DOI:10.22059/IJVM.2022.337920.1005233 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554

Evaluating the Competitiveness of Medicinal Plants in Comparison with Antibiotic on Performance, Blood Parameters, Meat Oxidation and Cecal Microbiota's Response in Broilers Challenged with *Salmonella Enterica* Serovar Typhimurium

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Running Headline: Effect of Herbal Medicines on Salmonellosis

Abstract

BACKGROUND: Salmonellosis is one of the important diseases in poultry industry as well as public health concerns. **OBJECTIVES:** Study 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 allocated to six 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, herbal medicines: A, B, and C were: 1, 1, 1, and 2 mL/L in drinking water, respectively; which were prescribed on days 16 to 21. On day 10, all groups except negative control were challenged with 1 mL suspension containing 1×10^7 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* ($P<0.05$). Medicine B had better effect on performance traits ($P<0.05$). Medicine A had the lowest amount of malondialdehyde in meat. Medicines A and B had the lowest concentration of cholesterol and triglyceride in serum ($P<0.05$). **CONCLUSIONS:** The above mentioned herbal medicines can be used as beneficial additives in poultry nutrition to improve growth performance, reduce *Salmonella* in gastrointestinal tract, and reduce cholesterol, triglycerides and meat oxidation.

KEYWORDS: Cinnamon, Licorice, Marjoram, Organic acid, Thyme.

INTRODUCTION

Salmonella spp. can cause Salmonellosis in humans and animals, which as a zoonotic disease, is often transmitted to humans through poultry products (Afshari *et al.,* 2018). The disease caused by ST causes 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 are exposed to *Salmonella* and the bacteria can be transmitted to humans through consumption of the 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 and 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 (Alagbe, 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 getting better the performance of poultry (Khan and 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 have a positive impact on 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 *Salmonella 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 has shown good results. The addition of 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 gr/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 feed of broilers challenged with ST improved BWG, FCR, and regulated FI (Ibrahim *et al.,* 2021).

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), improve 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, mixture 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 addition the mixture

of OAs and marjoram extract in water (0.2%) and feed (0.8%) of chickens significantly reduced *Salmonella enterica* serovar Enteritidis at 22 and 42 days of 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 antibiotic alternative in poultry diets.

Few studies have investigated the effect of the mixture of OA and herbal medicines on performance, meat quality, and intestinal microbial population in broiler chicken 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.

MATERIALS AND METHODS

The study was performed in the Poultry Research Center, Faculty of Agriculture, Tarbiat Modares University, Tehran-Iran. Experiment was approved by the Ethics Committee for Animal Experimentation of Tarbiat Modares University in Iran, with the ethics code IR.MODARES.REC.1399.191.

Management and experimental groups

The experiment was performed upto six weeks on 240 male Ross 308 chickens in six treatments and four replicates and ten chickens per replicate. The experimental groups were as follows:

1- Negative control group (NC): diet without any additives and ST challenge; 2- Positive control group (PC): diet without any additives + ST challenge; 3- Enrofloxacin group: 1 mL/L in drinking water + ST challenge; 4- herbal medicine A: a mixture of marjoram and thyme extracts (1 mL/L in drinking water + ST challenge); 5- herbal medicine B: a mixture of marjoram, thyme, cinnamon and licorice extracts (1 mL/L in drinking water + ST challenge); 6- herbal medicine C: a mixture of marjoram extract and OAs (2 mL/L in drinking water + ST challenge). The NC group kept with similar environmental conditions in 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 six days from 16 to 21 (six days after ST challenge) according to the manufacturer's recommendation. Enrofloxacin were obtained from Rooyan Darou Pharmaceutical Co, (Iran) for veterinary products and herbal medicines from the Academic Center for Education, Culture and Research (ACECR).

Salmonella challenge

Salmonella enterica serovar Typhimurium received from the microbiology laboratory of the Department of Bacteriology and Immunology, Faculty of Veterinary Medicine, University of Tehran (Tehran-Iran). For 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 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 at the same day.

Growth performance

For 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, the amount of blood parameters was 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, one gram 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 mL of thiobarbituric acid (TBA) was 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 one gram 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 count lactic acid bacteria, xylose lysine deoxycholate (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, 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.

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 number of *Salmonella* ($P<0.05$). The tested herbal medicines increase 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 ($P<0.05$).

