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The Effect of *in ovo* Injection of Royal Jelly on the Hatchability, Quality of One Day Broiler Chickens, the Performance and some Immune Related Traits in Hatched Chickens

Research Article

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ABSTRACT

In order to investigate the effect of *in ovo* injection of different levels of royal jelly on the hatchability rate and the quality of one-day-old broiler chickens (Ross 308) and some traits of the immune system, we have conducted an experiment using 640 eggs in a completely randomized design with four levels of royal jelly injection including 1) 1 mL of physiological serum (control), 2) 0.5 mg of royal jelly, 3) 0.75 mg of royal jelly, 4) 1 mg of jelly. For each treatment, four replicates were considered and 40 eggs were used in each replicate. On the 14th day of incubation, a test solution was injected into an allantoic fluid using an insulin syringe with a 2 cm needle length. After 510 hours of incubation, all of the hatched chicks were assessed with good, moderate and poor scores. The parameters studied during the breeding of the hatched eggs included weight gain, feed intake, feed conversion ratio, as well as the immune system traits. The *in ovo* injection of royal jelly did not include any difference in the hatching rate, but it significantly increased the quality of the one-day-old broiler chickens ($P<0.05$). In addition, the weight gain, feed intake and feed conversion ratio developed significantly as compared to the control group. The antibody titers was not affected by the experimental treatments, but it increased as a result of royal jelly injection. Regarding the performance of the birds throughout the breeding season, it can be inferred that the best performance and response of the immune system in hatched chicks was achieved when 0.5 mL of royal jelly was injected.

KEY WORDS broiler chickens, immune system, *in ovo* injection, royal jelly.

INTRODUCTION

The increased population of the world has gradually increased the human's need for protein products, and this has caused many animals to become domesticated, and their industrial production has helped resolve some of the human beings' protein needs. Among different foods, protein products provide the most important part of human needs. Due to the shortage of pastures and the lack of animal protein supplied through cattle and sheep in Iran, it seems necessary to develop the poultry industry in this country. The lack of nutrients in eggs can be one of the most important factors for the reduced hatchability in the final stages of incubation. In addition, the chicks hatched from weak eggs die due to food shortages and the immune system's disability, and if survived, they will be weak at the onset of growth and will need a longer breeding period. Mark's vaccine was the first compound that was injected into the egg using the *in ovo* injection method. Many studies have been conducted on the improvement of the immune system and the compensation of food shortages during the embryonic period of birds. Scientists have so far studied the effect of

injections of amino acids, hormones, antioxidants, vitamins and carbohydrates on broiler chickens. Injection of food into eggs reduces the effect of the inappropriate nutrition of the galemadaron the produced chicks. Therefore, the *in ovo* injection method can be used as an effective way to increase the hatchability percentage, boost the immune system and produce healthier chicks. In general, the applications of this technology include vaccination of birds, prebirth nourishment of chicks, production of modified birds, and medical applications (Bhanja *et al*. 2007). Considering the importance of the quality of one-day-old broiler chickens in the performance of broiler flocks, one of the important issues in the management of the incubation plant is the evaluation of hatching eggs. It is necessary to use quantitative and qualitative methods for detecting the success of the hatch in the incubation plant. The good quality of the chick can be different and have different meanings for different people depending on their perspective. The use of good eggs also usually results in high hatching rates and often high-quality one-day chicks (Tona *et al*. 2003). Royal jelly is secreted from the hypoparinx glands of the worker bees, and all the larvae in the colony feed on it and is like the mother's milk for the bee (Graham, 1992). Royal jelly's total composition is 67% water, 12.5% crude protein, a small amount (5%) of various amino acids and 11% simple sugar (monosaccharides), as well as high amounts (5%) of fatty acids. Most experiments showed that the injection of nutrients into eggs improves the quantitative and qualitative structures affecting hatchability. Therefore, this method can be used as a strategy for producing healthier chicks. To achieve this goal, we need to identify and test the factors increasing the growth, immune system and hatchability (Kocamis *et al*. 1999). The *in ovo* injection method can be used as an effective way to increase the hatchability percentage, boost the immune system and produce healthier chicks. One of the things that can be injected inside the egg is royal jelly. Therefore, the aim of this study is to evaluate the effect of royal jelly injection on the quality of one-day-old broiler chickens and the immune system's function in hatched eggs. Therefore, the aim of this study is to evaluate the effects of royal jelly injection on the quality of one-day-old broiler chickens and the immune system's function in hatched eggs. The aim of this study is to evaluate the hatchability rate, the quality of one-day-old broiler chickens and performance of broiler chickens (weight gain, feed intake, and feed conversion ratio) hatched from eggs subjected to different levels of royal jelly.

