

RESPONSE OF BROILER CHICKEN TO INOVO ADMINISTRATION OF DIFFERENT LEVELS OF ROSEMARY OIL(ROSMARINUS OFFICINALIS)K. M. Sulaiman
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Email: ihsan.tayeb@hotmail.com**ABSTRACT**

This study was conducted to determine the percentage of hatchability, post-hatch growth performance, immune response of broiler subjected to in- ovo injection of different doses of rosemary oil. A total of 300 eggs of ross broiler strain were obtained. The experiment distributed into five treatment groups: T1, Negative Control T2, positive control (in ovo injection of 0.05 ml distilled water) T3, in ovo injection of 0.05ml of rosemary oil; T4, in ovo injection of 0.075 ml of rosemary oil; T5, in ovo injection of 0.1 ml of rosemary oil. Highest hatchability percentage was shown in 0.1ml rosemary oil -injected hatching eggs. The final weight of birds from 0.075 and 0.1 ml of rosemary oil-injected eggs was significantly ($P < 0.05$) highest through the experiment. Feed intake and feed conversion ratio were improved with in ovo administration of rosemary oil at 0.075 and 0.1 ml doses. In addition, the level of blood cholesterol and blood glucose were decreased ($p < 0.05$) in birds from rosemary oil-injected eggs. The obtained data showed a positive effect of in-ovo injection of rosemary oil on stimulating bird's immunity.

Key words: hatchability ,rosemary oil; broiler performance , immunity.

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استجابة أفراخ اللحم لحقن مستويات مختلفة من زيت إكليل الجبل (ROSMARINUS OFFICINALIS)

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المستخلص

أجريت هذه الدراسة لتقدير نسبة الفقس، وأداء النمو بعد الفقس، والاستجابة المناعية لدجاج اللحم التي خضع الى جرعات مختلفة من زيت إكليل الجبل. تم الحصول على 300 بيضة من امهات دجاج التسمين عرق روز تم توزيع التجربة على خمس معاملات: T1 (بدون حقن البيض)، T2(تم حقن البيض ب 0.05 مل ماء مقطر)، T3 حقن البيض 0.05 مل من زيت إكليل الجبل، T4 حقن البيض 0.075 مل من زيت إكليل الجبل، T5 حقن البيض 0.1 مل من زيت إكليل الجبل. أظهرت النتائج أعلى نسبة للفقس في بيض التفريخ المحقون بزيت إكليل الجبل 0.1 مل، من خلال التجربة كان الوزن النهائي للطيور من 0.075 و 0.1 مل من بيض إكليل الجبل المحقون بالزيت أعلى بشكل ملحوظ. تم تحسين كفاءة التحويل الغذائي مع حقن البيض لزيت إكليل الجبل بجرعات 0.075 و 0.1 مل بالإضافة إلى انخفاض مستوى الكوليسترول في الدم وغلوكوز الدم ($p < 0.05$) في الطيور من بيض إكليل الجبل المحقون بالزيت. أظهرت البيانات التي تم الحصول عليها تأثيرًا إيجابيًا للحقن داخل البيض من زيت إكليل الجبل على تحفيز مناعة

الكلمات المفتاحية: نسبة الفقس، حقن البيض، إكليل الجبل، الكفاءة الانتاجية للدجاج، الاستجابة المناعية.

INTRODUCTION

Antibiotics assist controlling infections in gut mucus, and reduce disease susceptibility, therefore they have been used as a principal growth promoting substances in poultry feed for many years(5) . However, in 1996, the European Union banned the use of antibiotics as a growth promoters in animal and poultry diets (34). The main reason behind this trend is the risk of antibiotics cross-resistance among pathogens and residues in tissues for human health (31). The scientists were looking for an alternative natural growth promoting substances such essential oils and medicinal plants. These substances have antimicrobial effect. Component of essential oils possess antibacterial , antioxidant and digestive stimulant properties (26) . Medicinal plants also have stimulating effects on digestive system of animals. Numerous researchers have used medical plants and essential oils in poultry diets have been published.Addition of essential oil mixture to animal diet significantly improve feed conversion efficiency and raise egg production (14) .Moreover , diet supplemented with essential oils of (rosemary, thyme and sage) improved performance, immunity, the quality of eggshell, and egg weight. It also, they affected positively on eggshell thickness and egg mass (13,32).Rosemary is one of the most widely used medicinal plants in many countries (17). Biologically, feed conversion ratio of broiler was improved when fed diet supplemented with rosemary extract (32). Nielsen *et al.*, (28) reported that due to its content of high amounts of a rosmaric acid,

