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Original article

The Effects of In Ovo Injection of Propolis Extract to Breeder Eggs on Hatchability and Early Performance in Broilers

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Abstract

This research was conducted to determine the effects of in ovo injection of propolis extract into the amnion to breeder eggs on hatchability and early performance in broilers. For this purpose, a total of 250 Ross 308 broiler breeder hatching eggs were obtained from a commercial hatchery. Eggs were divided into four groups randomly, isotonic serum sodium (0.9%) was injected at the level of 0.2 ml into the first group and there were used as positive control (PC) group, whereas there was no treatment on negative control (NC) group. Where the amount of 0.2 ml prepared mixture which is included %3 propolis extract were injected into low propolis (LP) group, 0.2 ml prepared mixture which is included %6 propolis extract were given into the high propolis group (HP). In ovo propolis extract injection resulted in a shorter hatching time compared with the control groups. The hatchability, hatching weight and survival of newly hatched chicks were not affected by in ovo injection of propolis extract according to our results. There were no differences between the control and treatmet groups in hatchability and embryonic mortality. The results indicated that except for the PC group chicks' weights were similar at hatching day, but the later stage weights of chicks were started to significantly differ from each other and propolis groups have shown higher live weights than the controls at 5th and 10th day ($P<0.05$). All treatment groups exhibited greater weight gain from hatch to 10 d compared to the control groups. The results indicate that in ovo feeding of broiler embryos with propolis extract may have beneficial effects on day old chick weights and early growth rate.

Keywords: Broiler, Hatching, In ovo injection, Performance, Propolis.

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INTRODUCTION

The productivity in poultry production has been increased as improvement of the characteristics of poultry due to genetic selection. Hybrids used in conventional broiler production when after hatching about 5 weeks can reach the slaughter age, they are nearly 2.0-2.5 kg body weight today. Due to the decrease in slaughter age, the share of the incubation period in the total lifespan has approximately raised to 45% as well. The body weight of broilers increased by over 400% from 1957 to 2005 (Zuidhof et al., 2014), they reach 50 to 60-fold higher body weight, counting from hatching to slaughter (Druyan, 2010). Over the years, the demand of embryos for nutrients changed but the chemical composition of an egg has remained and therefore, it began providing the embryos with suitable substances with the help of in ovo technology (Kucharska-Gaca et al., 2017). The major intention of pre-hatch feeding is to equip the embryo with the nutrients necessary to continue intestinal development post-hatch at or close to the same rate as pre-hatch (Kordali, 2020). Some experiments with the use of in ovo feeding technology have shown that the injection of nutrients affects the physiological state of broiler embryos before and after hatching. Proper injection improves not only hatching but also the nutritional status of the chicks and results in a greater predisposition to growth (Kucharska-Gaca et al., 2017). In ovo feeding is a way for feeding chick embryos by inoculating nutrients into the breeder eggs' amniotic fluid, method was first used by Sharma and Burmester (1982) to vaccination of turkey hatching eggs for Marek's disease. This process, administered in the last stage of embryonic development, has been shown to improve the vitality of chicks at birth. If the eggs were inoculated with nutrients when the chick is ingesting the amniotic fluid around three days before hatching, the nutrients inoculated in that fluid could reach the embryonic intestine without any problem (Johnson, 2018). Many nutritional supplements can have injected into the egg by this method manually or automatically. The gradients such as amino acids (Ohta et al., 1999; Kadam et al., 2008; Johnson, 2018), carbohydrates (Tako et al., 2004; Uni et al., 2005; Zhai et al., 2011c), electrolyte solutions (McGruder et al., 2011), hormones (Henry and Burke, 1999; Kocamis et al., 1999, 2000), nucleotides (Dalloul et al., 2005), stimulants (Zhai et al., 2008; Keralapurath et al., 2010), and vitamins (Gore and Qureshi, 1997; Bhanja et al., 2007; Bello et al., 2013) can be given by in ovo injection technique. The in ovo application continues to expand and provide further benefits to the poultry industry (Peebles, 2018).

Natural products present a large variety of biological and pharmacological activities and are considered to have beneficial effects on nutrition for a long time. Propolis is a natural resinous complex collected from different plants by bees. Propolis has many biological activities thanks to more than 300 active compounds it contains. These active compounds in the content of propolis vary depending on the plants that are the source of propolis, the region where the bees are and the season. Due to its healing properties, propolis has been used in traditional medicine as an antiseptic, wound healer and therapeutic substance from ancient times to the present (Kılıç Altun and Aydemir, 2020).

