

THE EFFECT OF USING MARJORAM EXTRACT AS NATURAL GROWTH PROMOTER ON THE PERFORMANCE AND INTESTINAL BACTERIA OF BROILER CHICKENS



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ABSTRACT

The present study was performed in order to examine the effect of using marjoram extract as a natural growth promoter on performance and intestinal bacteria of broiler chickens. Marjoram extract was prepared by the maceration method. Then, an *in vitro* trial was conducted to determine the antibacterial activity of marjoram extract against six bacterial strains. The results of the antibacterial disk diffusion test showed that marjoram extract has a high inhibitory effect against both Gram-positive and Gram-negative bacteria. Later on, an *in vivo* feeding trial was done using 90 day-old Ross broiler chicks. The chicks were divided into two groups of three replicates each. The first group served as a control without any supplementation while the second group was supplemented with marjoram extract as a growth promoter. The results showed that addition of marjoram extract led to a significant ($p < 0.05$) improvements in final live body weight, total weight gain and total feed intake of broiler chickens. On the other hand, the relative weights of internal organs were not affected by marjoram extract supplementation. The total aerobic intestinal bacteria count of the broilers fed the marjoram extract supplemented diet was significantly lower ($p < 0.01$) than that of the control group. The overall results suggest that marjoram extract could successfully be used as a natural growth promoter alternative to antibiotic in broilers' diets.

Keywords: Marjoram extract; broilers; performance; carcass; intestinal bacteria

INTRODUCTION

Several decades ago, antibiotics have been introduced in broiler production as growth promoters. The growth promoting effect of antibiotics was initially attributed to the reduction of pathogenic bacteria in the intestinal tract of chicks (Graham and Boland, 2007). Recently, the use of antibiotics as growth promoters in broilers' diet has been prohibited by the European Union (Regulation 1831/2003/EC) (Ocak *et al.*, 2008). As a result of this prohibition of antibiotics in poultry production, there has been an urgent quest for suggesting other alternatives. Various substances such as probiotics, prebiotics, organic acids, and plant extracts were employed as alternatives to antibiotics in poultry diets (Buchanan *et al.*, 2008).

Plant extracts, mainly those prepared from herbs and spices, contain several bioactive ingredients, have high amounts of phenolic secondary metabolites, and have antimicrobial activity (Ho *et al.*, 2010; Djeussi *et al.*, 2013). The mode of action of these plant extracts is based on an alteration in

the intestinal microbiota, an increase in enzyme secretion, an enhancement in the immune response and maintenance of the morpho-histological characteristics of the gastrointestinal tract (Petrolli *et al.*, 2012).

Several plant extracts have been studied for their antimicrobial and growth promoting activities for broilers. In this connection, Sharifi *et al.* (2013) proved that the addition of cumin, peppermint, yarrow and poley herbs to the diets of broilers as alternative to antibiotic growth promoters improved the broilers' growth performance. Moreover, Goodarzi *et al.* (2014) found that supplementing broiler diet with 30 g of fresh onion/kg improved broiler performance and positively influenced ileal microflora composition. The results of Yazdi *et al.*, (2014) revealed that dietary inclusion of 10 g anise/kg can be used as an alternative to antibiotics for broiler diets. Turmeric rhizome extract supplemented to broiler chickens had a positive effect on their performance, carcass characteristics, antioxidant capability and meat quality (Wang *et al.*, 2015).

Marjoram (*Origanum majorana L.*) is one of the most familiar herbs used in food flavoring and for therapeutic applications (Toghyani *et al.*, 2010). Marjoram species, have been reported to produce significant antimicrobial activity (Al-Harbi, 2011) and strong antioxidant activities (Badee *et al.*, 2013).

The aim of the present study was to evaluate the effect of marjoram extract as a natural growth promoter on performance and intestinal bacteria of broiler chickens.

MATERIALS AND METHODS

Preparation of Marjoram extract

Marjoram plant was purchased from a local market in Egypt in powder form. The plant powder was soaked in ethanol (80%) and stirred on a magnetic stirrer overnight. The dry extract used in the present experiment was then obtained by filtration and evaporation of the solvent. Marjoram plant extract (MPE) was kept frozen until later use.

***In vitro* determination of the antibacterial effect of marjoram extract**

The antibacterial effect of marjoram extract was determined *in vitro* using the "disk diffusion method" as recommended by Chang *et al.*, (1997). In brief, a suspension of the tested bacteria (100 µl of suspension containing 10⁸ CFU/ml) was spread on nutrient agar. Then, filter paper discs were impregnated with known concentration of the extract and placed onto the inoculated plates. The plates were incubated at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested bacteria. The diameters of the inhibition zones were measured in millimeters. The antibiotic "Ampicillin" was used as a positive control.

