Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2016

Special Issue 2 Incubation / 087-094

http://dx.doi.org/10.1590/1806-9061-2015-0198

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

■Author(s)

Aygun A[I]

[I] Faculty of Agriculture, Department of Animal Science, Selcuk University, Konya, 42075, Turkey.

■Mail Address

Corresponding author e-mail address
Ali Aygun
Selcuk Univesity, Faculty of Ahriculture,
Department of Animal Science
42075 Konya, Konya, Turkey
Phone: +90 (332) 2232905
Email: aaygun@selcuk.edu.tr

■Keywords

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

Submitted: August/2016
Approved: September/2016

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 (Elibol *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; Sforcin *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 of16 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. The1% propolis solution was prepared by mixing 99 mL of distilled waterwith1g 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^2.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 the10 days of the rearing period. During the 10 days of rearing, mortality was recorded daily, and livability was calculated as a 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 Version14 (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±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±1.11	83.57±2.01^{a}	89.02±2.41^{a}	8.80±1.94	0.00±0.00^{c}	2.18±1.26^{b}
Water	93.92±0.93	78.69±3.48^{a}	83.87±4.28^{a}	8.21±2.32	4.47±2.25^{bc}	3.45±3.45^{b}
1% Propolis	92.78±0.19	70.93±3.81^{ab}	76.43±3.98^{a}	12.79±2.11	5.79±1.05^{bc}	5.00±2.52^{b}
2% Propolis	94.67±1.44	44.30±1.67^{c}	46.75±1.19^{b}	7.34±2.15	18.48±3.63^{a}	27.43±2.81^{a}
3% Propolis	94.70±1.00	57.42±4.83^{bc}	60.65±5.21^{b}	4.67±1.77	11.25±2.69^{ab}	23.43±3.37^{a}
p-value	0.673	0000	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

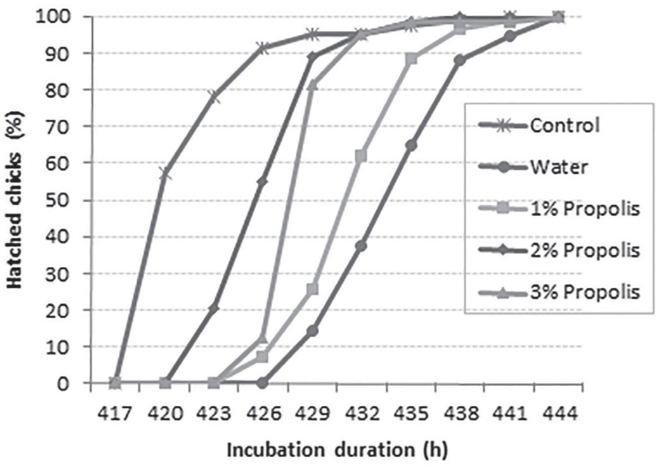

Figure 1 – Effects of in ovo injection of propolis on spread of hatch (p<0.05 range from 417 h to 435 h, and p>0.05 between 438 h and 441 h according to contrast comparisons).

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 led 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±SE)

Group	Body Weight, g (1 d)	Body Weight, g (10 d)	Body Weight Gain, g
Control	7.93±0.05	35.28±0.76	27.36±0.80
Water	7.88±0.15	34.04±1.39	26.17±1.29
1% Propolis	7.80±0.03	33.34±2.45	25.54±2.42
2% Propolis	7.64±0.17	34.89±1.13	27.25±1.10
3% Propolis	7.76±0.09	34.94±0.91	27.18±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, Salmanzadeh *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 the1% 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.

REFERENCES

Acikgoz Z, Yucel B, Altan O. The effects of propolis supplementation on broiler performance and feed digestibility. Archiv Für Geflügelkunde 2005;69:117-122.

Akpinar GC, Canogullari S, Baylan M, Alasahan S, Aygun A. The use of propolis extract for the storage of quail eggs. The Journal of Applied Poultry Research 2015;24:427-435.

