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#### ■ Keywords

In-ovo feeding, propolis, hatching traits, quail.

## The Effects of In-Ovo Injection of Propolis on Egg Hatchability and Starter Live Performance of Japanese Quails

### ABSTRACT

The purpose of this study was to determine the effects of *in-ovo* injection of a propolis water extract on hatchability, embryonic mortality, starter live performance, and livability of Japanese quails. In total, 500 fresh hatching eggs were randomly distributed into five treatment groups of 100 eggs per treatment with four replicates of 25 eggs each. On day 14 of incubation, eggs from group 1 were not injected (control), group 2 was injected with distilled water (water), group 3 was injected with 1% propolis water extract (1% propolis), group 4 was injected with 2% propolis water extract (2% propolis), and group 5 was injected with 3% propolis water extract (3% propolis). A completely randomized design was applied, and data were analyzed using the least-square methodology. Hatchability and embryonic mortality in the 2% propolis and 3% propolis treatment groups were significantly lower compared with the control group, but no significant differences were observed between the 1% propolis and control groups. There were no significant bodyweight gain, feed intake, feed conversion ratio, or livability differences among treatments. The results of this study demonstrated that *in-ovo* injection of propolis water extract, especially at doses of 2% and 3% propolis, had negative effects on hatchability and embryonic mortality, but 1% propolis had no detrimental effects on hatchability or embryonic mortality. In all treatment groups, propolis did not negatively affect body weight gain, feed intake, feed conversion ratio, or livability.

### INTRODUCTION

*In-ovo* injection is a method to administer exogenous substances into the amnion during embryo development with the objective of promoting positive effects on hatchability, post-hatch growth performance, immune response, and carcass quality (Uni & Ferket, 2004). The *in-ovo* method was first used by Sharma & Burmester (1982) for the vaccination of turkey hatching eggs against Marek's disease. Recently, the *in-ovo* method has been investigated by researchers for administering ascorbic acid (Elbol et al., 2001; Ipek et al., 2004; Sgavioli et al., 2015), carbohydrates (Zhai et al., 2011; Salmanzadeh, 2012; Ipek et al., 2004; Tako et al., 2004), amino acids (Bhanja et al., 2014; Ohta et al., 1999; Kermanshahi et al., 2015), vitamins (Bello et al., 2013; Salary et al., 2014), minerals (Yair et al., 2013; Oliveira et al., 2015), pollen (Coskun et al., 2014), hormones (Moore et al., 1994; Kocamis et al., 1999), and royal jelly (Moghaddam et al., 2014).

Propolis is a resinous mixture produced by honeybees from resins collected from various plants. (Greenaway et al., 1990; Krell, 1996; Schmidt, 1997). Propolis has antibacterial (Kujumgiev et al., 1993; Sforzin et al., 2000; Silici & Kutluca, 2005; Aygun & Sert, 2013), antifungal (Kartal et al., 2003; Longhini et al., 2007; Soylu et al., 2008;



Aygun *et al.*, 2012), antiviral (Serkedjieva *et al.*, 1992; Marcucci, 1995), antioxidant (Russo *et al.*, 2002; Gregoris *et al.*, 2011), and preservative effects (Copur *et al.*, 2008; Akpinar *et al.*, 2015). Propolis contains pollen, essential and aromatic oils, sugar, amino acids, vitamin and mineral elements (Schmidt and Buchmann, 1992; Krell, 1996; Hegazi, 1998; Burdock, 1998). There are several studies reporting the positive effects of the use of propolis in poultry diets on performance (Denli *et al.*, 2005; Shalmany & Shivazad, 2006; Galal *et al.*, 2008; Seven, 2008; Kleczek *et al.*, 2014). Therefore, the biological activity of propolis is expected to positively impact hatchability and performance of poultry embryos.

The aim of this study was to investigate the effects of *in-ovo* injection of propolis water extract on the hatchability, embryonic mortality, spread of hatch, and chick performance in Japanese quails (*Coturnix coturnix japonica*).

