**Lactobacilli** spp. and **Zataria multiflora** essence as antibiotic substituent on broiler health and performance parameters

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**ABSTRACT**

This experiment was conducted to evaluate the effects of commercial probiotics (Lactobacillus spp.) (LS) with **Zataria multiflora** essential oil (ZEO) and an antibiotic growth promoter (AGP) on broiler health and performance. In experiment 1, 300 one-day-old chicks (Ross 308) were assigned to one of 4 treatments, comprising five replicates per treatment in a completely randomised design. Dietary treatments included: basal diet (control), 0.25 and 0.50 g/kg LS, or 0.50 g/kg Flavomycine added to basal diet. In experiment 2, 225 one-day-old chicks, were assigned to one of 3 treatments, comprising five replicates per treatment in a completely randomised design. Dietary treatments included: basal diet (control), 0.50 g/kg LS, or 1 g/kg ZEO added to the basal diet. The inclusion of LS increased body weight gain significantly (P ≤ .05) at 42 days of age. Both AGP and LS reduced feed intake and feed conversion ratio compared to the control diet (P ≤ .05). The lowest triglyceride, cholesterol, LDL and ALT were observed in chicks administered by LS compared to control and AGP (P ≤ .05). The inclusion of LS decreased triglyceride, cholesterol, LDL and ALT, and increased immune titre against IBD and IB compare to control and AGP (P ≤ .05), and Intestinal health index were increased in LS group compared to control and AGP. The addition of ZEO, decreased LDL and ALT, and increased the immune titre against AI, ND and IBD compared to LS significantly (P ≤ .001). These results confirmed that LS and ZEO have effective on broiler chicken and can be considered a potent antibiotic replacer.

**HIGHLIGHTS**

- Use of antibiotic growth promoters (AGP) in poultry diets, has been banned worldwide.
- To control and eliminate of intestinal pathogens, and increase body weight and improve FCR, alternative procedures as adding phytogenic compounds and probiotics in the poultry diet.
- **Lactobacillus** spores and **Zataria multiflora** essence can decrease the microbial population of the caecum, and increase the intestinal health index.

**Introduction**

Numerous studies have demonstrated the positive effects of antibiotic growth promoters (AGP) on broiler survival and growth (Ferket 2004; Mountzouris et al. 2007). Nonetheless, antibiotic growth promoter (AGP) usage has been banned worldwide. Therefore, in recent years, additives such as probiotics, prebiotics, and phyto-stimulators as well as herbal essences have been paid attention to widely (Markowaik and Slizewska 2018), currently, probiotics research has mainly focused on livestock. In the most recent reports (Fuller 1997) live microbial organisms, which beneficially affect the host animal by improving its intestinal microbial balance have been named as probiotic. Probiotics act through pH reduction of the upper part of the GI tract, consequently reducing the presence of pathogenic bacteria sensitive to low pH such as *E. coli*, *Salmonella* and *Clostridia spp.* in the intestine and improving digestion in young birds (Izat et al. 1990). The use of medicinal plants as non-hazardous additives in poultry feed and/or even for the treatment of animal diseases are considered as low-cost and non-hazardous additives, without side effects and complications for the environment. Phenolic and Non-phenolic essential oils of the aromatic plant as natural antibacterial agents have taken such an interest in recent decades to control the growth of pathogenic bacteria both in humans and animals (Sevim and
Ayasan 2020). *Zataria multiflora* is an aromatic plant rich of monoterpenes, such as thymol and carvacrol which several studies have revealed its antimicrobial properties in humans, rat and fish (Avaei et al. 2015). Several studies have demonstrated *Zataria multiflora* essences effects against a variety of micro-organisms in in vitro and in vivo trials (Azizkhani et al. 2013). However, fewer studies have focussed on the antibacterial activities of *Zataria multiflora* essences in poultry (Romero et al. 2012) from poultry, alternatives such as antibiotics, probiotics, and plant essential oils have received considerable attention. Therefore, increasing knowledge about, these alternatives that could reduce or replace the use of antibiotics is fundamental (Carnevali et al. 2017), this encourages nutritionists to identify and characterise the low cost alternatives with no chronic side effects to improve poultry performance. However, growing studies and reports on the application of probiotics in poultry diets have demonstrated adequate amounts (Hedayati and Manafi 2018) to improve the health status. Therefore, in the present study, high dose of *Lactobacillus spores* compared with antibiotic growth promoters and also with a low cost plant essential oil to assess the influence of probiotics via the analysis of growth, immunity, survival and intestinal parameters between the probiotics, plant essential oils and antibiotic administrated in chicken.

