

Effects of *in Ovo* Application of Resveratrol on Hatchability, Hatching Time, Yolk Sac Weight and Chick Performance in Quail

Mohammed Kamil Kattami Alsadoon¹ , Ali Aygün^{1,*} 

¹Selçuk University, Agriculture Faculty, Department of Animal Science, 42250, Konya, Turkey

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*Corresponding Author

Tel: +903322232905
E-mail: aaygun@selcuk.edu.tr

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Abstract

The purpose of this study was to investigate the effects of *in ovo* resveratrol in hatching quail eggs on hatching traits, organ weights and chick performance. A total of 640 hatching quail eggs were used in the study. The eggs were randomly distributed into four groups as non-injected group (PC; Positive control), 0.2 ml saline (NC; Negative control), 0.2 ml solution containing 1 nmol of resveratrol (R1) and 0.2 ml solution containing 4 nmol of resveratrol (R4). *In ovo* injection was performed on the 14th day of incubation. Hatchability of set eggs (HSE) and hatchability of fertile eggs (HFE), embryonic mortality (EM), chick hatching time, yolk sac weight (YSW), and chick performance characteristics were investigated. The effects of *in ovo* resveratrol administration on the HSE were insignificant. HFE decreased significantly with *in ovo* resveratrol treatment ($P < 0.05$). All injected groups were adversely affected in terms of embryonic deaths ($P < 0.05$). The effect of *in ovo* resveratrol application on chick hatch weight, and YSW (g, %), chick performance was insignificant. It was observed that *in ovo* resveratrol treatment had no significant effect on the hatching time, except at 408 h of incubation. As a result, *in ovo* resveratrol application had a negative effect on hatchability and EM, but did not have affected the hatching time, yolk sac weight and chick performance.

Introduction

In ovo application is a method to apply exogenous substances into the amnion during embryo development in order to promote positive effects on egg hatching, post-hatching performance and immune response (Uni and Ferket 2004). This method was first used to administer a marek vaccine (Sharma and Burmester 1982).

Researchers have used ascorbic acid, carbohydrates, amino acids, vitamin-minerals, hormones and bee products (Kocamis et al., 1999; Salmanzadeh et al., 2012; Coşkun et al., 2014a; Coşkun et al., 2014b; Moghaddam et al., 2014; Sgavioli et al., 2015; Yair et al., 2015; Coskun et al., 2017; Maman et al., 2019; Alizadeh et al., 2022) in the *in ovo* method in recent years. Resveratrol is a polyphenolic phytoalexin synthesized by plants in response to environmental stress or

pytopathogene attacks. Phytoalexins are chemicals that are synthesized to protect plants against pathogenic microorganisms (Langcake and Pryce 1977). Resveratrol protects body cells against naturally occurring free radicals (Lin and Tsai 1999). It has been stated that resveratrol has antioxidant, antiplatelet, cardioprotective, vascular relaxant, anticancerogen and anti-inflammatory effects (Soleas et al., 1997; Tan et al., 2015).

El-Fakhrany et al., (2021) stated that *in ovo* resveratrol application to broiler eggs has a positive effect on kidney and liver functions, immune function except chick performance. Resveratrol is mostly given in the form of additions to feed in poultry (Sahin et al., 2010; Sridhar et al., 2015; Mohebodini et al., 2019; Zhang et al., 2020; Wang et al., 2021), and the

application of *in ovo* is limited (Tan *et al.*, 2015; El-Fakhrany *et al.*, 2021).

The aim of this study was to investigate the effect of *in ovo* injection of resveratrol on hatching results, yolk sac weight, hatching time and chick performance.

Materials and Methods

A total of 640 hatching quail eggs were used in the study. Eggs were randomly allocated to four treatment group with 160 eggs per treatment with four replicates of 40 eggs each. Experimental groups were arranged as the group eggs not injected with solution (Positive control; PC), the second group eggs were injected with saline (Negative control; NC), the third group eggs were injected with 1nmol resveratrol (R1) and the fourth group eggs was injected with 4 nmol resveratrol (R4) (Extrasynthese, France, Purity (HPLC) $\geq 99\%$). The application doses were diluted to be every 0.20 mL. On the 14th day of incubation, the blunt end of the eggs was opened with the device. Then, the prepared doses (0.20 mL) were injected into the amniotic fluid with a needle (26-gauge syringe). After the application, the eggs were transferred to the hatching unit after the hole was closed with wax.

