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Effect of different levels of *Satureja hortensis* essential oil on performance, carcass characteristics, acidity and intestinal microflora population in broilers

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This experiment was carried out to study the effects of dietary supplementation of *Satureja hortensis* essential oil on growth, carcass parts, pH and luminal microbe in broiler chicken. A total of 400 one week old ROS 308 male chicks were used in a completely randomize design model with 5 treatments and 4 replicates, each composing of 20 chicks. Duration of experiment was 35 days until the age of 42 days. Treatments were a basal diet supplemented with 50, 100 and 150 mg/kg of *S. hortensis* essential oil with a negative (no supplementation) and a positive control (containing 300 g/1000 kg) of Orego-Stim (a commercial compound). Feed intake and body weight gain were measured weekly and feed conversion ratio was calculated accordingly. Chicks were reared on litter and were fed *ad libitum* with antibiotic and coccidiostat free diet. At 35th day, litter samples was collected and sent to laboratory for determining its moisture and bacterial total count. At the 42nd day, two chicks per cage were slaughtered for determining carcass parts ratio, ileum digesta pH and bacterial count by dilution method. Results showed that *S. hortensis* essential oil and Orego-Stim compound had no effects on broiler growth. Liver relative weight increased by 50 and 150 ppm *S. hortensis* essential oil as compared to the control group. No change of pH was observed due to treatments. Chicks that received 150 ppm *S. hortensis* essential oil showed higher litter bacterial count as compared to the control and other treatments.

Key words: Broiler chicken, *Satureja hortensis* essential oil, growth performance, bacterial count.

INTRODUCTION

The wide use of antibiotics and other chemical compounds have been experienced throughout the last 50 years in which research have been directed back to natural antimicrobial products as indispensable resources. Different additives such as enzymes, organic acids, probiotics, prebiotics and phytogenics are used to

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improve performance (Jamroz et al., 2005).

Herbs and plant extracts represent a new class of additives in poultry feed. Their uses are still limited in relation to their mode of action and aspects of application. In addition, complications may be encountered due to various changes in botanical origins, transformations and compositions of plants and their extracts. Most of the investigations have studied the interactions of various active compounds and their physiological impacts and effects on production performance (Figueiredo et al., 2008).

The hypothesis that phytogenic compounds could improve the food palatability has not been demonstrated. Furthermore, it is believed that the phytogenic compounds can improve the digestive enzyme activity and nutrient absorption. Many studies have demonstrated their antioxidant and antimicrobial activity *in vitro* but *in vivo*, these results are limited. In addition, other effects such as anti-inflammatory, anti-fungal, anti-infectious and anti-toxigenic have been confirmed in some researches (Giannenas et al., 2003; 2004; Lopez-Bote, 2004; Burt, 2004; Ahmed et al., 2013; Khan, 2014).

At present, the scientists are working to improve feed efficiency and growth rate of livestock using useful herbs (Bunyapraphatsara, 2007). Researches on the use of herbal mixtures in poultry diets have produced inconsistent results (Fritz et al., 1993). In an experiment, broilers fed with 0.5% of peppermint, grow faster and performed better than those fed with 1.5% peppermint.

There was no significant effect with the addition of the peppermint to the diet on blood traits (PCV, RBC, Hb and WBC), but liver weight decreased, whereas the heterophile/lymphocyte ratio significantly increased in treatments when compared with the control (Al-Kassie, 2010). Using 0.5% of *Mentha pulegium* L. aerial parts powder significantly improved the performance and carcass traits and reduced the blood glucose of broilers. Nobakht et al. (2011) reported that broilers fed plant extracts showed higher body weight gain when compared with the control group (Jamroz et al., 2005). Supplementation of essential oils from medicinal plants improves the immune defense in poultry (Lavina et al., 2009).

Mixture of Mentha, Zizphora and peppermint aerial parts powder did not have any considerable effects on broiler serum biochemical measures such as total cholesterol, triglycerides and glucose, but mixture such as 1% Mentha, 0.5% Zizphora and and 0.5% peppermint stimulated immune system (Narimani-Rad et al., 2011).

Satureja hortensis L. is an annual, herbaceous aromatic and medicinal plant belonging to the Lamiaceae family. It is known as summer savory, native to southern Europe and naturalized in parts of North America (Sefidkon et al., 2006). It is widely distributed in different parts of Iran as one of the most important of classified 12 *Satureja* species. It's essential oil contains considerable amounts of two phenolic ketones, that is, carvacrol and thymol (Ghannadi, 2002).