## MATERIALS AND METHODS

### Hatching Eggs

A total of 500 fresh hatching eggs was obtained from Japanese quails (*Coturnix coturnix japonica*; 22 week of age) reared on a local commercial farm (Konya, Turkey). Quails were housed in battery cages (1 male: 2 females) under a photoperiod of 16 h of light (artificial): 8 h of dark. The quails were fed a breeder diet containing 2,900 kcal metabolizable energy/kg and 20% crude protein. Feed and water were provided *ad libitum*. The eggs were randomly distributed into five treatment groups with 100 eggs per treatment with four replicates of 25 eggs each. A completely randomized design was applied.

### Incubation Management

Eggs were incubated in a commercial incubator (Cimuka Co., Turkey) at dry-bulb temperature of 37.5°C and 60-65 % relative humidity (RH) until d 14 of incubation, when incubator conditions were changed to 37.2°C and 75% RH. Eggs were automatically turned 90° once every 2 h until 14 days of incubation.

### Preparation of the solutions

Propolis samples were collected from Konya (Turkey) in 2015, and extracted according to the method of Krell (1996) with some modifications. Propolis was frozen in liquid nitrogen and then crushed into a powder. Then, 1%, 2% and 3% water extracts of

propolis were prepared. The 1% propolis solution was prepared by mixing 99 mL of distilled water with 1g of propolis; the 2% propolis solution was prepared by mixing 98 mL of distilled water with 2g of propolis; and the 3% propolis solution was prepared by mixing 97 mL of distilled water with 3g of propolis. The propolis solutions were then stirred using a magnetic stirrer (Heidolph MR 3001, Germany) at 1000 rpm at 25 °C for 2h. The extracts were stored in sealed glass flasks, shaken twice daily for one week, and then maintained in an ultrasonic bath at 35 kHz for 15 minute. Each solution was filtered (coarse filter) separately and kept in the dark-glass flasks at 4°C until use.

### Injection Procedure

After the blunt end of the eggshell was disinfected with 70% ethanol, a hole for injection was opened with a micromotor (Strong 210, Korea). The prepared extracts were injected (0.20 mL) into the amnion with a 26-gauge plastic disposable syringe. After injection, the hole was sealed with wax and transferred to the hatch basket.

### Hatching

Between 408 and 444 h of incubation, the transferred eggs were individually checked every 3 h, and the number of hatched chicks were recorded. After 18.5 days of incubation, all hatched chicks were removed from each hatch basket, unhatched eggs were opened, and embryos were classified according to guidelines of Aygun *et al.* (2012) to establish the stage of embryonic mortality, as d 1-9 (black-eye visible and embryo without feathers), d 10-16 (embryo with feathers and embryo with yolk out), and d 17-18 (dead fully-grown embryo and with internalized yolk). Fertility was calculated as the percentage of set eggs. Hatchability of both set (groups) and fertile eggs was calculated.

### Chick Performance

Forty hatchlings per group (10 chicks/pen) were randomly selected to measure their performance for 10 days. Chicks were weighed at the beginning (1 day old) and end of the experiment (10 days old). Chicks were reared (four pens/ group) in different pens with 10 chicks per 0.22 m<sup>2</sup>. During the 10 days of rearing, a grower diet (2,910 kcal metabolizable energy/kg and 24.1% crude protein) was provided *ad libitum*. Room temperature was set at 33°C until the end of the rearing period (10 day). The photoperiod was 24L:0D. At the end of 10 days, all chicks were weighed per



pen basis. Feed intake was determined by subtracting feed residues from total feed offered during the entire rearing period (10 days). Feed conversion ratio (g feed /g weight gain) for the 10 days of the rearing period. During the 10 days of rearing, mortality was recorded daily, and livability was calculated as the percentage of live chicks relative to the number of dead chicks during the rearing period.