Chick hatching were recorded every 3 h from the 390th h of incubation until the time when chick hatching was completed. The chicks were transferred to the cages for their growth performance after they completed hatching, unhatched eggs were opened, and dead embryos were determined according to guidelines of (Aygün *et al.*, 2012). The following characteristics were calculated from these data.

Fertility (%) = Fertile eggs / total eggs * 100

HFS (%) = Hatched chicks/ fertile eggs * 100

HSE (%) = Hatched chicks/total eggs * 100

After hatching, yolk sac weight was determined in 5 chicks from each group. For this purpose, the chicks were weighed and killed, the yolk sac was removed and weighed to the nearest 0.01 g. The yolk sac weight was divided by the body weight and expressed as %.

In order to measure chick performance, 10 chicks (40 chicks/group) randomly selected from each subgroup were individually weighed, wing banded and placed in brooding cages. Chicks were fed with grower diet (24% CP and 2,900 kcal ME/kg) during the 10 d of rearing period. The room temperature is adjusted to be 33-35°C and continuous light is preferred as photoperiod until the end of the rearing period. The chicks were individually weighed at the end of the 10 d rearing period and the relative growth was calculated using these data (Tona *et al.*, 2003). Chick deaths were recorded daily.

Relative Growth (RG) = [Body weight of d 10 (g) - Initial body weight (g)] / Initial body weight (g) * 100

The data of hatching traits, hatch time, yolk sac weight, and chick performance characteristics were analyzed using variance analysis, and performed by

Tukey's multiple range test for comparison mean values of treatment groups. Statistically significant level (P) was 0.05.

Results and Discussion

The HSE, HFE and EM of the experiment are summarized in Table 1. No significant differences were found among treatments group for HSE. In terms of HFE, the PC group (84.13%) was higher than the NC (62.26%), R1 (64.05%) and R4 (57.90 %) treatment groups. However, no statistically significant difference was observed among the NC, R1 and R4 groups in terms of HF. There are studies showing that *in ovo* injection of different substances has positive or negative effects on hatchability. Hatchability were adversely affected by *in ovo* injection propolis (Aygün 2016), amino acids (Groff-Urayama *et al.*, 2019; Nazem *et al.*, 2019), organic trace minerals (Oliveira *et al.*, 2015), and vitamin D₃ (Maman *et al.*, 2019).

On the other hand, hatchability was improved by *in ovo* injection with histidine (Xu *et al.*, 2019), naringin (Ranjbar *et al.*, 2019), vitamin C (Zhu *et al.*, 2020), and carbohydrates (Dong *et al.*, 2013)

Beck *et al.*, (2019), Khaligh *et al.*, (2018); Ncho *et al.*, (2021), and Coşkun *et al.*, (2014a) stated that hatchability was not affected when eggs were injected with probiotics, quercetin, gaba and pollen, respectively. Giving different substances into egg with *in ovo* application can create nutrient imbalances in the egg and this can cause embryo death.

Embryonic mortality at 1-9 d of incubation in the PC (10.77%) and R4 (9.24%) groups were higher than in the NC (3.16%) and R1 (3.16%) groups (P<0.05). Embryonic mortality at 10-16 d of incubation were the lowest in the PC group (5.11%) (P<0.05). There were no significant differences among the groups for embryonic mortality on days 17-18 of incubation. The use of resveratrol may be toxic for the embryo development. *In ovo* administration may cause an allergic reaction due to changes in the amniotic fluid and cause embryos to die (Salmanzadeh *et al.*, 2012).