This experiment was carried out to study the effects of dietary supplementation *of S. hortensis* essential oil on growth, carcass parts, pH and luminal microbe in broiler chicken.

MATERIALS AND METHODS

Animals and dietary treatments

After reducing the moisture content of *S. hortensis*, its essential oil was extracted from the steam distillation or steam method which was used.

S. hortensis essential oil was obtained from the Kashan Golkaran Company. The essence used in current experiment was analysed using gas chromatography with mass spectrometry. Ten compounds were detected among Monotherpens which were the most and the others were y-terpinene (39.5%), carvacrol (38.5%), p-cymene (3.97%), α-terpinene (3.54%) and thymol (2.34%).

Four hundred, seven-day-old broiler chickens of male sex (Ross 308) at the Research Station Shahid Khorakian Qum (Qum - Iran), were used in a completely randomized design model with 5 treatments and 4 replicates, each composing of 20 chicks. Duration of experiment was 35 days until the age of 42 days. Treatments were a basal diet supplemented with 50, 100 and 150 mg/kg of *S. hortensis* essential oil with a negative (no supplementation) and a positive control (containing 300 g/1000 kg) of Orego-Stim (a commercial compound).

The diets were formulated (Table 1) to meet the nutrient requirements according to Ross 308 rearing guideline. Because the *S. hortensis* essential oil are very well soluble in vegetable oil, *S. hortensis* essential oil was dissolved in total vegetable oil of diets, then it was mixed with other feed ingredients.

Performance parameters

Body weight, feed intake and feed conversion were determined weekly on bird bases. Mortality was also recorded. Then the feed conversion ratio was calculated for all experimental units.

Carcass components

At 42 days of age, two birds per replicate were randomly chosen, slaughtered and carcass percentage and carcass components percentage including a thigh, breast, liver, heart and intestine were determined for live weight.

Acidity and microbial population of ileum

To determine the pH and microbial population of the ileum of broiler chickens, the whole lower ileum which was from Meckel's tag to the branching point of the blind gut, and its contents were removed with scissors into sterile cans, were unloaded and transported to the laboratory and homogenized. To determine the pH, the sample container was taken to the pH meter device and the recorded number was registered as an intestinal pH.

For counting of bacteria in ileal digesta, one gram of sample digestion contents available in this area was added to a test tube containing 9 ml of physiologic serum and was thoroughly mixed and homogenized. Afterwards, the sample was plated on 2 different media: blue agar (for total bacteria) and Levine eosin methylene blue agar (used to determine the number of bacteria gram-

Table 1. The ingredients and nutrition composition diets of broilers.

Ingredients of diet (%)	Diets	
	7 to 21 days	22 to 42 days
Yellow corn	53.8	60.7
Soybean meal	38.7	32.2
Vegetable oil	3	3
Calcium carbonate	1.63	2.03
Dicalcium phosphate	1.72	1.13
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
Salt	0.44	0.23
DL- Methionine	0.14	0.06
L- Lysine	0.07	0.05
Calculated composition		
Metabolisable energy (kcal/kg)	3000	3055
Crude protein (%)	21.54	19.09
Calcium (%)	0.93	0.85
Available phosphorous (%)	0.45	0.42
Energy to protein ratio	139.27	160.03
Methionine (%)	0.44	0.4
Lysine (%)	1.21	1.07

[1]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg ; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg. Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4.5H_2O$), 10 mg; I (KI, 58% I), 1mg; Se ($NaSeO_3$, 45.56% Se), 0.2 mg. The diets and water was provided with *ad libitum*. The lighting program during the experimental period consisted of a period of 23 h light and 1 h of darkness. Environmental temperature was gradually decreased from 33 to 25°C on day 21 and was then kept constant.

negative).

After counting the number of colonies per plate, the obtained number was multiplied by contrast dilution factor and the result was recorded as the number of colony forming units per gram of sample.

Litter moisture content

Individual litter samples were separated and weighed (15 g) before drying each sample for 24 h at 105°C (Barker et al., 2011). To determine the dry weight of each sample, samples were weighed again after drying. Moisture content (MC) was determined using the wet and dry weights (W) (American Society of Agricultural and Biological Engineers, 2007):

$$MC\ (\%) = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100$$

Litter microbial population

To check microbial population of broiler litter under test, in the thirty-fifth day of rearing, using clean and sterile containers, an approximately 10-g sample (one gram of litter sample was mixed by 9 ml of physiologic serum) was taken and transported to the laboratory in sterile conditions and was quite homogenized. The process was carried out similarly with the process of determining microbial population of ileum.

