

## Effects of *in ovo* injection of naringin into fertile broiler chicken eggs on hatchability and hatchling weight and length

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### Abstract

Naringin is an important flavonoid found in the fruit or peels of fruit from species of the Rutaceae family, such as grapefruit. The aim of this study was to investigate the effects of *in ovo* naringin injection on hatchability, hatchling weight, and hatchling length. A total of 180 fertilised Ross 308 broiler eggs were purchased from broiler flocks and randomly assigned to control (no *in ovo* injection), negative control (injection of 0.1 mL deionised water), and naringin (injection of 15 mg naringin in 0.1 mL deionised water) treatment groups. The eggs were incubated at 37.5 °C and 56% relative humidity and *in ovo* injections were made on the 18th day of incubation. Data were analysed using SPSS version 13.0, with the chi-square test used to determine the effects on hatchability, and hatchling weight and length compared using analysis of variance. The hatchability rates of the eggs in the control, negative control, and naringin treatment groups were 83.3%, 80%, and 88.3%, respectively, indicating that 15 mg naringin provided a 6% increase in hatchability. Hatchling weights were 46.26 g, 46.83 g, and 46.56 g, and hatchling lengths were 16.95 cm, 17.17 cm, and 17.28 cm, in the control, negative control, and naringin groups, respectively. *In ovo* injection with naringin insignificantly decreased hatchling weight, while significantly increasing hatchling length and insignificantly increasing hatchability compared to the control group. Additional research is needed to determine the most effective naringin level to obtain optimum results from *in ovo* injection during the incubation process.

**Keywords:** broiler chicken, chick length, hatchability, *in ovo* injection, naringin

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### Introduction

The poultry sector makes an important and continuous contribution to human nutrition, and reproductive and feeding models in poultry farming therefore try to produce the healthiest product in the shortest possible time (Inci *et al.*, 2014; Inci *et al.*, 2016). The *in ovo* injection technique was first used in the late 1970s and has been an important research topic in recent years. This technology has become widespread, particularly for vaccination against Newcastle disease, and *in ovo* injection has become the standard procedure for the embryonic vaccination of broiler chickens for the Marek's disease virus and infectious bursal disease in the United States (Sharma & Burmester, 1984; Johnston *et al.*, 1997; Gagic *et al.*, 1999; Williams, 2007). *In ovo* injection provides advantages in fields such as disease control, as

it can facilitate management practices, save labour, and improve hatchability results (Oladokun & Adewole, 2020; Tainika & Şekeroğlu, 2020; Shehata *et al.*, 2021; Arain *et al.*, 2022). *In ovo* injection, as one of the early feeding methods in poultry farming, also allows the investigation of the biological effects of phytobiotics, as well as other nutrients such as vitamins, carbohydrates, and proteins.

Naringin is an important flavonoid found in the fruits and peels of fruit from species of the Rutaceae family, such as grapefruit, and contributes to the bitter taste of citrus fruits (Li *et al.*, 2018). Citrus fruit extracts contain large amounts of flavonoids and are the most important sources of flavonoids such as nobiletin, hesperidin, narirutin, naringenin, and naringin. Naringin, a flavone glycoside, is a product of the combination of the flavone naringenin and the disaccharide neohesperidose (El-Hady *et al.*, 2024). *In vivo* and *in vitro* studies have shown that these citrus flavonoids have anti-inflammatory and antioxidant activities (Tripoli *et al.*, 2007). Furthermore, flavonoids, which are compounds obtained from vegetables and fruits, are claimed to have positive effects on human health because of their biochemical and pharmacological properties (Lautraite *et al.*, 2002; Türkoğlu *et al.*, 2019). Flavonones such as naringin and naringenin are also strong scavengers of free radicals and prevent lipid peroxidation (Cavia-Saiz *et al.*, 2010; Guimarães *et al.*, 2010; Ranjbar *et al.*, 2019).

