Savory (Satureja hortensis L.) powder and extract effects on broiler chicken ileal Escherichia coli and Lactobacillus bacteria

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1. Introduction

Antibiotics and growth promotants have been used to improve poultry performance (Dibner and Buttin 2002; Miles et al. 2006; Nosrati et al. 2017; Rostami et al. 2017). However, veterinary drugs may result in residues that may be harmful substances in poultry products for human health. Using herbs has gained increasing interest as a feed additive and possible alternative to antibiotics in poultry production (Hosseinzadeh et al. 2014; Seidavi et al. 2017). Satureja hortensis is an aromatic and medicinal plant species from the family Lamiaceae and is related to rosemary and thyme, which can be substituted for veterinary drugs as growth promoters. It is cultivated as culinary herbs and available in North Africa, Southern and Southeastern Europe, the Middle East, and Central Asia (Sefidkon et al. 2006). It is widely distributed in Iran and is one of the important 12 Satureja classified species (Ebrahimii et al. 2013). It has medicinal properties such as anti-inflammatory (Hajhashemi et al. 2002; Karabay-Yavasoglu et al. 2006), antibacterial (Sahin et al. 2003; Mihajilov-Krstev et al. 2009), and antifungal (Sahin et al. 2003; Boyraz and Özcän 2006; Diba et al. 2013), has antioxidant properties (Dorman et al. 2004), and improves broiler chicken performance (Jang et al. 2007; Khosravinia 2015). Stef et al. (2009) found that medicinal plants and plant essential oils had significant effects on duodenum morphology and the immunological profile from broiler chickens. It has been reported that different Satureja hortensis treatments had significant effects on performance, carcass traits, immune response, and serum biochemical parameters in broiler chickens (Ghalamkari et al. 2013; Souri et al. 2015; Yeganeparast et al. 2016). However, when feeding different savory powder and extract levels, results are limited and have been inconclusive when fed to broiler chickens and on evaluating the ileal microflora. Furthermore, Satureja hortensis’s mode of action on the small intestine microflora has not been fully clarified. Therefore, the aim of this study was to evaluate savory (Satureja hortensis L.) powder (SP) and savory methanol extract (SE) levels of ileal microbial population when fed to 1-day-old to 6-week-old broiler chicks.

2. Materials and methods

Two hundred and twenty-five newly hatched male Ross-strain 308 broiler chickens were randomly allocated to 15 pens (200 x 100 cm) with 15 chickens per floor pen. Five test diets were randomly allocated to each pen, such that there were 3 replicates per dietary treatment (15 chickens per pen). The birds were housed in an insulated temperature-controlled room. The initial brooding temperature was held at 32°C for the first three days and then gradually lowered to 23°C by the end of the experiment. Photoperiods were maintained at 24 h/d during the first week and decreased to 23 h/d for the remainder of the trial. The chicks had ad libitum access to a starter mash diet for the 21 d experiment, and a grower mash diet from the 22nd to the 42nd days. Additionally, chicks were provided ad libitum access to water throughout the trial.

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ABSTRACT The objective of this study was to evaluate savory (Satureja hortensis L.) powder (SP) and savory methanol extract (SE) levels on ileal microbial population when fed to 1-day-old to 6-week-old broiler chicks. A total of 225-day-old broiler chicks were randomly allocated to 5 treatments with 3 replicates and fed for 42 days. Treatments included no supplement (control), 1.0% and 2.0% SP in feed, 50 and 100 ppm SE in drinking water. Data analysis was performed using SAS software and means were separated using Tukey’s pairwise method. The results showed that including 1.0% or 2.0% SP in broiler feed or 50 and 100 ppm SE in the drinking water significantly (P < .05) reduced the ileal Escherichia coli population when compared to birds receiving the control treatment. The ileal Lactobacillus population was significantly (P < .05) greater from birds receiving the 50 and 100 ppm SE treatments when compared to birds receiving the control treatment. Furthermore, there were no significant effects on ileal E. coli and Lactobacillus populations among the four treatments of savory. The results revealed that SP or SE can be used as an alternative to antibiotics in broiler chicken feeds.
Table 1. Experimental diet (starter and finisher) ingredients and chemical composition fed to Ross-strain 308 broiler chicks from 1d to 42 d of age in a study evaluating Savory (Satureja hortensis L.) powder and extract on ileal microbial populations.

