsulated FA and SEO formulations against *Salmonella enterica* infection. Therefore, the present study aimed to develop spray-dried co-encapsulated FA-SEO nanocapsules and evaluate their physicochemical characteristics, antibacterial activity, and anti-inflammatory effects using both in vitro antibacterial assays and an In vivo Salmonella-infected mouse model.

2. Materials and methods

2.1. Chemicals and reagents

Formic acid (FA), maltodextrin, modified starch, whey protein concentrate (WPC), Tween 80, Brain Heart Infusion (BHI) broth, Mueller–Hinton agar, and other analytical-grade chemicals were purchased from Merck (Darmstadt, Germany). *Satureja hortensis* essential oil (SEO) was obtained from a commercial medicinal plant supplier in Mashhad, Iran. The chemical composition of SEO was characterized based on gas chromatography–mass spectrometry (GC–MS) analysis provided by the manufacturer. The major constituents identified in the essential oil were carvacrol (41.26%), thymol (18.74%), p-cymene (14.32%), γ-terpinene (11.48%), β-caryophyllene (4.17%), and α-terpinene (2.63%). These compounds are widely recognized for their antibacterial, antioxidant, and anti-inflammatory properties.

2.2. Preparation of FA-SEO Nanocapsules

FA-SEO nanocapsules were prepared using a spray-drying method. Briefly, maltodextrin, modified starch, and whey protein concentrate were dissolved in distilled water under continuous magnetic stirring at room temperature. Tween 80 was added as an emulsifying agent, followed by the gradual addition of formic acid and SEO into the aqueous phase during homogenization. The mixture was then homogenized at high speed to obtain a stable emulsion before spray drying. The emulsion was dried using a laboratory-scale spray dryer under controlled operating conditions. The resulting powder was collected and stored in airtight containers at 4 °C until further analysis (Moharreri et al., 2022a).

2.3. Physicochemical characterization of nanocapsules

The morphology and surface characteristics of the FA-SEO nanocapsules were examined using scanning electron microscopy (SEM). Dried particles were mounted on aluminum stubs using conductive carbon tape and coated with a thin layer of gold before imaging. SEM images were obtained using a Zeiss scanning electron microscope operated at an accelerating voltage of 15 kV under high-vacuum conditions. Particle size distribution, polydispersity index (PDI), and zeta potential were determined using dynamic light scattering (DLS) (Malvern Zetasizer, UK).

2.4. Bacterial strains and antibacterial activity

The antibacterial activity of the FA-SEO formulation was evaluated against *Salmonella enterica* PTCC 1709, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603. The identity of *Salmonella enterica* PTCC 1709 was confirmed using colony morphology on selective media and standard biochemical tests, including triple sugar iron agar (TSI), citrate utilization, urease production, indole production, and motility assays. Antibacterial activity was initially evaluated using the disk diffusion method on Mueller–Hinton agar plates. Sterile disks impregnated with the FA-SEO nanocapsule suspension were placed on inoculated agar surfaces and incubated at 37 °C for 24 h. The diameters of inhibition zones were subsequently measured in millimeters. Minimum inhibitory concentration (MIC) and minimum bactericidal

concentration (MBC) values were determined using the broth microdilution method in BHI broth medium. Serial dilutions of the formulation were prepared within a concentration range of 0–400 μg/mL. MIC values were defined as the lowest concentration inhibiting visible bacterial growth, whereas MBC values were defined as the lowest concentration showing no bacterial colony growth after subculture on agar plates. Because diffusion-based assays may underestimate the antibacterial activity of encapsulated essential oils due to the limited diffusion of hydrophobic compounds through agar media, MIC and MBC values were considered more reliable indicators of antibacterial activity in the present study (Najafi et al., 2021; Mogana et al., 2020).

2.5. Experimental animals and study design

Twenty male BALB/c mice (20 ± 5 g) were obtained from the Razi Vaccine and Serum Research Institute (Mashhad, Iran). Animals were maintained under controlled environmental conditions (22 ± 1 °C, relative humidity 60–70%, and a 12 h light/dark cycle) with free access to water and standard laboratory feed. Following a one-week acclimatization period, mice were randomly assigned to four experimental groups ($n = 5$ animals/group):

T1: Healthy control group receiving a standard diet.

T2: *Salmonella enterica*-infected group receiving a standard diet.

T3: Infected group receiving diet supplemented with FA-SEO nanocapsules.

T4: Infected group treated with gentamicin.

The experimental period lasted 30 days. Salmonella infection was induced according to previously reported procedures (Moharreri et al., 2022a). Each mouse was considered an experimental unit. Due to ethical considerations and the exploratory nature of the study, five animals per group were used. The ethical committee of Islamic Azad University of Mashhad approved the mice trial, adhering to international animal ethics laws, norms, and regulations under the number IR.IAU.MSHD.REC.1402.026.

2.6. Biochemical and antioxidant analyses

At the end of the experimental period, blood samples were collected from anesthetized animals for biochemical analyses. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) concentrations were measured using commercial diagnostic kits according to the manufacturer's instructions. Antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), were determined using standard spectrophotometric methods. Serum immunoglobulin levels were also evaluated to assess immune-related responses.

2.7. Histopathological and morphometric analyses

Jejunum and liver tissues were collected and fixed in 10% buffered formalin. Samples were dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Histopathological changes and intestinal morphometric parameters, including villus height and crypt depth, were evaluated using light microscopy (Navarrete et al., 2015).

