# Original Article

# Evaluating the Competitiveness of Medicinal Plants With Antibiotics to Control *Salmonella Enterica* Serovar Typhimurium in Broiler Chickens

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## ABSTRACT

**Background:** Salmonellosis is one of the important diseases in the poultry industry, which also causes public health concerns.

**Objectives:** We studied the effects of enrofloxacin and herbal medicines on growth performance, blood parameters, meat oxidation, and cecal microbial population in broilers challenged with *Salmonella enterica* serovar Typhimurium (ST).

**Methods:** A total of 240 one-day-old (male) Ross 308 broiler chicks were randomly divided into 6 groups: negative and positive control, enrofloxacin, and three herbal medicines (A, B, and C) containing different proportions of cinnamon, thyme, licorice, and marjoram extracts with compounds of organic acids. The dosage of enrofloxacin and A, B, and C herbal medicines were 1, 1, 1, and 2 mL/L in drinking water, respectively, prescribed from days 16 to 21. On day 10, all groups except negative control were challenged with 1 mL suspension containing $1\times10^7$ CFU/mL ST. Performance traits were measured in intervals of 1-10, 11-24, 25-42, and 1-42 days. Blood parameters, meat oxidation, and cecal microbial population were measured on day 21.

**Results:** Among the challenged groups, medicine C and enrofloxacin showed the lowest levels of *Salmonella* infection ($P<0.05$). Medicine B had a better effect on performance traits ($P<0.05$). Medicine A had the lowest amount of malondialdehyde in meat. Medicines A and B caused the lowest cholesterol and triglyceride concentration in serum ($P<0.05$).

**Conclusion:** The above-mentioned herbal medicines can be used as beneficial additives in poultry nutrition to improve growth performance, reduce the *Salmonella* population in the gastrointestinal tract, and cholesterol, triglycerides, and meat oxidation.

**Keywords:** Cinnamon, Licorice, Marjoram, *Salmonella Typhimurium,* Thyme

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## 1. Introduction

*Salmonella* spp. can cause Salmonellosis in humans and animals, a zoonotic disease often transmitted to humans through poultry products (Afshari et al., 2018). The disease is caused by *Salmonella enterica* serovar Typhimurium (ST) and results in serious damage to the poultry industry through stunted growth and increased mortality rates (Dar et al., 2017). *Salmonella* infection is very common at a young age due to the underdevelopment of the chickens' immune system (Abudabos et al., 2018). Poultry is exposed to *Salmonella* spp., and the bacteria can be transmitted to humans through consuming contaminated meat and egg (Wilson et al., 2016). Therefore, ensuring the microbial safety of poultry products is of great importance due to their increased production and consumption (Rouger et al., 2017; Thames et al., 2022).

Antibiotics have been used to control bacterial infections in poultry (Wibisono et al., 2020). Unreasonable use of these compounds to control Salmonellosis has led to the emergence and spread of antibiotic-resistant *Salmonella* spp. This resistance has increased the pressure on poultry producers to reduce antibiotic use (Schwartz & Vetvicka, 2021). Thus, poultry producers and researchers are looking for alternative feed additives to amend the performance and quality of poultry meat in the encounter of microbial infection. In the post-antibiotic era, organic acids (OAs) and medicinal plants are considered alternatives to safe and pathogen-free food production (Akintayo-Balogun Omolere & Álagbe, 2020; Rouger et al., 2017).

The effect of OAs on microorganisms focuses on their ability to withstand acid stress (Broom, 2015). Dietary organic acid supplementation can prevent competition between intestinal bacteria and host for nutrients and reduce bacterial toxins. This action improves the digestibility of nutrients, thus improving poultry performance (Khan & Iqbal, 2016). The positive effects of extracts of medicinal plants are probably due to the antimicrobial effects of the active ingredients in their composition (Chun et al., 2005), which positively impact the performance and gut health of broiler chickens. The active ingredients in the extracts of herbs, such as eugenol, thymol, carvacrol, and cinnamaldehyde, have antibacterial effects against *S. enterica* and *Campylobacter jejuni* (Du et al., 2015). Also, thymol, cinnamaldehyde, and carvacrol have beneficial effects on the oxidative stability of muscles in broiler chickens (Gholami-Ahangaran et al., 2022; Hashemipour et al., 2013).