Broilers' growth experiment

Experimental design and diet formulation

The experiment was performed using 90 day old Ross broiler chicks. The chicks were assigned into two groups of three replicates each. Each replicate consisted of fifteen chicks. The first group was considered as a control group without any supplementation. The second group was fed a diet

supplemented with 14g marjoram extract/ 100 kg diet (as a natural growth promoter).

During the first four weeks of life, the chicks from day1 to day 28 were fed a starter diet (22% crude protein and a metabolizable energy of 3000 kcal /kg). During the period from day 29 to day 36, the chickens were fed a finisher diet (19% crude protein and a metabolizable energy of 3100 kcal /kg). The ingredient composition of the starter and finisher diets are given in Table 1. The diets were formulated to meet the nutrients requirements of the broiler chicks (NRC, 1994).

Table 1: Composition and determined analysis of the basal starter and finisher diets

Ingredient (%)	Starter diet	Finisher diet
Yellow corn 7.3%	56.000	62.075
Soybean meal44%	28.500	24.000
Corn gluten meal 60%	8.735	6.000
Vegetable Oil	2.740	3.730
Di-calcium phosphate	2.030	1.820
Limestone	0.570	0.990
Vitamin and mineral*	0.400	0.400
Sodium chloride	0.300	0.300
Choline Chloride 70%	0.075	0.075
DI-Methionine	0.260	0.270
L-Lysine HCl	0.390	0.340
Calculated analysis (%)		
Crude protein	22	19
ME (kcal/kg)	3000	3100
Lysine	1.40	1.13
Methionine	0.53	0.47
Methionine + Cystine	1.04	0.90
Calcium	0.95	0.85
Non-phytate P%	0.47	0.43
Determined analysis (%)		
Crude protein	22.1	19.2
Calcium	1.05	0.91
Total phosphorus	0.74	0.68
Ether extract	2.90	3.40
Ash	6.20	5.35
Crude Fiber	2.55	2.48

(*) vitamin and mineral supplied per Kg of diet: Vit. A, 12000 I.U., Vit. D3, 2000I.U. ; Vit.E, 10mg ;Vit.K3 , 2mg; Vit.B1, 1 mg; Vit.B2, 5 mg; Vit. B6, 1.5 mg; Vit. B12, 10 ug; Biotin, 50ug; Choline chloride,500mg; Pantothenic acid , 10 mg; Niacin,30mg; Folic acid,1mg; Manganese, 60mg; Zinc,50mg; Iron,30mg; Copper,10mg; Iodine,1mg; Selenium,0.1mg and Cobalt,0.1mg (According to NRC;1994).

Performance and carcass traits

During the growth experiment: the chickens' live body weight, weight gain, feed consumption and feed conversion were recorded at days 14, 28 and 36 of birds' age (end of experimental period).

For carcass characteristics determination, 6 chickens from each experimental replicate were slaughtered. The head, viscera, shanks, spleen, gizzard, heart and liver were removed and the rest of the body was weighed to obtain the dressed weight. The edible organs (heart, empty gizzard and liver) were then individually weighed. All weights were recorded to the nearest 0.01 g and expressed as a percentage of fasted weight.

Intestinal bacterial count

To determine the total count of intestinal bacterial, the intestine of chickens was collected at day 21 and day 36. The samples were collected in sterile bags and cooled until their delivery to the laboratory for bacterial count examination. The total aerobic bacterial count was determined using a standard plate count agar medium. The plates were incubated at 30°C, aerobically, for 24 - 48h. All the data are expressed as CFUxlog/g.

Statistical analysis

A completely randomized design was design was used in this study. Data were statistically analyzed using the CoStat program. Significant differences between the two means of each variable were detected by Student's t test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

***In vitro* antibacterial effect of Marjoram extract**

The *in vitro* antibacterial activity of marjoram plant extract (MPE) was assessed by measuring the diameters of zones of inhibition for some bacterial strains and the results are shown in Table 2. The effect of marjoram extract was compared to a standard antibiotic "Ampicillin".

The obtained antibacterial activities were classified compared to the control antibiotic as follows: (a) strong: for inhibition > 70%, (b) moderate: for inhibition 50–70% or (c) weak: for inhibition <50% (El-Chaghaby *et al.*, 2014). According to the results obtained, the extract of marjoram produced strong antibacterial activity against the three tested Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*) as well as the three tested Gram-negative bacteria (*Escherichia coli*, *Neisseria gonorrhoea* and *Pseudomonas aeruginosa*). It can be noticed also that the antibacterial effect of 1mg of Marjoram extract is equivalent to about 0.7 mg from the standard antibiotic "Ampycillin".