### Statistical Analysis

Data were submitted to analysis of variance to compare the means of the studied traits (hatchability, embryonic mortality, spread of hatch, chick body weight, body weight gain, feed intake, feed conversion ratio, and livability) among the control, water, 1% propolis, 2% propolis, and 3% propolis treatment groups. Linear, quadratic, and cubic models were applied in regression analyses to determine the effect of propolis levels. Contrast analysis was applied to demonstrate the differences of the means among treatment groups. All statistical analyses were carried out using Minitab Version 14 (Minitab Inc., State College, PA).

## RESULTS

The effects of propolis water extract on hatchability and embryonic mortality are given in Table 1. The rates of hatchability of set eggs varied significantly, between 57.42% and 83.57%, among all groups ( $p < 0.01$ ). A linear ( $p < 0.001$ ) and cubic ( $p < 0.01$ ) effect was observed on the hatchability of both set and fertile eggs. The hatchability of set eggs in the 2% propolis treatment group was significantly lower than in the control, water, and 1% propolis treatment groups, but was not different from that of the 3% propolis treatment group. No significant differences were observed among the control, water, and 1% propolis treatment groups for hatchability of set eggs. The

hatchability of fertile eggs in the control (89.02%), water (83.87%), and 1% propolis (76.43%) treatment groups was higher than in the 2% propolis (46.75%) and 3% propolis (60.65%) treatment groups. There were no significant differences in the hatchability of fertile eggs among the control, water, and 1% propolis treatment groups.

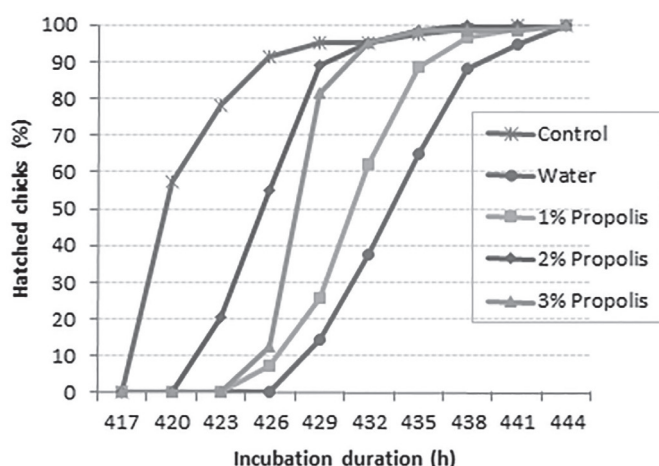
There was no significant effect of treatments on embryonic mortality between days 1 and 9 of incubation. A linear effect ( $p < 0.01$ ) on embryonic mortality was found between days 10 and 16 of incubation. Embryonic mortality between days 10 and 16 was higher in the 2% propolis (18.48%) treatment group than in the control (0.00%), water (4.47%) and 1% propolis (5.79%) treatment groups. A linear ( $p < 0.001$ ) and a cubic ( $p < 0.05$ ) effect were observed on embryonic mortality between days 17 and 18 of incubation. The control (2.18%), water (3.45%), and 1% propolis (5.00%) groups presented a lower embryonic mortality between days 17 and 18 of incubation than the 2% propolis (27.43%) and the 3% propolis (23.43%) treatment groups. No significant differences were found between control and water treatments group for the hatchability of fertile eggs, hatchability of set eggs, and embryonic mortality.

Hatching began at 420, 423, 426, 426, and 429 h of incubation in the control, 2% propolis, 1% propolis, 3% propolis, and water groups, respectively (Figure 1). Hatching ended at 438, 441, 444, 444, and 444 h of incubation in the 2% propolis, control, water, 1% propolis, and 3% propolis groups, respectively. A linear effect ( $p < 0.01$ ) was detected only on the hatching rates at 420 and 423 h of incubation. A quadratic effect ( $p < 0.05$ ) was found on hatching rates for all incubation durations, except at 435 and 438 h of incubation. A cubic effect ( $p < 0.01$ ) on hatching rates was observed for all incubation durations.

