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Effect of some dietary herbal supplements as growth promoters on productive and physiological performance of broilers

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ABSTRACT

Proscription of synthetic growth promoters (SGP) in poultry production worldwide has encouraged scientists to seek out natural alternatives, such as herbal additives. This research aimed to investigate the possible impact of adding garlic, thyme, sage and their combination on performances of broiler chickens. A total of 180 unsexed, one-day-old Cobb 500 chicks were randomly dispensed into five treatments (3 replicates × 12 chicks per replicate). Dietary treatments were as follows; T1: control group; T2: garlic powder (*Allium sativum*, 5g /kg); T3: thyme powder (*Thymus vulgaris L.*, 5g /kg); T4: sage powder (*Salvia officinalis,* 5g /kg); T5: garlic + thyme + sage powder (5g /kg for each). Growth performance was evaluated throughout the 35-day trial. Results obtained reveal that tested supplements thyme and sage, significantly increased final body weight (FBW) and improved feed conversion ratio (FCR) compared to other treatments. While the sage or garlic groups significantly ($P<0.05$) had the highest carcass yield %, without holding significant differences ($P > 0.05$) for other carcass variables. All studied supplements had a significant appreciable lowering impact in decreasing blood serum, total cholesterol and lipids compared to the control. In addition, enhanced immunological indices (H/L ratio and antibody titer against Newcastle disease) and beneficial bacteria count in the small intestine of birds provided by supplemented phytogenics. In respect of the results, it can be concluded that adding 0.5% of the proposed additives to broiler diets could be utilized as an alternative to SGP due to the affirmative productive performance and immune-system of broilers.

Keywords: Broiler; growth performance; microbiota; garlic; thyme; sage.

INTRODUCTION

Since the 1970s, there has been a lot of attention paid to the utilization of antibiotic growth promoters (AGPs) in modern livestock diets due to their significant advantages in improving the health and growth status of birds. Antibiotics as growth promoters are prohibited by the European Union due to the potential for antibiotic-resistant microorganisms to thrive in chicken products resulting from the prolonged sub-therapeutic use of AGPs in the industry (Lepczyński *et al*., 2024). Researchers were prompted by the previously mentioned situation to look for antibiotic alternatives, and they suggested using botanical products known as phytogenic feed additives, which include herbs and spices and have antimicrobial, antioxidant, anti-inflammatory, immune-modulatory, and digestion-stimulating properties (Oso *et al*., 2019).

One promising phytogenic feed additive is garlic (*Allium sativum*). Its main active component, allicin, quickly disintegrated into a number of volatile organo-sulfur compounds with beneficial bioactivities (Oluwafemi *et al*., 2020). Numerous studies conducted on broiler chickens have demonstrated that garlic has growth-promoting capabilities (Pagrut *et al*., 2018; Puvaca *et al*., 2019; Kairalla *et al*., 2022).

Another noteworthy plant is thyme (*Thymus Vulgaris L*.), which is a medicinal herb commonly grows along the Mediterranean coast. Thymol is the main component of the essential oils from thyme, which have antioxidant and antibacterial features (Vassiliou *et al*., 2023). The phenolic OH group in thymol donates hydrogen to peroxy radicals, which may account for the inhibition of the production of free radicals (Chen *et al*., 2023). Furthermore, nutritional researchers have shown that thymol can alter the gut microbiota and prevent grave compounds from interacting with cellular biological components.

Sage (*Salvia officinalis L.*) is a perennial shrub with woody shanks and grayish leaves that is a member of the *Labiatae* family. It possesses a broad range of pharmacological activities, including anti-inflammatory, antioxidant, and antibacterial characteristics (Zhumaliyeva *et al*., 2023), which makes it a viable natural alternative to antibiotics. As reported by Farhadi *et al*. (2020), the inclusion of sage powder in broiler diets boosted the growth performance and immune system of birds.

In this direction, the following study was conducted to ascertain the effect of the dietary additives of garlic, thyme, sage or their combination on the productive and physiological performances of broiler chickens.

MATERIALS AND METHODS

All experimental protocols and methodologies, including animal husbandry and handling, were approved by the Faculty of Agriculture's Animal Research Ethics Committee at Assiut University in Egypt. (Approval number 2023-12-1).