2.8. Quantitative real-time PCR analysis

The mRNA expression level of inducible nitric oxide synthase (iNOS) in jejunum tissue was evaluated using quantitative real-time PCR (RT-qPCR). Total RNA was extracted from approximately 20 mg of jejunum tissue using a commercial RNA extraction kit according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and samples with A260/A280 ratios between 1.8 and 2.0 were considered acceptable. RNA integrity was further confirmed by agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized from 100 ng of total RNA using a reverse transcription kit (Parstous, Iran). Quantitative PCR was performed using SYBR Green master mix (Thermo Fisher Scientific, USA) on a Bio-Rad real-time PCR system. GAPDH was used as the housekeeping gene. Relative gene expression levels were calculated using the 2ˆ−ΔΔCt method after confirmation of amplification specificity by melt-curve analysis. The primer sequences used for RT-qPCR were as follows: GAPDH: Forward: 5′-gcagggggagccaaaacggt-3′; Reverse: 5′-gggtggcagtgatggcatgg-3′; iNOS: Forward: 5′-caccttggagttcacccagt-3′; Reverse: 5′-accactcgtacttgggatgc-3′ (Moharreri et al., 2022a).

2.9. Statistical analysis

All experiments were performed in triplicate unless otherwise stated. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS software version 21. Data normality was evaluated using the Shapiro–Wilk test before analysis. Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical characterization of FA-SEO nanocapsules

The morphology and physicochemical properties of the co-encapsulated formic acid (FA) and *Satureja hortensis* essential oil (SEO) nanocapsules were evaluated using scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential analysis. SEM images revealed that the spray-dried particles were predominantly spherical with relatively smooth surfaces and low aggregation, indicating successful encapsulation and proper formation of the carrier matrix. The average particle diameter observed by SEM under dry-state conditions was approximately 442.91 nm (Fig. 1). Similar spherical morphologies have been reported for spray-dried essential oil nanocapsules prepared using polysaccharide- and protein-based wall materials, where smooth and compact particle structures were associated with improved stability and encapsulation performance (Kaushalya and Rupasinghe, 2024; Klojdová et al., 2023). Particle size distribution was further characterized using DLS analysis. The FA-SEO nanocapsules exhibited a hydrodynamic diameter of 346.93 nm with a polydispersity index (PDI) of 0.246, indicating relatively uniform particle distribution. In addition, the zeta potential value of −20.4 mV suggested moderate colloidal stability of the formulation (Fig. 2). Previous studies have shown that nanocapsules with PDI values below 0.3 generally exhibit acceptable homogeneity and dispersion stability for biological applications (Dima et al., 2021). The relatively close agreement between DLS and SEM measurements further confirmed the successful production of particles within the nanoscale range. Minor differences between the two methods were expected because DLS measures the hydrodynamic diameter of particles dispersed in liquid medium, whereas SEM evaluates particle dimensions in the dry state.

The encapsulation efficiency of the FA-SEO formulation was 81.47 ± 2.16%, indicating efficient incorporation of the active compounds within the carrier matrix. However, some formulation-related parameters, including loading capacity, release kinetics, moisture content, water activity, storage stability, and retention efficiency of individual compounds, were not evaluated in the present study and should therefore be considered limitations of the formulation characterization.

Comparable encapsulation efficiencies have previously been reported for encapsulated essential oil systems prepared by spray drying. For example, Klojdová et al. (Klojdová et al., 2023) reported encapsulation efficiencies ranging from 70% to 85% for spray-dried thyme and oregano essential oils, while Kaushalya and Rupasinghe (Kaushalya and Rupasinghe, 2024) demonstrated that polysaccharide-protein carrier

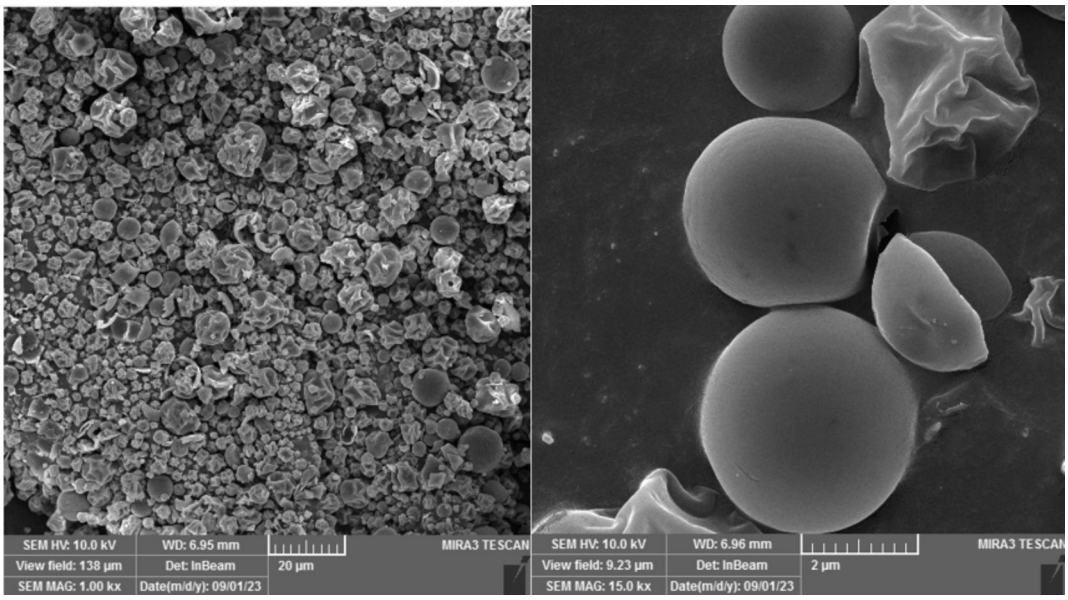

Fig. 1. SEM image of the *nanocapsules* containing FA and SEO.