| Ingredient (%) | Starter | Finisher |
|---------------|---------|----------|
| Corn          | 57.80   | 59.00    |
| Soybean Meal  | 34.75   | 32.00    |
| Corn Oil      | 3.50    | 3.50     |
| CaCO3         | 0.0     | 1.30     |
| Anzymite      | 0.0     | 1.30     |
| NaCl          | 0.20    | 0.20     |
| DL-Methionine | 0.15    | 0.15     |
| Lysine-Hydo-Chloride | 0.15 | 0.15 |
| Mineral Mixture | 0.5   | 0.50     |
| Vitamin Mixture | 0.5  | 0.50     |

Chemical analysis

- Energy (kcal/kg): 3019 ± 3025
- Protein (%): 20.48 ± 19.39
- Calcium (%): 1.00 ± 0.85
- Available phosphorous (%): 0.50 ± 0.42
- DCAB (mEq/kg): 236 ± 202
- Lysine SID (%) : 1.15 ± 0.96
- Methionine SID (%) : 0.50 ± 0.48
- Methionine + Cystine SID (%): 0.83 ± 0.78
- Threonine SID (%): 0.79 ± 0.71

aCu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g.

bVitamin A: 5000 IU/g; Vitamin D3: 500 IU/g; Vitamin E: 3 mg/g; Vitamin K3: 1.5 mg/g; Vitamin B2: 1 mg/g; Calcium Pantothenate: 4 mg/g; Niacin: 15 mg/g; Vitamin B6: 13 mg/g.

**SID**: Standardized Ileal Digestible.

**SEM**: Standard error of means.

Pre-experimental starter and grower feed composition are shown in Table 1. The basal diet was formulated according to broiler Ross 308 catalogue recommendations (Aviagen 2015). Five experimental diets in a completely randomized experimental design were used, treatment 1 with no Satureja hortensis supplementation in the diet, treatments 2, 3, 4, and 5 were supplemented with 1.0% and 2.0% SP in the diet, 50 and 100 ppm SE in the drinking water, respectively.

Dried savory powder and methanol extract savory were obtained from the local market and their compositions were determined according to AOAC (2005). Commercial preparation methods were as follows: Fresh aerial Satureja hortensis parts were purchased commercially and sun-shade dried and ground to obtain powder. The extract from the dried and powdered plant materials was obtained using methanol in a Soxhlet extractor for 72 h at a temperature not exceeding the solvent’s boiling point (Lin et al. 1999). Microflora measurement was conducted based on Dibaji et al. (2014). Briefly, at 42 d of age, 15 birds (3 birds from each treatment) were euthanized by cervical dislocation. When the birds were completely immobilized, the body cavity was opened, the ileum was excised, and digesta removed into pre-weighed, sterile sampling tubes, and immediately transferred on ice to the laboratory for microbial examinations. The sterile tubes with samples were weighed at the laboratory to determine the sample weight. The tubes were shaked for approximately 30 min. to isolate bacteria from digesta contents. One millilitre was removed from the prepared suspension and added to 9 mL of PBS in another tube. The suspension was prepared from 10⁻¹ dilutions and serial dilutions were made (10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻⁹). Next, 100 µL were removed from dilutions (10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻⁹) and poured into a pre-prepared petri dish containing a medium. The sample from each dilution was completely distributed to all parts of the medium within each petri dish. The culture media were prepared 24 h before the collected samples were poured into the petri dishes. Lactobacilli was cultured using MRS agar (Man Rogosa Sharpe agar, 1.10660.500) and Eosin Metillan Blou (EMB, 1.01347.0500) agar was used to culture Escherichia coli. The cultures were incubated at 37°C in anaerobic conditions for 72 h. At the end of the incubation periods, the colony-forming unit number (cfu) was numerated using a colony counter. Bacterial counts were reported as logarithm number of colony-forming units per gram of the sample.

**Statistical analysis**

The ileum microbial population data were analysed by one-way analysis of variance SAS, version 8 (2000). The completely randomized design model included 5 treatments containing savory powder and savory extract as main effects. The Tukey pair-wise test was used to separate means when model effects were significant. The statistical model used: $X_{ij} = \mu + T_j + e_{ij}$ where $\mu$ the common mean, $T_j$ the effect of the savory, and $e_{ij}$ the effect of the error. Before performing the statistical analysis, all of the data were tested by a normality test.

The experimental protocol was approved by the Animal Ethic Committee of the Islamic Azad University, and the experiment conducted in respect to the International Guidelines for research involving animals (Directive 2010/63/EU).