phenolic acids and flavonoids, rosemary has an antioxidant capacities. Additionally, rosemary extract postpone the rancidity occurrence in poultry products(24) . Tekeliet *al.* (33) demonstrated that blood glucose could be reduced when rosemary leaves add to the diet. . Furthermore, the growth of *L. innocua*, *E. coli*, and *S. Indiana*, could be inhibited by essential oil of rosemary (15).This study was aimed to investigate the effect of in-ovo injection of different doses of rosemary oil on hatchability percentage, chick quality, production performance, some physiological characteristics and the immunity of the newly hatched chicks.

MATERIALS AND METHODS

The first practical part of the experiment was conducted at Jeen hatchery in Marina,Sumail district, Duhok province, Kurdistan Region of Iraq and the second part of the experiment was conducted at the poultry farm of Animal Production Department, College of Agricultural Engineering sciences , University of Duhok, Kurdistan Region, Iraq.

Hatching of Eggs

Ross broiler strain eggs (360) were obtained from broiler breeder fed diet supplemented with 1ml/kg of rosemary oil. Eggs were stored in a cool-humid storage area with a temperature of 17°C and 75% relative humidity.Cool eggs were warmed up slowly to room temperature before placing them in the incubator. Eggs were fumigated before setting them into the incubator. On the day18th of incubation, eggs showing viable embryo after candling them were injected with different doses of rosemary oil (Table 1) .

Table 1. Show treatments of eggs for in-ovo injection

Treatment G.	in-ovo injection
T1	Negative Control (No injection)
T2	Positive control(Injection with distilled water (0.05ml)
T3	Injection with Rosemary oil, using dose (0.05ml)
T4	Injection with Rosemary oil, using dose (0.075 ml)
T5	Injection with Rosemary oil, using dose (0.1 ml)

Post-hatch chicks

Post-hatch chicks were distributed into 5 treatments and 4 replicates of 12 chicks per replicate, and reared in floor cages with a provision of wood shaving for bedding. Feeders and waterers were provided based on standard management condition. Broiler starter (0-21 d), and finisher (22-35 d) diets were provided . The experiment lasted for 35

days ,during the experiment feed and water were provided ad libitum.

Fedieng

All diets were prepared approximately one week before the beginning of the experiment. The dietary formulation was based on the recommendation of Rose 308 management handbook (2018). All birds were fed mash feeds ad libitum throughout the duration of the

experiment. All feeds were weighed and recorded prior to distribution to each pen and supplied by hanging tube feeders. The experimental diets were the same for all groups. Standard broiler starter (0-21 d), and

finisher (22-35 d) diets were provided(Table 2).The major ingredients of diet were corn and soybean meal. All of the birds had a constant access to drinking water by typical hanging bell drinkers.

Table 2. Composition and calculated chemical analyses of experimental diets

Ingredients	Percentage (%)	
	Starter (0-21days)	Finisher (22-35days)
Corn	56	62.6
Soybean(44%)	34	28
Dical. P	4	3.15
Sunflower oil	4	4.35
Common salt	1	1
Vit.Mineral.Premix*	0.50	0.50
DL.Methionine	0.35	0.25
L.Lysine	0.15	0.15
Total	100.00	100.00
Calculated Nutrient Composition		
ME (kcal/kg)	2,920	3,215
Crude protein (%)	22.91	20.24
Calcium (%)	1.10	1.17
Methionine (%)	0.59	0.56
Lysine (%)	1.30	1.14
Available p. (%)	0.67	0.70