**Table 1** – Effects of in-ovo injection of propolis on hatchability and embryonic mortality (Mean $\pm$ SE)

| Group                               | Fertility        | Hatchability of set eggs       | Hatchability of fertile eggs  | Embryonic mortality (% of fertile eggs) |                                |                               |
|-------------------------------------|------------------|--------------------------------|-------------------------------|---|--------------------------------|-------------------------------|
|                                     |                  |                                |                               | 1 to 9d                                 | 10 to 16d                      | 17 to 18d                     |
| Control                             | 93.91 $\pm$ 1.11 | 83.57 $\pm$ 2.01 <sup>a</sup>  | 89.02 $\pm$ 2.41 <sup>a</sup> | 8.80 $\pm$ 1.94                         | 0.00 $\pm$ 0.00 <sup>c</sup>   | 2.18 $\pm$ 1.26 <sup>b</sup>  |
| Water                               | 93.92 $\pm$ 0.93 | 78.69 $\pm$ 3.48 <sup>a</sup>  | 83.87 $\pm$ 4.28 <sup>a</sup> | 8.21 $\pm$ 2.32                         | 4.47 $\pm$ 2.25 <sup>bc</sup>  | 3.45 $\pm$ 3.45 <sup>b</sup>  |
| 1% Propolis                         | 92.78 $\pm$ 0.19 | 70.93 $\pm$ 3.81 <sup>ab</sup> | 76.43 $\pm$ 3.98 <sup>a</sup> | 12.79 $\pm$ 2.11                        | 5.79 $\pm$ 1.05 <sup>bc</sup>  | 5.00 $\pm$ 2.52 <sup>b</sup>  |
| 2% Propolis                         | 94.67 $\pm$ 1.44 | 44.30 $\pm$ 1.67 <sup>c</sup>  | 46.75 $\pm$ 1.19 <sup>b</sup> | 7.34 $\pm$ 2.15                         | 18.48 $\pm$ 3.63 <sup>a</sup>  | 27.43 $\pm$ 2.81 <sup>a</sup> |
| 3% Propolis                         | 94.70 $\pm$ 1.00 | 57.42 $\pm$ 4.83 <sup>bc</sup> | 60.65 $\pm$ 5.21 <sup>b</sup> | 4.67 $\pm$ 1.77                         | 11.25 $\pm$ 2.69 <sup>ab</sup> | 23.43 $\pm$ 3.37 <sup>a</sup> |
| p-value                             | 0.673            | 0.000                          | 0.000                         | 0.142                                   | 0.000                          | 0.000                         |
| Linear effect of propolis levels    | 0.464            | 0.000                          | 0.000                         | 0.212                                   | 0.001                          | 0.000                         |
| Quadratic effect of propolis levels | 0.419            | 0.376                          | 0.454                         | 0.089                                   | 0.275                          | 0.484                         |
| Cubic effect of propolis levels     | 0.830            | 0.004                          | 0.005                         | 0.732                                   | 0.060                          | 0.024                         |

SE: Standard Error



**Figure 1** – Effects of in ovo injection of propolis on spread of hatch ( $p < 0.05$  range from 417 h to 435 h, and  $p > 0.05$  between 438 h and 441 h according to contrast comparisons).

The highest hatching rates were obtained in the control group (57.29%, 78.39%, and 91.52%) at 420, 423, and 426 h of incubation, respectively. The lowest rate of hatching was observed in water group (65.04%) at 435 h of incubation, but no significant differences were found among the control (97.67%), 1% propolis (88.33%), 2% propolis (98.33%), and 3% propolis (98.33%) groups. There were no significant ( $p > 0.05$ ) differences among groups at 438 and 441 h of incubation.

The effect of treatments on body weight at d 1, body weight at d 10, and body weight gain are shown in Table 2. No linear, quadratic, or cubic effects of propolis levels on body weight at d 1, body weight at d 10, and body weight gain were observed ( $p > 0.05$ ). There were no significant differences between treatments in terms of body weight at d 1, body weight at d 10, and body weight gain.