172 The results of BWG, FI, and FCR are shown in Table 3. According to the results, until the
173 challenge with ST on day 10 of the experiment, there was no difference between the
174 experimental treatments in terms of performance (P>0.05). At 11 to 24 days, the negative control
175 had the highest, and the positive control had the lowest BWG (P<0.05). Among the challenged
176 groups, enrofloxacin and medicine B had better BWG than the other two medicines and positive
177 control (P<0.05). The enrofloxacin group had the highest, and the positive control group had the
178 lowest FI during this period (P<0.05). Also, the negative control group and medicine B had the
179 best, and the positive control had the weakest FCR (P<0.05). The difference in the performance
180 of treatments in the range of 25 to 42 days was not significant (P>0.05). Throughout the
181 experiment period, negative control groups and medicine B had the highest BWG and the best
182 FCR (P<0.05). The enrofloxacin group had the highest, and the positive control group had the
183 lowest FI and the worst FCR (P<0.05).

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

190 According to the results of Table 5, the comparison between negative and positive control groups
191 shows that challenge with ST did not affect the oxidation of chicken meat (P>0.05). Also, the
192 use of antibiotic did not affect the concentration of MDA in the meat of challenged chickens.

193 However, the use of herbal medicines significantly reduces the oxidation of meat in the thighs
194 and chest. The effect of medicine A was more substantial ($P<0.05$).

195 **DISCUSSION**

196 In the present study, enrofloxacin showed the best effect in reducing cecal *Salmonella*
197 populations in GI tract of the broilers. This result is supported by a study by Randall *et al.* (2005)
198 that reported the treatment of chickens with enrofloxacin reduced ST excretion from 10^5
199 CFU/swab to 40 and 2 CFU/swab on days 1 and 7 after treatment, respectively. Herbal
200 medicines have not been as effective as antibiotic in reducing *Salmonella*. However, anti-
201 S*almonella* effect was more significant in medicine C with the combination of marjoram extract
202 and OAs than the other medicines and appeared somewhat similar to the antibiotic. Khatibjoo *et*
203 *al.* (2020) showed that experimental supplements with marjoram oil reduced the population of *E.*
204 *coli* and *Salmonella* in broilers. Also, Amerah *et al.* (2012) reported that, *Salmonella*
205 colonization in broiler cecum was affected by the addition of cinnamaldehyde and thymol to the
206 diet which are abundant in marjoram. Usually, herbal extracts due to antibacterial effect of their
207 active ingredients, such as carvacrol and thymol, can be effective in reducing *Salmonella*.
208 Helander *et al.* (1998) demonstrated the inhibitory effect of carvacrol and thymol, two
209 components that are present in the essential oil obtained from marjoram against *E. coli* and ST.
210 Active ingredients in the extracts of medicinal plants use disrupt the structure of the bacterial cell
211 membrane and increase its permeability, leading to leakage of ions and other cellular contents
212 and ultimately the death of bacteria (Calo *et al.,* 2015; Ultee *et al.,* 2002). The anti-*Salmonella*
213 effect of medicine C may be due to the presence of a mixture of OAs in its composition. It has
214 been reported that OAs reduce *Salmonella* populations by producing an acidic environment in

215 the gut (El-Saadony *et al.*, 2022; Sultan *et al.*, 2015). In confirmation of our results, Cerisuelo *et*
216 *al.* (2014) stated that the combination of essential oils with butyrate organic acid effectively
217 controls the proliferation of *Salmonella* in broilers.

218 Our results showed that the decrease in the population of harmful bacteria was accompanied by
219 an increase in the population of lactic acid bacteria. The role of these bacteria in protecting the
220 intestinal environment against the invasion of pathogens is known (Mead, 2000). Herbal
221 remedies in our study increased lactic acid bacteria and decreased the total number of aerobic
222 bacteria, but medicines B and C were slightly more effective than medicine A. Giannenas *et al.*
223 (2014) stated that organic acids and essential oils may increase the bacterial population of lactic
224 acid and prevent the growth of coliforms, which confirms our results. Lactic acid bacteria
225 compete with pathogens for nutrients and binding sites, thereby reducing the population of
226 pathogens such as ST in the intestine (Mead, 2000). This action will improve the health and well-
227 being of the intestine and is effective in improving performance (Jazi *et al.*, 2016).

228 As shown in Table 2, ST reduced broilers performance after challenge at 11 to 24 days of age
229 and the entire experimental period. These results are coordinate with Vandeplas *et al.* (2009)
230 which have reported ST cause to a significant decrease in the performance of broilers due to
231 inhibition of digestion and absorption of nutrients in the intestine. Compared to the positive
232 control group, treatment of challenged broilers with herbal medicines and enrofloxacin improved
233 their performance and chickens receiving medicine B had similar BWG with enrofloxacin and
234 better FCR than medicine A, C and enrofloxacin groups. These results are supported by
235 Abudabos *et al.* (2016), which BWG and FCR were similar in ST-challenged broilers treated
236 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 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 is in agreement 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 be the result of improving FI and reducing growth retardation disorders by stimulating the secretion of digestive enzymes and stabilizing the intestinal microflora ecosystem (Franz *et al.,* 2010; Lee *et al.,* 2003). The effects of medicines on FI are quite variable. Contrary to our findings, some studies did not find any difference in FI between control group and therapies applied against *Salmonella* (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 had no effect on 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 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 Bolukbasi *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-Ghany (2015). Licorice and cinnamon extracts are other compounds in medicine B. Addition of licorice extract to drinking water (0.1, 0.2 or 0.3 g/L) reduced 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 reduction of blood lipids can be due to active ingredients such as carvacrol and thymol. The results of Lee *et al.* (2003) showed that adding carvacrol in the diet significantly reduced triglycerides, which is comsistent 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 and DeBose-Boyd, 2021). Contrary to the results of this study, Amad *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 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 the other two medicines. The concentration of MDA in thigh meat was much higher than 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 and 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 and 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, a study by 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.