Vitamin-mineral premix supplied per kg. of diet: vitamin A, 2,200,000 I.U.; vitamin D, 374,000 I.U.; vitamin E, 4,400 I.U.; menadione, 292.6 mg.; riboflavin, 1,760 mg.; pantothenic acid, 2,720 mg.; niacin 8,800 mg.; vitamin B12, 6.6 mg.; vitamin B6, 506 mg.; folic acid, 264 mg.; thiamine, 308 mg.; choline, 81,840 mg.; biotin, 79.2 mg.; calcium min, 7.5%; calcium max, 8.4%; Sulfur, 4.5%; cobalt, 290.4 ppm.; copper, 4840 ppm.; iodine, 123.2 ppm.; iron, 2.18%.; manganese, 2.38%; selenium, 264 ppm.; zinc, 3.96%.

Studied traits

Egg weight, chick weight and hatchability percentage were recorded. Performance parameters include the body weight, feed intake, feed conversion ratio (27). Birds were being weighed at 7,21 and 35 days of age. The feed conversion has been calculated depending on the following equation (as a ratio of feed intake and body weight gain)

FCR= Feed intake (gm) / body weight gain (gm):

Mortality rate was as calculated as the number of mortal birds from a total number of broilers during whole period of the experiment At the last day of the experiment, blood samples (4.0

ml) were collected in a test tube to obtain serum .Blood samples were received from 8 broilers of each treatments which collected from the wing vein of birds. Serum was analyzed for immunoglobulin (IgG, and IgM) , T3, T4, total cholesterol, triglyceride content, total protein and glucose. Serum biochemical parameters were determined by colorimetric enzymatic methods following the procedures provided in the used corresponding commercial kits provided by (Randox laboratories limited, United Kingdom).

Statistical Analysis

For determining the significance of main effects, The statistical package, SAS (PROC GLM) was used (SAS, 2013). To indicate the differences between means of individual element, Duncan's multiple range tests was used. The chosen level of significance was $P \leq 0.05$.

RESULTS AND DISCUSSION

Hatchability percentage and post hatched chicks body weight: Effect of in-ovo injection of different doses of rosemary oil on hatchability percentage and post hatch chicks weight is shown in Table 3. The effect of experimental groups was non-significant on post hatched chicks body weight. While,

hatchability percentage was highest ($p < 0.05$) in eggs injected with (0.1ml) of rosemary oil (95%), followed by those injected with (0.05 ml and 0.075ml) of rosemary oil (90%, 89%) compared to control group (81%). While, the hatchability percentage was decreased to 76% in eggs injected with the distilled water. These results are in line with Radwan *et al.* finding who concluded that hens receiving 1% thyme in their diet followed by 1.0% rosemary had the highest percentage of hatchability respectively

Table 3. Effect of in- ovo injection of rosemary oil on hatchability (%), and body weight the of chicks at hatching

Items	Egg weight (g)	Hatchability %	Body weight of chicks (g)
T1	61.1	81c	44.6
T2	61.4	76d	44.8
T3	60.6	90 b	43.7
T4	59.9	89 b	42.9
T5	60.7	95.a	43.8
SEM	3.14	2.21	0.30

ab Treatment means with different superscripts are significantly different ($P < .05$).

Growth performance

At day 7 of broiler age, a significant ($p < 0.05$) effect was been detected among the experimental groups in regards to body weight indicating the highest body weight of bird from (0.1ml) of rosemary oil injected eggs compared to compared to other treatments groups (Table 4). While, no significant difference were recorded between other treatment groups. At 21 days of age the body weight gain of birds from all eggs groups injected with rosemary oil were increased compared to control group with the highest body weight of birds from (0.1, and 0.075 ml) followed by 0.05 ml of rosemary oil injected eggs compared to control groups. At 35 days of age the final body weight of birds from both (0.1, and 0.075 ml) rosemary oil injected eggs groups were improved compared to other groups. These results agree with Ghazalah and Ali (21) finding who showed that broiler diet supplemented with rosemary leaves at a rate of 0.5% improved broiler performance, as well as the quality of meat was highly acceptable by consumer. Same results were recorded by Mathlouthi *et al.* (26) when added rosemary essential oils to the diet which increased the body weight gain of broiler chicken. Al-Kassie *et al.* (6) stated that supplementation of