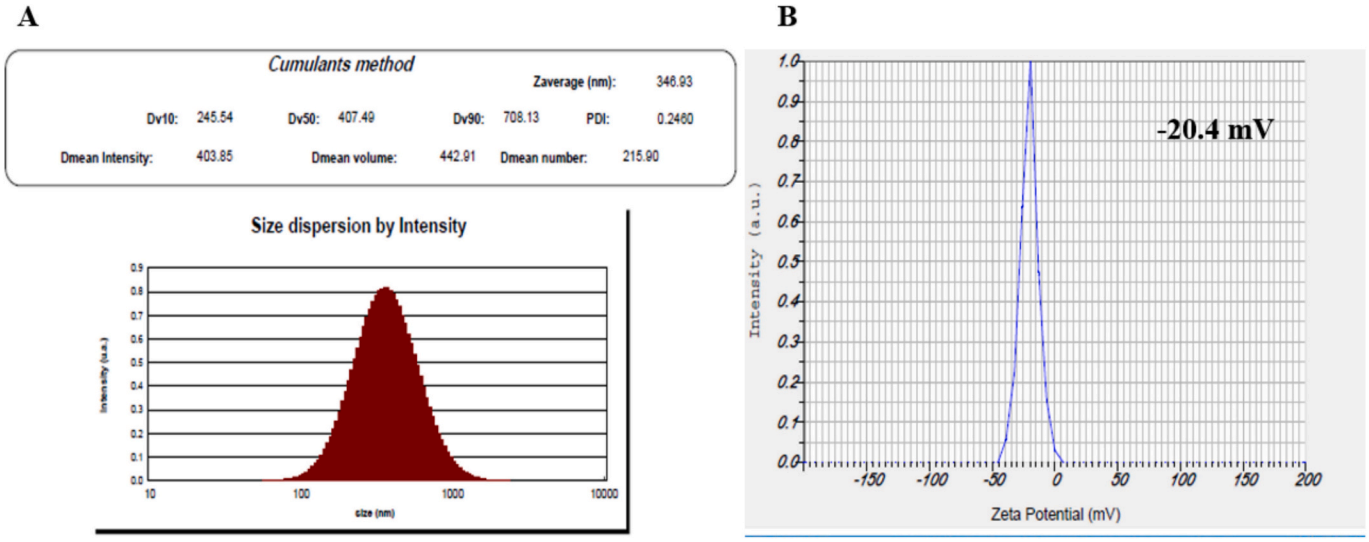

Fig. 2. The DLS and Zeta potential of the *nanocapsules* containing FA and SEO.

systems could effectively retain volatile bioactive compounds during thermal processing. The relatively high encapsulation efficiency observed in the present study may be attributed to the combined use of maltodextrin, modified starch, and whey protein concentrate, which likely enhanced emulsion stability and reduced volatilization of SEO constituents during drying.

Encapsulation is known to improve the physicochemical stability and controlled release of volatile compounds by protecting them against oxidation, evaporation, and environmental degradation (Dima et al., 2021). In addition, nanoencapsulation may enhance the dispersibility and bioavailability of hydrophobic essential oil components, thereby improving their biological activity (Weisany et al., 2022). The nanoscale particle size observed in the present study may therefore contribute to improved interaction of the encapsulated compounds with bacterial cell membranes and gastrointestinal tissues.

3.2. Antibacterial activity of FA-SEO nanocapsules

The antibacterial activity of the FA-SEO nanocapsules was evaluated against several clinically important Gram-positive and Gram-negative bacterial strains using disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The results demonstrated that the encapsulated formulation inhibited the growth of all tested bacterial strains, including *Salmonella enterica*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Klebsiella pneumoniae* (Fig. 3, Tables 1 and 2).

In the disk diffusion assay, the FA-SEO nanocapsules produced inhibition zones ranging from 10.66 to 14.33 mm. The highest inhibitory activity was observed against *S. enterica* (14.33 ± 0.3 mm), whereas the lowest inhibition zone was recorded against *S. aureus* (10.66 ± 0.1 mm). As expected, gentamicin exhibited larger inhibition zones than the nanocapsule formulation due to its strong broad-spectrum antibacterial activity. Nevertheless, the results confirmed that the FA-SEO nanocapsules possessed measurable antibacterial activity against both Gram-positive and Gram-negative bacteria.

The antibacterial effects observed in the present study may be associated with the complementary mechanisms of action of formic acid and the major bioactive compounds identified in SEO, particularly

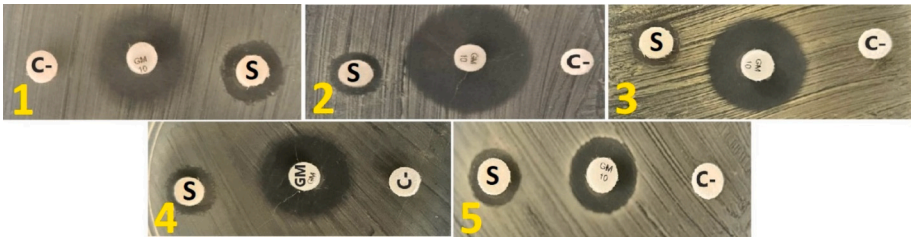

Fig. 3. Disk diffusion assay of FA-SEO nanocapsules against pathogenic bacterial strains. 1: *Salmonella enterica*; 2: *Staphylococcus epidermidis*; 3: *Staphylococcus aureus*; 4: *Escherichia coli*; 5: *Klebsiella pneumoniae*; S: FA-SEO nanocapsules; C−: Negative control; GM: Gentamicin.