The results of feed intake, feed conversion ratio, and livability are presented in Table 3. No linear, quadratic, or cubic effects of propolis levels on feed intake, feed conversion ratio, or livability were observed ( $p > 0.05$ ). There were no significant feed conversion ratio

differences among the control (1.89), water (2.23), 1% propolis (1.78), 2% propolis (2.02), and 3% propolis (1.94) treatment groups. Similarly, treatments had no effect on livability in the control (95.0%), water (85.0%), 1% propolis (97.5%), 2% propolis (95.0%), and 3% propolis (97.5%) treatment groups.

## DISCUSSION

To the best of our knowledge, no previous studies have been conducted on the effects of *in-ovo* injection of propolis on hatching eggs. Hatchability was adversely affected in the 2% propolis and 3% propolis treatment groups, but not in the 1% propolis treatment group. The results of different studies report both negative and positive effects of the *in-ovo* injection of substances on hatchability. Hatchability was increased by *in-ovo* injection with ascorbic acid (Ipek *et al.*, 2004), L-arginine (Al-Daraji *et al.*, 2012), and carbohydrates (Dong *et al.*, 2013). However, hatchability was reduced by *in-ovo* injection with ascorbic acid (Sgavioli *et al.*, 2015), organic trace minerals (Oliveira *et al.*, 2015), glucose (Ebrahimnezhad *et al.*, 2011), and glucose and magnesium (Salmanzadeh *et al.*, 2012). In contrast, Bhanja & Mandal (2005), Nowaczewski *et al.* (2012), Moore *et al.* (1994), Shafey *et al.* (2012), and Coskun *et al.* (2014) reported that hatchability was not affected when eggs were injected with amino acids, vitamin C, hormones, carbohydrates, and pollen extract, respectively. *In-ovo* injection of some nutrients may cause nutrient imbalance inside the eggs, and consequently may limit maximal growth and development of the embryo during incubation (Uni, 2014). *In-ovo* injection into the albumen may cause an allergic reaction that may prevent the respiration of the developing embryo, and this may lead to the death of the chicks (Salmanzadeh *et al.*, 2012).

The 2% propolis and 3% propolis treatments negatively affected embryonic mortality between

**Table 2** – Effects of in-ovo injection of propolis on chick body weight and body weight gain (Mean $\pm$ SE)

| Group                               | Body Weight, g (1 d) | Body Weight, g (10 d) | Body Weight Gain, g |
|-------------------------------------|----------------------|-----------------------|---------------------|
| Control                             | 7.93 $\pm$ 0.05      | 35.28 $\pm$ 0.76      | 27.36 $\pm$ 0.80    |
| Water                               | 7.88 $\pm$ 0.15      | 34.04 $\pm$ 1.39      | 26.17 $\pm$ 1.29    |
| 1% Propolis                         | 7.80 $\pm$ 0.03      | 33.34 $\pm$ 2.45      | 25.54 $\pm$ 2.42    |
| 2% Propolis                         | 7.64 $\pm$ 0.17      | 34.89 $\pm$ 1.13      | 27.25 $\pm$ 1.10    |
| 3% Propolis                         | 7.76 $\pm$ 0.09      | 34.94 $\pm$ 0.91      | 27.18 $\pm$ 0.97    |
| p- value                            | 0.437                | 0.876                 | 0.863               |
| Linear effect of propolis levels    | 0.109                | 0.971                 | 0.869               |
| Quadratic effect of propolis levels | 0.522                | 0.364                 | 0.385               |
| Cubic effect of propolis levels     | 0.373                | 0.655                 | 0.601               |

SE: Standard Error





**Table 3** – Effects of in-ovo injection of propolis on feed conversion and livability (Mean±SE).

| Group                               | Feed Intake, g | Feed Conversion Ratio (g feed/g gain) | Livability (%) |
|-------------------------------------|----------------|---------------------------------------|----------------|
| Control                             | 51.41±3.51     | 1.89±0.15                             | 95.0±2.89      |
| Water                               | 57.79±4.61     | 2.23±0.23                             | 85.0±5.00      |
| 1% Propolis                         | 45.85±5.83     | 1.78±0.07                             | 97.5±2.50      |
| 2% Propolis                         | 54.52±3.59     | 2.02±0.19                             | 95.0±2.89      |
| 3% Propolis                         | 52.80±3.44     | 1.94±0.09                             | 97.5±2.50      |
| p- value                            | 0.414          | 0.358                                 | 0.088          |
| Linear effect of propolis levels    | 0.973          | 0.836                                 | 0.217          |
| Quadratic effect of propolis levels | 0.799          | 0.821                                 | 0.487          |
| Cubic effect of propolis levels     | 0.598          | 0.379                                 | 0.146          |