The antibacterial activity of marjoram extract is attributed to its bioactive chemical constituents including carvacrol as main component, sabinene, terpinene, γ -cymene, α -terpineol, linalool and other phenolics compounds (Toghyani *et al.*, 2010; De Moraes *et al.*, 2014). The genus of marjoram plant is rich in phenolic compounds with powerful antibacterial and antifungal properties (Baranauskaitė *et al.*, 2015).

Table 2: *In vitro* evaluation of antibacterial activity of marjoram extract on intestinal bacteria in broiler chickens

Bacteria (Gram reaction)	Inhibition zone (mm/mg sample)	
	Marjoram extract	Standard antibiotic
<i>Bacillus subtilis</i> (G ⁺)	14 (70%)	20
<i>Staphylococcus aureus</i> (G ⁺)	13 (72%)	18
<i>Streptococcus faecalis</i> (G ⁺)	13 (72%)	18
<i>Escherichia coli</i> (G ⁻)	14 (70%)	20
<i>Neisseria gonorrhoea</i> (G ⁻)	15 (75%)	20
<i>Pseudomonas aeruginosa</i> (G ⁻)	13(76%)	17

The values in parentheses are the inhibition percentages of MPE extract compared to the standard antibiotic Ampicillin

Broilers' performance

The effect of supplementation of marjoram plant extract (MPE) on the broilers' performance parameters at different age intervals are presented in Table 3. The results showed that during the first four weeks of age; the addition of MPE significantly ($p < 0.01$) improved the broilers' body weight, weight gain and feed intake. On the other hand; no significant ($p > 0.05$) differences ratio were observed in feed conversion between the control group and MPE group. During the last stage of the experiment (day 29-36), the body weight of the group supplemented with MPE was significantly higher ($p < 0.05$) than control group, Whereas no significant differences ($p > 0.05$) were observed for other performance parameters. These results suggested that the effect of MPE supplementation was more pronounced in the first stage of broilers' growth than at the later growth phase (Ocak *et al.*, 2008).

The data of the whole experimental period (1- 36 days old) clearly demonstrated a beneficial effect of adding MPE to the chickens' diet. The MPE group recorded significantly ($p < 0.05$) higher body weight and weight gain as well as significantly higher ($p < 0.01$) feed intake. Whereas, there were no significant differences ($p > 0.05$) in the feed conversion ratio of the treated group in comparison to the control group.

It can be inferred that the addition of marjoram extract as natural growth promoter into chickens' diet resulted in a significant improvement of broilers' growth performance parameters. This result could be due to the role of the herbal extract applied herein, in improving the nutrient digestibility, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes which in turn can result in improvement of broiler performance parameters (Ocak *et al.*, 2008).

The chemical composition of marjoram extract as previously mentioned showed that it contains a variety of secondary metabolites and mainly carvacrol (Jamroz *et al.*, 2003). These bioactive ingredients of the extract are reasons for improvement of gut health and nutrients absorption and utilization which ultimately enhance the health of broilers and increase their performance (Ali, 2014). These results are in agreement with the results of Demir *et al.*, (2003) who reported that herbal extracts could be successfully used as natural growth promoters in poultry production.

Table 3: Broilers' performance parameters fed marjoram extract supplemented diet

Treatment Parameter (1-14 day)	Control group	MPE group	p-value	Sig.
Body weight (g)	375.67±0.88	391.00±2.08	0.0025	**
Body weight gain (g)	332.67±0.88	348.00±2.08	0.0025	**
Feed intake (g)	459.67± 1.20	481.33±2.85	0.0022	**
Feed conversion ratio (g feed: g gain)	1.38±0.033	1.38±0.017	0.8500	NS
(15-28 day)				
Body weight (g)	1181.00±2.31	1257.33±6.17	0.0003	**
Body weight gain (g)	805.33± 2.73	866.33±4.63	0.0003	**
Feed intake (g)	1570.33±11.55	1661.67±14.50	0.0079	**
Feed conversion ratio (g feed: g gain)	1.9467±0.0133	1.9167±0.0067	0.1145	NS
(29-36 day)				
Body weight (g)	1653.67±20.00	1749.67±8.29	0.0114	*
Body weight gain (g)	472.67±19.15	492.33±10.41	0.4180	NS
Feed intake (g)	929.00±25.63	970.33±11.84	0.2170	NS
Feed conversion ratio (g feed: g gain)	1.9633±0.0384	1.9667±0.0384	0.9541	NS
(1-36 day)				
Body weight (g)	1653.67±20.00	1749.67±8.29	0.0114	*
Body weight gain (g)	1610.67±20.00	1706.67±8.29	0.0114	*
Feed intake (g)	2959.00±15.1	3113.33±2.67	0.0005	**
Feed conversion ratio	1.8333±0.0145	1.8233±0.0067	0.5655	NS

Means ± standard error (SE)

Carcass traits

The effect of adding marjoram extract to broilers' diet on their carcass characteristics is shown in Table 4. The results revealed that there were no significant differences ($p>0.05$) in relative weights of the total organs parts, liver, gizzard and heart between the control group and MPE group. Whereas, the final body weight of broilers in MPE group was significantly ($p<0.01$) higher than that of the control group. Similar results were obtained by Hernández *et al.*, (2004) who found no differences in gizzard, liver and pancreas weights of broiler chickens fed diets supplemented with two plant extracts the first is composed of sage, thyme and rosemary and the second from oregano, cinnamon and pepper.