**Materials and methods**

**Animal care**

Experiments were carried out based on procedures and guidelines approved by the Animal Care Committee of the Iranian Council of Animal care 1995. In *Lactobacillus spp.*, $1 \times 10^9$ cfu/g of lactobacillus spores, was obtained from Lallemand Animal Nutrition®, France and, *Zataria multiflora* essence (ZEO) was supplied in malayer university lab.

**Bird management and experimental design**

**Experiment 1**

Three hundred Ross-308 male broiler chicks with an initial BW of $38 \pm 1.1$ grams (parent stock age, 38 weeks) were obtained from a local. Experiment 1 was performed as a completely randomised design with four treatments and five replicates of 15 chicks per each replicated pens ($1^\text{st}1 \text{m}^2$). Experimental groups were included: control group (no probiotics and no antibiotic growth promoter) treatment 2: basal diet supplemented with antibiotic growth promoter of pharmaceutical company Huvepharma®, Sofia, Bulgaria (0.05%), treatments 3 and 4 basal diet supplemented with lactobacillus spores (0.1 and 0.25 FCU/kg of feed) respectively.

Experimental diets were formulated according to the nutrient specification of Ross 308 (2014) for three rearing phases: starter (1–10 day), grower (11–24 day) and finisher (25–42 day) (Table 1). The performance parameters such, Bodyweight (BW) and cumulative feed intake (FI) and feed conversion ratio (FCR) were measured based on the feeding periods described above.

**Blood analysis**

At the end of the rearing period, two birds from each experimental unit were selected and five cc blood of

| Ingredients | Starter (1–10 d) | Grower (11–24 d) | Finisher (25–42 d) |
|-------------|-----------------|-----------------|-------------------|
| Corn        | 495.00          | 598.00          | 662.70            |
| Wheat       | 55.80           | 50.00           | 50.00             |
| Soybean meal, 440 g/kg CP | 268.60          | 160.50          | 101.20            |
| Corn Gluten meal, 500 g/kg CP | 100.00          | 114.80          | 115               |
| Soybean oil | 33.30           | 33.40           | 33.20             |
| Lime stone  | 14.50           | 12.30           | 10.00             |
| Di calcium phosphate (DCP) | 18.50          | 18.00           | 18.00             |
| Sodium chloride (NaCl) | 3.60            | 3.60            | 3.60              |
| Vitamin and mineral premix$^2$ | 5.00            | 5.00            | 5.00              |
| DL – methionine (99%) | 3.20            | 2.80            | 2.20              |
| L- Lysine HCl (78%) | 2.50            | 1.60            | 1.40              |

| Calculated composition |
|-------------------------|
| Metabolizable energy (MJ/kg) | 12.59         | 13.23          | 13.44          |
| Analysed composition (g/kg) |
| Crude protein (CP) | 230.00         | 210.00         | 190.00         |
| Calcium | 11.00          | 9.00           | 9.00           |
| Available phosphorus | 5.00           | 4.50           | 4.50           |
| Lys | 14.10          | 11.60          | 10.50          |
| Met + Cys | 10.90         | 8.10           | 7.80           |

| Vitamin and mineral mix supplied the following per kg of diet: transretinol: 13 mg; cholecalciferol: 0.5 mg; a tocopherol acetate: 80 mg; menadione: 3 mg; thiamine: 3 mg, riboflavin: 8 mg; pyridoxine: 5 mg; cyanocobalamin: 0.024 mg; nicotinic acid: 60 mg; folic acid: 2 mg; Ca pantothenate: 15 mg; choline: 1000 mg; Mn: 120 mg; Zn: 1100 mg; Cu: 16 mg; Se: 0.3 mg; I: 1 mg; and Fe: 40 mg.
wing vein were obtained for immunity and blood serum parameters. Investigation of immunity titre of Newcastle virus (ND) and Avian Influenza (AI) virus were performed with hemagglutination-inhibition (HI) test according to (Hedayati and Manafi 2018), and Infectious Bronchitis (IB) virus and infectious Bursal disease (IBD) (Gumboro) using Elisa assay on blood serum of hens were carried out in Serology laboratory (IDEXX, IBDV Antibody ELISA; IBELISA kit). Evaluation of serum biochemical parameters including triglyceride, cholesterol, glucose, albumin, globulin, total protein, low-density lipoprotein (LDL), high-density lipoprotein (HDL), as well as liver enzymes including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) was analysed with an automatic biochemical analyser (Boehringer Mannheim Hitachi 704 automatic analyser, Japan), which had been standardised for analysis of chicken blood by using commercial laboratory kits (Pars Azmoon, Iran) to examine traits.