Statistical analysis

The data were subjected to analysis of variance using General Linear Model Procedures of SPSS 16 (2007) in the computer for a completely randomized design with 5 treatments and 4 replicates. Duncan multiple range test was used to test significant differences in treatments. Statements of statistical significance are based on $P<0.05$.

$$Yijk = \mu + Aj + Bk + ABjk + Eijk$$

Where Yijk is the dependent variable; μ is the overall mean; Aj is the treatment effect; Bk is the period effect; A × Bjk is the interaction effect of treatment and period; and Eijk is the residual error.

RESULTS AND DISCUSSION

Feed consumption, body weight gain, feed conversion and mortality

In comparison with the Duncan's test, there was also no significant difference in the amount of feed intake, weight gain, feed conversion and mortality which were observed between the control and experimental treatments (Tables 2, 3, 4 and 5).

The addition of *S. hortensis* essence and Orego-Stim commercial combination (in amounts determined in this experiment) to the male broiler diets did not improve the productive performance of the broilers and had no deleterious effect on productive broilers ($p<0.05$).

The results of this study were in line with the findings of Samadian et al. (2013), Kirkpinar et al. (2011), Khaligh et al. (2011), Amad et al. (2013), Erdogan et al. (2010), Botsoglou et al. (2002), Ghalamkari et al. (2011), Zamanimoghaddam et al. (2007), Jang et al. (2007), Hernandez et al. (2004), Tekeli et al. (2006) and Lee et al. (2003) who observed no positive effects and significant improvements in productivity. On the other hand, findings of the present study disagreed with the studies conducted by Ocak et al. (2008), Peric et al. (2010), Jafari et al. (2011), Giannenas et al. (2003), Ciftci et al. (2005), Al-Kassie (2009) and Cross et al. (2007), Ertas et al. (2005), Hashemi et al. (2009 a, b), Jia-Chi et al. (2012), Hosseini (2011), Khodaei (2011b), Pish Jang (2011), Pooryousef and Hosseini (2011), Jamroz et al. (2003) and Tiihonen et al. (2010) which maintained that in the case of growth performance of broiler chickens, there were significant improvements in body weight, weight gain, feed conversion and reduced fatalities due to addition of various compounds of plant origin.

Table 2. Feed intake of broilers fed the experimental diets.

Stages of rearing	Control diet	Diets containing *Satureja hortensis* essential oil			Diets containing Orego-Stim	P-value
		50 parts per million	100 parts per million	150 parts per million		
3 Wk	8±545	13±571	23±545	11±533	21±543	0.57
4 Wk	18±612	78±650	50±675	29±597	53±662	0.52
5 Wk	19±1086	33±1092	44±1160	53±1090	56±1192	0.32
6 Wk	56±1376	40±1387	42±1446	44±1404	66±1421	0.88
Total experimental period	66±3620	86±3701	94±3825	107±3624	178±3817	0.55

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

Table 3. The weight gain of broilers fed the experimental diets.

Stages of rearing	Control diet	Diets containing *Satureja hortensis* essential oil			Diets containing Orego-Stim	P-value
		50 parts per million	100 parts per million	150 parts per million		
3 Wk	13±306	24±316	14±333	11±317	7±331	0.68
4 Wk	15±402	32±393	22±420	17±408	23±401	0.93
5 Wk	14±489	23±482	23±480	29±462	23±513	0.63
6 Wk	36±663	49±609	26±621	25±586	34±582	0.50
Total experimental period	29±1860	51±1800	70±1854	56±1773	43±1827	0.73

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

Table 4. The feed conversion ratio of broilers fed the experimental diets.

Stages of rearing	Control diet	Diets containing *Satureja hortensis* essential oil			Diets containing Orego-Stim	P-value
		50 parts per million	100 parts per million	150 parts per million		
3 Wk	0.08±1.79	0.13±1.72	0.04±1.64	0.10±1.81	0.07±1.64	0.53
4 Wk	0.07±1.53	0.07±1.53	0.04±1.60	0.07±1.60	0.04±1.64	0.59
5 Wk	0.04±2.23	0.07±2.26	0.03±2.42	0.18±2.40	0.11±2.33	0.61
6 Wk	0.11±2.09	0.18±2.34	0.10±2.34	0.09±2.38	0.12±2.46	0.32
Total experimental period	0.05±1.95	0.02±2.01	0.04±2.07	0.10±2.10	0.06±2.09	0.41

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

Table 5. Percent mortality of chickens fed the experimental diets.