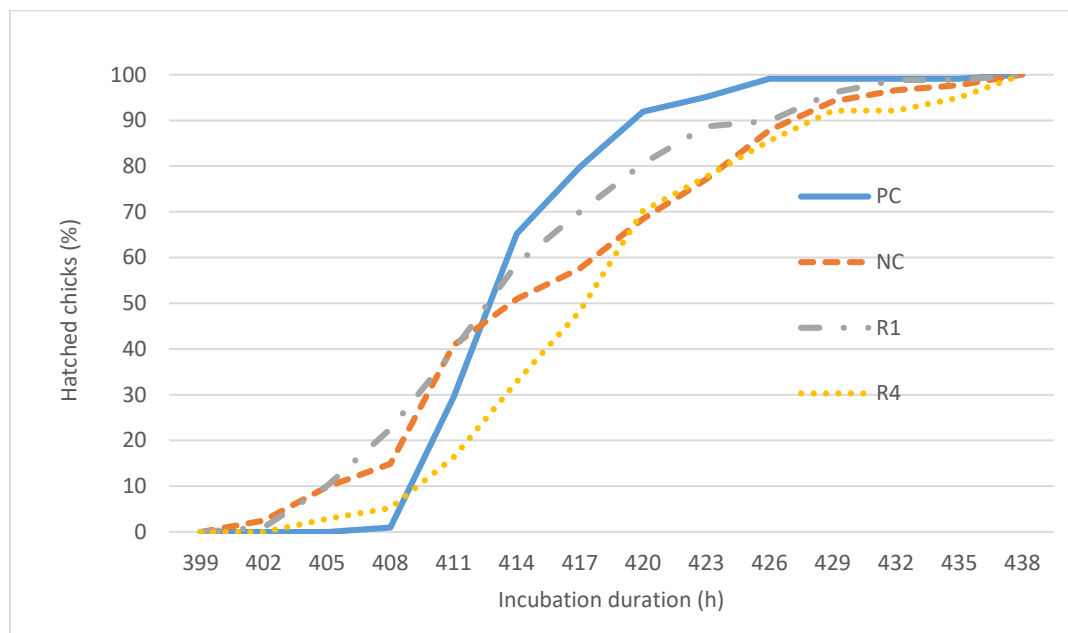
The effect of *in ovo* resveratrol application on spread of hatch is given in Figure 1. The first chick hatching times were observed in the PC, NC, R1 and R4 groups at 408, 402, 402 and 405 hours, respectively. Chick hatching was completed in all groups at 438 h of incubation. R1 group (22.49%) had significantly higher hatching percentage than that of PC group (0.96%) at 408 h of incubation, but no significant differences were observed among NC (14.89%), R1 (22.49%), and R4 (5.24%) group at 408 h of incubation. There were no significant differences among groups at other hatching times.

Table 1. Effects of *in ovo* resveratrol on HSE, HFE and EM (mean \pm SE)

| Group | Fertility | HSE | HFE | EM (% of fertile eggs) | | |
|---------|------------------|------------------|-------------------------------|-------------------------------|-------------------------------|-----------------|
| | | | | 1-9 d | 10-16 d | 17-18 d |
| PC | 75.81 \pm 3.79 | 63.71 \pm 2.75 | 84.13 \pm 0.91 ^a | 10.77 \pm 1.15 ^a | 5.11 \pm 1.07 ^b | 0.00 \pm 0.00 |
| NC | 81.71 \pm 1.45 | 50.10 \pm 3.49 | 62.26 \pm 3.29 ^b | 3.16 \pm 1.24 ^b | 31.36 \pm 2.90 ^a | 3.23 \pm 0.18 |
| R1 | 84.65 \pm 1.64 | 54.11 \pm 2.84 | 64.05 \pm 3.88 ^b | 3.16 \pm 1.24 ^b | 25.93 \pm 5.93 ^a | 0.78 \pm 0.78 |
| R4 | 82.29 \pm 1.75 | 47.41 \pm 5.85 | 57.90 \pm 7.82 ^b | 9.24 \pm 2.45 ^{ab} | 33.74 \pm 6.36 ^a | 2.21 \pm 2.20 |
| P-value | 0.10 | 0.06 | 0.00 | 0.04 | 0.00 | 0.37 |

^{a-b} Different letters in the same column indicate significant differences (P<0.05)

PC: Positive control, no injection; NC: Negative control, only saline injection; R1: 1nmol resveratrol injection; R4: 4 nmol resveratrol injection, SE: Standard error

**Figure 1.** Effects of *in ovo* resveratrol injection on spread of hatch

PC: Positive control, no injection; NC: Negative control, only saline injection; R1: 1nmol resveratrol injection; R4: 4 nmol resveratrol injection

It can be stated that the narrower the time between the first and last chicks hatching, the higher the homogeneity in chicks. The longer the chicks stay in the incubator, the delayed access to feed, dehydration, and reduce post-hatch performance (Gonzales *et al.*, 2003; Careghi *et al.*, 2005; Powell *et al.*, 2016; Özlü *et al.*, 2018).