In recent years, numerous scientific studies have reported the beneficial health effects of naringin as a supplement and feed additive. These effects have included improving production and meat quality by regulating lipid metabolism, increasing antioxidant capacity, and producing a favourable fatty acid profile, which are desirable characteristics in the broiler production industry (Goliomytis *et al.*, 2015; Jiang *et al.*, 2020; Hager-Theodorides *et al.*, 2021; Bao *et al.*, 2022). When examining published research on the supplementation of naringin to poultry, Hamady (2024) found that the addition of naringin to the diet at 1.25 and 1.5 g/kg improved body weight and feed utilisation at four and five weeks of age. Additionally, an increase in live weight was achieved by the addition of 0.2% *Chelidonium majus* or *Lonicera japonica* extract, containing 7.75 and 12.16 mg/kg naringin, respectively, to the diets of broilers (Park *et al.*, 2014). Furthermore, Goliomytis *et al.* (2019) reported that the addition of naringin to the diets of laying hens could improve egg yolk colour without any adverse effects on other egg quality traits. Wong *et al.* (2013) also reported that naringin stimulated cell proliferation and the differentiation of osteoblasts.

The injection of plant-derived phytochemicals into poultry eggs has shown various benefits, including increased weight gain, feed efficiency, and growth rate, and decreased morbidity and mortality of embryos, as well as increased immunity and improved health status (Arain *et al.*, 2022). Nonetheless, few studies have investigated the effects of naringin supplementation during incubation. The aim of this study was thus to evaluate the effect of the *in ovo* injection of naringin on hatchability results.

## Materials and methods

Ethical approval for this study was obtained from the Afyon Kocatepe University Animal Experiments Local Ethics Committee (approval number: AKU HADYEK-33-22). Fertile chicken eggs (Ross 308) were purchased from broiler flocks and incubated at 37.5 °C and 56% relative humidity. On the 18th day of incubation, they were divided into the control (60 eggs), negative control (60 eggs), and naringin treatment groups (60 eggs). The eggs in the three groups were treated as described in Table 1. Before the injection process, the blunt end of each egg was cleaned with alcohol and a small hole was opened using a micromotor. The air sac was detected with the help of a light, and the relevant solutions were injected into the air sac through the hole opened by the micromotor using a syringe. The hole in the shell was then closed with paraffin wax. After the injection process, the eggs were transferred to the hatching section.

**Table 1** Experimental groups

Groups	Application	n
Control	Nothing was injected into the eggs	60
Negative control	0.1 mL deionised water was injected into each egg	60
Naringin (15 mg)	0.1 mL deionised water containing 15 mg naringin was injected into each egg	60

The hatchability percentage was calculated as the number of live chicks hatched after 21 days of incubation, divided by the number of fertile eggs that were *in ovo* injected, multiplied by 100 (Joshua *et al.*, 2016). Hatchling weight (g) was determined by weighing the chicks on a digital scale with a sensitivity of 0.01 g. Hatchling length (cm) was determined by lying the chick on a ruler and measuring the distance between the tip of the beak and the tip of the toe (Wolanski *et al.*, 2005). Data were analysed using SPSS version 13.0 software, with the chi-square ( $X^2$ ) test being used to determine the effects on hatchability, while chick weight and length were compared using analysis of variance. The threshold for significance was  $P < 0.05$ .

## Results and discussion

There were no significant differences in hatchling weights between the treatment groups (Table 2). However, the mean weights of the chicks in the control, negative control, and naringin groups were 46.26, 46.83, and 46.56 g, respectively.

