Available online at **http://bjas.bajas.edu.iq**
https://doi.org/10.37077/25200860.2022.35.1.05
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 **Basrah J. Agric. Sci. 35(1), 61-70, 2022** **E-ISSN: 2520-0860**

The effects of *In Ovo* Injection of Garlic (*Allium sativum* L.) Extract on Hatchability, Liver Enzymes and Antioxidant status of Broiler Chickens

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Received 1st April 2021; Accepted 5th August 2021; Available online 4th March 2022

Abstract: The objective of this study was to investigate the effect of *in ovo* injection of alcoholic garlic extract on hatching traits, liver enzymes, and antioxidant status of broiler chickens post-hatch. In total, 625 broiler breeder eggs (ROSS 308 strain with an average weight of 58.5 g) were divided into 5 treatments (each treatment had three replicates). At 0 embryonic day, the eggs were injected as follows: the first treatment without in ovo injection (negative control), eggs of second treatment were injected with 100 $\mu L.egg^{-1}$ distilled water (sham control). The third, fourth, and fifth treatments were injected with 50, 100, and 150 $\mu L.egg^{-1}$ of an alcoholic garlic extract respectively. The chicks were reared for 4 weeks after hatching according to *in ovo* treatments. Hatchability, embryonic mortality, chicks' weight, serum AST, ALT, GPx, SOD activities, and MDA concentration were all measured. The results showed that the hatchability of the fifth treatment was higher ($P\leq 0.05$) than those of the other treatments. On the other hand, the embryonic mortality of the fifth treatment was recorded less ($P\leq 0.05$) than those of the other treatments. The results of serum AST, ALT, GPx, SOD activities, and MDA concentration were non different significance ($P>0.05$) among treatments. It is concluded that a garlic extract injection with 150 $\mu L.egg^{-1}$ improved hatchability and reduced embryonic mortality. Moreover, it did not affect the chick's weight, serum liver enzymes, and antioxidant status post-hatch.

Keywords: Garlic extract, Hatchability, Liver enzymes, Antioxidant status.

Introduction

The formation of free radicals, which can cause oxidative stress, is linked to the rapid growth of chicken embryos (Deeming & Pike, 2013). In ovo injection of nutrients can provide poultry producers a new way to improve hatchability and the weight of newly hatched chicks (Ohta *et al*., 2001). Many researchers have pointed out the importance of medicinal herbs and its extracts in stimulating growth and production and its work as antioxidants (Oke *et al*., 2017; Oke, 2018; Al-Ashoor & Al-Salhie, 2020; Al-Mosawy & Al-Salhie, 2021). Garlic contains a variety of antioxidants, including flavonoids and sulfur-containing compounds (Leonarduzzi *et al*., 2002). Garlic contains thirty-three sulfur compounds, as well as proteins and minerals (Togashi *et al*., 2008). Sulfurous organic compounds (Aliin, Ajoene, Allicin, and others) are the most essential components of garlic (Mansoub, 2011). Garlic has been commonly used as a feed additive or growth enhancer material since antiquity (Teshika *et al*., 2019). Garlic has also been known as a medicinal plant that can be used to prevent and cure a variety of illnesses (Kim *et al*., 2009). According to Astal & Younis (2003), garlic extract at a level of 750-1000 $\mu g.ml^{-1}$ had a very good antimicrobial activity against both gram-positive and gram-negative bacteria. Garlic has an antioxidant effect, which lowers the thiobarbituric acid reactive substance value and can protect against lipid oxidation (Hanieh *et al*, 2010). Hatchability is one of the most important factors to consider in the hatchery industry. Hatchability improvement and chick quality are the critical steps in increasing the productivity of poultry production (Abou El-Ghar, 2013). Copur *et al*. (2011) found that treating eggs with allicin (3600 mg. l^{-1} and 7200 mg. l^{-1}) is a safe replacement for formaldehyde/ fumigation, enhancing hatchability and lead to decrease contamination and embryonic mortality.

