Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

The impact of encapsulated savory (*Satureja hortensis*) essential oils versus antibiotics on growth performance, ileal microbiota, and immune response in broiler chickens

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Received: 08 Dec 2024,
Received in revised form: 11 Mar 2025,
Accepted: 24 Apr 2025,
Published online: 25 Apr 2025,

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Abstract This study compared the effects of non-encapsulated (SEO) savory essential oils, encapsulated savory essential oils (ESEO) and antibiotics (ANT) on growth performance, ileal microbiota, and immune responses in broiler chicks. A total of 360 one-day-old male Ross 308 broiler chicks was randomly assigned to six treatments, five replicates, and 12 chickens per replicate. The dietary treatments included a control group (CON), an antibiotic group (ANT, 650 ppm flavophospholipol), and groups receiving SEO and ESEO at the levels of 150 and 300 ppm. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), production efficiency factor (PEF), ileal microbiota, and immune response were measured on day 42. Results indicated an increase ($P<0.01$) in BWG for birds receiving 150 ppm SEO, while FCR improved ($P<0.01$) in birds receiving either 150 ppm SEO or ESEO compared to the control and ANT groups. Birds that were fed with 150 ppm SEO showed significantly higher PEF than other treatments except for those fed with ESEO150. Additionally, both SEO and ESEO at 150 ppm level positively influenced the ileal microbial population ($P<0.05$). Dietary supplementation with varying levels of savory essential oils (SEO or ESEO) increased ($P<0.05$) the relative weight of the Bursa of Fabricius compared to the ANT group. Specifically, inclusion of 150 ppm SEO led to a notable increase ($P<0.05$) in anti-SRBC antibody levels at both 35 and 42 days of age, and elevated the IgG level at 35 days of age, when compared to the control birds. Furthermore, supplementation of SEO or ESEO, particularly at the 150ppm level, was effective in enhancing the populations of beneficial bacteria in the gastrointestinal tract, along with improving the growth performance and antibody response in broilers.

Keywords: broiler chickens, encapsulation, essential oils, savory

Introduction

Synthetic antibiotics have traditionally played a crucial and inseparable role in intensive production systems, serving as a key component of the industrial feed technology. Inclusion of antibiotics in broiler chicken diets enhances growth performance, aids in disease control, and promotes overall bird health (Ayalew et al., 2022). However, the use of antibiotics as growth promoters has contributed to the emergence of antibiotic resistance among certain bacterial pathogens. Consequently, there is a growing interest in natural alternatives, such as plant essential oils, which are increasingly utilized for similar purposes. Essential oils contain a variety of active compounds that primarily serve to protect plants from damage caused by insects and bacteria (Maurya et al., 2022). Each compound may exhibit distinct mechanisms of action, and these compounds can work synergistically. Thus, the efficacy of essential oils is closely linked to their chemical composition. For instance, thymol and carvacrol are known to possess similar antimicrobial properties (Diaz-Sanchez et al., 2015). Savory (*Satureja hortensis*) is one of the most important medicinal plants in Iran, cultivated across various regions of the country.

Savory possesses biological properties, including antioxidant and antimicrobial activities, which are attributed to its major essential oil components, thymol, and carvacrol (Vitanza et al., 2019). In addition to its medicinal applications, savory can be utilized as a feed additive in poultry diets, offering benefits such as improved performance, enhanced immune response, and better overall health of broilers (Ghalamkari et al., 2011; Nobakht et al., 2012; Montazeri et al., 2014; Yeganeparast et al., 2016). However, there are limitations associated with the direct use of herbal extracts in poultry diets. These limitations stem from the hydrophobicity, reactivity, and volatility of the biologically active compounds found in these extracts. When exposed to oxygen, these components are susceptible to peroxidation and oxidative damage (Meimandipour et al., 2017). Additionally, the impact of herbal extracts on intestinal microflora may be constrained by rapid absorption or metabolism upon entering the duodenum, resulting in reduced bioavailability (Piva et al., 2007). Such destructive factors can diminish the biological activity of plant extracts and essential oils. Furthermore, using large quantities of these substances in the diet may alter the feed taste, leading to decreased palatability and reduced feed intake. Therefore, it is essential to preserve the biological activity of these plant extracts and essential oils for optimal effectiveness (Meimandipour et al., 2017).