The use of essential oils (EOs) and extracts of medicinal plants and their active ingredients in studies have shown good results. Adding thyme and cinnamon extracts at levels of 100 and 200 mg/kg to the broiler chicken diet increased the growth performance compared with the control group (Al-Kassie, 2009). Supplementation of chicken feed with marjoram extracts at a rate of 14 g/100 kg improved body weight gain (BWG) and feed intake (FI) (Abdel-Moneim et al., 2015). Also, adding licorice extract to broilers drinking water has shown an important role in poultry performance by stimulating digestion and appetite (Alagawany et al., 2019). In addition, the use of thymol in the feed of broilers challenged with ST improved BWG, feed conversion rate (FCR), and regulated FI (Ibrahim et al., 2021).

A standard diet with EOs and extracts of medicinal plants in poultry nutrition can be one of the practical nutritional strategies to improve the quality of poultry meat (Stamilla et al., 2020), maximize overall performance (Kang et al., 2010), enhance the digestibility of poultry diets (Oluwafemi et al., 2020), and reduce *Salmonella* colonization (Chaney et al., 2022). OAs can reduce the amount of ST in the cecum by acidifying drinking water. Therefore, a mixture of the extracts of medicinal plants together or with OAs can increase their effects (Basmacioğlu-Malayoğlu et al., 2016; Du et al., 2015). Machado et al. (2014) reported that the addition of the mixture of OAs and marjoram extract in water (0.2%) and feed (0.8%) of chickens significantly reduced *S. enterica* serovar Enteritidis at 22 and 42 days of the rearing period. Thus, the combination of antimicrobial agents is suggested for controlling pathogenic bacteria agents (Scandorieiro et al., 2016). Due to the effects of these medicinal plants and their active ingredients, the combination of OAs and extracts of these plants as herbal medicines can be used as an antibiotic alternative in poultry diets.

Few studies have investigated the effect of OA and herbal medicines on the performance, meat quality, and intestinal microbial population in broiler chickens infected with ST. Therefore, this study was conducted to investigate the effect of several herbal medicines (under-commercialization) containing a mixture of OAs and herbal extracts on intestinal microflora, performance, oxidation of meat, and blood parameters of broiler chickens challenged with ST.

## 2. Materials and Methods

The study was performed in the Poultry Research Center, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

### Management and experimental groups

The experiment was performed for 6 weeks on 240 male Ross 308 chickens in 6 treatments, 4 replicates, and 10 chickens per replicate. The experimental groups were as follows:

**Negative control group (NC):** diet without any additives and ST challenge;

**Positive control group (PC):** diet without any additives+ST challenge;

**Enrofloxacin group:** 1 mL/L in drinking water+ST challenge;

**Herbal medicine A:** a mixture of marjoram and thyme extracts (1 mL/L in drinking water+ST challenge);

**Herbal medicine B:** a mixture of marjoram, thyme, cinnamon, and licorice extracts (1 mL/L in drinking water+ST challenge);

**Herbal medicine C:** a mixture of marjoram extract and OAs (2 mL/L in drinking water+ST challenge).

The NC group was kept in similar environmental conditions in an isolated room than other groups. All broilers received the same diet during the experiment. The diet was prepared based on the recommended nutrient requirements of NRC 1994 (Table 1). The chickens had free access to feed and water throughout the experiment. The management conditions of the poultry house, ventilation, humidity, and lighting program were applied according to Ross 308 catalog (www. Aviagen.com). Enrofloxacin and herbal medicines were used for 6 days from day 16 to 21 (6 days after the ST challenge) according to the manufacturer's recommendation. Enrofloxacin was obtained (Rooyan Darou Pharmaceutical Co, Iran) for veterinary products and herbal medicines from the Academic Center for Education, Culture, and Research (ACECR).

### *Salmonella* challenge

*S. enterica* serovar Typhimurium was obtained from the Microbiology Laboratory of the Department of Bacteriology and Immunology, Faculty of Veterinary Medicine, University of Tehran (Tehran, Iran). For the preparation of the inocula, ST was incubated in a brain heart infusion broth (Merck, Germany) culture medium at 37°C for 24 hours. The viable cell concentration of the inoculums was determined on xylose lysine deoxycholate (XLD) agar (Merck, Germany) plates (Bjerrum et al., 2003). Chickens of all groups except negative control at 10 days of age were challenged with 1 mL of broth medium containing ST ($10^7$ CFU/mL) by oral gavage (Cox et al., 2020). The negative control chickens were given 1 mL of sterile nutritional broth medium on the same day.