Fouad *et al*. (2018) indicated that garlic oil solution for spraying on Japanese quail eggs as a natural disinfectant is a better method to enhance embryonic growth, hatchability, and reduce bacterial contamination of the eggshell surface. Garlic activates the immune organs, induces quantitative improvements in the white blood cells, improves digestion, and is used as a growth promoter and enhances the productive performance when added to broiler rations (Onibi *et al*., 2009). In the last decade, garlic has been added to poultry feed due to its impact on gains (Khan *et al*., 2012). Its impact is greater throughout the first weeks of a bird's life (Togashi *et al*., 2008). Garlic is also high in aromatic oils, which aid digestion and have a beneficial effect on the respiratory organs of birds (Gardzielewska *et al*., 2003). The objective of this study was to examine the effect of in-ovo injection of alcoholic garlic extract on hatching characterizes liver enzymes and antioxidant status of broiler chickens post-hatch.

Material & Methods

Alcoholic extract of garlic preparation

Garlic extract was obtained using an alcoholic extraction process. We bought the bulbs of garlic (*Allium sativum L.*) from a local market in a nutshell. These were then washed off any dried material that had adhered to them. Peeled garlic bulbs were washed, dried on paper towels, and finely ground. In a clean beaker, 50 grams of powder were mixed with 250 ml of ethanol. A water bath (37 °C) was used to cover the beaker. After twenty-four hours, the mixture was stirred for one hour with a magnetic stirrer. After it has dried, scrape it off and store it in the refrigerator for later use (Harborne, 1973). Finally, 5 grams of an alcoholic garlic extract were obtained and dissolved in 50 milliliters of distilled water.

In ovo injection

Six hundred, and twenty-five eggs (ROSS 308 strain with an average weight of 58.5 g) were divided into 5 treatments (each treatment had three replicates). At 0 embryonic day, the eggs were injected by using a sterile syringe for each egg. The needle gauge of the syringe was 29 mm. The eggs were injected as follows: the first treatment without *in ovo* injection (negative control), eggs of the second treatment were injected with 100 $\mu L.egg^{-1}$ distilled water (sham control). The third, fourth, and fifth treatments were injected with 50, 100, and 150 $\mu l.egg^{-1}$ of an alcoholic garlic extract, respectively. At 0 embryonic days, the eggs were injected into the blunt end (Weber *et al*., 2004; Bertin *et al*., 2009). Injection sites on the eggs were cleaned with 10% formaldehyde and then sealed by medical clay.

Eggs and incubation conditions

The eggs were incubated in an incubator (made in Egypt). Automatic egg turning was performed every 1 hour from days 1 to 18, eggs were incubated under suitable incubation conditions (37.5 °C and 52% relative humidity). The eggs were transferred from the setter tray to the hatcher basket (three replicates for each treatment) on the 18th day of incubation, at a temperature of 37°C and relative humidity of 75%.

Management and feeding

The chicks were reared for 4 weeks after hatching according to *in ovo* treatments. One-day-old hatched chicks were divided into five treatments, each with three replicates. According to the Ross 308 broiler management manual, the chicks were reared for four weeks under similar required conditions. At 32°C in the first week, then it was then reduced by 3°C each week until the end of the four weeks. Then, the temperature remained constant at 23°C until the end of the experiment. The lighting schedule was 23 hours of light and 1 hour of darkness on the first day till 28 days. Two basal rations were used to fed hatched chicks. The chemical composition of the rations was calculated based on NRC (1994, Table 1). The chicks were given *ad libitum* pellet rations and freshwater.

Table (1): Nutritional composition of rations.

Ingredient %	Starter ration (1-21 days) (%)	Grower ration (22-28 days) (%)
Yellow corn	45	53
Wheat	15	12
Soybean meal (48%)	32	25
[1]Protein concentrate (40%)	4	4
Plant oil	0.5	3
Limestone	2	1.5
Salt (NaCl)	0.2	0.2
[2]Premix (29%)	1	1
L-Lysine	0.2	0.2
Methionine	0.1	0.1
Total	100	100
Calculated chemical composition		
Metabolizable energy (kcal.kg^{-1})	2873	3119
Crude protein (%)	23.33	20.24
Calorie: protein ratio	123.14	154.10
Ether extract (%)	3.29	5.97
Crude fiber (%)	3.61	3.21
Calcium (%)	1.04	0.84
Available Phosphorus (%)	0.29	0.26
Lysine (%)	1.30	1.12
Methionine + Cysteine (%)	0.84	0.76