Also results reported here in are in agreement with those obtained by Sarica *et al.*, (2005) and Yazdi *et al.*, (2014) and others who reported that the addition of growth promoters had no effect on the internal organs weights.

Table 4: Carcass Characteristics of broilers fed Marjoram extract-supplemented diets

	Control group	ME group	p-value	significance
Body weight (g)	1659.33±6.120	1754.33±7.88	0.0007	**
Total organs parts	6.7899±0.0889	6.7733±0.0809	0.8964	NS
Liver (%)	2.6533±0.0260	2.6467±0.0219	0.8541	NS
Gizzard (%)	3.5733±0.0612	3.5633±0.0664	0.9172	NS
Heart (%)	0.5633±0.0186	0.5633±0.0203	1.0000	NS

Mean ± SE

Microbiological analysis

The concentrations of total aerobic bacteria in the small intestine of the chickens in the control and MPE groups are shown in Table 5. It was noticed that the addition of MPE as a natural growth promoter caused a highly significant ($p < 0.01$) reduction in the total intestinal bacteria of the chickens fed the MPE- supplemented diet as compared to the control group. This result was expected since many previous studies confirmed that the pathogenic intestinal microflora is controlled by the direct antimicrobial action of phytogetic extracts (Jamroz *et al.*, 2003).

Table 5: Total bacterial count (CFUxlog/g) in intestine of 21 and 36-day-old chickens fed marjoram extract – supplemented diet

	21 day old			36 day old		
	Total bacterial count	p-value	significance	Total bacterial count	p-value	significance
Control group	7.1267±0.0088	0.0001	**	7.2133±0.0145	0.0001	**
ME group	6.6900±0.0058			6.8833±0.0033		

Mean ± SE

CONCLUSION

The results of the present study showed that the use of marjoram extract as natural alternative antibiotic growth promoter in broilers' diets has a positive effect on chickens' performance. The microbiological data of the study have also confirmed the beneficial effect of the marjoram extract. Further studies are needed to investigate the *in vitro* and *in vivo* effects of other plant extracts as natural and safe alternatives to the prohibited antibiotic growth promoters on broilers' performance.

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تأثير استخدام مستخلص البردقوش كمنشط نمو طبيعي على مقاييس الاداء و
بكتريا الامعاء لدجاج التسمين
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تم إجراء الدراسة الحاليه من أجل بحث تأثير استخدام مستخلص البردقوش كمنشط نمو طبيعي على مقاييس الأداء و بكتيريا الأمعاء لكتاكيت اللحم. تم إعداد مستخلص البردقوش بطريقة النقع. ثم اجريت تجربة معملية لإختبار النشاط المضاد للبكتيريا لمستخلص البردقوش ضد ستة من السلالات البكتيرية. أظهرت نتائج الاختبار أن المستخلص له تأثير تثبيطي مرتفع لثلاثة انواع من البكتيريا الموجبة لجرام و ثلثه اخرى من البكتريا السالبة لجرام. تم بعد ذلك إجراء تجربة تغذية باستخدام ٩٠ من كتاكيت اللحم من نوع "روس" عمر يوم. تم تقسيم الكتاكيت إلى مجموعتين من ثلاثة مكررات لكل منهما. كانت المجموعة الأولى "كونترول" بدون أي اضافات والمجموعة الثانية كانت مجموعة " المعامله" التي تم إضافة مستخلص البردقوش لها. أظهرت النتائج في نهاية الفترة التجريبية (٣٦ يوماً) أن إضافة مستخلص البردقوش أدى الي تحسين معنوي ($p > 0.05$) لوزن الجسم النهائي وزيادة الوزن الكليه واستهلاك العلف الكلي لكتاكيت اللحم. و على الجانب الاخر لم تتأثر الاوزان النسبيه للأعضاء الداخلية بالمعامله. كما أظهرت النتائج أن إضافة مستخلص البردقوش إلى علائق كتاكيت اللحم أدى إلى إنخفاض معنوي ($P > 0.01$) في تعداد البكتريا الهوائية في الأمعاء مقارنة بالمجموعه الكونترول. وتشير النتائج الإجمالية إلى أن مستخلص البردقوش يمكن استخدامه بنجاح كمادة طبيعيه منشطه للنمو في علائق دجاج التسمين.