Table 1
Disk diffusion assay (mm) of FA-SEO nanocapsules against tested bacterial strains.

Material	*S. enterica*	*S. epidermidis*	*S. aureus*	*E. coli*	*K. pneumoniae*
FA-SEO nanocapsules (100 μg/mL)	14.33±0.3	11.33±0.2	10.66±0.1	12.66±0.1	13.33±0.1
Gentamicin (10 μg)	21±0.10	27±0.23	22±0.14	21±0.11	16±0.09

Table 2
MIC, MBC, and MBC/MIC ratio of FA-SEO nanocapsules against bacterial strains.

	Nanocapsules containing FA and SEO		
Bacteria	MIC (μg/mL)	MBC (μg/mL)	MBC/MIC ratio
S. enterica	200±0.2^{c}	300±00^{b}	1.5 (+)^{b}
S. epidermidis	300±0.1^{a}	350±00^{a}	1.16 (+)^{c}
S. aureus	250±0.1^{b}	350±00^{a}	1.4 (+)^{b}
E. coli	300±0.1^{a}	350±00^{a}	1.16 (+)^{c}
K. pneumoniae	200±0.3^{c}	350±00^{a}	1.75 (+)^{a}

Letters a, b, and c in each column indicate significant differences between treatments.

carvacrol and thymol. Formic acid can penetrate bacterial cells in its undissociated form, leading to intracellular acidification and disruption of essential metabolic processes (Ricke et al., 2020). In parallel, phenolic monoterpenes such as carvacrol and thymol can interact with bacterial cell membranes, alter membrane permeability, and induce leakage of intracellular components (Sivamaruthi et al., 2024). Previous studies have shown that combining organic acids with essential oils may enhance antibacterial activity through simultaneous disruption of membrane integrity and intracellular homeostasis (Abd El-Hack et al., 2025).

The MIC and MBC results further confirmed the antibacterial efficacy of the FA-SEO nanocapsules. MIC values ranged between 200 and 300 μg/mL, while MBC values ranged from 300 to 350 μg/mL (Table 2). The lowest MIC values were observed against *S. enterica* and *K. pneumoniae* (200 μg/mL), indicating greater sensitivity of these bacteria to the encapsulated formulation. In contrast, *S. epidermidis* and *E. coli* exhibited slightly higher MIC values (300 μg/mL). The MBC/MIC ratios ranged from 1.16 to 1.75, suggesting that the formulation exerted bactericidal rather than merely bacteriostatic effects against the tested microorganisms.

The relatively strong activity against *S. enterica* is particularly important considering the growing prevalence of multidrug-resistant Salmonella strains associated with foodborne infections. Similar antibacterial effects have been reported for encapsulated essential oil formulations rich in carvacrol and thymol. Bouaouina et al. (Bouaouina et al., 2022) demonstrated that nanoencapsulated phenolic essential oils exhibited enhanced antibacterial activity against Gram-negative bacteria due to improved stability and dispersion of hydrophobic compounds. Likewise, Klojdová et al. (Klojdová et al., 2023) reported that spray-dried essential oil systems could effectively inhibit pathogenic bacteria while improving the physicochemical stability of volatile compounds.

The nanoscale size of the FA-SEO particles may also have contributed to the observed antibacterial activity. Smaller particles generally provide a larger surface area and closer interaction with bacterial membranes, potentially enhancing cellular uptake and membrane disruption (Kaushalya and Rupasinghe, 2024). In addition, encapsulation may protect volatile compounds from oxidation and evaporation, thereby improving their retention and biological effectiveness during antimicrobial testing (Zhu et al., 2021).

It should be noted that diffusion-based methods may underestimate the antibacterial activity of encapsulated essential oils because hydrophobic compounds often exhibit limited diffusion through agar media. Therefore, MIC and MBC assays were considered more reliable indicators of antibacterial activity in the present study. In addition, several important control groups, including blank nanocapsules, free FA, free SEO, and physical mixtures of FA and SEO, were not included. Consequently, the individual contribution of each component and the precise effect of co-encapsulation could not be fully distinguished. Future studies incorporating these controls are needed to better clarify the synergistic interactions between FA and SEO and to further optimize the antibacterial performance of the formulation.

3.3. Effects of FA-SEO nanocapsules on growth performance and biochemical parameters

The In vivo findings demonstrated that *Salmonella enterica* infection markedly impaired growth performance and altered serum biochemical parameters in infected mice. Animals in the infected untreated group (T2) showed significantly lower body weight gain and reduced feed intake compared with the healthy control group (T1), indicating the negative impact of Salmonella-associated intestinal inflammation and metabolic disturbance on nutrient utilization and overall physiological

Table 3
Effects of FA-SEO nanocapsules on body weight gain and food intake in *Salmonella enterica*-infected mice.