### Growth performance

To investigate the effect of experimental treatments on growth performance, BWG, FI, and FCR were measured in the intervals of 1 to 10, 11 to 24, 25 to 42, and 1 to 42 days.

### Blood parameters

Cholesterol, triglyceride, total protein, glucose, and uric acid levels in broiler serum samples were measured on day 21 of the experiment. For this purpose, blood was taken from the brachial veins and centrifuged at 3000 rpm for 10 minutes. After separating the serum, blood parameters were measured using an ELISA kit (Pars Azmoun-Iran), and absorbance was measured at 546 nm.

### Oxidation of meat

Malondialdehyde (MDA) concentration was measured as a marker of fat peroxidation in meat samples on day 21 of the experiment. First, 1 g of the meat sample was homogenized in 4 mL of trichloroacetic acid (TCA) and 2.5 mL of butylated hydroxytoluene (BHT). Next, the samples were centrifuged at 3000 rpm for 3 minutes. After centrifugation, the hexane layer was discarded, and the aqueous phase was filtered with smooth Whatman No. 1 paper and increased volume to 5 mL with TCA. Three milliliters of thiobarbituric acid (TBA) were added to standard tubes and samples; then, they were placed in a water bath at 70°C for 30 minutes. Then, the absorbed light was read at 532 nm with a spectrophotometer.

### Cecum microflora

On day 21, one chick was randomly selected from each replicate and euthanized. After necropsy, its ceca was removed, and 1 g of cecal contents was diluted in 9 mL of saline phosphate buffer (PBS). Diluted samples were cultured on three media: Lactobacillus MRS agar (Merck, Germany) for counting lactic acid bacteria, XLD agar (Merck, Germany) for counting *Salmonella*, and plate count agar (PCA; Merck, Germany) for counting all aerobic bacteria. Colonies were counted in each plate after incubation at 37°C for 24 hours by the counter colony (Hashemzadeh et al., 2010).

### Statistical analysis

All data obtained through the experiment were analyzed in a completely randomized design. All data were analyzed using the one-way ANOVA, general linear model (GLM) PROC of SAS. Mean comparison was performed by Duncan's multiple range test methods to investigate the differences between treatments, and all values P<0.05 were considered significant.

## 3. Results

The results for the cecal microbial population are reported in Table 2. The negative control group did not have Salmonella. Among the groups challenged with ST, enrofloxacin and medicine C had the lowest, and positive control had the highest counting of *Salmonella* populations (P<0.05). The tested herbal medicines increased the number of beneficial lactic acid bacteria in the ceca, and the lowest number of these bacteria was observed in the positive control group (P<0.05). Antibiotic treatment had the lowest total number of aerobic bacteria in the ceca, and the positive control group had the highest number of aerobic bacteria (P<0.05).

The results of BWG, FI, and FCR are presented in Table 3. According to the results, until the challenge with ST on day 10 of the experiment, there was no difference between the experimental treatments in terms of performance (P>0.05). From days 11 to 24, the negative

**Table 1.** Composition and calculated analysis of the basal diet of broiler chickens

| Item | | Starter | Grower | Finisher |
|---|---|---|---|---|
| Ingredients (% diet) | Corn | 49.62 | 52.21 | 47.04 |
| | Soybean meal | 40.04 | 35.05 | 30.96 |
| | Wheat | 4.00 | 8.09 | 14.60 |
| | Soybean oil | 1.34 | 1.00 | 4.23 |
| | Dicalcium phosphate | 2.46 | 2.20 | 2.60 |
| | DL-methionine | 0.34 | 0.27 | 0.16 |
| | L-lysine | 0.23 | 0.19 | 0.03 |
| | Vitamin premix[1] | 0.25 | 0.25 | 0.25 |
| | Mineral premix[2] | 0.25 | 0.25 | 0.25 |
| | Limestone | - | 0.05 | - |
| | Salt | 0.27 | 0.28 | 0.28 |
| Calculate analysis | ME (kcal/kg) | 2820 | 2950 | 3045 |
| | Crude protein (%) | 21.53 | 18.85 | 18.01 |
| | Crude fiber (%) | 4.7 | 5.09 | 4.82 |
| | Fat (%) | 2.04 | 2.45 | 2.57 |
| | Calcium (%) | 0.93 | 0.83 | 0.80 |
| | Available phosphorus | 0.47 | 0.41 | 0.40 |
| | Methionine+Cysteine | 0.9 | 0.82 | 0.72 |
| | Lysine | 1.28 | 1.14 | 0.95 |