MATERIALS AND METHODS

Location and time of the experiment

Eggs were prepared from parent flocks (at 44 weeks of age) and transferred in a standard condition (the temperature of 20 ˚C and humidity of 70%) to the place where the design was to be done (Golpayegan city) by a special machine used for leghorn egg transfer. The parent flock had a good status in terms of health and the flock was negative in *Mycoplasma synoviae* (MS), *Mycoplasma gallinarum* (MG), *Salmonella pullorum* (SP), and *Salmonella gallinarum* (SG).

Preparing the incubator

The incubator was pre-disinfected by detergent and formalin and all the egg-laying rocks were checked so that no problems would be encountered during the rotation of the eggs during the incubation period and the appropriate conditions (temperature and humidity) were observed before preparing the eggs for the hatch. The ventilation fans were also checked so that no problem would occur in terms of ventilation and oxygenating the fetus. The eggs remained in the setter for 18 days. The temperature of the setter was adjusted to an appropriate level before the eggs was set. In addition, the eggs were put into the setter machine at a certain time and all of the sensors and electrical and mercury markers were controlled carefully. The temperature of the setter machine was 37.5 ˚C and the temperature of the hatcher was 37 ˚C. The relative humidity was between 65 and 70%, and it was between 70 and 75% in the hatcher during the incubation period. We used a special commercial solution for washing the machine, which is one of the main principles of health to be observed in incubation. Prior to disinfection, the surface contaminations should be cleaned as much as possible, as otherwise, the disinfectant materials used could have no effect. The inside parts of the incubators were completely cleaned and disinfected using formalin and antiseptic commercial disinfection solution. The inner surface of the incubators was smooth and it was cleaned with water and brushes. The outer surface of the incubator was also cleaned carefully. The top of the incubator was also cleaned every time and the setter trays, chick plastic boxes, grading tables, and special candling devices were cleaned with water and brushes. The incubator's capacity was 1000 eggs, and the machine controlled the proper conditions (humidity, temperature and ventilation) automatically.

Experimental treatments

The experimental treatments included: 1) 1 mL of physiological serum (control); 2) 0.5 mg of royal jelly; 3) 0.75 mg of royal jelly and 4) 1 mg of royal jelly. The eggs were weighed and numbered and randomly placed in experimental treatments. There was no significant difference between the weights of the eggs placed in the experimental treatments. A total of 640 Ross 308 leghorn eggs were used for the test. The eggs were individually weighed and divided

into 4 experimental groups with normal dispersion (each group consisting of 4 replicates and each replicate containing 40 eggs). To prepare the desired doses of royal jelly, we used the injectable physiologic serum as a solvent. A certain amount of royal jelly was mixed in a certain volume of physiological serum in order to obtain a homogeneous solution of a certain dosage of royal jelly to be injected into experimental treatments. This experiment was conducted in a completely randomized design with four treatments and four replicates for each treatment. Experimental treatments consisted of eggs injected with physiological serum with levels of 0.5, 0.75 and 1 mg. Royal jelly is watersoluble. To evaluate the effect of the injected physiological serum, the eggs of one treatment were injected with one milliliter of physiological serum solution for each egg. The eggs were incubated for 21 days (from day zero to day 18 in the setter and from day 18 to day 21 in the hatcher) and injected in the fourteenth incubation. On the 14th day, the injection site (Hepatitis Allantoic Bag) was determined using the candling method, and it was disinfected with alcohol, and then 1 ml of experimental solution was injected into an allantoic fluid using an insulin syringe with a 2 cm long needle syringe. After injection, the perforated area was closed by glue. On the 14th day of incubation, the egg carbohydrates reserves came to an end and the fetus was more likely to have energy shortages and lower energy levels than ever before, as the factor that limits the fetal development is primarily energy and energy shortage causes chick embryo death more than does any other factor. Containing 11% simple carbohydrates, royal jelly can compensate for this shortage. To measure the hatchability percentage, we used the ratio of hatched (healthy and non-healthy) chicks to the whole eggs from a bird. After 510 hours of incubation, all of the hatched chicks were measured using good, medium, and poor scores. Then the poultry equipment was first moved out of the hall. The salon was subsequently completely cleaned from the remains of the dust remaining in the previous period, and the floor and walls of the hall were washed with water pressure and detergents. After the hall was dried, the disinfection performed using a glutar solution at a dilution of 1:100 (as recommended by the manufacturing company). In the next step, a 24-pen wire with dimensions of 120 × 100 cm was made and was covered with cardboard rolls. The devices needed for breeding (including feeder, drinker, etc.) were transferred to the hall after washed and disinfected. All of the pores were then closed and the hall was fumigated with formalin and permanganate. After 24 hours, the air penetration ways were opened and the ventilators were turned on in order to allow gas to escape from the hall. Before the chicks were brought to the hall, the temperature was adjusted to the optimum temperature (32 ˚C).