The first week of broiler life is considered as a fluctuation period and the real start of the rearing period at broiler farms is supposed to be the end of this period (Decuypere, 1979; Deeming, 1995). It's the most critical period in a chick's life, which has a significant bearing on the survivability and growth of the bird (Sanniyasi et al., 2012). Thus, the qualitative aspects of day-old chicks can be partly linked to their viability and performance. Day-old weight of broiler chicks is not correlated to the broiler weight at slaughter age but 7 days old weights are, therefore this weight can be considered as a parameter for predicting performance and for confirming day-old quality as this may be an expression of intrinsic factors (Tona et al., 2005). On the other hand, chicks after the incubation cannot consume feed or water for the first 24-72 hours generally because of the post-incubation procedures like sex determination, vaccination, box and transport, so up to 72 hours old they are classified as a 'day-old chick. Prolonged hunger and thirst may result in negatively affect the early growth of the chicks and finally the performance of the broilers (Wang et al., 2018). Therefore, the few days pre and post-hatch are critical for the development and survival of commercial chickens (Uni and Ferket, 2004). Newly hatched chicks can seem like completely their anatomic growth, but the digestive, immune, and thermoregulatory systems need further development and maturation. Early feeding may useful for the development of the gastrointestinal tract and the growth of the bird. Some innovative approaches and new hatching concepts have been improved for made of early feeding in commercial conditions and they had started to be using to produce high-quality broiler chicks.

Propolis is an important substance that honeybees collect from the resins of plants and form them with their own enzymes. Therefore, it's expected that the biological activity of propolis to positively impact the hatchability and performance of poultry embryos (Aygun, 2016). In ovo administration of propolis to the air cell increased the hatchability but was tended to reduce the hatching weight of chicks (Kop Bozbay et al., 2016). This research was conducted to determine the effects of in ovo injection of propolis extract into the amnion to breeder eggs on hatchability and early performance in broilers. Because of the positive correlation with 7 days old and the slaughter weights, it's expected that in ovo propolis extract administration better effects on the further performance of the broilers.

MATERIALS and METHODS

In total, a number of 250 broiler breeder (Ross 308) hatching eggs obtained from a commercial hatchery were randomly distributed into four treatment groups of 60 eggs per each. Eggs were incubated under common commercial incubation conditions. On day 18 of incubation, each egg was treated manually one by one explained below:

Group NC (negative control) was no injection, Group PC (positive control) was injected with physiological saline (0.9% NaCl isotonic), Group LP (low dose propolis) was injected with 0.2 ml propolis extract diluted with physiological saline (containing 3% propolis), Group HP (high dose

propolis) was injected 0.2 ml propolis extract (Kop Bozbay et al., 2016) diluted with physiological saline (containing 6% propolis).

After the blunt end of the eggshell was disinfected with 70% ethanol, a hole was opened with a 20-gauge plastic disposable syringe for injection and the prepared extracts were injected (0.20 mL) into the amnion. After the injection, the hole was sealed with wax and eggs were transferred to the hatch cases. Between 471-501 hours of incubation, the incubator opened every 3 hours, hatched chicks in groups were counted and average hatching time was calculated.

Twenty-seven hatchlings were randomly selected for each group (9 chicks/per pen) with three replicates to measure growth performance over 10 days. All chicks were weighed individually before transferring to the house and on the 5^{th} and 10^{th} day of the experiment. Chicks were reared in different pens (three pens per group) with 0.12 m^2 floor area per chick. Room temperature was set below under 35 °C until the end of the rearing period and photoperiod set as 24L:0D. During the rearing period, drinking water and a starter diet (3000 kcal metabolic energy/kg and 22% crude protein) were provided as ad libitum. Data were recorded by excel and they were analyzed with JMP statistical software when comparing the differences of means it was deemed to be significant at $P<0.05$. ANOVA procedures were used for balanced data; percentage data was conducted using chi-square tests (JMP, Version 10).