[1]Protein concentrate used Jordanian Origin, to provide the following per kg of diet: 44% protein, 2800 kcal.kg^{-1}ME, 12% fat, 25% ash, 5% calcium, 2.9% phosphorus, 2.55% methionine + Cysteine, 2.8% lysine. [2]Premix contents: vitamins in amounts per kg diet: vit.A: 2500 IU, vit.D3: 5000IU, vit.E: 75mg, vit.K: 3mg, vit B1: 3 mg, vit B2: 8 mg, vit B6: 5 mg, vit B12: 0.016 mg, folic acid: 2mg, biotin: 0.20 mg, pantothenic acid: 13mg, Nicotinic acid :55 mg, Choline chloride 1600mg. Mineral composition (mg kg diet): Cooper :16 mg, Iodin:1.25mg, Iron:40mg, Manganese:120 mg, Selenium: 30mg, Zinc 100mg.

Hatching characterizes:

On one day of hatching, the hatched chicks were weighted by the electronic scale. The hatchability of the fertilized eggs was measured and expressed as a percentage of the total number of fertilized eggs (Dooley *et al*., 2011) as follows:

$$\text{Hatchability of the fertilized eggs} = \frac{\text{The total number of hatched chicks}}{\text{The total number of fertilized eggs}} \times 100$$

The embryonic mortality was calculated as follows:

$$\text{The embryonic mortality} = \frac{\text{The total number of dead embryos}}{\text{The total number of fertilized eggs}} \times 100$$

Liver and antioxidant enzymes

Eight birds were selected from each treatment at 28 days old for blood drawn. To extract serum, blood samples from the Jugular vein were taken and centrifuged at 3000 RPM for 10 minutes. The activity of AST and ALT enzymes was calculated using kits made by Biomerieux. Calculate enzyme activity using a regular curve prepared for this purpose and a spectrophotometer calibrated to 505 nm wavelength. The Malondialdehyde (MDA) concentration was calculated using the Malondialdehyde (MDA) reaction with the Thiobarbituric acid process. The reaction takes place in an acidic setting. Using a spectrophotometer with a wavelength of 353 nm, the concentration of Malondialdehyde was measured (Yagi, 1998). According to Sanja *et al*. (2015) superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes were determined.

Statistical analysis

The data were analyzed using one-way variance analysis (One –way ANOVA) in accordance to Completely Randomized Design (CRD). Duncan (1955) was used to make a 0.05 level comparison between means (SPSS, 2016). Each treatment had three replicates.

Results & Discussion

Hatchability, embryonic mortality, and chick weight are provided in table (2). Garlic extract injection did not affect body weight at 0 days post-hatch. These findings are in agreement with Fazli *et al*. (2015) who showed *in ovo* injection of the garlic extract did not affect the chicks' weight post-hatch. The hatchability of the fertilized eggs had a significant difference ($P \leq 0.05$) among treatments. The fifth treatment caused the highest hatchability (95.53%, $P \leq 0.05$).The lowest embryonic mortality was observed in the fifth treatment (4.47%) compared to other treatments. On the other hand, the highest embryonic mortality was recorded in the negative and sham control treatments (19.20 and 16.75%) respectively. While, the third and fourth treatments have not a significantly different (13.65 and 13.43%) ($P > 0.05$) from the negative, sham control and fifth treatments. These findings may be due to positive effects of garlic extract on embryonic development. Garlic contains antibacterial, antioxidant, and antihypertensive qualities, making it helpful to both human and animals. These effects have been attributed to garlic's bioactive components, one of which is a sulphur-containing organic molecule known as diakylpolysufides, which has antibacterial action and may be responsible for garlic's growth-promoting impact (Sivam, 2001). The hatchability findings in this study indicate that the injected solutions were healthy for developing embryos. The extract had a dose-dependent effect on the hatchability. Despite a large number of studies on the effects of garlic powder, oil, and extract in chicken diets, there is a paucity of knowledge on the effect of in ovo injection of garlic extract. The use of in ovo is one way to improve chicken hatchability and post-hatch efficiency (Bello *et al*., 2013; Li *et al*., 2016). The improvement of chicken hatchability in the present study may be due to garlic extract or its components. These compounds may have antioxidant properties (Fanelli *et al*., 1998; Siegers *et al*., 1999). Garlic's antioxidant stress properties may be attributable to its sulfur component in various stages. These findings may be attributable to the embryos good health, which could be caused by treatment with garlic oil solution, as mentioned by (Fadlalla *et al*., 2010). The lower embryonic mortality rate in the garlic extract treatments may compare to the negative and sham control treatments is due to the reduction amount of microbial by garlic extract injected. Allicin, the main chemical in garlic, is effective against a wide variety of gram-positive and gram-negative bacteria, including *E. coli*, and also has antimicrobial properties, according to Ankri & Mirelman (1999). Copur *et al.* (2011) found that using allicin to disinfect hatching broiler eggs decreased the microbial load on the eggshell surface, increasing hatchability and decreasing embryonic mortality. Contrary to the results of our study, some researchers have reported no changes in hatchability after garlic extract was injected. Fazli *et al*. (2015) showed an in ovo injection of the garlic extract did not affect the hatchability of the eggs.