(30). These results could bring to an end that rosemary had a favorable effect on hatchability, and antioxidant activity might be the main reason behind this trend (19, 18, 16, and 22). Carnosic acid, an antioxidant compounds present in rosemary pass on to eggs and deposit into yolk which increase the mechanism of adaptation to treat the over production of free radicals; which consequently increase hatchability (12, 25, 22, 30).

rosemary at levels 0.5 and 1.0% to the diet increased growth rate of broiler at age 42 days. On the other hand, Yildirim *et al.* (36) concluded that diet supplemented with rosemary decrease the feed consumption and body weight in comparison to control group and it also does not have any influence on feed conversion rate. Growth performance of broiler chicken might be improved as a result of increase in the apparent digestibility of dietary protein and the digestive capability of prececal, because these raises the availability of nutrients in the intestine for absorption and as a result birds grow faster (45). Furthermore, the ecosystem of the gastrointestinal microbiota could be stabilized by essential oils as a result of diminishing microbial activity, and controlling infective microorganisms within the digestive tract of birds (15, 35). Moreover, the production of volatile fatty acids decrease by diminishing microbial activity, and this led to the stabilization of the intestinal pH and, therefore, confirmed an ideal activity of digestive enzymes (23).

Feed intake, feed conversion ratio and mortality%: The data in Table 4 indicate that feed intake and feed conversion efficiency at starting period were not significantly ($P > 0.05$) affected by in-ovo injection of rosemary oil, while both were improved during the growing

period. As a result, FCR was significantly ($P < 0.05$) better in T4 and T5 than T1, T2 and T3. While, FI was the best in T5 treatment followed by T3 and T4. During the finishing period, data showed an improvement in FI and FCR with the best results recorded with T5 and T4. These results are in agreement with Gazalah and Ali (21) finding, who concluded that feed intake can be improved by adding rosemary leaves to the chicken diet. There is a big differences in antimicrobial and antioxidant activity of rosemary's oils received from natural plants (27). It has been reported in previous studies that dietary antioxidants,

such as flavonoids, phenolic compounds, vitamin E, and vitamin C, could reduce the oxidative damage in animals that created by different sources of stress (7). Improved feed conversion in groups of in-ovo administration of rosemary oil may be due to the combined impact of all these active ingredients in a positive way (17). Treatments from in-ovo injection with 0.1 and 0.05ml of rosemary oil recorded the lowest mortality percentage (2.3%) followed by treatments from in-ovo injection with 0.075ml of rosemary oil, positive control, and negative control respectively (4.7%, 4.7% and 7.14%).

Table 4. Effect of in- ovo injection of rosemary oil on the performance of broiler chicks

Treatments	Body weight (g)	Feed intake (g)	FCE	No. of dead birds
7 days old				
T1	172b	178.2	1.03	1
T2	169b	177.0	1.04	2
T3	175b	170.6	0.97	-
T4	173b	175.4	1.01	1
T5	180a	182.5	1.01	-
SEM	3.326	2.351	0.10	
21 days old				
T1	885b	1183a	1.34a	1
T2	881b	1189a	1.35a	-
T3	888ab	1176b	1.32a	1
T4	906a	1175b	1.29b	-
T5	922a	1179c	1.27b	1
SEM	7.564	13.647	0.024	
35 days old				
T1	1864.2b	2996.4a	1.61a	-
T2	1853.9b	2988.1a	1.61a	1
T3	1901.4b	3047.0a	1.60a	-
T4	2004.2a	3026.5a	1.50b	1
T5	2029.2a	2932.9b	1.44c	-
SEM	.84017	22.935	0.014	

ab Treatment means with different superscripts are significantly different ($P < .05$).