Birds, experimental design, diets and management:

For this experiment, one hundred and eighty unsexed a-day-old broiler chicks (**Cobb 500**) with an average starting body weight of 42.2 0.5 g/chick were obtained from a nearby respectable commercial hatchery. All chicks were randomly distributed into 15 littered pens (1 m^2) with five treatments (3 replicates each, 12 birds per replicate). The experimental treatments were T1: control group, T2: garlic powder (*Allium sativum*, 5 g/kg diet), T3: thyme powder (*Thymus vulgaris L.*, 5 g/kg diet), T4: sage powder (*Salvia officinalis,* 5 g/kg diet), and T5: garlic + thyme + sage powder (5 g/kg diet from each additive). All birds were fed commercial basal diets mixed with additives suggested for starter (d 0 to 14), grower (d 15 to 28), and finisher (d 29 to 35) periods (**Table 1**). Chicks were grown for 35 days at 33°C of room temperature and progressively decreased to 24°C (with 60% humidity). *Ad libitum* consumption of feed and water was offered. The birds were exposed to 24 hours of light on day one, and then the light was reduced by 1 hour for each day until they had 18 hours of light, which was maintained until the completion of the experiment. Individual body weights were recorded each week. Feed intake per bird was determined at the end of each growing phase. The feed conversion ratio was calculated at the end of each growing phase by dividing feed intake by weight gain. The mortality rate was recorded during the experimental periods.

Table 1. Feed ingredients and nutrient levels of basal diets.

Ingredient (%)	Starter (0-14 d)	Grower (15-28 d)	Finisher (29-35 d)
Yellow Corn	52.27	59.10	63.20
Soybean meal (44%CP)	34.01	26.68	22.49
Corn gluten (60% CP)	6.00	7.00	6.31
Sunflower Oil	3.00	3.00	4.00
Di-Calcium phosphate	1.83	1.63	1.57
Lime stone	1.44	1.19	1.10
L- Lysine HCl	0.33	0.33	0.28
Dl -Methionine	0.27	0.21	0.17
Sodium Chloride	0.23	0.23	0.25
Sodium bicarbonate	0.22	0.23	0.23
Vitamins Premix *	0.10	0.10	0.10
Minerals Premix**	0.30	0.30	0.30
Nutrients levels %			
Crude Protein	23.17	21.25	19.14
Metabolizable Energy (Kcal/Kg)	3100	3110	3205
Crude fiber	3.81	3.46	3.23
Crude fat	5.56	5.77	5.86
Calcium	1.04	0.90	0.84
Av. Phosphorus	0.50	0.45	0.43
Lysine	1.44	1.24	1.09
Methionine	0.68	0.60	0.54

* Supplied per kg of diet: Vit. A, 11000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3mg; Vit. B1, 2 mg; Vit. B2 6 mg; B6 3 mg; B12, 14 mcg; Nicotinic acid 60 mg; Folic acid 1.75 mg, Pantothenic acid 13 mg; and Biotin 120 mcg.

** Supplied per kg of diet: Choline 600 mg; Copper 16 mg; Iron 40 mg; Manganese 120 mg; Zinc 100 mg; Se 0.20 mg and Iodine 1.25 mg.

Characteristics of carcass and relative organs weight of broilers:

At 35 days of age, after fasting for 6 hours before slaughtering, two birds/replicate were randomly selected (six birds for each treatment) and individually weighed, euthanized followed by bleeding, de-feathering, and

evisceration. Carcass cut parts (breast, thighs, wings and neck), organs (liver, heart, spleen and gizzard) and abdominal fat were weighed and calculated as percentages of the pre-slaughter live body weight of the birds.

Blood parameters:

At slaughtering, blood samples were collected from the birds at 35 days of age using vacuum blood collection tubes. The samples were then centrifuged at 3,000× g for 15 minutes to obtain serum, and then they were frozen at -20 degrees Celsius until analysis. Serum, total protein, albumin, glucose, total cholesterol, total lipids and aminotransferases aspartate (AST) and alanine (ALT), all these measurements were carried out following the kit manufacturing guidelines. Antibody titers against the new castle vaccine were measured using the Haemagglutination inhibition test (Suartha *et al*., 2018). The percentage of heterophils (H) and lymphocytes (L) were also determined to calculate the H/L ratio.