It has been previously reported that encapsulation can be effective in delaying the rapid degradation of drugs in the upper gastrointestinal tract. Encapsulation involves coating of the particles or droplets of solid or liquid substances with a continuous film of polymeric materials, such as gums and chitosan, to create capsules (El Asbahani et al., 2015). However, the effects of encapsulated essential oils (ESEO) on broiler growth performance have not been well documented. Therefore, the objective of this study was to evaluate the effects of different dosages of SEO and ESEO compared to antibiotics on growth performance, ileal microbiota, and immune responses in broiler chicks.

Materials and methods

Preparation of Savory essential oils

Hydrodistilled savory essential oils were purchased from Barij Essence Pharmaceutical Company in Kashan, Iran. The oil was analyzed using gas chromatography-mass spectrometry (GC-MS) to identify its components. A total of eighteen compounds were identified, with carvacrol (50.46%), γ-terpinene (16.91%), thymol (11.74%), and p-cymene (8.14%) being the major constituents. The essential oils were stored at 4 °C in dark glass bottles until use.

Encapsulation of SEO

Encapsulation of the SEO was performed by ionic gelation according to the procedure of Sawtarie et al. (2017). Briefly, chitosan (medium molecular weight, derived from crab shell, purchased from Sigma-Aldrich) was dissolved at a concentration of 1 mg/mL in 1% (*w/v*) acetic acid and sonicated until the solution became transparent. The dropwise addition of 10 mL TPP (sodium triphosphate pentabasic) solution (1 mg/mL) to a 25 mL chitosan solution (pH=5), under constant stirring at room temperature, created the formation of chitosan -TPP particles by ionic gelation. For the preparation of chitosan -TPP particles loaded with SEO, 20% (*w/v*) SEO was added to the chitosan solution before adding the TPP solution.

Diets and experimental design

A total of 360 one-day-old Ross 308 male broiler chicks (initial body weight of 37.2 ± 2.6 g) was sourced from a local commercial hatchery (Mahan Hatcheries, Kerman). The chicks were divided into six treatment groups, each with five replicates and twelve chicks per replicate, following a completely randomized design. They were housed in a facility with 30 pens (1.2 × 1.2 m). The temperature was maintained at 33 ± 1°C during the first week and it was subsequently decreased by 3°C each week until reaching 21°C. The chicks were provided with mash diets and had *ad libitum* access to both feed and fresh water throughout the experiment.

The six dietary treatments included a control group (CON), an antibiotic group (ANT, 650 ppm flavophospholipol), and groups receiving SEO and ESEO at the levels of 150 and 300 ppm. The diets were formulated to meet the nutrient specifications for the starter (days 1 to 10), grower (days 11 to 24), and finisher (days 25 to 42) phases (Table 1) as outlined in the Ross 308 Broiler Nutrition Specification (Aviagen, 2014).

Growth performance

On day 42, the birds were weighed, and the amount of feed remaining in each pen was recorded. The data were used to calculate the body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Mortality rate was recorded daily. The production efficiency factor (PEF) was calculated as follows (Aviagen, 2018):

$$PEF=\frac{[\text{viability d0}-42\ (\%) \times \text{LBW d42 (Kg)}]}{[\text{age (d42)} \times \text{FCR d0}-42]} \times 100$$

Intestinal microflora population

At the end of the rearing period, the ileal digesta of two birds per pen were carefully collected in sterilized glass tubes and immediately transferred to the laboratory for counting total coliforms and lactic acid bacteria. The ileal contents were diluted in normal saline. Plates containing MacConkey agar were prepared for counting total coliform bacteria, and plates containing MRS agar were prepared for counting lactobacillus bacteria (Yang et al., 2012). After culturing the bacteria, plates were incubated for 24 h at 37 °C temperature (Adaszyńska-Skwirzyńska and Szczerbińska, 2018). Results were expressed as $\log_{10}$ CFU/g ileal digesta.