Table (2): The effects of in-ovo Injection of garlic extract on hatching characterizes (Mean± SE)

Traits Treatments	Hatchability %	Embryonic mortality %	Chicks weight g
T1	80.80^{b} ± 2.73	19.20^{a} ± 2.15	39.16^{a} ± 1.37
T2	83.25^{b} ± 2.41	16.75^{a} ± 1.84	39.46^{a} ± 0.65
T3	86.35^{ab} ± 3.31	13.65^{ab} ± 3.19	39.14^{a} ± 0.85
T4	86.57^{ab} ± 5.47	13.43^{ab} ± 5.08	39.61^{a} ± 0.42
T5	95.53^{a} ± 2.30	4.47^{b} ± 2.23	40.08^{a} ± 0.23

^{ab} Means within main effects with no common superscripts are different significantly($P \leq 0.05$), ^{a}Means in the same column with a common superscript are no significant differences ($P>0.05$).

Liver and antioxidant enzymes and malondialdehyde concentration are presented in table (3). The results of serum AST, ALT, GPx, SOD activities, and MDA concentration were non different significance ($P>0.05$) among treatments. The reason that the liver enzymes and antioxidant status were not affected significantly by the injection treatments may be due to the ending of the effectiveness of garlic extract injected into the eggs at the age of 28 days, and its effect was limited to the newly hatched chicks. Some researchers found that supplementing garlic essential oils at 10, 20, and 40 $mg.kg^{-1}$ diet had no important impact on the activities of serum ALT in broiler chickens (Dieumou *et al*. 2009). El-Latif *et al*. (2013) found that the ALT activity of broilers provided 200 $mg.kg^{-1}$ garlic oil remained unchanged. Garlic essential oils, according to these researchers, had no harmful effects on liver and kidney functions. Our findings corroborated those of Pourali *et al*. (2014), who found that adding garlic to the broiler diet did not affect SOD activity. Contrary to the results of our study, some researchers have reported changes in antioxidant enzyme activities after garlic treatment. According to Lee *et al*. (2016), broilers provided 0.1 % fermented garlic had lower serum AST and ALT activities. Eidi *et al.* (2006) found that garlic improved lipid profiles and reduced serum AST levels. The use of garlic powder and probiotics, according to Rastad (2020), greatly reduced the activity of liver enzymes, including AST, ALT, and ALP. Also, Alagawany *et al*. (2015) found that dietary supplementation of garlic in the diet increased SOD activity. According to Pourali *et al*. (2014), adding garlic to the broiler diet reduced MDA concentrations by 30% as compared to birds fed a normal diet. According to Ismail *et al*., (2021), antioxidant status in terms of SOD was enhanced in birds fed garlic powder, where broilers fed with 0.50 and 0.75 $g.kg^{-1}$ of garlic powder having the highest SOD levels. In addition, as compared to control, MDA levels were lower in groups receiving feed additives, with the lowest value being 0.75 g garlic powder per kilo. These discrepancies could be caused by a variety of reasons, such as dosage, duration of usage, treatment procedures, or chicken breed.

Table (3): The effects of in ovo injection of garlic extract on liver and antioxidant enzymes and malondialdehyde concentration (Mean± SE).