[1]Per kilogram of feed: Vitamin A: 11000 IU; Vitamin D3: 1800 IU; Vitamin E: 36 mg; Vitamin K3: 5 mg; Thiamine: 1.53 mg; Riboflavin: 7.5 mg; Calcium pantothenate: 12.40 mg; Niacin: 30.40 mg; Pyridoxine: 1.53 mg; Folic acid: 1.26 mg; Vitamin B12: 1.6 mg; Biotin: 5 mg; Choline chloride: 1100 mg; Antioxidant: 100 mg.

[2]Mn: 16.3 mg; Zn: 84.5 mg; Fe: 250 mg; Cu: 20 mg; I: 1.6 mg; Co: 0.48 mg; Se: 20 mg.

**Table 2.** Effect of herbal medicines and antibiotics on Ceca microflora population (day 21) in broiler chickens challenged with *Salmonella enterica* Serovar Typhimurium

| Parameters | Treatment | | | | | | SEM | P |
|---|---|---|---|---|---|---|---|---|
| | NC | PC | ENR | Medicine A | Medicine B | Medicine C | | |
| Salmonella (log CFU/g) | $0^{d}$ | $6.19^{a}$ | $3.76^{c}$ | $4.56^{b}$ | $4.23^{b}$ | $3.92^{c}$ | 0.03 | 0.000 |
| Lactic acid bacteria (log CFU/g) | $7.23^{b}$ | $6.45^{d}$ | $6.67^{c}$ | $7.45^{ab}$ | $7.74^{a}$ | $7.65^{a}$ | 0.09 | 0.020 |
| Total count of aerobic bacteria (log CFU/g) | $8.12^{b}$ | $8.63^{a}$ | $7.54^{d}$ | 7.89c | $8.04^{bc}$ | $7.94^{c}$ | 0.13 | 0.032 |

a-d Means or percentages with different superscripts within a column differ significantly (P<0.05)

Abbreviations: SEM: Standard error of the mean; NC: Negative control; PC: Positive control; ENR: Enrofloxacin.

Notes: Medicine A: A mixture of marjoram and thyme extracts; Medicine B: A mixture of marjoram, thyme, cinnamon, and licorice extracts; Medicine C: A mixture of marjoram extract and OAs.

control had the highest, and the positive control had the lowest BWG (P<0.05). Among the challenged groups, enrofloxacin and medicine B had better BWG than the other two medicines and positive control (P<0.05). The enrofloxacin group had the highest, and the positive control group had the lowest FI during this period (P<0.05). Also, the negative control group and medicine B had the strongest, and the positive control had the weakest FCR (P<0.05). The difference in the performance of treatments from 25 to 42 days was not significant (P>0.05). Throughout the experiment period, negative control groups and medicine B had the highest BWG and the

**Table 3.** Effect of herbal medicines and antibiotics on performance results in broiler chickens challenged with *Salmonella enterica* Serovar Typhimurium