Table 1. Ingredients and nutrient composition of the starter, grower and finisher diets (%)

Ingredients	Starter (d 1–10)	Grower (d 11–24)	Finisher (d 25–42)
Corn	48.42	52.30	58.22
Soybean meal (44%)	42.00	37.84	32.20
Vegetable oil	5.00	5.70	5.70
Limestone	1.15	1.05	1.00
Dicalcium phosphate	1.75	1.60	1.40
Common salt	0.43	0.43	0.43
DL-methionine	0.40	0.32	0.30
HCl-lysine	0.25	0.18	0.19
L-threonine	0.10	0.08	0.06
Vitamin & mineral premix[1]	0.50	0.50	0.50
Calculated chemical composition			
Metabolizable energy (kcal kg^{-1})	3000	3100	3200
Crude protein (%)	23	21.5	19.5
Methionine (%)	0.74	0.64	0.60
Methionine + cysteine (%)	1.08	0.99	0.91
Lysine (%)	1.44	1.29	1.16
Threonine (%)	0.80	0.69	0.60
Calcium (%)	0.96	0.87	0.79
Available phosphorous (%)	0.48	0.44	0.40
Sodium (%)	0.17	0.16	0.16

[1]Mineral and vitamin premix provided per kilogram of diets: A: 10 000 IU, D3: 5000, E: 50 IU, K: 3 mg, B_1: 2 mg, B_2: 6 mg, niacin: 60 mg, pantothenic acid: 15 mg, B_6: 3 mg, biotin: 0.1 mg, folic acid: 1.75 mg, B_{12}: 0.016; Cu: 16 mg, I: 1.26, Fe: 40 mg, Mn: 120 mg, Se: 0.3 mg, and Zn: 100 mg.

Lymphoid organ weights

At the end of the experiment (42 days of age), two birds from each pen were randomly selected, weighed, and slaughtered. The spleen and Bursa of Fabricius were then collected. The relative organ weights were calculated and expressed in grams per 100 grams of live body weight.

Immunological tests

To assess the primary and secondary humoral immune responses at 28 days of age, two chicks from each replicate were injected with 0.5 mL of a 10% saline suspension of sheep red blood cells (SRBC) into the wing vein. A second injection was administered to the same chicks after 7 days to promote a stable immune response for the subsequent sampling. Blood samples (2 mL per hen) were collected seven days post-injection. These samples were centrifuged to separate the serum, which was then stored at -20°C until further analysis. Hemagglutination titers were expressed as the $\log^2$ of the reciprocal of the highest dilution that demonstrated 100% agglutination (Habibian et al., 2014). Additionally, mercaptoethanol-sensitive (MES, presumably IgM) and mercaptoethanol-resistant (MER, presumably IgY) anti-SRBC antibody titers were measured using a microhemagglutination technique as described by Qureshi and Havenstein (1994). The IgM and IgG levels were assessed following the method outlined by Cheema et al. (2003).

Statistical analysis

All data were subjected to ANOVA using the General Linear Models procedure of SAS software (SAS, 2001). Mean separation was performed by the Duncan's multiple range tests. A level of P<0.05 was used as the criterion for statistical significance.

Results

Growth performance

The effects of SEO and ESEO on BWG, FI, and FCR are presented in Table 2. The BWG improved (P<0.01) in birds fed with 150 ppm SEO compared with the control and ANT groups. The FI was not influenced by the dietary treatments but FCR was improved (P<0.01) in birds fed with 150 ppm SEO or ESEO compared with the control and ANT groups. Birds fed with 150 ppm SEO showed significantly higher PEF than other treatments except for those fed with ESEO150.