Experimental diets	Mortality in total experimental period
Control diet	1.3±1.3
Diet containing 50 ppm *Satureja hortensis* essential oil	3.2±8.9
Diet containing 100 ppm *Satureja hortensis* essential oil	2.5±3.9
Diet containing 150 ppm *Satureja hortensis* essential oil	0±5
Diets containing Orego-Stim	2.9±5.1
P-value	0.31

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

Table 6. Percent of carcass components of broiler chickens fed the experimental diets.

Carcass trait (%)	Control diet	Diets containing Satureja hortensis essential oil			Diets containing Orego-Stim	P-value
		50 parts per million	100 parts per million	150 parts per million		
Carcass	[ab] 0.22±70.97	[b] 0.48±69.94	[ab] 0.64±70.39	[b] 0.53±69.58	[a] 0.32±71.63	0.03
Liver	[b] 0.1±2.19	[a] 0.11±2.56	[ab] 0.13±2.30	[a] 0.06±2.51	[ab] 0.09±2.45	0.08
Heart	0.02±0.48	0.03±0.51	0.06±0.55	0.03±0.55	0.02±0.49	0.52
Intestine	0.12±1.11	0.05±1.08	0.06±1.06	0.06±1.11	0.06±1.03	0.92
Breast	0.70±24.83	0.41±24.81	0.72±23.74	0.51±24.06	0.48±24.70	0.58

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

Carcass composition

Comparison of the mean for the separation of components from the carcass into pieces is presented in Table 6. The findings revealed that the addition of *S. hortensis* essential oil and commercial drug Orego-Stim did not cause any improvements on the carcass traits of broilers. The only significant difference between the experimental and control group was the higher liver weight in broilers receiving diets containing 50 and 150 ppm of the *S. hortensis* essential oil which is in line with the study conducted by Khodaei (2011a). On the contrary, some studies including Pish Jang (2011), Kirkpinar et al. (2011), Nobakht et al. (2011), Jang et al. (2007) and Hernandez et al. (2004) concluded that the liver of broilers' fed with herbal additives were not affected.

Considering other carcass traits, according to the data given in Table 6, the results of this study (maintaining the inefficacy of added *S. hortensis* essential oil and commercial drug Orego-Stim in male broiler diet regarding the yielded carcass and relative weights of the heart, bowel, breast and thigh as compared to the weight of the live tested chickens), were in conformity with the results reported by Pish Jang (2011) reporting no impacts on the relative weights of gizzard and heart, Kirkpinar et al. (2011) showing no improvements in carcass and relative weight of gizzard, small intestine and heart, Vukic-Vranjes et al. (2013) maintaining no positive effects in breast and thigh, Scheuermann et al. (2009) showing no significant difference in carcass composition, Nobakht et al. (2011) revealing insignificant effects on thigh, gizzard and intestine weight, Ghalamkari et al. (2011) stating no influences on the weight of internal organs and carcass characteristics, Jang et al. (2007) indicating lowers firm-level which did not affect the weight of the intestine, Hernandez et al. (2004) mentioning no relevant effects on the weight of the gizzard and intestines, Khaligh et al. (2011) declaring no significant improvements in the characteristics of carcass and even loss of yielded carcass, and finally Lee et al. (2003) who concluded that lower firm-level had no effect on carcass composition of broiler chickens fed with herbal additives. However, the findings of the present study were not in agreement with the studies carried out by Pooryousef and Hosseini (2011) who showed an increase in the relative weight of the breasts and improved indicators of carcass traits, Jamroz et al. (2005) reporting 1.2% weight increase when comparing the breast muscle weight to body weight, Khodaei (2011a) showing increases in relative weight of the breast muscle, and Hosseini (2011) concluding improved breast weight and carcass traits in broilers fed with herbal additives.

pH and microbial population of the gut contents

pH comparison of ileal digesta and microbial population of the lower small intestine of broiler chickens is presented in Table 7. Comparing the means of these traits via Duncan's test, there was also no significant difference between the experimental diets with each other and with control groups.