| Day | Parameters | Treatment | | | | | | SEM | P |
|---|---|---|---|---|---|---|---|---|---|
| | | NC | PC | ENR | Medicine A | Medicine B | Medicine C | | |
| 1-10 day | BWG (g) | 125.10 | 123.82 | 122.87 | 122.53 | 122.34 | 125.79 | 0.19 | 0.461 |
| | FI (g) | 198.28 | 197.08 | 194.05 | 199.59 | 197.08 | 204.14 | 3.17 | 0.530 |
| | FCR | 1.58 | 1.59 | 1.57 | 1.62 | 1.61 | 1.62 | 0.03 | 0.081 |
| 11-24 day | BWG (g) | $787.93^{a}$ | 663.33d | 766.62b | $744.58^{c}$ | 760.80b | $747.84^{c}$ | 11.83 | 0.022 |
| | FI (g) | $1285.90^{b}$ | $1221.81^{c}$ | $1330.60^{a}$ | $1265.77^{b}$ | $1251.82^{b}$ | $1277.03^{b}$ | 15.72 | 0.013 |
| | FCR | $1.63^{c}$ | $1.84^{a}$ | $1.73^{b}$ | $1.69^{b}$ | $1.64^{c}$ | $1.70^{b}$ | 0.05 | 0.017 |
| 25-42 day | BWG (g) | 1787.26 | 1730.72 | 1770.73 | 1783.80 | 1779.82 | 1754.54 | 34.23 | 0.063 |
| | FI (g) | 3315.29 | 3283.16 | 3423.58 | 3330.84 | 3296.04 | 3315.76 | 51.43 | 0.114 |
| | FCR | 1.85 | 1.90 | 1.93 | 1.86 | 1.85 | 1.88 | 0.08 | 0.201 |
| 1-42 day | BWG (g) | $2700.29^{a}$ | $2517.88^{c}$ | $2660.23^{ab}$ | $2650.92^{b}$ | $2662.96^{a}$ | $2628.18^{b}$ | 42.56 | 0.012 |
| | FI (g) | $4799.48^{b}$ | $4702.06^{c}$ | $4948.24^{a}$ | $4796.21^{b}$ | $4744.95^{b}$ | $4796.93^{b}$ | 61.63 | 0.043 |
| | FCR | $1.77^{c}$ | $1.87^{a}$ | $1.86^{a}$ | $1.80^{b}$ | $1.78^{c}$ | $1.82^{b}$ | 0.07 | 0.049 |

a- dMeans or percentages with different superscripts within a column differ significantly (P<0.05).

Abbreviations: SEM: Standard error of the mean; NC: Negative control; PC: Positive control; ENR, enrofloxacin; BWG, body weight gain; AFG, Average feed intake; FCR, feed conversion rate.

Notes: Medicine A: A mixture of marjoram and thyme extracts; Medicine B: A mixture of marjoram, thyme, cinnamon, and licorice extracts; Medicine C: A mixture of marjoram extract and OAs.

**Table 4.** Effect of herbal medicines and antibiotics on the serum biochemical indices in broiler chickens challenged with *Salmonella enterica* Serovar Typhimurium

| Parameters | Treatment | | | | | | SEM | P |
|---|---|---|---|---|---|---|---|---|
| | NC | PC | ENR | Medicine A | Medicine B | Medicine C | | |
| Glucose (mg/dL) | 205.85 | 171.79 | 175.48 | 196.72 | 182.22 | 195.49 | 16.70 | 0.406 |
| Cholesterol (mg/dL) | 129.14[a] | 125.90[a] | 134.86[a] | 108.33[bc] | 100.47[c] | 112.24[b] | 11.22 | 0.049 |
| Triglyceride (mg/dL) | 169.74[a] | 171.20[a] | 166.47[a] | 131.06[c] | 128.21[c] | 149.13[b] | 13.97 | 0.036 |
| Total protein (g/dL) | 4.35 | 4.08 | 4.18 | 4.78 | 4.28 | 4.40 | 0.31 | 0.121 |
| Uric acid (mg/dL) | 7.59 | 6.33 | 7.24 | 8.58 | 7.64 | 8.14 | 0.51 | 0.114 |

[a-c]Means or percentages with different superscripts within a column differ significantly ($P<0.05$).

Abbreviations: SEM: Standard error of the mean; NC: Negative control; PC: Positive control; ENR: enrofloxacin.

Notes: Medicine A: A mixture of marjoram and thyme extracts; Medicine B: A mixture of marjoram, thyme, cinnamon, and licorice extracts; Medicine C: A mixture of marjoram extract and OAs.

best FCR ($P<0.05$). The enrofloxacin group had the highest, and the positive control group had the lowest FI and the worst FCR ($P<0.05$).

The effects of herbal medicines and antibiotics on the blood parameters of broiler chickens challenged with ST are reported in Table 4. The results showed that the challenge with this bacterium and the use of antibiotics and herbal medicines did not affect the serum concentrations of uric acid, glucose, and protein in chickens ($P>0.05$). However, all herbal medicines reduced serum cholesterol and triglyceride levels in broilers ($P<0.05$). The decreasing effect of medicine A and B were more severe than medicine C ($P<0.05$).

According to Table 5, the comparison between negative and positive control groups shows that challenge with ST did not affect the oxidation of chicken meat ($P>0.05$). Also, antibiotic use did not affect the concentration of MDA in the meat of challenged chickens. However, using herbal medicines significantly reduced the oxidation of meat in the thighs and chest. The effect of medicine A was more substantial ($P<0.05$).