**Ileal morphology**

To intestinal morphology assay, two samples from each unite were collected in sterile conditions from mid-section of ileum by 5 cm, then samples were fixed in formalin 10% and sent to pathology lab using and after preparation, the samples were cut using a microtome and stained by using the Hematoxylin-Eosin H&E and Periodic acid–Schiff (PAS) methods, then after villi length and crypt depth were measured with optical microscope observation and analysed using Toupe viewer software (version 1.3 2007) (Xu et al. 2003).

**Experiment 2**

In a second experiment, the inclusion level of 500 mg/kg of lactobacillus spores was selected according to the results obtained from experiment 1 and compared with a plant essential oil. As in experiment 1, 225 male broilers (BW of 40 ± 1.1 grams (parent stock age, 38 weeks)). The experiment was performed, as a completely randomised design with five replicates of 15 chicks in each pen. Three treatments were formulated by adding 0.25 g/kg of lactobacillus spores and 1 g/kg of ZEO to the basal diet. Blood characteristics were measured using the same facilities and same procedure described in experiment 1.

**Microbial analysis**

On day 42, the caeca contents of two birds from each pen were pooled and used for serial dilution. Microbial populations were counted by serial dilution ($10^{-3}$ to $10^{-9}$) of caecal samples in anaerobic diluents (saline solution 9%) before inoculation onto Petri dishes of sterile agar as described by (Hedayati and Manafi 2018). Salmonella Shigella agar (SS agar) is used for *salmonella* culture and McConkey agar, and Eosin Methylene Blue (EMB) respectively, for *E. coli* and coliforms spp. Plates were incubated at 37°C and were counted between 24 until 48 hours after inoculation. Colony-forming units were measured immediately after removal from the incubator (GmbH, D-91126, and Germany).

**Preparation of hydroalcoholic extract of Zataria multiflora (ZEO)**

Collective samples of *Zataria multiflora* in malayer region of Hamedan province in Iran, were collected during the June 2016. Collected plant materials were dried in the shade, and the plant leaves were separated from the stem and grounded in a grinder to small particles.

The powder of *Zataria multiflora* (leaves and stems) young flowers macerated with 70% ethanol (1:20, w/v) at room temperature for 2 days and filtered through a Whatman no.42 filter paper. Other portions of the solvent were added to the marc and the extraction was repeated until the last extract was colourless. The extracts were combined and concentrated under reduced pressure at 75°C, 30 rpm and 110 minutes, using a rotary vacuum evaporator. The crude extract was then evaporated on a boiling water bath (HANSHIN, Scientific Co, South Korea) until a constant weight was obtained to afford the maceration extract. It was poured into sterile containers and sealed in a package, and after 24 hours of refrigeration for use for broilers, it was transferred to Malayer University Research Farm (Manafi et al. 2014).

**Statistical analysis**

Collected data were analysed by SAS software (version 9.2; 2009). Comparison of treatments means was performed using Duncan-range Test (1995) and the significance level was considered at $P \leq .05$.

**Results**

**Experiment 1**

**Performance**

The effect of *Lactobacilli spores* on broiler performance parameters is shown in Table 2. Inclusion of
Lactobacilli spores increased BW significantly \((P \leq 0.05)\) at 42 d, and the increase in body weight gain was more pronounced and comparable to antibiotic with supplementation of 500 mg/kg Lactobacilli spores in the diet. Both antibiotic and Lactobacilli spores reduced feed intake and FCR compared to the control diet \((P \leq 0.05)\).

**Blood characteristics**

There were significant differences in the blood lipid criteria of chicken fed antibiotic, and Lactobacilli spores supplemented diets compared to the control diet (Table 3; \(P \leq 0.05\)). Among the treatments, the lowest plasma triglyceride, cholesterol, and LDL were observed in chicks administered by Lactobacilli spores \((P \leq 0.05)\). Interestingly, liver ALT, AST, and ALP activity were reduced considerably when chickens received a diet supplemented with Lactobacilli spores (Table 3; \(P \leq 0.05\)).