Intestinal microbial populations:

At 35 days of age, two birds were randomly picked from each replicate and euthanized via cervical dislocation The distal half of the small intestine, which includes the area between Meckel's diverticulum and the ileo-cecal junction, was quickly removed and the culture were repaired according to the methods outlined by Hu *et al.* **(**2012**)**. The contents were collected into glass containers under CO2 and frozen at -80°C to determine the counts of E. coli and Lactobacillus. The Ileal contents (0.2 g) were diluted in 2 ml sterilized saline, and three 10-fold serial dilutions (10−4, 10−5 and 10−6) were made. A 100 ml portion of the last three dilutions was evenly distributed onto plates. Lactobacillus were enumerated on MRS agar at 37°C for 48 h, and E. coli colonies were counted on MacConkey agar and incubated for 24 hours at 37°C. The colony forming units (CFUs) on plates with countable colonies were numbered and averaged to express 1 g CFU per gram of ileal contents.

Statistical analysis:

The statistical analysis was conducted using SAS **(**2013**)** General Linear Model (GLM). Duncan's Multiple Range Test (Steel and Torrie**,** 1980) was used to examine the notable variations in treatment means. Before analysis, all percentages were transformed using arcsine to approximate normal distribution. The information is displayed as average values and SEM. Probabilities below 0.05 ($p < 0.05$) were considered significant. One-way ANOVA was utilized to assess how different treatments impacted a variety of measured parameters. The data were analyzed according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Y_{ij}= Observation, μ = average mean, T_i = Effect of treatments, e_{ij} = random errors.

RESULTS

The experiment did not result in any mortality. Results of dietary additives proposed on broilers performance indices presented in **Table (2)** shows that birds provided with feeds supplemented with individual forms of tested herbal plants significantly (P<0.05) enhanced final body weight (FBW), average gain (AVG) and feed conversion ratio (FCR) compared to birds in the control group. Feed intake (FI) was noticed to be lower for the additives treatments, except for thyme powder group. It was assumed that birds in the combination group would exhibit the best values for FBW, AVG and FCR. However, the highest FBW and AVG were registered for broilers in the thyme group, while the sage treatment resulted in an improved FCR.

Table 2. Growth performance of broiler chickens fed experimental diets.

Treatment Items	Control	Garlic	Thyme	Sage	Combination	SEM	*P*-value
Initial W g/bird	42.6	42.7	42.4	42.5	42.2	0.01	0.83
FBW g/bird	2195^{c}	2232^{bc}	2303^{a}	2256^{b}	2213^{c}	6.16	<.0001
AVG g/bird	2153^{c}	2190^{bc}	2261^{a}	2214^{b}	2171^{c}	7.10	<.0001
FI g/bird	3766^{b}	3754^{b}	3781^{a}	3565^{d}	3723^{c}	3.91	<.0001
FCR	1.75^{a}	1.71^{b}	1.67^{c}	1.61^{d}	1.72^{b}	0.01	<.0001

FBW= final body weight, AVG= average gain, FI= feed intake, FCR= feed conversion ratio. ($^{a-c}$) Different superscripts indicate significant differences between groups at a certain point in time ($P < 0.05$).

Table (3) shows the impact of different herbal additives on carcass yield and anatomical parts development. A significant increase was noticed in carcass yield (%) in the supplemented groups, especially in garlic or sage groups compared to other groups. There were no significant ($P > 0.05$) differences in carcass cuts and organs (%) among all experimental treatments.

Table 3. Carcass characteristics of broiler chickens fed experimental diets.

Treatment Items (%)	Control	Garlic	Thyme	Sage	Combination	SEM	*P*-value
Carcass yield	67.82^{b}	70.11^{a}	69.04^{ab}	70.57^{a}	69.13^{ab}	0.58	0.048
Carcass cuts:							
Breast	25.82	26.50	26.23	26.44	26.28	0.42	0.864
Thigh	24.41	24.49	24.15	24.70	24.36	0.25	0.668
Wing	5.31	5.86	5.42	5.72	5.35	0.14	0.061
Nick	2.39	2.36	2.37	2.38	2.37	0.07	0.998
Organs:							
Liver	2.92	2.69	2.78	2.71	2.74	0.08	0.405
Heart	0.39	0.41	0.39	0.39	0.39	0.01	0.111
Gizzard	0.75	0.77	0.77	0.78	0.79	0.02	0.743
Abdominal fat	1.55	1.46	1.52	1.51	1.52	0.03	0.568

($^{a-c}$) Different superscripts indicate significant differences between groups at a certain point in time (P < 0.05).

Concerning blood parameters evaluated in this study, the data in **Table (4)** reveal no significant (P > 0.05) variations between the experimental treatments in serum total protein and liver functions of AST and ALT. Serum albumin was significantly higher in birds fed a garlic powder supplemented diet compared to other treatments. Moreover, reduced serum cholesterol and total lipids figures were significantly recorded in all herbal-supplemented birds compared to the control. Birds fed diets supplemented with various herbal additives had a significantly (P<0.05) lower H/L ratio than the control group. Additionally, there was a significant increase in AbNDV levels of broilers within the same dietary groups.