Al-Daraji HJ, Al-Mashadani AA, Al-Hayani WK, Al-Hassani AS, Mirza HA. Effect of *in-ovo*injection with L-arginine on productive and physiological traits of Japanese quail. South African Journal of Animal Science 2012;42:139-145.

Aygun A, Sert D, Copur G. Effects of propolis on eggshell microbial activity, hatchability, and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs. Poultry Science 2012;91:1018–1025.

Aygun A, Sert D. Effects of prestorage application of propolis and storage time on eggshell microbial activity, hatchability, and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs. Poultry Science 2013;92:3330–3337.

Bello A, Zhai W, Gerard PD, Peebles ED. Effects of the commercial *in-ovo* injection of 25- hydroxycholecalciferol on the hatchability and hatching chick quality of broilers. Poultry Science 2013;92:2551–2559.

Belloni M, Almeida Paz ICL, Nääs IA, Alves MCF, Garcia RG, et al. Productive, qualitative, and physiological aspects of layer hens fed with propolis. Brazilian Journal of Poultry Science 2015;17:467-472.

Bhanja SK, Mandal AB. Effect of *In-ovo*Injection of critical amino acids on pre- and post-hatch growth, immunocompetence and development of digestive organs. Asian_Australasian Journal of Animal Sciences 2005;18;524-531.

Bhanja SK, Sudhagar M, Goel A, Pandey N, Mehra M, Agarwal SK, et al. Differential expression of growth and immunity related genes influenced by *in-ovo*supplementation of amino acids in broiler chickens. Czech Journal of Animal Science 2014;59:399-408.

Biavatti, MW, Bellaver, MH, Volpato, L, Costa, C, Bellaver, C. Preliminary studies of alternative feed additives for broilers:Alternanthera brasiliana extract, propolis extract and linseed oil. Revista Brasileira de Ciência Avícola 2003;5:147-151.

Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food and Chemistry Toxicology 1998;36:347–363.

Canogullari S, Baylan M, Sahinler N, Sahin A. Effects of propolis and pollen supplementations on growth performance and body components of Japanese quails (Coturnix coturnix japonica). Archiv Für Geflügelkunde 2009;73:173-178.

Careghi C, Tona K, Onagbesan O, Buyse J, Decuypere E, Bruggeman V. The effects of the spread of hatch and interaction with delayed feed access after hatch on broiler performance until seven days of age. Poultry Science 2005;84:1314-1320.

Casteel ET, Wilson JL, Buhr RJ, Sander JE. The influence of extended posthatch holding time and placement density on broiler performance. Poultry Science 1994;73:1679-1684.

Copur G, Camci O, Sahinler N, Gul A. The effect of propolis eggshell coatings on interior egg quality. Archiv für Geflügelkunde 2008;72:35–40.

Coskun I, Cayan H, Yılmaz O, Taskin A, Tahtabicen E, Samli HH. Effects of *In-ovo* Pollen Extract Injection to Fertile Broiler Eggs on Hatchability and Subsequent Chick Weight. Turkish Journal of Agricultural and Natural Sciences 2014;1:485-489

Denli M, Cankaya S, Silici S, Okan F, Uluocak AN. Effect of Dietary Addition of Turkish Propolis on the Growth Performance, Carcass Characteristics and Serum Variables of Quail (*Coturnix coturnix japonica*). Asian-Australasian Journal of Animal Sciences 2005;18:848-854.

Dong XY, Jiang YJ, Wang MQ, Wang YM, Zou XT. Effects of *in-ovo* feeding of carbohydrates on hatchability, body weight, and energy status in domestic pigeons (*Columba livia*). Poultry Science 2013;92:2118–2123.

Ebrahimnezhad Y, Salmanzadeh M, Aghdamshahryar H, Beheshti R, Rahimi H. The effects of *in-ovo*injection of glucose on characters of hatching and parameters of blood in broiler chickens. Annals of Biological Research 2011;2:347-351.

Elibol O, Turkoglu M, Akan M, Erol H. Effects of ascorbic acid injection during incubation on the hatchability of large broiler eggs. Turkish Journal of Veterinary and Animal Sciences 2001;25:245–248. (In Turkish, with English abstract).