Average	T1	T 2	T3	T 4
Average daily weight gain (mg)	123 ±4.35^{a}	51 ±3.82^{d}	115.1 ±5.27^{b}	97 ±6.11^{c}
Average daily food consumption (g)	5.6 ±0.11^{b}	3.8 ±0.09^{c}	6.2±0.07^{a}	5.0 ±0.06^{b}

T1: mice with normal diet; T2: mice with normal diet + infection with *S. enterica*; T3: Mice with a diet enriched with *nanocapsules* containing FA and SEO + infection with *S. enterica*; T4: Mice with normal diet + *S. enterica* infection + antibiotics; Letters a, b, c, and d indicate significant differences between different treatments.

status (Table 3). In contrast, dietary supplementation with FA-SEO nanocapsules significantly improved both weight gain and daily food intake in infected animals.

Mice receiving FA-SEO nanocapsules (T3) exhibited substantially higher average daily weight gain and feed consumption compared with the infected untreated group. The improvement in growth performance may be associated with the antibacterial activity of the formulation, which likely reduced intestinal microbial burden and alleviated infection-related intestinal damage. In addition, the antioxidant and anti-inflammatory properties of the encapsulated compounds may have contributed to improved nutrient absorption and metabolic efficiency. Similar findings have been reported in recent studies showing that organic acids and phytogenic compounds can enhance intestinal integrity, nutrient digestibility, and growth performance under bacterial challenge conditions (Wang et al., 2024; Abd El-Hack et al., 2025).

The FA-SEO-treated group also demonstrated reduced serum ALT, AST, and ALP concentrations compared with the infected untreated group, suggesting attenuation of hepatic injury induced by Salmonella infection. Elevated liver enzyme levels are commonly associated with systemic inflammation, oxidative stress, and hepatocellular damage during enteric bacterial infections (Bello et al., 2024). The reduction in serum hepatic enzymes observed in the present study may therefore indicate a protective effect of the formulation against infection-associated liver injury.

The hepatoprotective effects of the FA-SEO nanocapsules may be linked to the biological activities of both formic acid and the phenolic constituents of SEO, particularly carvacrol and thymol. These compounds have been reported to exhibit strong free radical scavenging activity and the ability to modulate inflammatory signaling pathways, thereby reducing oxidative tissue damage (Ejaz et al., 2023). Recent evidence has further demonstrated that phenolic monoterpenes can suppress pro-inflammatory cytokine production and improve antioxidant defense systems during bacterial infections (Gopalsamy et al., 2026). Moreover, formic acid has been shown to improve gut microbial balance and intestinal health, which may indirectly reduce systemic inflammatory responses and metabolic stress (Abd El-Hack et al., 2025).

Interestingly, in several evaluated parameters, the FA-SEO-treated group demonstrated biological responses comparable to or slightly better than those observed in the gentamicin-treated group. This finding may reflect the multifunctional properties of the encapsulated formulation. Unlike conventional antibiotics, which primarily target bacterial growth, the FA-SEO nanocapsules may simultaneously exert antibacterial, antioxidant, and anti-inflammatory effects. The nanoencapsulation system may also improve the stability and bioavailability of the active compounds, thereby enhancing their biological efficacy. Similar multifunctional effects have recently been reported for nanoencapsulated phytogenic formulations used against enteric pathogens and inflammation-associated disorders (Kaushalya and Rupasinghe, 2024). Nevertheless, some limitations should be acknowledged. The present study did not evaluate bacterial load in intestinal tissues or quantify inflammatory cytokines, which could provide additional mechanistic insight into the observed biological effects. Furthermore, the absence of treatment groups receiving free FA, free SEO, or blank nanocapsules limits the ability to distinguish the individual contribution of each component and the specific effect of nanoencapsulation. Future studies incorporating these analyses are warranted to better clarify the mechanisms underlying the protective effects of the FA-SEO formulation.

3.4. Antioxidant and immunological responses

Salmonella infection induced marked oxidative stress in infected mice, as evidenced by the significant reduction in antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in the untreated infected group (T2) compared with healthy controls (Table 4). These findings are consistent with previous reports demonstrating that *Salmonella enterica* infection promotes excessive production of reactive oxygen species (ROS), leading to oxidative damage, cellular dysfunction, and impairment of endogenous antioxidant defense systems (Bello et al., 2024).

Table 4
Blood biochemical, antioxidant, and immunological parameters in mice receiving different treatments.

Parameters	T1	T2	T3	T4
AST (U/L)	107 ± 4.12^{c}	182 ± 3.20^{a}	114 ± 2.07^{b}	83 ± 1.67^{d}
ALT (U/L)	109 ± 2.11^{b}	120 ± 3.42^{a}	102 ± 3.20^{b}	95 ± 2.43^{c}
ALP (U/L)	344 ± 5.91^{b}	545 ± 6.62^{a}	276 ± 4.37^{c}	262 ± 3.65^{c}
GPX (U/mL)	189 ± 2.05^{b}	151 ± 3.44^{c}	199 ± 1.76^{a}	202 ± 2.94^{a}
SOD (U/mL)	331 ± 6.39^{a}	172 ± 7.12^{d}	272 ± 5.49^{b}	268 ± 4.63^{c}
CAT (U/mL)	139 ± 3.55^{a}	116 ± 2.71^{c}	130 ± 3.15^{b}	128 ± 1.86^{b}
IgM (mg/dl)	6.9 ± 0.11^{a}	3.7 ± 0.09^{c}	5.8 ± 0.12^{b}	5.9 ± 0.14^{b}
IgA (mg/dl)	5.5 ± 0.07^{a}	3.9 ± 0.06^{c}	4.8 ± 0.10^{b}	5.1 ± 0.17^{b}
IgG (mg/dl)	7.8 ± 0.04^{a}	5.1 ± 0.14^{b}	7.2 ± 0.08^{a}	7.6 ± 0.10^{a}

Treatment 1: mice with normal diet; Treatment 2: mice with normal diet + infection with *S. enterica*; Treatment 3: Mice with a diet enriched with *nanocapsules* containing FA and SEO + infection with *S. enterica*; Treatment 4: Mice with normal diet + *S. enterica* infection + antibiotics; Letters a, b, c, and d in the same row indicate significant differences between different treatments.