**Immunity titer**

The Lactobacilli spores had significant effects on the immune titre against IBD and IB viruses (Table 4; \(P \leq 0.05\)). Compared to control, Lactobacilli spores increased but antibiotic reduced humoral immunity titre against IBD and IB viruses (Table 4; \(P \leq 0.05\)). Avian Influenza and Newcastle disease immune titre did not differ among treatments.

**Ileal morphology**

The Lactobacilli spores inclusion increased villi length and villi to crypt ratio and reduced crypt depth in comparison with the control and antibiotic fed chicks (Table 5; \(P \leq 0.05\)). The number of goblet cells per each villus was increased by the inclusion of Lactobacilli spores \((P \leq 0.05)\).

### Experiment 2

**Performance**

The influence of Lactobacilli spores and ZEO on broiler performance is shown in Table 6. The inclusion of Lactobacilli spores and ZEO had no significant effect on body weight, feed intake and FCR of chicks at 42 days of age \((P \geq 0.05)\). However, the weight gain and FCR were numerically improved by the inclusion of Lactobacilli spores and ZEO.

### Table 2. Effect of Lactobacilli spores and Flavomycine on broiler performance.

| Experimental groups | BW (g)   | FI (g)    | FCR (g of feed / BW) | Ability to survive (%) |
|---------------------|---------|----------|-----------------------|------------------------|
| Control             | 2580.00 | 4890.00  | 1.85                   | 97.50                  |
| Flavomycine 500 mg/kg | 2711.00 | 4683.00  | 1.72                   | 99.00                  |
| Lactobacilli spores, 250 mg/kg | 2604.00 | 4720.00  | 1.81                   | 99.50                  |
| Lactobacilli spores, 500 mg/kg | 2670.00 | 4620.00  | 1.73                   | 98.00                  |
| P-value             | 0.043   | 0.014    | 0.051                 | 0.162                  |
| SEM                 | 489.22  | 658.21   | 0.24                  | 17.34                  |

\(a\)–\(c\) Means within a column without a common superscript significantly differ \((P < 0.05)\).

BW: Body Weight; FI: Feed Intake; FCR: Feed Conversion Ratio.

### Table 3. Effect of Lactobacilli spores and Flavomycine on plasma lipid profile and liver Enzyme activity.

| Experimental groups | Triglyceride (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | Cholesterol (mg/dL) | Total protein (g/100 ml) | AST (IU/L) | ALT (IU/L) |
|---------------------|----------------------|-------------|-------------|---------------------|--------------------------|------------|-----------|
| Control             | 126.00\(^{a}\) | 50.00\(^{a}\) | 56.00\(^{a}\) | 193.00\(^{a}\) | 3.60                     | 125.50\(^{a}\) | 5.20\(^{a}\) |
| Flavomycine 500 mg/kg | 117.00\(^{b}\) | 55.00\(^{b}\) | 54.00\(^{b}\) | 180.00\(^{b}\) | 3.80                     | 125.60\(^{b}\) | 4.80\(^{b}\) |
| Lactobacilli spores, 250 mg/kg | 89.00\(^{c}\) | 65.00\(^{c}\) | 50.00\(^{c}\) | 150.00\(^{c}\) | 3.90                     | 120.80\(^{c}\) | 4.50\(^{c}\) |
| Lactobacilli spores, 500 mg/kg | 82.00\(^{c}\) | 75.00\(^{c}\) | 48.00\(^{c}\) | 145.00\(^{c}\) | 4.15                     | 118.75\(^{c}\) | 4.15\(^{c}\) |
| P-value             | 0.03             | 0.025       | 0.032       | 0.018                | 0.246                   | 0.035       | 0.024     |
| SEM                 | 2.58             | 12.47       | 8.45        | 26.49                | 0.68                    | 32.28       | 0.94      |

\(a\)–\(c\) Means within a column without a common superscript significantly differ \((P < 0.05)\).

HDL: high density lipoprotein; LDL: Low Density Lipoprotein; AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase.
**Immune titer against IBD, AI and ND**

The Lactobacilli spores significantly reduced humoral antibody titre against IBD and AI viruses (Table 7; \( P \leq .001 \)). However, humoral antibody titre against IBD, AI and ND were increased by the inclusion of ZEO (Table 7; \( P \leq .001 \)).