SE: Standard Error

10-16 and 17-18 days of incubation. The use of 2% propolis and 3% propolis may be toxic for the embryo, particularly during these incubation ages. However, Nowaczewski *et al.* (2012), Sgavioli *et al.* (2015), Shafey *et al.* (2012), and Ipek *et al.* (2004) reported that *in-ovo* injection with vitamin C, ascorbic acid, carbohydrates, and glucose, respectively, had no significant effect on embryonic mortality.

Chick body weight on d 10 and body weight gain were not affected by the *in-ovo* injection of propolis. Salary *et al.* (2014) reported no significant weight gain differences between chicks submitted to *in-ovo* injection of vitamin E and the control group. On the other hand, Al-Daraji *et al.* (2012) reported that the chicks from eggs injected with L-arginine presented higher weight gains than control groups. Researchers (Biavatti *et al.*, 2003; Ziaran *et al.*, 2005; Acikgoz *et al.*, 2005; Canogullari *et al.*, 2009) observed that the addition of propolis to broiler diets did not significantly influence broiler body weight and body weight gain, or the performance of laying hens (Belloni *et al.*, 2015). In the current study, the amount of propolis biological material may have been insufficient to promote positive broiler performance because, according to Biavatti *et al.* (2003), the effects of propolis on broilers body weight and body weight gain are observed only after 14 days of age, depending on the level of concentrate.

The *in-ovo* injection of propolis had no effect on feed intake, feed conversion ratio, or livability during the first 10 days of life. However, different results are reported in literature. Al-Daraji *et al.* (2012) reported no significant feed intake differences between Japanese quails injected or not *in-ovo* with L-arginine, but the *in-ovo* injection of L-arginine resulted in better feed conversion ratio. Similarly, no significant effect of the *in-ovo* injection of broiler embryos with selected substances on feed conversion ratio were detected

by Bhanja & Mandal (2005) and Salary *et al.* (2014). In contrast, Salmazadeh *et al.* (2012) reported that the broilers submitted to *in-ovo* injection of glucose presented better feed conversion ratio during the rearing period than the control group. Feed intake and feed conversion ratio were not affected by supplemental propolis in broiler (Ziaran *et al.*, 2005; Acikgoz *et al.*, 2005; Canogullari *et al.*, 2009; Mahmoud *et al.*, 2013) and quail diets (Sahin *et al.*, 2003).

Our results showed that chicks of the 1% propolis, 2% propolis, and 3% propolis treatment groups started to hatch later than those of control group, but the end of chick hatch was almost the same. Therefore, the *in-ovo* injection of propolis may be advantageous for the prevention of dehydration of chicks. A narrow hatch window (spread between early- and late-hatched chicks) promote better flock uniformity. Casteel *et al.* (1994) reported that extended hatching time decreased immune response of broiler chicks. Also, the growth rate of chicks after hatch is adversely affected by a delay in access to feed after hatch (Careghi *et al.*, 2005).

## CONCLUSIONS

The periods of embryonic development are approaching 40-50% of the rearing period of most of meat-type poultry species, and therefore, the incubation period matters for high performance of birds. The results of this study demonstrated that the *in-ovo* injection of propolis water extract, especially at doses of 2% and 3% propolis, had negative effects on hatchability and embryonic mortality, but 1% propolis had no detrimental effects on hatchability or embryonic mortality. In all treatment groups, propolis did not negatively affect body weight gain, feed intake, feed conversion ratio, or livability. Further studies should be performed to determine the effects of different solvents and the propolis dose to be applied in hatching eggs.



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