Galal A, Abd El-Motaal AM, Ahmed AMH, Zaki TG. Productive performance and ımmune response of laying hens as affected by dietary propolis supplementation. International Journal of Poultry Science 2008;7:272-278.

Greenaway W, Scaysbrook T, Whatley FR. The composition and plant origins of propolis. A report of work at Oxford. Bee World 1990;71:107–118.

Gregoris E, Fabris S, Bertelle M, Grassato L, Stevanato R. Propolis as potential cosmeceutical sunscreen agent for its combined photoprotective and antioxidant properties. International Journal of Pharmaceutics 2011;405:97–101.

Hegazi AG. Propolis an overview. Journal Bee Informed 1998;5/6:22-28.

Ipek A, Sahan U. Yilmaz B. The effect of *in-ovo* ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. Archiv für Geflügelkunde 2004;63:132-135.

Kartal M, Yıldız S, Kaya S, Kurucu S, Topcu G. Antimicrobial activity of propolis samples from different regions of Anatolia. Journal of Ethnopharmacology 2003;86:69–73.

Kermanshahi H, Daneshmand A, Khodambashi Emami N, Ghofrani Tabari D, Doosti M, et al. Effect of *in-ovo*injection of threonine on Mucin2 gene expression and digestive enzyme activity in Japanese quail (*Coturnix japonica*). Research in Veterinary Science 2015;100:257-262.

Kleczek K, Wilkiewicz-Wawro E, Wawro K, Makowski W, Murawska D, Wawro M. The Effect of Dietary Propolis Supplementation on the Growth Performance of Broiler Chickens. Polish Journal of Natural Sciences 2014;29:105-117.

Kocamis H, Yeni YN, Kirkpatrick-Keller DC, Killefer J. Postnatal growth of broilers in response to *in-ovo* administration of chicken growth hormone. Poultry Science 1999;78:1219-1226.

Krell R. Value-added products from beekeeping [Agricultural Services Bulletin No124]. Rome: Food and Agriculture Organization of the United Nations; 1996.

Kujumgiev A, Bankova V, Ignatova A, Popov S. Antibacterial activity of propolis, some of its components and their analogs. Pharmazie 1993;48:785–786.

Longhini R, Raksa SM, Oliveira ACP, Terezinha I, Svidzinski E, Franco SL. Obtenção de extratos de propolis sob diferentes condições e avaliação de sua atividade antifungica. Brazilian Journal of Pharmacognosy 2007;17:388–395.

Mahmoud UT, Abdel-Rahman MA, Darwish MHA. The effect of chinese propolis supplementation on ross broiler performance and carcass characteristics. Journal of Advanced veterinary Research 2013;3:154-160.

Marcucci MC. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 1995;26:83–99.

Moghaddam AA, Borji M, Komazani D. Hatchability rate and embryonic growth of broiler chicks following *in-ovo* injection royal jelly. British Poultry Science 2014;55:391-397.

Moore RW, Dean CE, Hargis PS. Effects of *in-ovo* hormone administration at day eighteen of embrygeesis on posthatch growth of broilers. The Journal of Applied Poultry Research 1994;3:31-39.

Nowaczewski S, Kontecka H, Krystianiak S. Effect of *in-ovo* injection of vitamin C during incubation on hatchability of chickens and ducks. Folia Biologica 2012;60:93-97.

Ohta Y, Tsushima N, Koide K, Kidd MT, Ishibashi T. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poultry Science 1999;78:1493–1498.

Oliveira TFB, Bertechini AG, Bricka RM, Kim EJ, Gerard PD, Peebles ED. Effects of *in-ovo* injection of organic zinc, manganese, and copper on the hatchability and bone parameters of broiler hatchlings. Poultry Science 2015;94:2488-2494.

Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 2002;73 Suppl1:21-29.

Sahin A, Baylan M, Sahinler N, Canogullari S, Gul A. The effects of propolis on fattening performance and carcass traits of japanese quails. Apicultural Research 2003;11:42-44.

