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Effect of royal jelly *in ovo* injection on embryonic growth, hatchability, and gonadotropin levels of pullet breeder chicks

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ABSTRACT

The objectives of this study were to compare the following: (1) hatchability, (2) chicks' body and internal organs weights, and (3) plasma gonadotropin levels of hatchlings after *in ovo* administration of royal jelly (RJ) on Day 7 of incubation. Fertile eggs (n = 270) were injected into the air sac or yolk sac with 0.5 mL normal saline solution consisting of four formulations (normal saline solution with antibiotics, ultrafiltrate RJ, pure RJ, and RJ with antibiotics). The eggs were randomly divided into nine groups of 30 eggs each: (i) C: the control eggs received no injection, (ii) ASA: air sac–injected eggs received normal saline solution with antibiotics, (iii) ARJ: air sac–injected eggs received pure RJ, (iv) ARJA: air sac–injected eggs received RJ with antibiotics, (v) ARJF: air sac–injected eggs received RJ ultrafiltrate solution, (vi) YSA: yolk sac–injected eggs received normal saline solution with antibiotics, and (vii) YRJ: yolk sac–injected eggs received pure RJ, (viii) YRJA: yolk sac–injected eggs received RJ with antibiotics, and (ix) YRJF: yolk sac–injected eggs received ultrafiltrate RJ solution. Hatchability rate was lower in ARJ (46.7%), ARJA (43.3%), ARJF (43.3%), and YRJF (46.7%) groups than in the control (80.0%; $P < 0.05$). Hatchability rate in ASA (70.0%), YSA (66.7%), YRJ (66.7%), and YRJA (63.3%) groups were comparable to the control ($P > 0.05$). *In ovo* injection of RJ and or RJ with antibiotics in both sacs increased chicks' body weight (CWT), heart weight (HWT), and liver weight (LWT) and FSH and LH levels compared with control ($P < 0.05$). CWT in YRJ (37.02 g), YRJA (37.03 g), ARJ (36.82 g), and ARJA (36.89 g) groups were higher than control (34.9 g; $P < 0.05$). Similarly, HWT significantly increased in YRJ (0.22 g), YRJA (0.21 g), ARJ (0.20 g), and ARJA (0.20 g) in comparison to control (0.18 g; $P < 0.05$). In addition, LWT were higher in YRJ (0.83 g), YRJA (0.82g), ARJ (0.81g), and ARJA (0.81g) than control (0.72 g; $P < 0.05$). Six hours post-hatch, the mean plasma FSH and LH levels in ARJ (1.13 and 2.80 mIu/mL), YRJ (1.32 and 3.36 mIu/mL), ARJA (1.23 and 2.95 mIu/mL), and YRJA (1.31 and 3.28 mIu/mL) groups were higher than in the control (0.56 and 1.48 mIu/mL, $P < 0.05$). We concluded that *in ovo* administration of RJ or RJ with antibiotics might be an effective method to increase CWT, chicks' internal organs weights, and LH and FSH secretion rate without deleterious effect on hatchability. However, further research should be conducted to determine the putative endocrine disruptive effects of RJ and its byproducts.

1. Introduction

The avian embryo develops in an environment with a finite amount of *in ovo* energy and nutrients to support

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embryonic growth and hatching [1]. *In ovo* feeding (IOF) is the administration of exogenous nutrients into avian fertile eggs [2]. Because the embryo orally consumes the amniotic fluid (primarily water and albumen protein) before piping of the air cell, supplementing the amnion with nutrients is fundamentally feeding the embryo an exogenous diet before hatch. Hence, IOF may serve as a tool to overcome early growth constraints during embryonic and post-hatch development in domestic poultry. Supplying embryos with exogenous nutrients *in ovo* may improve hatchability and increase hatched chick weight and post-hatch development [2].

Royal jelly (RJ) is a functional food secreted by the hypopharyngeal and mandibular glands of worker honeybees for the sole nourishment of the Queen bee [3]. It is a mixture of many constituents including proteins (27%–41%), free amino acids (AAs; 0.6%–1.5%), sugars (~30%), lipids (8%–19%), enzymes, antibiotic components, vitamins, mineral salts, sterols, phosphorous compounds, acetylcholine, and hormone-rich substance [4–9]. Major RJ proteins constitute ~90% of total RJ protein [10–12]. The Major RJ proteins can be multifunctional, performing a nutritional role as a component of RJ, although the physiological significance of their roles remains to be determined [3]. The predominant AAs in RJ are proline, lysine, glutamic acid, ß-alanine, phenylalanine, aspartate, serine, cystine, lysine, and arginine [13–15]. RJ carbohydrates comprise mainly fructose, glucose, and sucrose, with some traces of maltose, trehalose, melibiose, ribose, and erlose also being found [15,16]. Eighty to ninety percent of RJ lipids consist of free fatty acids, the rest being neutral lipids, sterols, and hydrocarbons [15,17–20]. Vitamins A, B1, B2, B5, B6, B8, B9, C, D, E, PP, and H, folic acid, pantothenic acid, and mineral salts like K, Ca, Na, Zn, Fe, Cu, and Mn were found in RJ [14–16,21–24]. Testosterone, progesterone, prolactine, estradiol, and insulin-like growth factor-1 (IGF-1) have been found in RJ [25]. In addition, several estrogenic compounds were identified in RJ such as 10-hydroxy-*trans*-2-decenoic acid, 10-hydroxydecanoic acid, *trans*-2-decenoic acid, and 24-methylene cholesterol [8,14,15]. All these compounds inhibited binding of 17β-estradiol to ERβ with less power than diethylstilbestrol or phytoestrogens [8]. 10-Hydroxydecenoic acid and the other fatty acids of RJ have antibacterial properties [26]. Generally, the ether-soluble fraction of RJ, which contains 10-hydroxydecenoic acid as its major component, has strong antibacterial effect [27–31]. Several studies have reported that RJ has antioxidant potency against oxygen free radicals weaker than that of vitamin E [32].