Serum analysis

The data illustrated in table (5) represented the effect of in- ovo injection of rosemary oil on blood parameters of broiler chicken. The average value of total protein and globulin fractions were significantly increased ($p < 0.05$) with in-ovo injection of rosemary oil at (0.075 and 0.1ml) doses compared to in- ovo injection of rosemary oil at 0.05 ml dose and control groups. This reflects the ability of chicks to store reserve protein for depositing in to the tissues even after the body has reached its maximum proportion. Moreover, the increase in the globulin shows the effective role of rosemary in increasing immunity due to its role in inhibiting non-enzymatic oxidation and

developing and protecting cells (34). These results came in line with that obtained by other researchers (20, 21). Cholesterol, triglyceride, and glucose were significantly ($p < 0.05$) reduced with in-ovo injection of rosemary oil. These outcomes are in disagreement with Bugdayci and Ergun (13) finding, who reported that total cholesterol and triglyceride levels in blood serum and humoral immune response did not affected by adding rosemary oil and probiotics to broiler rations. While, these results agree with Belenli et al (8) who reported that rosemary has decreased blood cholesterol. In a study, Gazalah and Ali (21), added rosemary leaves to broiler diets at a level of 0.5%. They determined that rosemary decreased plasma glucose, total lipid and cholesterol content. In another study done by

Polat et al. (29), rosemary oil and rosemary itself were added to broiler rations. They have observed that rosemary additives had a significant effect on decreasing serum albumin/globulin ratio, and total cholesterol

levels. The reduced plasma content of total cholesterol may reflect the hypocholesterolemic effect of rosemary oil as it contains α -pinene, camphor, cineole, borneol (20).

Table 5. Effect of in- ovo injection of rosemary oil on blood parameters of broiler chicken

Treatments	T.protein	Albumin	Globulin	A/G ratio	Glucose	Chol	Trig
T1	3.51b	1.810b	1.70b	1.06	174a	137a	97a
T2	3.49b	1.82b	1.67b	1.08	176a	138a	95a
T3	3.46b	1.860b	1.60b	1.16	162b	129b	86b
T4	3.88a	2.14a	1.84a	1.22	148c	118c	72c
T5	4.02a	2.19a	1.85a	1.19	144c	116c	66c
SEM	0.091	0.037	0.045	0.051	13.25	10.37	6.11

ab Treatment means with different superscripts are significantly different ($P < 0.05$).

Effect of in- ovo injection of rosemary oil on thyroid gland activity and immunoglobulin levels in broiler chicken is showing in Table 6. The effect between the experimental groups was significant ($p < 0.05$) on thyroid gland activity of broiler chicken. As a result, a significantly higher T3 and T4 were recorded in the birds subjected to in-ovo injection with 0.075 and 0.1% rosemary oil compared to other experimental groups. It has been established that thyroid hormones play a great role in adjusting oxidative metabolism of broiler. The metabolically active thyroid hormone is triiodothyronine (T3), which plays an energetic role in the metabolism of energy and metabolic rate. Metabolic rate could be altered by any declared alteration in thyroid function (no matter if it is hyperthyroidism or hypothyroidism) (1). These results indicated

that in-ovo injection of rosemary oils resulted in a superior metabolic and growth rate. The obtained data showed a positive effect of rosemary on stimulating bird's immunity through significant increases in the serum levels of IgM and IgG in rosemary-treated groups. These results are in conformity with that received by Ghozlanet *al*(20) who concluded that the supplementing of rosemary to broiler diet had remarkably improved the serum immunoglobulins (IgG, IgM and IgA). Same results are obtained in the study of El-Naggar et al. (18) who pointed out that the inclusion of rosemary to the basal diet at different concentrations had notably improved the serum immunoglobulins (IgY, IgM, and IgA). Compared the control diet, supplementing rosemary to layer diets could improve the concentration of serum IgM (2).

Table 6. Effect of in- ovo injection of rosemary oil on thyroid gland activity and immunoglobulin levels in broiler chicken

Treatments						
Variable	T1	T2	T3	T4	T5	SEM
T3 (ng/ml)	1.62ab	1.57b	1.65ab	1.81a	1.79a	0.032
T4(ng/ml)	6.21b	5.97b	7.11ab	7.34ab	8.67a	0.11
IgG(mg/ml)	1.84ab	1.71b	2.04a	2.35a	2.41a	0.020
IgM(mg/ml)	0.95b	0.88b	1.23ab	1.48a	1.47a	0.012

ab Treatment means with different superscripts are significantly different ($P < .05$).

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