Dietary supplementation with FA-SEO nanocapsules significantly improved antioxidant enzyme activities in infected animals. Mice receiving the FA-SEO formulation showed higher SOD, CAT, and GPx activities compared with untreated infected mice, indicating attenuation of infection-associated oxidative stress. The observed antioxidant effects may be largely attributed to the phenolic constituents of SEO, particularly carvacrol and thymol, which are known to possess strong free radical scavenging and lipid peroxidation inhibitory activities (Bello et al., 2024). Recent studies have further demonstrated that phenolic monoterpenes can enhance cellular antioxidant defense mechanisms through modulation of oxidative stress-related signaling pathways, including Nrf2-mediated antioxidant responses (Grigore-Gurgu et al., 2025).

In addition to the direct antioxidant properties of SEO constituents, the reduction in oxidative stress may also be associated with the antibacterial activity of the formulation. By decreasing bacterial colonization and limiting inflammatory tissue injury, the FA-SEO nanocapsules may reduce ROS generation during infection. Similar findings have recently been reported for phytogenic nanoformulations and organic acid-based feed additives, which improved antioxidant status and reduced oxidative tissue damage in bacterial challenge models (Wang et al., 2024; Abd El-Hack et al., 2025). The FA-SEO-treated group also demonstrated improved serum immunoglobulin concentrations compared with infected untreated animals. Serum IgM, IgA, and IgG levels were significantly reduced following Salmonella infection, reflecting impaired immune function and physiological stress. However, supplementation with FA-SEO nanocapsules partially restored immunoglobulin concentrations toward normal values. Improvement of humoral immune responses may be associated with reduced intestinal inflammation, improved gut integrity, and the biological activities of the phenolic compounds present in SEO.

Previous studies have shown that carvacrol- and thymol-rich essential oils may influence immune-related pathways by modulating oxidative stress and inflammatory mediators (Gopalsamy et al., 2026). Furthermore, preservation of intestinal barrier integrity may enhance mucosal immune function and improve immunoglobulin production during enteric infections. The improved IgA levels observed in the present study are particularly important because secretory IgA plays a key role in intestinal immune defense against pathogenic bacteria.

Interestingly, the antioxidant and immunological responses observed in the FA-SEO-treated group were generally comparable to those of the gentamicin-treated group. This finding may suggest that the FA-SEO formulation exerted broader biological effects beyond direct antibacterial activity. Unlike conventional antibiotics, the encapsulated

phytogenic formulation may simultaneously reduce oxidative stress, support intestinal health, and attenuate inflammation.

Nevertheless, several limitations should be acknowledged. The present study evaluated only a limited number of immunological biomarkers, and inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-10, and NF-κB signaling pathways were not investigated. Therefore, the use of the term "immunomodulatory" should be interpreted cautiously. Future studies involving comprehensive cytokine profiling and molecular inflammatory analyses are needed to better clarify the immunological mechanisms underlying the observed protective effects.

3.5. Histopathological and morphometric findings

Histopathological examination of jejunum and liver tissues revealed substantial pathological alterations in the Salmonella-infected untreated group (T2). Infected animals exhibited inflammatory cell infiltration, epithelial disruption, villus shortening, and hepatic tissue degeneration, confirming the severe intestinal and systemic effects of *Salmonella enterica* infection (Fig. 4). These pathological changes are consistent with previous studies demonstrating that Salmonella infection can impair intestinal barrier integrity, induce oxidative tissue injury, and promote hepatic inflammation through bacterial translocation and systemic inflammatory responses (Wang et al., 2024; Moharreri et al., 2022a).

In contrast, mice treated with FA-SEO nanocapsules showed marked improvement in intestinal and hepatic tissue architecture. Histological sections from the FA-SEO-treated group demonstrated reduced inflammatory infiltration, improved epithelial integrity, and partial restoration of normal villus morphology. These findings suggest that the encapsulated formulation exerted protective effects against infection-associated tissue injury.

Morphometric analysis further supported the histopathological observations. The infected untreated group exhibited significant reductions in villus height and villus width, along with increased crypt depth, indicating intestinal mucosal damage and elevated epithelial turnover during infection (Table 5). However, treatment with FA-SEO nanocapsules significantly improved villus height and villus width compared with infected untreated mice. Improvement in intestinal morphology may enhance nutrient digestion, absorptive capacity, and mucosal barrier function, thereby contributing to the improved growth performance observed in treated animals.

Table 5
Morphometric analysis of the jejunum in the mice receiving different treatments.

	Villus Height (μm)	Villus Width (μm)	Crypt Depth (μm)
T1	650±6.98^{a}	246±7.33^{a}	164±9.32^{b}
T2	438±9.11^{c}	164±5.49^{c}	200±6.27^{a}
T3	506±7.36^{b}	209±9.08^{b}	195±4.58^{a}
T4	625±8.41^{a}	223±6.62^{a}	171±6.73^{b}

T 1: mice with normal diet; T2: mice with normal diet + infection with *S. enterica*; T3: Mice with a diet enriched with *nanocapsules* containing FA and SEO + infection with *S. enterica*; T 4: Mice with normal diet + *S. enterica* infection + antibiotics.
Different letters in the same column indicated a significant difference ($p<0.05$). The analysis was performed in triplicates.