RESULTS and DISCUSSION

In experiments in which eggs were administrated with 0.2 ml propolis extract at the embryonic day 18 (E18), there were no differences between the hatchability (97.19%) and embryonic mortality (95.97%) rates in average of the control or treatmet groups in. Broiler chicks hatch during a time interval of 480 to 510 hours and used incubation times of approximately 504 hours in commercial hatcheries, which are considered optimal for the complete hatching of the incubated eggs. But, in ovo propolis extract injection resulted in a shorter hatching time compared with the control groups (Table 1). In ovo saline application (positive control) resulted in a marked increase on hatching time of approximately 2 hours. The hatching time of the propolis groups was significantly reduced compared to the control (negative or positive) groups. In ovo propolis extract application has led to a decrease in hatching time of approximately 2 hours. Chicks hatching early in the hatch window seem to have higher potential for posthatch growth compared to later hatching chicks (Hager and Beane, 1983). Whereas earlier hatching chicks seem less developed than later hatching chicks that were fasted for a shorter period, they are physiologically more developed.

Table 1. The effects of in ovo propolis injection on hatching time.

Parameters	Negative Control	Positive Control	Propolis Low (3%)	Propolis High (6%)	Chi-Sq
Fertility, %	96.67	100.00	96.81	96.84	0.6091
Total embryonic mortality, %	0.00	6.67	5.38	3.16	0.3184
Hatching time, h	483.40^{b}	485.11^{a}	481.62^{c}	482.02^{c}	0.0004
Hatchability, %	100.00	86.67	90.43	94.74	0.1508

$^{a, b, c}$: The differences between the means marked with different letters on the same line are significant (Chi-Sq=0.0004).

The results of the present study, in ovo injection applied groups birds had higher day-old chick weights. In ovo injection of 0.2 ml applied groups chicks had approximately 2 g heavier than the others on the first day. But these differences negatively influenced the 5th day live weight of the negative control group. In ovo propolis extract injected groups have shown better performance in terms of average live weight at first, 5th and 10th days of age (Table 2). It has been shown that the injection-induced weight gain, which is reflected in the hatching weight, could not be maintained in the following periods, therefore it's understood that, unlike propolis, extract isotonic serum sodium (0.9%) could not be metabolized. The changes in volume and composition of both allantoic and amniotic fluids are closely associated with the water metabolism of the avian egg (Romanoff and Hayward, 1943). Some experiments with the use of in ovo feeding technology have shown that the injection of nutrients affects the physiological state of broiler embryos before and after hatching. Proper injection improves not only hatching but also the nutritional status of the chicks and results in a greater predisposition to growth (Kucharska-Gaca et al., 2017). Providing in ovo propolis into the amnion has been shown to improve chick weight and posthatch growth.

Table 2. The effects of in ovo propolis injection on live weight and weight gain (WG) 0-10th day.

Weight, g	**Negative Control**	**Positive Control**	**Propolis Low (3%)**	**Propolis High (6%)**	**P Values**
Day old	40.79^{b}±0.51	42.01^{a}±0.55	42.64^{a}±0,30	42.46^{a}±0.30	0.016
5th day	75.93^{b}±1.68	73.16^{a}±1.83	82.23^{a}±0,98	84.21^{a}±0.94	0.001
10th day	109.76^{b}±3.45	106.31^{b}±3.71	122.66^{a}±1,93	121.56^{a}±1.88	0.001
WG 0-10 d	69.66^{b}±2.97	64.19^{b}±3.71	80.67^{a}±1.67	79.65^{a}±1.62	0.001

$^{a, b}$: The differences between the means marked with different letters on the same line are significant (P<0.05).

At the early phase of the growth is the relative growth rate of the chicks in propolis groups was highest, with an approximately three-fold increase of the live weight at the 10th day old.

Conclusions

Broilers have a short life cycle, and they increase their body weight approximately 50-fold within 40 days in this reason growth during early ages is very important and it can be assessable as an indicator for the next performance as well. The quality of a newly hatched chick is a major factor in determining its health and growth. The results of this study demonstrated that the in ovo injection of propolis extract at both doses had positive effects on hatchability and performance. In ovo feeding by propolis extract seems to be a reliable method to promote health and performance of the newly hatched chicks, but the best dose or combination is unknown in ovo in practice yet. Our results indicated that in ovo propolis injection can be one of the highest economic returns not only the weight, health and vitality of the newly hatched chicks but also growth throughout their lives. Further research is needed to understand how early nutrition affects intestinal health, overall performance traits, specific genes responsible in poultry, and how to adapt the in ovo feeding technique for commercial scale conditions.

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