**3. Results and discussion**

Savory powder or savory extract effects on ileal microflora parameters from Ross-strain 308 broiler meat chickens from 1 d to 6 wk of age are presented in Table 2. The results from the present study showed the Escherichia coli population number ranged from 5.78 to 8.78 (log_{10} CFU/gr) and Lactobacillus from 6.49 to 8.47 (log_{10} CFU/gr) from the experimental treatments. The control sample had significantly ($P < .05$) more E. coli numbers when compared to the four other treatments. The results from the current study are in agreement with Ebrahimi et al. (2013) who reported that the control diet (without SP or SE) had significantly ($P < .05$) greater mean E. coli values when compared to diets containing 1% SP, 50 and 100 ppm SE. Among the four dietary treatments in the present study, there was no SP or SE treatments’ effect on E. coli at 42 d of age. These results are similar to those reported by Ebrahimi et al. (2013) who used similar treatments (dietary inclusion of 1%, 2% SP, 100, 200 ppm SE) in broiler chickens. The Lactobacillus digesta...
population was significantly ($P < .05$) lower in chickens fed the control diet at 42 d of age when compared to the chickens fed diets supplemented with 50 or 100 ppm SE in drinking water. Akbarian et al. (2013); and Hosseinizadeh et al. (2014) reported similar conclusions. Vidanarachchi et al. (2006) found that the lactose-negative Enterobacteria and Bifidobacteria numbers in ileum of broiler chickens were unaffected by dietary inclusion plant extract level. Lactobacillus bacteria are considered beneficial bacteria that contribute to a balanced gut microflora and may provide an optimal precondition for effective protection against pathogenic microorganisms and an intact immune system (Vidanarachchi et al. 2006). The present results showed that supplementing 100 ppm SE in the drinking water provided to chickens significantly ($P < .05$) increased Lactobacillus bacteria number when compared to the control chickens. Si et al. (2006) stated that it is possible to select plant bioactive compounds with a strong antimicrobial action against gut pathogens without harming beneficial bacteria such as Lactobacillus bacteria. Savory extracts can lead to decreased harmful bacteria populations such as E. coli and increase the beneficial bacteria populations such as Lactobacillus (Ebrahimi et al. 2013). The results reported by Ebrahimi et al. (2013) are in agreement with the present results. The exact antimicrobial mechanism that Satureja hortensis L. possesses is unknown. Since it is difficult to identify a specific action site where many interactions take place between the plant extract and bacteria, Satureja hortensis L. antimicrobial activity may be attributed to the essential oils present in the plant (Skandamis et al. 2001; Carson et al. 2002; Güllüce et al. 2003; Sahin et al. 2003).

As Sahin et al. (2003) stated, methanol extract has a stronger and broader antimicrobial activity spectrum when compared to hexane extract. Eloff (1998) reported methanol is a better solvent for extracting antimicrobial substances from medicinal plants than water, ethanol, and hexane. There are some reports demonstrating Satureja hortensis contains substances with antibacterial properties (Leung and Foster 1996). Meanwhile, Dorman and Deans (2000) stated the components with phenolic structures, such as carvacrol, eugenol, and thymol, were highly active against test microorganisms. Members from this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used.

The researcher emphasized that the broiler performance can be affected, other than feed traits, mainly by health and immune status factors. In fact, Pirgozliev et al. (2014) observed that the broiler rearing/hygienic conditions influenced nutrient availability when a blend of essential oils (XT 6930; Pancosma S.A., Geneva, Switzerland) including 5% carvacrol was used, suggesting that the rearing conditions should be taken into account for a correct interpretation of studies involving essential oils.

Plants have long been known to produce a variety of antimicrobial actions derived from their specific bio-active components, mostly secondary metabolites. Some plants including savory exerted a weak antimicrobial activity. Savory extract showed in vitro antibacterial activity against some pathogens. Our results showed that intestinal microbiota was significantly altered by the experimental diets and waters with savory, which indicate a healthy housing microflora. Some bacteria disturbances in the gut of broilers may lead to high feed conversion ratio and reduce animal performance.

The hydrophobicity observed from these essential oils enables them to partition lipids in the bacterial cell wall, disturbing the structures and rendering them more permeable, which has a positive influence on the gastrointestinal microflora (Brul and Coote 1999; Gauthier et al. 2007). Savory extract may improve endogenous enzymes' secretion, appetite stimulation, nutrient digestion and absorption, and gastrointestinal microflora balance, which reduces the E. coli population and stimulates the Lactobacillus bacterial population. Additionally, it can increase gastrointestinal villi proliferation and protect them and stimulate the immune system (Jamroz et al. 2005). Further, it has antiseptic, antifungal, and antioxidant properties and can be used as an antibiotic alternative. In conclusion, using 1.0%, 2.0% of SP in the diet, 50 or 100 ppm of SE in drinking water has positive effects on ileal microbial population from broiler chickens from 1 d to 42 d of age.

**Disclosure Statement**

No potential conflict of interest was reported by the authors.

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