**Table 2** Descriptive statistics for hatching weights of chicks hatched from eggs *in ovo* injected with naringin (15 mg), deionised water (negative control), or nothing (control) on the 18th day of incubation

Groups	n	Minimum (g)	Maximum (g)	Mean (g)	SD	P-value
Control	50	38.86	56.44	46.26	3.13	0.408
Negative control	48	37.92	53.40	46.83	3.43	
Naringin (15 mg)	53	37.81	53.00	46.56	3.26	

N: number of chicks, SD: standard deviation

Hatching weight and length are factors used to predict marketing weight in chicks (Ranjbar *et al.*, 2019). Previous studies have reported that the injection of various nutrients into eggs had no effect on hatchling weight (Hajati *et al.*, 2014; Al-Shamery & Al-Shuhaib, 2015; Joshua *et al.*, 2016; Abdulqader *et al.*, 2018; Şentürk *et al.*, 2018; Groff-Urayama *et al.*, 2019; Maman *et al.*, 2019). However, Tangara *et al.* (2010) and Zhai *et al.* (2011) reported that carbohydrate (glucose, fructose, sucrose, maltose, and dextrin) injections increased chick weight. Furthermore, Ranjbar *et al.* (2019) reported that the *in ovo* injection of 30 mg naringin improved chick weight by stimulating the development of the embryo. Similarly, Surai (1999) reported that natural antioxidants given externally to the breeder chicken embryo will affect the embryo, and thus higher-quality and healthier chicks will be obtained. However, hatchling weight is affected by egg weight, incubation temperature and humidity, dosage of the injected nutrient, and day of injection (Ohta *et al.*, 2001; Ferket *et al.*, 2005), and these factors could be responsible for the contradicting results of previous studies. Hristakieva *et al.* (2017) reported that egg weight had a significantly positive effect on hatchling weight, while there was a negative correlation between the weight loss during incubation and the relative hatchling weight. Sklan *et al.* (2003) observed that heavier eggs produced heavier chicks and heavier broilers at slaughter at 41 days of age.

Significant differences between the groups were found for hatchling lengths (Table 3), with the mean lengths of the hatchlings in the control, negative control, and naringin groups being 16.95, 17.17, and 17.28 cm, respectively. Ranjbar *et al.* (2019) injected 15 to 30 mg naringin/egg into the amniotic fluid on days 14 and 17.5 of incubation, and found that naringin injection significantly improved the blood concentration of antioxidant enzymes and biochemical indices; increased the height, bone length, and thyroid hormone levels of the day-old chicks; and stimulated antioxidant enzymes such as superoxide dismutase and alkaline phosphatase. Flavonoid injection also positively affected chondrocyte proliferation and bone growth, and increased hatchling length (Swarnkar *et al.*, 2012; Karimian *et al.*, 2013). These results concur with the results of this study.

Hatchling length reflects embryo development at hatching, and has been found to be positively correlated with performance at later ages. Hatchling length is also an indicator of yolk absorption rate and may vary depending on parent stock age, hatchling weight, or incubation conditions (such as temperature and humidity) (Gharahveysi & Kenari, 2018). According to the literature, hatching weight and length are the main factors for predicting marketing weight in chickens (Wolanski *et al.*, 2006;

Willemsen *et al.*, 2008). However, chick length is more important than chick weight in predicting the growth performance of broilers because chick weight is biased by the presence of unabsorbed residual yolk in the abdomen (Patbandha *et al.*, 2017). It has therefore been recommended that hatchling length be used as a rapid, repeatable, and harmless method of determining chick quality and predicting performance (Wolanski *et al.*, 2006). Furthermore, the number of saleable chicks is very important for the hatchery. It is thus important for hatcheries to not only have a high hatchability rate, but also to have good quality day-old chicks. According to research, there is a positive correlation between hatchling length and hatchling weight up to seven days of age. This relationship may indicate that the organs of taller chicks are better developed, and previous research has shown that the heart, liver, and spleen weights of tall chicks are higher than those of short chicks, and that the digestive system is better developed in tall chicks (Molenaar *et al.*, 2007; Petek *et al.*, 2008; Willemsen *et al.*, 2008; Meijerhof, 2009; Şeremet, 2012; Sözcü & İpek, 2013; Mukhtar *et al.* 2013; Kamanlı & Durmuş, 2014; Gharahveysi & Kenari 2018; Peşmen, 2023). This suggests that *in ovo* naringin injection improved the quality of the chicks produced.