Table 4. Blood parameters of broiler chickens fed experimental diets.

Treatment Items	Control	Garlic	Thyme	Sage	Combination	SEM	*P* value
Blood Protein (*g/dl*)	2.41	2.73	2.12	2.24	2.35	0.13	0.092
Blood Albumin (*g/dl*)	1.17^{b}	1.78^{a}	0.82^{c}	0.94^{bc}	0.88^{c}	0.06	<.0001
Blood Glucose (*mg/dl*)	124.2	133.4	147.7	130.6	117.2	9.34	0.301
Total Cholesterol (*mg/dl*)	126.6^{a}	106.6^{c}	115.5^{b}	114.71^{b}	118.8^{b}	1.74	<.0001
Total lipids (*mg/dl*)	791.2^{a}	713.3^{b}	702.7^{bc}	701.8^{bc}	635.1^{c}	22.13	0.031
AST (*u/l*)	74.4	95.32	87.8	93.3	95.8	4.43	0.184
ALT (*u/l*)	15.57	14.69	15.69	15.11	14.77	0.27	0.192
Immune indices:							
H/L Ratio	0.82^{a}	0.68^{b}	0.63^{b}	0.65^{b}	0.59^{b}	0.01	0.031
AbNDV	4.77^{b}	5.13^{a}	5.18^{a}	5.29^{a}	5.35^{a}	0.19	0.049

($^{a-c}$) Different superscripts indicate significant differences between groups at a certain point in time (P < 0.05), H/L= Heterophils/Lymphocytes, AbNDV = antibody titer against Newcastle diseases.

According to results given in **Table (5)**, A significant (P<0.05) increase in *Lactobacillus spp.* count observed, with decreased *E. coli* bacteria count in broilers at the supplementary treatments in comparison with control group.

Table 5. Microbial population of broiler chickens fed experimental diets.

Treatment Items	Control	Garlic	Thyme	Sage	Combination	SEM	*P* value
Lactobacillus spp. (*log^{10} CFU/g*)	33.08^{d}	56.14^{b}	47.09^{c}	53.48^{b}	62.81^{a}	1.96	<.0001
E. coli (*log^{10} CFU/g*)	24.33^{a}	12.51^{bc}	15.39^{b}	9.66^{cd}	8.41^{d}	0.77	<.0001

($^{a-c}$) Different superscripts indicate significant differences between groups at a certain point in time (P < 0.05).

Discussion

In the current study, the dietary inclusion of garlic, thyme and sage powder, as well as their combination in broiler diets promoted growth performance parameters. These observed improvements may have been facilitated by the antibacterial and antioxidant properties of the herbal plants used, which had the ability to ameliorate gut health and catalyze digestive enzymes, resulting in enhanced digestion of nutrients necessary for growth (Wang *et al.,* 2024). In a recent study (Vlaicu *et al.,* 2023), it was reported that dietary additives of thyme and sage essential oils (0.05%) succeeded in boosting the production performances of broiler chickens.

Similar findings were documented by Hayat *et al.* **(**2022**),** who found that broiler diets supplemented with garlic powder (0.6%) improved growth indices.

The findings in Table (3) are consistent with those of Atay *A.* (2023), who summarized that adding garlic powder (0.5 %) to broiler diets had no significant effects on carcass cuts or visceral organs weight; however, a significant effect on carcass yield (%) was noticed. Furthermore, Naderiboroojerdi *et al.* **(**2022**)** demonstrated that the inclusion of varying levels of thyme ether extracts (0.5 and 0.75 %) in broiler diets had no effect on carcass characteristics. Another study made by Vlaicu *et al.* **(**2023**)**, elucidated that the dietary inclusion of sage essential oil (0.05 %) improved carcass percentage without discernible impact on carcass cuts or organs weight.

In this study, results pertaining to blood constituents measured revealed reduced serum cholesterol and total lipids due to the inclusion of herbal additives suggested. The potential mechanism for the hypocholesterolemic and hypolipidemic effects of herbal plants may involve decreasing the activity of liver enzymes such as malic enzyme, glucose-6-phosphate dehydrogenase, and 3-hydroxy-3-methyl-glutaryl-CoA reductase (*Abaszadeh et al.,* 2023). Our findings are in line with those Kairalla *et al.* (2022); Yalcin *et al.* (2020); Farhadi *et al.* (2020), who documented that broiler diets supplemented with garlic, thyme and sage decreased cholesterol and total lipids values.