According to the properties of RJ described above, we hypothesize that *in ovo* administration of RJ may increase metabolic activity by increasing nutrient availability in the egg and subsequently may improve the hatchability outcome or possibly the endocrine *milieu* in chicken. This hypothesis was tested by comparing hatchability, body weights, and plasma gonadotropin levels of hatchlings administered RJ *in ovo* on Day 7 of incubation.

2. Materials and methods

Eggs of 45-weeks-old Hy-Line hens were purchased from a commercial hatchery (Morghak Co., Karaj, Iran) and delivered to our laboratory within 24 hours of lay. The eggs (n = 400) were weighed individually and then incubated at 37.8 °C and 60% relative humidity (RH). On Day 7 of incubation, eggs were candled, and 270 fertile eggs were chosen and divided into nine groups of 30 eggs each.

2.1. RJ *in ovo* exposure and treatments

There was no report regarding *in ovo* administration of ultrafiltrate RJ and/or RJ combined with antibiotics. To delete the possibility of microbial contamination during *in ovo* injection, we also used ultrafiltrate RJ beside RJ plus antibiotics in our experiments.

RJ solutions were prepared by dissolving 1.0 g of pure RJ (Ferdos Co., Karaj, Iran) in 1.5 mL of normal saline solution (SA; 0.9%) at low heat (37.0 °C) in a water bath for 15 minutes. To prepare the ultrafiltrate solution of RJ (RJF), 5 mL of thawed RJ was centrifuged at 12,000 rpm at 10.0 °C for 10 minutes. The supernatant was collected and passed through a sterilized filter with micropore diameter size of 0.45 μm.

One gram of streptomycin was dissolved in 5 mL of double-distilled water and the resulting suspension was again mixed with 800,000 IU of penicillin G. The resulting antibiotic solution was mixed vigorously in RJ solution (RJA) in the proportion of 12% to 88%. The final solution contained 12% antibiotics (160,000 IU of penicillin and 20 mg of streptomycin) plus 88% of RJ in normal saline RJ solution. In addition, antibiotic solution was mixed with SA in the proportion of 12% to 88%, which served as a vehicle of antibiotic-RJ solution.

In ovo administration of the above solutions at 0.5 mL per egg was performed at Day 7 of incubation through the blunt end of the eggs. Before injection, the blunt end of the egg was sterilized with 10% povidone-iodine solution and 96% ethanol. A single hole was made with a dental drill bit without penetrating the chorio-allantoic membrane. All solutions were injected into the air sac or yolk sac with a 22-gauge needle. The hole was sealed with 0.5 mL of paraffin and the eggs were incubated in an incubator/hatcher with a temperature of 37.0 °C ± 0.5 °C and RH of 86% to 87%. The eggs (n = 270) were randomly divided into nine groups of 30 eggs each as follows: the control eggs received no injection (C), air sac–injected groups received SA with antibiotic (ASA) or RJ (ARJ) or ultrafiltrate RJ solution (ARJF) or RJ with antibiotics (ARJA), or yolk sac–injected eggs received SA with antibiotics (YSA) or RJ (YRJ) or RJ ultrafiltrate solution (YRJF) or RJ with antibiotic (YRJA). The weight of eggs, percentage hatchability (i.e., number of chicks hatched/number of fertile eggs set), and body weights of hatched chicks as an absolute (CWT) and relative (CWT%) value were recorded. Six hours after hatching, blood sample was taken from newly hatched chicks to obtain plasma FSH and LH concentration. The heart, liver, and ovary were also removed and the absolute and relative weights of the heart (HWT and HWT%, respectively), liver (LWT and LWT%, respectively) and ovary (OWT and OWT%, respectively) were recorded. Hormone concentration was assayed by enzyme-linked immunofluorescent assay (ELIFA) method using kits (BioMerieux Co., France) and was read by VIDAS