## 4. Discussion

In the present study, enrofloxacin showed the best effect in reducing cecal *Salmonella* populations in the GI tract of the broilers. This result is supported by a study by Randall et al. (2005) that reported that the treatment of chickens with enrofloxacin reduced ST excretion from $10^5$ CFU/swab to 40 and 2 CFU/swab on days 1 and 7 after treatment, respectively. Herbal medicines were not as effective as an antibiotic in reducing the *Salmonella* population. However, the *anti-Salmonella* effect was more significant in medicine C with the combination

**Table 5.** Effect of herbal medicines and antibiotics on the concentration of malondialdehyde (MDA) in the thigh and breast meat (day 21) in broiler chickens challenged with *Salmonella enterica* Serovar Typhimurium

| Parameters | Treatment | | | | | | SEM | P |
|---|---|---|---|---|---|---|---|---|
| | NC | PC | ENR | Medicine A | Medicine B | Medicine C | | |
| Thigh | 1.06[a] | 1.15[a] | 1.11[a] | 0.47[c] | 0.56[b] | 0.53[b] | 0.03 | 0.001 |
| Breast | 0.58[a] | 0.59[a] | 0.47[ab] | 0.42[c] | 0.45[b] | 0.44[b] | 0.01 | 0.023 |

[a, b]Means or percentages with different superscripts within a column differ significantly ($P<0.05$).

Abbreviations: SEM: Standard error of the mean; NC: Negative control; PC: Positive control; ENR: Enrofloxacin.

Notes: Medicine A: A mixture of marjoram and thyme extracts; Medicine B: A mixture of marjoram, thyme, cinnamon, and licorice extracts; Medicine C: A mixture of marjoram extract and OAs.

of marjoram extract and OAs than the other medicines and appeared somewhat similar to the antibiotic. Khatibjoo et al. (2020) showed that experimental supplements with marjoram oil reduced the population of *E. coli* and *Salmonella* spp. in broilers. Also, Amerah et al. (2012) reported that *Salmonella* colonization in broiler cecum was affected by adding cinnamaldehyde and thymol to the diet, which is abundant in marjoram. Usually, herbal extracts, due to the antibacterial effect of their active ingredients, such as carvacrol and thymol, can be effective in reducing *Salmonella* infection. Helander et al. (1998) demonstrated the inhibitory effect of carvacrol and thymol, two components in the essential oil obtained from marjoram, against *E. coli* and ST. Active ingredients in the extracts of medicinal plants use to disrupt the structure of the bacterial cell membrane and increase its permeability, leading to the leakage of ions and other cellular contents and, ultimately, the death of bacteria (Calo et al., 2015; Ultee et al., 2002). The *anti-Salmonella* effect of medicine C may be due to a mixture of OAs in its composition. It has been reported that OAs reduce *Salmonella* populations by producing an acidic environment in the gut (El-Saadony et al., 2022; Sultan et al., 2015). In confirmation of our results, Cerisuelo et al. (2014) stated that the combination of essential oils with butyrate organic acid effectively controls the proliferation of *Salmonella* in broilers.

Our results showed that an increase in the population of lactic acid bacteria was accompanied by a decrease in harmful bacteria. The role of these bacteria in protecting the intestinal environment against the invasion of pathogens is known (Mead, 2000). Herbal remedies in our study increased lactic acid bacteria and decreased the total number of aerobic bacteria. However, medicines B and C were slightly more effective than medicine A. Giannenas et al. (2014) stated that organic acids and essential oils might increase the bacterial population of lactic acid and prevent the growth of coliforms, which confirms our results. Lactic acid bacteria compete with pathogens for nutrients and binding sites, thereby reducing the population of pathogens such as ST in the intestine (Mead, 2000). This action will improve the health and well-being of the intestine and is effective in improving performance (Jazi et al., 2016).