**Blood characteristics**

LDL, ALT and AST were reduced by the inclusion of ZEO compared to other groups (Table 8; \( P \leq .001 \)). Plasma cholesterol was reduced by ZEO and LS compared to a control diet. Also, triglyceride did not affect by supplementation of diets with Lactobacilli spores and ZEO.

**Microbial analysis**

In this study, the population of E. coli, Salmonella and coliform spp. were reduced by supplementation of Lactobacilli spores and ZEO (Table 9; \( P \leq .001 \)).

**Discussion**

In the present study, the beneficial effects of Lactobacilli spores treatments on broiler growth and feed consumption are in agreement with many other research studies using probiotics in broilers (Kalavathy et al. 2003). However, many factors can promote or attenuate the efficacy of a probiotic (Ewing and Cole 1994), such as species composition and viability, administration level, and type (e.g. live bacteria or its spores), frequency of application, diet composition, bird age and strain and situation of health and hygiene of farm (Mountzouris 2007). The intestinal health outcome of the current trial increased in villi length and a decrease E. coli, Salmonella and coliform population in the caecum. This finding suggests that Lactobacillus spores may beneficially affect the host animal by improving its intestinal microbiota balance.

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**Table 5.** Effect of different treatments on morphology of ileum.

| Experimental groups          | Villus height | Crypt depth | Goblet cell number | Height to depth ratio |
|-----------------------------|---------------|-------------|--------------------|----------------------|
| Control                     | 2.50 b        | 5.50 a      | 5.00 d             | 0.45 c               |
| Flavomycin 500 mg/kg        | 3.00 b        | 5.20 a      | 6.00 c             | 0.57 c               |
| Lactobacilli spores, 250 mg/kg | 3.15 b      | 3.50 b      | 7.00 b             | 0.90 b               |
| Lactobacilli spores, 500 mg/kg | 3.50 a      | 3.00 b      | 8.00 a             | 1.12 a               |
| \( P \)-value               | 0.017         | 0.034       | 0.042              | 0.034                |
| SEM                         | 0.48          | 0.59        | 0.94               | 0.96                 |

\( a-d \)Means within a column without a common superscript significantly differ (\( P < .05 \)).

The crypt depth and the height of the villi are calculated in micrometer units (\( \times 10^2 \)) and in Log 10.

The number of goblet cells per millimetre is calculated.

**Table 6.** Effect of Lactobacilli spores (LS) and Zataria multiflora essential oil (ZEO) on growth performance of broilers from one to 42 days of age.

| Treatments          | Control | LS, 500 mg/kg | ZEO | \( P \)-value | SEM |
|---------------------|---------|---------------|-----|--------------|-----|
| BW (g)              | 2388.40 | 2420.60       | 2395.50 | 0.369        | 9.56 |
| FI (g)              | 4280.00 | 4235.20       | 4220.00 | 0.063        | 11.22 |
| FCR (g of feed / BW)| 1.792   | 1.750         | 1.760   | 0.081        | 0.01 |

**Table 7.** Effect of Lactobacilli spores (LS) and Zataria multiflora essential oil (ZEO) on the immune response.

| Treatments          | Avian influenza (Log2) | Infectious bursal disease | Newcastle disease (Log2) |
|---------------------|------------------------|---------------------------|--------------------------|
| Control             | 4.58 b                 | 444.40 a                  | 5.01 b                   |
| LS, 500 mg/kg       | 4.08 b                 | 346.80 b                  | 5.04 b                   |
| ZEO                 | 5.07 a                 | 445.20 a                  | 6.01 a                   |
| \( P \)-value       | 0.002                  | 0.001                     | 0.003                    |
| SEM                 | 0.13                   | 3.92                      | 0.18                     |

\( a-c \)Means within a column without a common superscript significantly differ (\( P < .05 \)).