The beneficial effects of the FA-SEO formulation on intestinal morphology may be attributed to several complementary mechanisms. The antibacterial activity of formic acid and SEO likely reduced bacterial colonization and intestinal inflammation, while the antioxidant properties of carvacrol and thymol may have protected intestinal epithelial cells against oxidative damage. Recent studies have shown that phytogenic compounds rich in phenolic monoterpenes can preserve tight junction integrity, reduce inflammatory injury, and improve intestinal morphology during bacterial challenge conditions (Gopalsamy et al., 2026).Nanoencapsulation may also have contributed to the observed biological effects by improving the stability and controlled release of the active compounds in the gastrointestinal tract. Similar protective effects of encapsulated phytogenic compounds on intestinal morphology and tissue integrity have previously been reported in Salmonella-challenged animal models (Moharreri et al., 2022a; Moharreri et al., 2022b). Interestingly, the histopathological improvements observed in the FA-SEO-treated group were relatively comparable to those observed in the gentamicin-treated group, suggesting that the encapsulated phytogenic formulation may provide substantial protective effects during

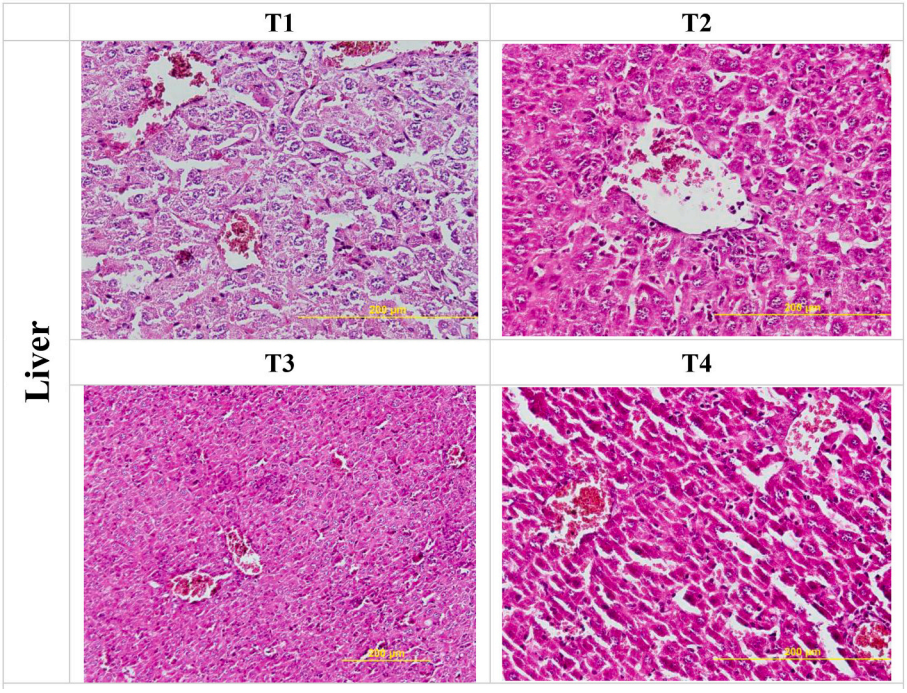

Fig. 4. Histopathological changes in liver tissues of mice receiving different treatments. T1: healthy control group; T2: *Salmonella enterica*-infected group; T3: infected group receiving FA-SEO nanocapsules; T4: infected group treated with gentamicin.

enteric bacterial infection. Nevertheless, the present study did not evaluate bacterial translocation, intestinal permeability markers, or inflammatory cytokines in tissue samples. Therefore, additional molecular and microbiological analyses are needed to further clarify the mechanisms underlying the observed tissue-protective effects.

3.6. iNOS gene expression

The relative expression of inducible nitric oxide synthase (iNOS) in jejunum tissue was significantly elevated in the *Salmonella enterica*-infected untreated group compared with healthy controls, indicating activation of intestinal inflammatory pathways during bacterial infection (Fig. 5). Increased iNOS expression is commonly associated with excessive nitric oxide (NO) production and inflammatory tissue injury during enteric infections. Previous studies have shown that Salmonella infection can stimulate macrophage activation and induce pro-inflammatory signaling cascades, leading to overexpression of iNOS and subsequent oxidative and nitrosative stress in intestinal tissues (Bello et al., 2024).

Dietary supplementation with FA-SEO nanocapsules significantly reduced iNOS gene expression compared with infected untreated mice, suggesting attenuation of infection-associated inflammatory responses. The reduction in iNOS expression observed in the present study may be related to both the antibacterial and antioxidant properties of the encapsulated formulation. By limiting bacterial proliferation and reducing intestinal inflammation, the FA-SEO nanocapsules may indirectly suppress activation of inflammatory signaling pathways associated with iNOS induction. The anti-inflammatory effects of the formulation may also be attributed to the phenolic constituents of SEO, particularly carvacrol and thymol. These compounds have been reported to modulate inflammatory mediators and oxidative stress pathways through inhibition of NF-κB activation and reduction of reactive oxygen species production (Gopalsamy et al., 2026). Recent evidence further suggests that phenolic monoterpenes can downregulate iNOS expression and suppress nitric oxide overproduction during inflammatory conditions (Grigore-Gurgu et al., 2025). In addition, formic acid may contribute to the improvement of intestinal microbial balance and the reduction of pathogen-associated inflammatory responses, thereby supporting intestinal homeostasis during bacterial challenge (Abd El-Hack et al., 2025).