As shown in Table 2, ST reduced broilers' performance after the challenge at 11 to 24 days of age and the entire experimental period. These results are coordinated with Vandeplas et al. (2009), which have reported that ST causes a significant decrease in the performance of broilers due to the inhibition of digestion and absorption of nutrients in the intestine. Compared to the positive control group, treatment of challenged broilers with herbal medicines and enrofloxacin improved their performance, and chickens receiving medicine B had similar BWG with enrofloxacin and better FCR than medicines A and C and enrofloxacin groups. These results are supported by Abudabos et al. (2016), in which BWG and FCR were similar in ST-challenged broilers treated with antibiotics, OAs, and phytogenes in the first and second weeks. Abdel-Wahab (2019) observed that feeding different levels of marjoram improved FCR and BWG compared to control chickens. Also, improvement in BWG from 7 to 35 days of age has been observed in broilers fed with mint and thyme (Ocak et al., 2008) or a mixture of marjoram essential oils and hops extract (Bozkurt et al., 2009). Our findings in this study indicate a reduction in FI in effect challenge and improvement due to treatments. This result agrees with Remus et al. (2014), who reported that broilers infected with *Salmonella* spp. showed a 9% reduction in FI and a 29% reduction in their growth. Improving BWG and FCR with medicines can improve FI and reduce growth retardation disorders by stimulating the secretion of digestive enzymes and stabilizing the intestinal microflora ecosystem (Franz et al., 2010; Lee et al., 2003b). The effects of medicines on FI are quite variable. Contrary to our findings, some studies did not find any difference in FI between the control group and therapies applied against *Salmonella* infection (Adhikari et al., 2020; Abudabos et al., 2016). The rationale for this can be attributed to the differences in the composition of different herbal additives and the concentration of their active ingredients.

In the present study, enrofloxacin and ST challenge did not affect blood parameters. However, cholesterol and triglyceride concentrations were reduced by medicines, and other blood parameters did not show a significant difference. Our findings agree with Yakhkeshi et al. (2011), who reported that serum triglyceride and cholesterol levels were reduced in broiler chickens by using herbal medicines. This reduction effect was more considerable in medicines B and A than in medicine C. Two of the main components of these two medicines are thyme and marjoram extracts. The results reported by Bölükbaşı et al. (2008) showed the decreasing effect of thyme, sage, and rosemary essential oils on serum cholesterol and triglyceride in laying hens. Also, the reducing effect of marjoram at a concentration of 0.4% and 0.8% in the diet on cholesterol was reported by Abdel-Moneim (2015). Licorice and cinnamon extracts are other compounds in medicine B. Adding licorice extract to drinking water (0.1, 0.2, or 0.3 g/L) reduces the total cholesterol of broiler chickens (Alagawany et al., 2019). Additionally, according to Sarica et al. (2009), adding cinnamon EO to the quail diet reduces total cholesterol and plasma triglyceride levels compared to the basal diet. The effect of medicines on the reduction of blood lipids can be due to active ingredients such as carvacrol and thymol. Lee et al. (2003b) showed that adding carvacrol to the diet significantly reduced triglycerides, which is consistent with our results. The active ingredients in the extracts of herbal medicines, such as thymol and carvacrol (Rathod et al., 2021), can be effective in reducing fat and total cholesterol by affecting the activity of the enzyme HMG-CoA reductase (Radwan, 2003), which is a key regulatory enzyme in cholesterol synthesis (Schumacher & DeBose-Boyd, 2021). Contrary to the results of this study, Amed et al. (2013) did not observe a change in triglyceride levels in broilers fed Biostrong®, a preparation of partially microencapsulated essential oils of thyme and star anise compared to control groups. A probable reason for the inconsistency of the results of different experiments may be due to differences in the level and type of herbal feed additives, nutrition, genetics, age, and experimental design.

Herbal medicines in this study reduced the amount of MDA in the thigh and breast meat as a marker of lipid oxidation and one of the most important factors in reducing the quality of meat (Zhai et al., 2018). This reducing effect was observed in medicine A with the combination of thyme and marjoram extracts more than in the other two medicines. The concentration of MDA in thigh meat was much higher than in breast meat. This concentration of MDA may be due to the higher content of unsaturated fatty acids in the thigh muscles, which oxidize to produce peroxides, lipids, or MDA (Tongnuanchan & Benjakul, 2014). The active ingredients in thyme and marjoram can be effective in reducing MDA as a result of using medicine A. Like natural antioxidants, active ingredients of extracts have several mechanisms that slow down oxidation reactions. Preventing the initiation of chain reactions and the continuation of oxidation, trapping free radicals, quenching single oxygen, and binding to metal ions are among the most important mechanisms of their action (Tungwanwanchan & Benjakol, 2014). In one experiment, the addition of thymol and carvacrol (200 mg/kg in feed) had a strong antioxidant effect (low MDA concentration, increased unsaturated fatty acids) on chicken thigh muscle lipids (Hashemipour et al., 2013). Similar to our results, Akbarian et al. (2014) showed that adding turmeric and oregano oil to the diet significantly reduced MDA levels in chicken muscles. According to the results of the present study, the antioxidant status of chicken meat can be increased by using natural antioxidants such as extracts of herbs as herbal medicine.