**Table 8.** Effect of Lactobacilli spores (LS) and Zataria multiflora essential oil (ZEO) on plasma lipid profile and liver enzyme activity.

| Treatments          | Triglyceride (mg/dl) | Cholesterol (mg/dl) | LDL (mg/dl) | HDL (mg/dl) | ALT (IU/L) | AST (IU/L) | ALP (IU/L) |
|---------------------|----------------------|---------------------|-------------|-------------|------------|------------|------------|
| Control             | 126.20               | 193.40 a            | 74.20 b     | 50.60 a     | 5.48 b     | 170.04 b   | 150.04 b   |
| LS, 500 mg/kg       | 118.80               | 190.20 a            | 67.40 b     | 45.40 a     | 5.01 b     | 190.72 a   | 160.48 a   |
| ZEO                 | 116.80               | 117.60 b            | 67.80 b     | 44.20 b     | 4.92 b     | 170.77 c   | 150.09 b   |
| \( P \)-value       | 0.027                | 0.001               | 0.0004      | 0.002       | 0.01       | 0.001      | 0.005      |
| SEM                 | 3.42                 | 1.95                | 1.21        | 0.98        | 0.06       | 0.97       | 1.32       |

\( a-b \)Means within a column without a common superscript significantly differ (\( P < .05 \)).

**Table 9.** Effect of Lactobacilli spores (LS) and Zataria multiflora essential oil (ZEO) on bacterial population in the caecum (Log10).

| Treatments          | E. coli | Salmonella | Coliform |
|---------------------|---------|------------|----------|
| Control             | 3.51 a  | 2.05 a     | 3.70 a   |
| LS, 500 mg/kg       | 2.52 b  | 1.65 a     | 2.09 a   |
| ZEO                 | 2.33 c  | 1.90 b     | 2.31 b   |
| \( P \)-value       | 0.001   | 0.001      | 0.01     |
| SEM                 | 0.04    | 0.03       | 0.03     |

\( a-d \)Means within a column without a common superscript significantly differ (\( P < .05 \)).
Plenty of researches have demonstrated that *Lactobacilli* can enhance beneficial microorganisms and ultimately enhance gut health (Rada and Rychly 1995).

Results of the current trial showed that *Lactobacillus* spores are very effective to reduce triglyceride and cholesterol concentration in chicken plasma. Also, the concentration of LDL and cholesterol was considerably decreased in birds fed *Lactobacillus* in both trials. The *Lactobacilli* spores significantly enhanced the immune titre against infectious bursal disease and infectious bronchitis. These results show that the *Lactobacilli* spores fed in the present study could effectively influence antibody titres against infectious bursal disease and infectious bronchitis. Similar results were obtained for Newcastle disease titre in bird which was administered with *Lactobacillus salivarius* (Zhang et al. 2015). Contrasting results were observed in the study with (Balevi et al. 2001) who reported no significant response to NDV vaccine antigen affecting specific antibody synthesis. This difference is probably due to competitive exclusion (Jin et al. 1998), in which beneficial bacteria isolated from the host were most efficient when being reintroduced back to the animal. In this study, *Lactobacilli* spores increased the villus height and villus height to crypt depth ratio in ileum which proposed an increased epithelial cell turnover due to feeding of probiotic in spores form. *Zataria multiflora* improved immune titre against vaccine in the current trial and reduced *E. coli*, *Salmonella* and *coliiform* population in the caecum.

Several studies have demonstrated *Zataria multiflora* essential oil’s effects against a variety of microorganisms in vitro and in vivo trials (Azizkhani et al. 2013). However, fewer studies have focussed on the antibacterial activities of *Zataria multiflora* essential oils in poultry. *Zataria multiflora* essential oil is rich in secondary metabolites such as thymol, carvacrol and para-cymene. Reported that higher concentration of phenol composition such as thymol stimulates the immune response. *Zataria multiflora* essential oils reduced the *E. coli*, *Salmonella* and *coliiform* population of caeca in the current trial. One of the main biological effects of essential oils is their antimicrobial effects which are related to their concentration of phenolic compounds, specially carvacrol or thymol have been reported against microorganisms (Cosentino et al. 1999). Essential oil with a high amount of carvacrol has higher antimicrobial activity, with carvacrol identified as a fatal compound for microorganisms as it interferes with membrane functions, increases the lipid membrane permeability and cellular adenosine triphosphate and finally causes death in the cell (Ultee et al. 1999). Thyme essential oils with a higher concentration of phenol exhibit greater antimicrobial effects (Burt 2004). Overall, considering the Effects of *Lactobacilli* spores and ZEO on intestinal health and immune response in the current study, it could be said that using high concentrations of *Lactobacilli* spores and ZEO in a broiler diet could lead to a reduction of intestinal pathogen bacteria and consequently could improve bird’s performance.