Interestingly, the gentamicin-treated group also demonstrated reduced iNOS expression compared with the infected untreated group, which was likely associated with decreased bacterial burden following antibiotic treatment. However, the reduction in iNOS expression observed in the FA-SEO-treated group may additionally reflect the antioxidant and anti-inflammatory properties of the phytogenic compounds present in the formulation. Similar reductions in inflammatory gene expression have recently been reported in animal models receiving encapsulated essential oils and phytogenic bioactive compounds during bacterial infection and intestinal inflammation (Wang et al., 2024). Although the present findings support the anti-inflammatory potential of the FA-SEO nanocapsules, several limitations should be considered. The evaluation of iNOS expression alone is not sufficient to support broad immunomodulatory claims. Additional inflammatory biomarkers, including TNF-α, IL-1β, IL-6, IL-10, cyclooxygenase-2 (COX-2), and NF-κB signaling pathways, were not investigated in the present study. Furthermore, oxidative stress-related molecular markers and bacterial load quantification were not evaluated. Therefore, the current findings should be interpreted primarily as evidence of anti-inflammatory activity associated with the FA-SEO formulation rather than comprehensive immunomodulation. Overall, the reduction in iNOS expression, together with the improvements observed in antioxidant status, histopathological parameters, and intestinal morphology, suggests that FA-SEO nanocapsules may help alleviate intestinal inflammatory damage during Salmonella infection.

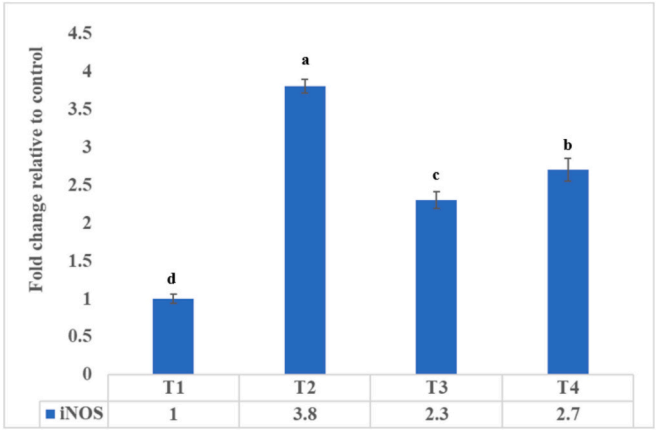

Fig. 5. *iNOS* gene expression in the jejunum tissue of mice receiving different treatments; T 1: mice with normal diet; T 2: mice with normal diet + infection with *S. enterica*; T 3: Mice with a diet enriched with nanocapsules containing FA and SEO + infection with *S. enterica*; T 4: Mice with normal diet + *S. enterica* infection + antibiotics; Letters a, b, c, and d indicate significant differences between different treatments.

4. Conclusion

The present study demonstrated that co-encapsulation of formic acid (FA) and *Satureja hortensis* essential oil (SEO) produced a stable nanocapsule formulation with promising antibacterial and anti-inflammatory properties against *Salmonella enterica*. The FA-SEO nanocapsules exhibited suitable physicochemical characteristics, including relatively uniform nanoscale particle distribution and high encapsulation efficiency, indicating effective incorporation of the active compounds within the carrier matrix.

The encapsulated formulation showed antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria and significantly alleviated infection-associated physiological disturbances in the Salmonella-infected mouse model. Dietary supplementation with FA-SEO nanocapsules improved growth performance, antioxidant enzyme activities, serum immunoglobulin levels, intestinal morphology, and liver-related biochemical parameters while reducing histopathological damage and intestinal iNOS gene expression. These findings suggest that the formulation may help attenuate oxidative stress and inflammatory responses associated with enteric bacterial infection.

The observed biological effects are likely associated with the complementary mechanisms of formic acid and the phenolic constituents of SEO, particularly carvacrol and thymol. While formic acid may contribute to bacterial growth inhibition and modulation of intestinal conditions, SEO-derived phenolic compounds may provide additional antioxidant and anti-inflammatory effects. Moreover, nanoencapsulation may enhance the stability, dispersibility, and biological availability of these bioactive compounds, thereby improving their overall efficacy.

Despite these promising findings, several limitations should be acknowledged. The study did not include comparative control groups receiving free FA, free SEO, blank nanocapsules, or individually encapsulated compounds, which limits precise evaluation of the contribution of each component and the specific effects of co-encapsulation. In addition, important formulation-related characteristics such as release kinetics, storage stability, loading capacity, and long-term stability were not investigated. The inflammatory assessment was also limited primarily to iNOS gene expression, and additional molecular biomarkers associated with inflammation, oxidative stress, and immune regulation were not evaluated.

Therefore, further studies involving expanded mechanistic analyses, inflammatory cytokine profiling, bacterial load quantification, microbiome assessment, and long-term safety evaluation are necessary to

better characterize the therapeutic potential of the formulation. Nevertheless, the present findings suggest that co-encapsulated FA-SEO nanocapsules may represent a promising natural supportive strategy for improving the biological efficacy of antimicrobial compounds against Salmonella-associated infections.

CRediT authorship contribution statement

Sarah Feyzi: Methodology, Investigation. **Ehsan Karimi:** Writing – review & editing, Supervision, Project administration. **Mahboobeh Nakhaei Moghaddam:** Project administration, Formal analysis. **Mahla Hosseinzadeh:** Methodology, Investigation. **Ehsan Oskoueian:** Writing – review & editing, Validation, Supervision, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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