## 5. Conclusion

Although none of the herbal medicines used in this experiment was as effective as an antibiotic in reducing *Salmonella* colonization in the intestine of the chickens, they can be used as an effective antibiotic alternative in the prevention of Salmonellosis.

## Ethical Considerations

### Compliance with ethical guidelines

The study was approved by the Ethics Committee for Animal Experimentation of Tarbiat Modares University in Iran (Code: IR.MODARES.REC.1399.191).


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### Authors' contributions

Conceptualization and supervision: Shaban Rahimi, and Taghi Zahraei Salehi; Methodology: Mohammad Amir Karimi Torshizi; Investigation and writing-original draft: Ahmad Gholipour-Shoshod; Writing–review & editing: Alireza Behnamifar; Data collection: Ahmad Gholipour-Shoshod, Alireza Behnamifar, Tahereh Ebrahimi, Mahmoud Valizadeh, and Faeze Ganjpoor; Data analysis: Ahmad Gholipour-Shoshod, and Alireza Behnamifar.

### Conflict of interest

The authors declared no conflict of interest.


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مقاله پژوهشی

# ارزیابی رقابت پذیری گیاهان دارویی با آنتی‌بیوتیک برای کنترل سالمونلا انتریکا سرووار تیفی‌موریوم در جوجه‌های گوشتی

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## چکیده

**زمینه مطالعه:** سالمونلوز یکی از مهم‌ترین بیماری‌های طیور و مورد توجه از نظر بهداشت عمومی است.

**هدف:** مطالعه حاضر اثر انروفلوکساسین و داروهای گیاهی را بر عملکرد رشد، فراسنجه‌های خونی، اکسیداسیون گوشت و جمعیت میکروبی روده کور در جوجه‌های گوشتی چالش‌شده با ST بررسی کرد.

**روش کار:** تعداد ۲۴۰ قطعه جوجه گوشتی یک‌روزه (نر) سویه راس ۳۰۸ به طور تصادفی به ۶ گروه تقسیم شدند: کنترل منفی، کنترل مثبت، انروفلوکساسین و سه داروی گیاهی (A، B و C) حاوی نسبت‌های مختلف عصاره دارچین، آویشن، شیرین‌بیان و مرزنجوش با ترکیبات اسیدهای آلی. دوز انروفلوکساسین، داروهای A، B و C به ترتیب ۱، ۱، ۱ و ۲ میلی‌لیتر در لیتر آب آشامیدنی بود که در روزهای ۱۶ تا ۲۱ تجویز شد. در روز ۱۰، تمام گروه‌ها به‌جز کنترل منفی با ۱ میلی‌لیتر سوسپانسیون حاوی $1\times10^{7}$ CFU/mL، ST چالش شدند. صفات عملکردی در فواصل ۱-۱۰، ۱۱-۲۴، ۲۵-۴۲ و ۱-۴۲ روزگی اندازه‌گیری شد. فراسنجه‌های خونی، اکسیداسیون گوشت و جمعیت میکروبی روده کور در روز ۲۱ اندازه‌گیری شد.

**نتایج:** در میان گروه‌های مورد چالش، داروی C و انروفلوکساسین کمترین میزان سالمونلا را نشان دادند ($P<0/05$). داروی B اثر بهتری بر صفات عملکردی داشت ($P<0/05$). داروی A کمترین مقدار مالون‌دی‌آلدئید در گوشت و داروی A و B کمترین غلظت کلسترول و تری‌گلیسیرید را در سرم داشتند ($P<0/05$).

**نتیجه‌گیری نهایی:** داروهای گیاهی فوق‌الذکر می‌توانند به عنوان افزودنی‌های مفید در طیور برای بهبود عملکرد، کاهش باکتری‌های مضر دستگاه گوارش، کلسترول، تری‌گلیسیرید و اکسیداسیون گوشت استفاده شوند.

**کلیدواژه‌ها:** آویشن، دارچین، سالمونلا تیفی‌موریوم، شیرین‌بیان، مرزنجوش

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