Traits Treatments	AST $IU.^{L}$	ALT $IU.^{L}$	GPx $U.^{L}$	SOD $U.^{ml}$	MDA $\mu m.^{L}$
T1	21.66^{a} ± 15.66	12.33^{a} ± 3.28	417.11^{a} ± 64.57	2.44^{a} ± 0.64	4.81^{a} ± 1.57
T2	32.66^{a} ± 10.17	14.33^{a} ± 0.88	396.56^{a} ± 17.43	2.31^{a} ± 0.04	5.30^{a} ± 1.84
T3	17.66^{a} ± 6.35	18.66^{a} ± 3.71	429.61^{a} ± 90.26	2.73^{a} ± 0.14	2.19^{a} ± 0.78
T4	15.66^{a} ± 3.71	10.33^{a} ± 1.45	458.47^{a} ± 24.24	2.90^{a} ± 0.32	3.78^{a} ± 1.79
T5	17.66^{a} ± 5.81	11.00^{a} ± 2.08	457.30^{a} ± 18.12	3.05^{a} ± 0.19	2.03^{a} ± 0.26

[a]Means in the same column with a common superscript are no significant differences (P>0.05).

Conclusion

The study concluded that garlic extract injection at the level of 100 or 150 µl.egg^{-1} improved hatchability and reduce embryonic mortality. Moreover, it did not affect the chick's weight, serum liver enzymes, and antioxidant status in the post-hatch.

Acknowledgments

The authors sincerely thank the staff of the poultry farm, College of Agriculture, the University of Basrah for their assistance in this study.

Conflict of interest

Regarding the publisher's policy criteria, the authors have no possible conflict of interest.

Ethical approval

All ethical guidelines related to poultry breeding and care issued by national and international organizations were implemented in this report.

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تأثير حقن البيض بمستخلص الثوم (*Allium sativum* L.) في نسبة الفقس وانزيمات الكبد وحالة مضادات الاكسدة لفروج اللحم (ROSS 308)

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المستخلص: اجريت الدراسة الحالية بهدف معرفة تأثير حقن البيض بالمستخلص الكحولي للثوم في نسبة الفقس والهلاكات الجنينية وإنزيمات الكبد وحالة مضادات الأكسدة لفروج اللحم بعد الفقس. استخدم 625 بيضة تفقيس من بيض امهات فروج اللحم (ROSS 308) بمتوسط وزن 58.5 غرام ، قُسمت على 5 معاملات تجريبية لكل منها 125 بيضة. حقن البيض قبل ادخاله الى الحاضنة على النحو التالي: المعاملة الأولى بدون حقن (سيطرة سالبة) ، وحقن بيض المعاملة الثانية بـ 100 ميكرولتر لكل بيضة من الماء المقطر (سيطرة موجبة) ، وحُقن بيض المعاملة الثالثة والرابعة والخامسة بـ 50 و 100 و 150 ميكرولتر لكل بيضة من مستخلص الثوم الكحولي. تم تربية الافراخ الفاقسة وفق معاملات البيض لمدة 4 أسابيع. تم جمع البيانات حول نسبة الفقس، نسبة الهلاكات الجنينية ، ووزن الافراخ بعد الفقس ، وفعالية انزيم AST وALT و الجلوتاثيون بيروكسيديز (GPx) وسوبر أوكسيد ديسموتاز (SOD) وتركيز مركب المالون ثنائي الالديهايد (MDA). أظهرت النتائج أن نسبة الفقس في المعاملة الخامسة كانت أعلى ($p\leq0.05$) من تلك الموجودة في المعاملات الأخرى. من ناحية أخرى ، سجلت المعاملة الخامسة أقل فرق معنوي ($p\leq0.05$) في نسبة الهلاكات الجنينية مقارنة بالمعاملات الأخرى. ولم يتأثر كل من وزن الافراخ بعد الفقس ، وفعالية انزيم AST وALT و الجلوتاثيون بيروكسيديز (GPx) وسوبر أوكسيد ديسموتاز (SOD) وتركيز مركب المالون ثنائي الالديهايد (MDA) معنوياً ($P>0.05$) بمعاملات الحقن. من خلال ما تقدم نستنتج أن حقن مستخلص الثوم بمقدار 150 ميكرولتر لكل بيضة يعمل على رفع نسبة الفقس من البيض المخصب ويقلل من نسبة الهلاكات الجنينية. ولم تؤثر معاملات الحقن بمستخلص الثوم الكحولي على وزن الافراخ بعد الفقس وانزيمات الكبد وحالة مضادات الأكسدة في مرحلة ما بعد الفقس.

الكلمات المفتاحية: مستخلص الثوم، نسبة الفقس، انزيمات الكبد، حالة مضادات الاكسدة.