Table 2. Effect of experimental diets on growth performance of broiler chickens from 1-42 d of age

Treatments	BWG (g/b/d)	FI (g/b/d)	FCR	PEF
CON	49.30^{b}	99.80	2.03^{a}	244.9^{b}
ANT	49.01^{b}	96.50	1.97^{a}	246.6^{b}
SEO150	55.25^{a}	98.37	1.78^{b}	312.1^{a}
SEO300	50.08^{b}	96.70	1.93^{ab}	263.9^{b}
ESEO150	52.99^{ab}	95.83	1.81^{b}	292.1^{ab}
ESEO300	52.62^{ab}	99.11	1.88^{ab}	263.9^{b}
SEM	0.929	1.046	0.036	10.97
P-value	0.001	0.083	0.001	0.001

BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio, PEF: production efficiency factor.
CON: control, ANT: antibiotic 650ppm Flavophospholipol, SEO150 and SEO300: 150 and 300 ppm savory essential oils, ESEO150 and ESEO300: 150 and 300 ppm encapsulated savory essential oils.
a,b: Within columns, means with common superscript (s) do not differ (P>0.05; Duncan's test).

Microbial populations

The effects of the experimental diets on the ileal microbial population are shown in Table 3. Dietary inclusion of 150 ppm ESEO, significantly decreased the concentration of *coliforms* in the ileal digesta in comparison to the control birds (P<0.05). Also, the number of *lactobacilli* was increased in birds fed with 150 ppm SEO compared to the control and ANT groups (P<0.05).

Lymphoid organ weights

Dietary supplementation with different levels of SEO and ESEO increased (P<0.05) the relative weight of Bursa of Fabricius in comparison to the antibiotic diet (Table 4). No significant effect of treatments was detected on the relative weight of the spleen.

Table 3. Effect of experimental diets on ileal microbial population (log_{10} CFU/g) of broiler chickens at 42 d of age

Treatments	Coliforms	Lactic acid
CON	5.52^{a}	6.88^{b}
ANT	5.25^{ab}	6.98^{b}
SEO150	4.25^{ab}	8.26^{a}
SEO300	4.62^{ab}	7.59^{ab}
ESEO150	3.72^{b}	7.82^{ab}
ESEO300	4.59^{ab}	7.26^{ab}
SEM	0.362	0.288

CON: control, ANT: antibiotic 650ppm Flavophospholipol, SEO150 and SEO300: 150 and 300 ppm savory essential oils, ESEO150 and ESEO300: 150 and 300 ppm encapsulated savory essential oils.
a,b: Within columns, means with common superscript (s) do not differ (P>0.05; Duncan's test).

Table 4. Effect of experimental diets on lymphoid organ weights (% of live weight) of broiler chickens at 42 d of age

Treatments	Bursa of Fabricius	Spleen
CON	0.13^{ab}	0.11
ANT	0.12^{b}	0.11
SEO150	0.15^{a}	0.10
SEO300	0.15^{a}	0.12
ESEO150	0.15^{a}	0.11
ESEO300	0.15^{a}	0.10
SEM	0.008	0.011
P-value	0.029	0.778

CON: control, ANT: antibiotic 650ppm Flavophospholipol, SEO150 and SEO300: 150 and 300 ppm savory essential oils, ESEO150 and ESEO300: 150 and 300 ppm encapsulated savory essential oils.
a,b: Within columns, means with common superscript (s) do not differ (P>0.05; Duncan's test).

Antibody response to SRBC

The results on the effect of experimental diets on anti-SRBC, IgG, and IgM titers at 35 and 42 days are presented in Table 5. Dietary inclusion of 150 ppm SEO increased (P<0.05) the anti-SRBC at 35 and 42 days of age, in comparison to the control birds. Also, dietary inclusion of 150 ppm SEO increased (P<0.05) the IgG at 35 days of age compared to the control and antibiotic-fed birds.