Salary J, Sahebi-Ala F, Kalantar M, Matin HRH. *In-ovo* injection of vitamin E on post-hatch immunological parameters and broiler chicken performance. Asian Pacific Journal of Tropical Biomedicine 2014;4:616-619.

Salmanzadeh M, Ebrahimnezhad YH, Aghdam S, Beheshti R. The effects of *in-ovo* injection of glucose and magnesium in broiler breeder eggs on hatching traits, performance, carcass characteristics and blood parameters of broiler chickens. Archiv Für Geflügelkunde 2012;76:277-284.

Schmidt JO, Buchmann SL. Other products of the hive. In: Graham JM, editor. The hive and the honey bee. Hamilton (IL): Dadant & Sons; 1992. p.928-977.

Seven PT. The effects of dietary turkish propolis and vitamin c on performance, digestibility, egg production and egg quality in laying hens under different environmental temperatures. Asian-Australasian Journal of Animal Sciences 2008;21:1164-1170.

Sforcin JM, Fernandes Jr A, Lopes CA, Bankova V, Funari SR. Seasonal effect on Brazilian propolis antibacterial activity. Journal of Ethnopharmacology 2000;73:243–249.

Sgavioli S, Matos Júnior JB, Borges LL, Praes MFFM, Morita VS, Zanirato GL. Effects of ascorbic acid injection in incubated eggs submitted to heat stress on incubation parameters and chick quality. Brazilian Journal of Poultry Science 2015;17:181-190.

Shafey TM, Alodan MA, Al-Ruqaie IM, Abouheif MA. *In-ovo* feeding of carbohydrates and incubated at a high incubation temperature on hatchability and glycogen status of chicks. South African Journal of Animal Science 2012;42:210-220.

Shalmany SK, Shivazad M. The effect of diet propolis supplementation on ross broiler chicks performance. International Journal of Poultry Science 2006;5:84-88.

Sharma JM, Burmester BR. Resistance to Marek's disease at hatching in chickens vaccinated as embryos with the turkey herpesvirus. Avian Diseases 1982;26:134-149.

Schmidt JO. Chemical composition and application. In: Mizrahi A, Lensky Y, editors. Bee Products: Properties, Applications and Apitherapy. Newyork: Plenum Press; 1997. p. 15-26.

Serkedjieva J, Manolova N, Bankova V. Anti-influenza virus effect of some propolis constituents and their analogues (esters of substituted cinnamic acids). Journal Natural Products 1992;55:294–302.

Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology 2005; 99:69–73.

Soylu EM, Ozdemir AE, Erturk E, Sahinler N, Soylu S. Chemical composition and antifungal activity of propolis against *Penicillium digitatum*. Asian Journal Chemistry 2008;20:4823–4830.

Tako E, Ferket PR, Uni Z. Effects of *in-ovo* feeding of carbohydrates and beta-hydroxy-beta-methylbutyrate on the development of chicken intestine. Poultry Science 2004;83:2023–2028.

Uni Z, Ferket RP. Methods for early nutrition and their potential. World's Poultry Science Journal 2004;60:101-111.

Uni Z. The effects of *in-ovo* feeding. 2014 [cited 2016 Jan 8]. Available from: http://www.thepoultryfederation.com/public/userfiles/files/Zehava%20Uni%20Presentation.pdf.

Yair R, Shahar R, Uni Z. Prenatal nutritional manipulation by *in-ovo* enrichment influences bone structure, composition, and mechanical properties. Journal of Animal Science 2013;91:2784-2793.

Zhai W, Gerard PD, Pulikanti R, Peebles ED. Effects of *in-ovo* injection of carbohydrates on embryonic metabolism, hatchability, and subsequent somatic characteristics of broiler hatchlings. Poultry Science 2011;90:2134-2143.

Ziaran HR, Rahmani HR, Pourreza J. Effect of dietary oil extract of propolis on immune response and broiler performance. Pakistan Journal of Biological Sciences 2005;8(10):1485-1490.