**Table 3** Descriptive statistics for hatching lengths of chicks hatched from eggs *in ovo* injected with naringin (15 mg), deionised water (negative control), or nothing (control) on the 18th day of incubation

Groups	n	Minimum (cm)	Maximum (cm)	Mean (cm)	SD	P-value
Control	50	15.30	18.30	16.95	0.62	
Negative control	48	16.00	18.00	17.17	0.49	0.004
Naringin (15 mg)	53	16.00	18.30	17.28	0.61	

n: number of chicks, SD: standard deviation

The average hatchability values in the control, negative control, and naringin groups were 83.3%, 80.0%, and 88.3%, respectively, and the chi-square test indicated that there were no significant differences in hatchability between the groups. Nonetheless, the naringin group had the highest hatchability, while the negative control group had the lowest hatchability, and 15 mg *in ovo* naringin provided a 6% increase in hatchability in this study. No negative effect of *in ovo* naringin application on hatchability was thus observed. Ranjbar *et al.* (2019) reported similar results, with the injection of 15 mg naringin on the 17.5th day of incubation causing a 4.33% increase in hatchability. In other studies, hatchability was improved by the *in ovo* injection of nutrients such as histidine (Xu *et al.*, 2019), grape seed extract (Hajati *et al.*, 2014), naringin (Ranjbar *et al.*, 2019), arginine (Hazim & Salih, 2012), vitamin C (Zhu *et al.*, 2020), and carbohydrates (Dong *et al.*, 2013). In contrast, Beck *et al.*, (2019), Ncho *et al.*, (2021), Khaligh *et al.*, (2018), and Coşkun *et al.*, (2014b) reported that the injection of probiotics,  $\gamma$ -aminobutyric acid, quercetin, and pollen into eggs did not affect hatchability. These contrasting results may have been caused by differences in the injection site used, the time of injection (Moosanezhad *et al.*, 2011), or the dosage of the nutrient used. In addition, administering different substances to the egg via *in ovo* injection may create nutritional imbalances in the egg, which may lead to embryo death (Alsadoon & Aygün; 2022). According to Ricks *et al.* (1999), the effectiveness of *in ovo* application depends on the incubation conditions, the equipment used, and the level of hygiene in solution preparation and application. Moosanezhad *et al.* (2011) further reported that the most appropriate time for the injection of various nutrients is between the 17th and 18th days of the incubation period. Since the fluids contained in different areas of the egg have different functions and contents, the response of the embryo can also vary depending on the site of *in ovo* injection (Williams, 2008). Coşkun *et al.* (2017) reported that the most important factor in the profitability of commercial poultry production is hatchability, and if the hatchability rate decreases after *in ovo* injection, the scientific results have no commercial significance (Coşkun *et al.*, 2017). This emphasises the importance of the lack of negative effects on hatchability of *in ovo* naringin injection found in this study.

## Conclusion

*In ovo* injection with naringin insignificantly decreased hatchling weight, significantly increased hatchling length, and insignificantly increased hatchability compared to the control group and negative control group. The study thus concluded that *in ovo*-administered naringin can be used to increase chick quality; however, the effect of different concentrations of naringin needs to be investigated to reach definitive conclusions. Knowledge of the value of the *in ovo* injection technique is rapidly progressing, as the effect of injecting different nutrients is determined. Nonetheless, further research needs to be conducted to determine standardised methods for *in ovo* injection (in terms of injection time and location, and the injected amount) for each nutrient (for example, carbohydrates, proteins, probiotics, or flavonoids).

## Conflict of interest declaration

The author declares no conflicts of interest.

## Data availability statement

Derived data supporting the findings of this study are available from the corresponding author, Günnür Peşmen, on request.

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