Discussion

Notably, the data indicated a significant improvement in BWG among birds that were administered with 150 ppm of SEO, when compared to control and ANT groups. This suggests that the inclusion of SEO in the diet can effectively enhance weight gain in broilers, potentially offering an alternative to the traditional antibiotic growth promoters. Also, the FCR and PEF exhibited a significant improvement in birds receiving both 150 ppm of SEO and ESEO when contrasted with the control and ANT groups. The improvement in FCR indicates that these natural additives improve the efficiency of converting feed into body mass, a crucial factor in optimizing broiler production. However, the literature presents a varied picture regarding the efficacy of various plant extracts and essential oils. For instance, Meimandipour et al. (2017) reported that while the addition of certain plant extracts did not influence BWG during the initial two weeks of growth, marked improvements were observed in the later periods, specifically from 28 to 42 days and over the entire growth span from 1 to 42 days. In contrast, studies involving essential oils derived from pennyroyal and savory demonstrated no significant impact on growth performance in quail diets and the application of thyme essential oils at a higher concentration of 400 ppm resulted in a decrease in feed intake (FI) and FCR, indicating a complex relationship between essential oils and growth parameters (Dehghani et al., 2018).

Further complicating the narrative, Yeganeparast et al. (2016) found that the inclusion of savory essential oils in male broiler diets did not enhance the growth performance. This sentiment is resounded by several other researchers, including Ghalamkari et al. (2011) and Montazeri et al. (2014), who reported no significant positive or negative effects of essential oils on poultry growth metrics. Such findings underscore the variability in response to essential oil supplementation, which may be influenced by factors such as the type of essential oils, dosage, and specific dietary contexts.

Table 5. Effect of experimental diets on anti-SRBC (total), IgG, and IgM titers in 35 and 42-day-old broilers chickens (Log^2)

Treatments	Anti-SRBC35	IgG35	IgM35	Anti-RBC42	IgG42	IgM42
CON	4.20^{b}	2.60^{b}	1.60	4.10^{b}	2.60	1.50
ANT	4.40^{ab}	2.60^{b}	1.80	4.70^{ab}	3.00	1.70
SEO150	5.80^{a}	3.40^{a}	2.40	5.70^{a}	3.50	2.20
SEO300	5.30^{ab}	3.30^{ab}	2.00	5.40^{ab}	3.30	2.10
ESEO150	5.10^{ab}	3.10^{ab}	2.00	5.20^{ab}	3.30	1.90
ESEO300	4.60^{ab}	2.80^{ab}	1.80	5.00^{ab}	3.10	1.90
SEM	0.359	0.180	0.25	0.300	0.204	0.175
P-value	0.037	0.012	0.341	0.016	0.070	0.096

CON: control, ANT: antibiotic 650ppm Flavophospholipol, SEO150 and SEO300: 150 and 300 ppm savory essential oils, ESEO150 and ESEO300: 150 and 300 ppm encapsulated savory essential oils.
a,b: Within columns, means with common superscript (s) do not differ (P>0.05; Duncan's test).

According to the results, SEO and ESEO at 150 ppm level had beneficial effects on ileal microbial population. The findings highlight their potential as an alternative dietary supplement to antibiotics in poultry nutrition by effectively reducing harmful coliforms and promoting the beneficial lactobacilli, thereby supporting the sustainability and efficiency of poultry production systems. The intricate balance of intestinal microflora is not only essential for the well-being of poultry but also for consumer safety, as gastrointestinal contents can act as a reservoir for pathogens that may contaminate the carcass (Choi et al., 2015). Factors influencing the intestinal microflora include environmental stressors, the age of the birds, and the composition of their feed. Hence, maintaining homeostasis within the intestinal ecosystem is crucial. Dietary interventions, specifically the supplementation of active components such as essential oils, have been shown to positively modulate the microbial populations within the intestines (Roberts et al., 2015). Several studies have corroborated the beneficial effects of essential oils on intestinal microbial flora. Cross et al. (2007), Tiihonen et al. (2010), Kirkpinar et al. (2011), Erhan et al. (2012), and Hong et al. (2012) demonstrated that the inclusion of these oils can selectively target and reduce pathogenic bacteria while preserving the beneficial lactic acid bacteria (Choct, 2009). This selective reduction is critical; a decreased pathogenic load enhances the regenerative capacity of intestinal villi, thereby improving nutrient absorption (Choi et al., 2015; Zeng et al., 2015).