CONCLUSION

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

Conflict of Interest

The authors declared no conflict of interest.

ACKNOWLEDGMENT

The authors are greatly appreciated for financial support of Tarbiat Modares University and Jehad Daneshghahi.

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Table 1. Composition and calculate 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 premix1	0.25	0.25	0.25
Mineral premix2	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 antibiotic 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		value
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]	7.89[c]	8.04[bc]	7.94[c]	0.13	0.032

[a- d] Means or percentages with different superscripts within a column differ significantly ($P<0.05$). SEM: Mean standard deviation. NC: Negative control; PC: Positive control; ENR: enrofloxacin; 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 3. Effect of herbal medicines and antibiotic on performance results in broiler chickens challenged with *Salmonella enterica* serovar Typhimurium

Parameters	Treatment						SEM	P-value
	NC	PC	ENR	Medicine A	Medicine B	Medicine C		
1 to 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 to 24 day								
BWG (g)	787.93[a]	663.33[d]	766.62[b]	744.58[c]	760.80[b]	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 to 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 to 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

$^{a-d}$ Means or percentages with different superscripts within a column differ significantly (P<0.05). SEM: Mean standard deviation. NC: Negative control; PC: Positive control; ENR: enrofloxacin. BWG: Body weight gain. AFG: Average feed intake. FCR: Feed conversion rate; 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 antibiotic on the serum biochemical indices in broiler chickens challenged with *Salmonella enterica* serovar Typhimurium

Parameters	Treatment						SEM	P-value
	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	7.59	6.33	7.24	8.58	7.64	8.14	0.51	0.114

(mg/dL)

[a-c] Means or percentages with different superscripts within a column differ significantly ($P<0.05$). SEM: Mean standard deviation. NC: Negative control; PC: Positive control; ENR: enrofloxacin; 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 5. Effect of herbal medicines and antibiotic 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-value
	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 ($P<0.05$). SEM: Mean standard deviation NC: Negative control; PC: Positive control; ENR: enrofloxacin; 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.

ارزیابی رقابت پذیری گیاهان دارویی در مقایسه با آنتی بیوتیک بر عملکرد، پارامترهای خون، اکسیداسیون گوشت و واکنش میکروبیوتای سکوم در جوجه های گوشتی در چالش با *سالمونلا انتریکا* سروار تیفی موریوم

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چکیده

زمینه مطالعه: سالمونلوز یکی از مهمترین بیماری های 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 240 قطعه جوجه گوشتی یک روزه (نر) سویه راس 308 به طور تصادفی به شش گروه تقسیم شدند. کنترل منفی، کنترل مثبت، انروفلوکساسین و سه داروی گیاهی (A، B و C) حاوی نسبت‌های مختلف عصاره دارچین، آویشن، شیرین بیان و مرزنجوش با ترکیبات اسیدهای آلی. دوز انروفلوکساسین، داروهای A، B

و C به ترتیب 1، 1، 1 و 2 میلی‌لیتر در لیتر آب آشامیدنی بود، که در روزهای 16 تا 21 تجویز شد. در روز 10، تمام گروه‌ها به جز کنترل منفی با 1 میلی‌لیتر سوسپانسیون حاوی 1×10^7 CFU/mL ST، چالش شدند. صفات عملکردی در فواصل 1-10، 11-24، 25-42 و 1-42 روزگی اندازه گیری شد. فراسنجه‌های خونی، اکسیداسیون گوشت و جمعیت میکروبی روده کور در روز 21 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 مالون دی‌آلدئید در گوشت و داروی A و B کمترین غلظت کلسترول و تری گلیسیرید را در سرم داشتند ($P<0/05$). **نتیجه گیری نهایی:** داروهای گیاهی فوق الذکر می‌توانند به عنوان افزودنی‌های مفید در طیور برای بهبود عملکرد، کاهش باکتری‌های مضر دستگاه گوارش، کلسترول، تری گلیسیرید و اکسیداسیون گوشت استفاده شوند.

کلمات کلیدی: اسید آلی، آویشن، دارچین، شیرین بیان، مرزنجوش.