Natural antioxidants like garlic, thyme and sage conserve cells against reactive oxygen overproduction and therefore can frustrate oxidative stress-mediated tissues deterioration. Identical to our results, Farhadi *et al.* **(**2020**)** and Behboodi *et al.* **(**2022**)** observed a lower H/L ratio in broilers fed diets supplemented with medicinal plants. As the H/L ratio is considered a credible stress response indicator in broilers, the reduction in the H/L ratio found in the present experiment indicates the immune-motivated impact of the herbal additives examined. Abovementioned influences are valuable from the consumers' standpoints because they are believed to be coveted health factors.

Regarding the influence of dietary additives suggested on birds' intestinal microbiota, an increased beneficial bacteria count *Lactobacillus spp.* and a decrease in *E. coli* bacteria count were noticed. Numerous publications have shown that herbal feed additives possess the ability to stimulate the intestinal health of broiler chickens, leading to improved nutrient absorption (Bonos *et al.,* 2022**;** Vlaicu *et al.,* 2023).

CONCLUSION

It is possible to draw the conclusion that adding garlic, thyme, sage powder and their combination at a level of (0.5%) as feed supplementation in broiler diets has a favorable impact on growth performance, intestinal microbiota and immune status. This affords a ratification to nutritional scientists that such herbal additives possess considerable potency in ameliorating the overall productive and physiological performances of broiler chickens.

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تأثير بعض الإضافات الغذائية العشبية كمحفزات نمو على الأداء الإنتاجي والفسيولوجي لدجاج اللحم

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شجع حظرإستخدام منشطات النمو الصناعية في إنتاج الدواجن بجميع أنحاء العالم علماء التغذية على محاولة ايجاد بدائل طبيعية مثل الإضافات العشبية. يهدف هذا البحث إلى معرفة التأثيرات المحتملة لإضافة الثوم، الزعتر، المريمية والتوليفة فيما بينهما على أداء دجاج التسمين. تم توزيع 180 كتكوتً تسمين (كب 500) غير مجنس عمر يوم عشوائيا إلي 5 معاملات مختلفة (3 مكررات × 12 كتكوت لكل مكررة) كالآتي: T1: مجموعة المقارنة، T2: مسحوق الثوم المضاف (*Allium sativum*، 5 جم / كجم)، T3: مسحوق الزعتر المضاف (*Thymus vulgaris L.*، 5 جم / كجم)، T4: مسحوق المريمية المضاف (*Salvia officinalis*، 5 جم / كجم) و T5: مسحوق ثوم + زعتر + الميرمية المضاف (5 جم/كجم من كل مادة مضافة). تم تقييم أداء النمو للمجموعات من خلال التجربة التي استمرت لمدة 35 يوما. كشفت النتائج التي تم الحصول عليها أن المكملات الغذائية العشبية التي تم اختبارها مثل الزعتر أو المريمية أدت إلى زيادة كبيرة في وزن الجسم النهائي (FBW) وتحسين نسبة التحويل الغذائي (FCR) مقارنة بالمعاملات الأخرى. في حين حققت مجموعات المريمية أو الثوم أعلى إنتاجية للذبيحة معنويا، دون وجود فروق معنوية في المعاملة لمواصفات الذبيحة الأخرى. جميع المكملات الغذائية المدروسة كان لها تأثير ملحوظ في خفض نسبة الكولسترول والدهون الكلية في مصل الدم مقارنة بمجموعة المقارنة. بالإضافة إلى ذلك، فإن المؤشرات المناعية المعززة (نسبة H/L وعيار الأجسام المضادة ضد مرض نيوكاسل) وزيادة أعداد البكتيريا المفيدة بالأمعاء الدقيقة للطيور التي تحفزها الإضافات العشبية. فيما يتعلق بالنتائج، يمكن الاستنتاج أن إضافة 0.5% من الإضافات المقترحة في علائق دجاج التسمين يمكن استخدامها كبديل لمنشطات النمو الصناعية بسبب الأداء الإنتاجي الإيجابي وتنشيط الجهاز المناعي لفروج اللحم.

الكلمات الدالة: دجاج التسمين، أداء النمو، الكائنات الحية الدقيقة، الثوم، الزعتر، الميرمية.