**Conclusions**

The results showed, the use of LS in the broiler diet, can increase the intestinal health index and reduce plasma triglyceride, cholesterol. Also Adding of LS and ZEO can increase the humoral immunity titre against common viral diseases and improve the performance parameters. The inclusion of ZEO can decrease triglyceride, cholesterol, and liver enzyme activity. These results confirmed that LS and ZEO have effective on broiler chicken and can be considered a potent antibiotic replacer.

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**Ethical approval**

This study was approved by the Animal Care Committee of the Iranian Council of Animal care 1995 (animal care number: 950095).

**Disclosure statement**

Authors have no conflict of interests to declare.

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Data availability statement

The results and analyses presented in this paper freely available upon request.

References

Avaei A, Mohammadi-Sani A, Mahmoodzadeh-Vaziri B. 2015. Chemical composition and antimicrobial effect of the essential oil of Zataria multiflora boiss endemic in khorasan-Iran. Asi Pacific J Tropi Dis. 5(3):181–185.

Azizkhani M, Misaghi A, Basti AA, Gandomi H, Hosseini H. 2013. Effects of Zataria Multiflora Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in Staphylococcus aureus ATCC 29213. Int J Food Microbiol. 163(2–3):159–165.

Balevi T, Uçan US, Cosğun B, Kurtogu V, Cetingül İS. 2001. Effect of dietary probiotic on performance and humoral immune response in layer hens. Br Poult Sci. 42(4):456–461.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol. 94(3):223–253.

Carnevali O, Maradonna F, Gioacchini G. 2017. Integrated control of fish metabolism, well-being and reproduction: the role of probiotic. Aqua Cult. 472:144–155.

Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. 1999. In-vitro antimicrobial activity and chemical composition of Sardinian thymus essential oils. Lett Appl Microbiol. 29(2):130–135.

Ewing WN, Cole DJA. 1994. The living gut: an introduction to micro-organisms in nutrition. Dungannon (UK): Context Graphics.

Ferket PR. 2004. Alternatives to antibiotics in poultry production: responses, practical experience and recommendations. Nottingham (UK): Nottingham University Press; p. 56–67.

Fuller R. 1997. Probiotics. Application and practical aspects. London (UK): Chapman and Hall; p. 10–39.

Hedayati M, Manafi M. 2018. Evaluation of an herbal compound, a commercial probiotic, and an antibiotic growth promoter on the performance, intestinal bacterial population, antibody titers, and morphology of the jejenum and ileum of broilers. Braz J Poult Sci. 20(2):305–316.

Izat AL, Tidwell NM, Thomas RA, Reiber MA, Adams MH, Colberg M, Waldroup PW. 1990. Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poult Sci. 69(5):818–826.

Jin LZ, Ho YW, Abdullah N, Jalaludin S. 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers diets containing lactobacillus cultures. Poult Sci. 77(9):1259–1265.

Kalavathy R, Abdulla N, Jalaludin S, Ho YW. 2003. Effects of lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br Poult Sci. 44(1):139–144.

Manafi M, Hedayati M, Yari M. 2014. Effectiveness of rosemary (Rosmarinus officinalis L.) essence on performance and immune parameters of broilers during aflatoxicosis. Advances in Life Sci. 4(3):166–173.

Markowiak P, Slizewska K. 2018. The role of probiotics, prebiotics and symbiotics in animal nutrition. Gut Patho. 10(21):1–20.

Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K. 2007. Evaluation of the efficacy of a probiotic containing lactobacillus, bifidobacterium, enterococcus, and pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci. 86(2):309–317.

Rada V, Rychly I. 1995. The effect of Lactobacillus salivarius administration on coliforms and enterococci in the crop and ceca of chicken broilers. Vet Med. 40:311–315.

Romero J, Feijoo CG, Navarette P. 2012. Antibiotics in aquaculture—use, abuse and alternatives. In: Carvalho, E, ed. Health and environment in aquaculture. Rijeka: Tech Europe Publication. p. 159–198.

Sevim B, Ayasan T. 2020. The use of jatropha (Jatropha curcas) in poultry nutrition. Turkish J Agr Res. 7(2):227–232.

Ultee A, Kets E, Smid E. 1999. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Appl Environ Microbiol. 65(10):4606–4610.

Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci. 82(6):1030–1036.

Zhang D, Lin T, Liu X. 2015. A comparison of growth, survival, and fatty acid composition of the lined seahorse, hippocampus erectus, juveniles fed enriched artemia and a calanoid copepod, Schumacker dubia. J World Aquacult Soc. 46(6):608–616.