Chick body weight at hatch, YSW (g, %) of treatments groups are summarized in Table 2. No statistical significant differences were observed among groups for chick body weight at hatch, YSW (g, %). Chick hatching weight and egg yolk weight were not affected by *in ovo* resveratrol application. This result is consistent with the studies of Abdulqader *et al.*, (2018); Senturk *et al.*, (2018); Maman *et al.*, (2019); Nazem *et al.*, (2019) that *in ovo* probiotic, Vitamin D₃, amino acids, and manganese injections do not affect the chick hatching weight, respectively.

Conversely, Tangara *et al.*, (2010) chick weight was significantly increased by *in ovo* injection of carbohydrates and arginine relative to control treatment in ducks. Ncho *et al.*, (2021) stated that *in ovo* application with 5% GABA had a higher hatching weight compared with control group.

No significant differences were found among treatments group for yolk sac weight (g, %). There are similar studies showing that the injection of *in ovo* substances has no effect on chick hatching weight (Abdulqader *et al.*, 2018; Groff-Urayama *et al.*, 2019; Maman *et al.*, 2019). On the other hand, Zamani *et al.*, (2018) reported *in ovo* carbohydrates injection significantly decreased the yolk sac residue compared with control group (P<0.05). The weight of the yolk sac in poultry varies between 10% and 25% of the hatching body weight. (Jamroz *et al.*, 2004; Abdulqader *et al.*, 2018).

Table 2. Effects of *in ovo* resveratrol on chick hatching weight and yolk sac weight (mean \pm SE)

| Group | Chick hatching weight, g | Yolk sac weight, g | Yolk sac weight (%) |
|---------|--------------------------|--------------------|---------------------|
| PC | 8.25 \pm 0.50 | 0.79 \pm 0.12 | 9.48 \pm 1.02 |
| NC | 8.34 \pm 0.64 | 1.05 \pm 0.14 | 12.46 \pm 1.09 |
| R1 | 8.58 \pm 0.18 | 1.13 \pm 0.25 | 13.20 \pm 2.10 |
| R4 | 8.79 \pm 0.54 | 1.41 \pm 0.25 | 16.30 \pm 3.41 |
| P-value | 0.81 | 0.14 | 0.16 |

PC: Positive control, no injection; NC: Negative control, only saline injection; R1: 1nmol resveratrol injection; R4: 4 nmol resveratrol injection, SE: Standard error

Table 3. Effects of *in ovo* resveratrol on chick body weight (1 d and 10 d) and relative growth (mean \pm SE)

| Group | Body weight, g, (1 d) | Body weight, g, (10 d) | Relative growth |
|---------|-----------------------|------------------------|--------------------|
| PC | 8.35 \pm 0.15 | 44.73 \pm 0.93 | 437.73 \pm 9.90 |
| NC | 8.14 \pm 0.14 | 42.76 \pm 1.17 | 430.11 \pm 1.96 |
| R1 | 8.38 \pm 0.12 | 44.37 \pm 0.88 | 430.26 \pm 8.83 |
| R4 | 8.27 \pm 0.190 | 44.07 \pm 1.46 | 433.98 \pm 15.21 |
| P-value | 0.69 | 0.59 | 0.97 |

PC: Positive control, no injection; NC: Negative control, only saline injection; R1: 1nmol resveratrol injection; R4: 4 nmol resveratrol injection, SE: Standard error

Egg yolk sac is very rich in nutrients necessary for the embryonic development and for feeding in the early post-hatching period (Noy and Sklan 1998; Jamroz *et al.*, 2004)

In ovo resveratrol administration on chick body weight at d 1, body weight at d 10, and relative growth are presented Table 3. It has been previously stated by some researchers that *in ovo* substances injection does not have a significant effect on body weight gain (Aygun 2016; Khaligh *et al.*, 2018; Zhu *et al.*, 2020; Ncho *et al.*, 2021).

The results of our study are inconsistent with Hassan *et al.*, (2021), which reported *in ovo* Nano Cu or Cu sulfate injection caused a significant increase in body weight at 35 days of age compared to the group. Similarly, Al-Daraji *et al.*, (2012) reported that *in ovo* L-arginine injection presented higher weight gain than control groups ($P < 0.05$).

Conclusions

According to our research results, *in ovo* resveratrol administration had a negative effect on hatchability and EM. However, it is seen that there is no negative effect on chick hatching weight, yolk sac weight, hatching time and post-hatch performance.

Highlights

- Resveratrol is applied as *in ovo* application into quail hatching eggs.

- In ovo* resveratrol application negatively affected hatchability and embryonic mortality.
- In ovo* resveratrol application has not had any effect on hatching time, yolk sac weight and chick performance.

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