Our results agreed with the findings reported by Jia-Chi et al. (2012) revealing the ineffectiveness of herbal additives on acidity of the intestinal flora. Moreover, the effect of experimental diets on microbial populations in the ileum contents of the lower small intestine of broilers under test was not significant. This finding was in line with Kirkpinar et al. (2011), Corduk et al. (2008), Cross et al. (2007) and Cao et al. (2010) stating the insignificant effect of herbal additives on the microbial population of the gastrointestinal tract. On the other hand, it was unlike the results reported by Vukic-Vranjes et al. (2013), Jia-Chi et al. (2012) and Roofchaee et al. (2011) who showed a significant reduction in the total population of gut flora by adding some additives to the diet of broiler chickens.

Litter moisture content and microbial population

Comparing the means for these traits using Duncan's

Table 7. pH and microbial population of the contents of the ileum of the small intestine of broiler chickens.

pH and microbial population of ileum	Control diet	Diets containing *Satureja hortensis* essential oil			Diets containing Orego-Stim	P-value
		50 parts per million	100 parts per million	150 parts per million		
pH of intestinal contents	0.39±5.60	0.18±5.21	0.23±5.82	0.27±4.93	0.41±5.19	0.35
Bacterial(billion per gram)	8224±15762	8628±15057	8652±15014	8449±15376	938±1208	0.61
Gram-negative bacteria (million per gram)	387.2±388.4	55±69	11.6±18.9	1.2±1.3	10±10	0.50

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

Table 8. Percentage of moisture and microbial population of broiler litter.

Moisture and microbial population of litter	Control diet	Diets containing *Satureja hortensis* essential oil			Diets containing Orego-Stim	P-value
		50 parts per million	100 parts per million	150 parts per million		
Litter moisture content (%)	4.92±50.96	0.84±51.18	2.95±53.45	2.72±52.72	3.29±48.63	0.86
Bacterial(billion per gram)	[b] 1064±1213	[b] 2154±2472	[b] 197±210	[a] 7630±17130	[b] 1038±1449	0.02
Gram-negative bacteria (Million per gram)	13.2±22.6	39.5±54.5	3.5±5	0±0	12.3±21.3	0.35

Table numbers in percentages and as mean ± standard error (SEM ±) are inserted. Values in the same row not sharing a common superscript differ significantly (P<0.05).

test, no significant difference was observed between the experimental diets with each other and with the control ones (Table 8). Considering the litter characteristics of the broilers, the only significant difference was the higher bacterial population in the context of broilers receiving diets containing 150 ppm of oils which was considerably greater than the control and experimental groups. Investigating the results of the previous studies, no study has been found to examine the microbial load in broiler litter.

In this study, the reason that no positive effects were observed due to addition of oils to broiler chickens' diet may be related to the appropriate conditions of the chickens, environmental conditions, and the composition of the diet of the broiler chickens. According to some researchers, the effectiveness of herbal dietary additives might be affected by internal and external factors such as infections and environmental conditions (Giannenas et al., 2003; Lee et al., 2004a, b, c). Furthermore, the effects of medicinal plants depends largely on the health status of animals (Acamovic and Brooker, 2005) and it is generally expected that the growth-stimulating effects of additives are most evident in situations where chickens are kept in a less clean environment (Samadian et al., 2013). Chick quality, health conditions and environmental management also play important roles in the effectiveness of herbal additives (Hashemi and Davoodi, 2012). Inasmuch as this experiment benefited the proper quality of chicks, favorable environmental conditions, and appropriate diet, the chickens' performance was at its peak, therefore, it is likely that no room was left for further improvement.

Conclusion

Since our knowledge is still limited in practical usages of herbal additives, and since there is a great disparity in herbal compounds, the process and geographical origin of the plants in additives, the complexity of the issue expands. Therefore, before the widespread use of herbal additives in poultry nutrition, thorough investigations should be carried out on mechanisms, compatibility with other components of the diet, toxicity and safety evaluation.

In conditions similar to this study, the addition of *S. hortensis* essence and Orego-Stim commercial combination (in amounts determined in this experiment) to the male broiler diets did not improve their productivity, nor did it cause harmful effects on their productivity. Thus, unreasonable use of herbal additives may just be a waste of money for aviculturists.

Conflict of Interests

The authors have not declared any conflict of interests.

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