The study of dietary effects on the relative weight of immune organs is crucial for understanding the interplay between nutrition and immunological health in various species. The results elucidated the potential of SEO and ESEO as effective dietary supplements that can enhance immune organ development, specifically the Bursa of Fabricius. The Bursa of Fabricius plays a pivotal role in the maturation of B-lymphocytes, thereby influencing the overall immune competence of an organism. Alipour et al. (2015) corroborated these findings, revealing that diets supplemented with 200 ppm of thyme extract exhibited an increase in the weight of the Bursa of Fabricius in broilers. The relationship between lymphoid organ weights and immune response capabilities is well-established, as highlighted by Akter et al. (2006). The relative weights of both the spleen and Bursa of Fabricius serve to indicate an animal's capacity to produce T and B lymphocytes, crucial components of the immune system.

The immune response in poultry is a critical parameter for maintaining health and productivity within the industry. Based on the results, the use of 150 ppm SEO had a positive impact on anti-SRBC and IgG levels. This observation underscores the efficacy of SEO in promoting the humoral immunity, as IgG is a critical antibody for long-term immune defense. The results collectively suggest that dietary SEO not only stimulates immediate immune responses but also enhances adaptive immunity, thereby offering a viable alternative to traditional antibiotics in poultry nutrition. This study warrants further investigation into the mechanisms underlying these immune enhancements and the long-term implications for poultry health and productivity. The work of Stef et al. (2009) emphasized that essential oils derived from medicinal herbs can be instrumental in augmenting immune responses while simultaneously inducing beneficial alterations in the duodenal mucosa of animals. Several detrimental factors including inadequate vaccination protocols, immune-suppressive infections, and the misuse of antibiotics can culminate in immunodeficiency. The introduction of immune system stimulants represents a viable strategy for enhancing immunity and diminishing vulnerability to various pathogens. Flavonoid-rich plants, such as thyme, have been recognized for their immunostimulatory effects (Acamovic and Brooker, 2005). Noteworthy constituents of savory essential oils, namely thymol and carvacrol, have been documented to possess significant antibacterial, antiviral, and antioxidant properties. As expressed by Botsoglou et al. (2002), the enhancement of immune responses in chickens through these components is not only plausible but expected. Furthermore, the elevation of antioxidant levels serves to mitigate free radical reactions, which can adversely affect cellular activities (Pourhossein et al., 2019). The SEO is enriched with antioxidants that, upon absorption and metabolism by the poultry's body, contribute to a robust immune response. The pronounced anti-SRBC titer response observed in chickens receiving the SEO-enriched diets underscores the potential of essential oils to bolster immunity, thereby reinforcing their role as strategic additives in broiler nutrition.

Conclusion

In summary, the findings of this study indicated that dietary supplementation of SEO or ESEO, at a concentration of 150 ppm significantly enhanced the populations of beneficial bacteria within the gastrointestinal tract of broilers. Additionally, these supplementation improved growth performance and PEF and bolstered the antibody response in these broiler chickens. Despite these positive outcomes, the current data did not establish a definitive superiority of ESEO over SEO. Therefore, further research must be conducted to explore the comparative effectiveness of these dietary supplements, as well as to elucidate the underlying mechanisms responsible for the observed enhancements in health and performance. This will facilitate a more comprehensive understanding of the potential benefits of essential oils in poultry nutrition and health management.

Acknowledgment

The authors are grateful to Shahid Bahonar University of Kerman, for supports.

Conflict of interests

No potential conflict of interest was reported by the authors.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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