When chicks arrived, water containing 5% sugar and multivitamins was used and the initial food was given to them immediately after they were divided. A tray-like feeder and a conical feeder were used during the first week, and a pendant barrel feeder was used from the second week until the end of the breeding season. The height of the drinker was also increased based on the age of the chicks. The drinkers were washed on a daily basis using water and detergents.

The hall's temperature was reduced by 3 degrees each week until it reached 24 ˚C in the fourth week. In this experiment, two diets were used for the initial period (0-14 days) and the growth period (14-28 days). The experimental diets were regulated based on the nutritional requirements recommended by the National Research Council (NRC, 1994). Diets composition is summarized in Table 1.

Measuring the performance parameters of hatched chicks

Feed intake

The feed for each experimental unit was put in special bags on which the pen number and the type of treatment had been recorded. The feed intake was measured on a weekly basis and reported periodically. The chicks of each experimental unit were collectively weighted at the beginning and the end of the period and their number was recorded. The weight changes in grams during the period were calculated using the following formula.

Weight gain

The daily weight gains of the chicks of the experimental unit (g)= [total weight of the chicks of the experimental unit at the beginning of the period (g) - weight of the losses (g)] + total weight of chicks per test unit at the end of the period (g) / [number of chicks in the pen in that week × average daily weight gains per week (g)] .

Feed conversion ratio

The feed conversion ratio was calculated based on the weight gain and feed intake in each experimental unit during each period.

Food conversion ratio= feed intake of each test unit / weight gains of that experimental unit

Immune system traits

Injection of the sheep red blood cells (SRBC) solution to chicks

In the second and third weeks of the breeding period, a 0.2 mL of 5% SRBC solution was injected into the breast muscle of two chicks from each experimental unit by insulin syringe.

Table 1 The diet composition in terms of the percentage of dry matter[1]

Ingredients (%)	0-21 days	21-42 days
Corn	53.70	60.16
Soybean meal	38.63	32.41
Soy oil	3.77	4.02
Calcium carbonate	1.29	1.38
Di-calcium phosphate	1.47	1.09
Salt	0.44	0.33
Vitamin supplement[2]	0.25	0.25
Mineral supplement[3]	0.25	0.25
DL-methionine	0.16	0.07
Coccidiostate	0.02	0.02
Antioxidant	0.02	0.02
Calculated analysis		
Metabolizable energy (kcal/kg)	3000	31.0
Protein (%)	21.56	19.37

[1] The experimental diet contains at least the nutrients recommended by the NRC (1994).
[2] Each kilogram of vitamin supplement supplies the following: vitamin A: 3500000 IU; vitamin D_3: 1000000 IU; vitamin E: 9000 IU; vitamin B_1: 900 mg; vitamin B_2: 3300 mg; vitamin B_3: 5000 mg; vitamin B_5: 15000 mg; vitamins B_6: 150 mg; vitamin B_9: 500 mg; vitamin B_{12}: 7.5 mg; Choline: 2500000 mg and Biotin: 500 mg.
[3] Each kilogram of mineral supplements supplies the following: Mn: 50000 mg; Fe: 25000 mg; Zn: 50000 mg; Cu: 5000 mg; I: 500 mg and Se: 100 mg.

Seven days after each injection (days 21 and 28 of the breeding period), blood samples were taken from the vein under the chicks' wings and, after the serums were separated, the samples were transferred to a freezer of -20 ˚C and kept at this temperature until the day the antibody titers were measured. It should be noted that the first and second stages injections were performed on two distinct chicks from each pen for obtaining the secondary antibody titer response.

Blood cell count

Around 0.1 mL of blood was used for spreading. After the lam was dried in the vicinity of the air, it was fixed with 99.5% methanol, and then, the diluted GIMSA color was added to the dried extent of blood in a ratio of 3 to 1, with urban water, to the extent that the lam is lymphocyte, and it was performed for 50 minutes. Then, using the running or distilled water, we slowly washed the color on the lam until the lam dried in air. The heterophilic to lymphocytes ratio was determined in a colored area using immersion oil and 100x objective lens, in search of a spiral shaped lam, and the white blood cell type in each segment was detected. We continued this till counting 100 RBCs when the heterophile to lymphocyte ratio was determined.

Statistical analysis

The data obtained from this research were analyzed in a completely randomized design with 4 treatments and 4 replicates. The mean of treatments was compared using the Duncan test and a significant level of 5%. The statistical software of SAS (2005) is used to analyze the data and Excel software is used to plot the graphs. Considering that this research is implemented in a completely randomized design, the design model is as follows:

$$Y_{ij} = \mu + a_i + e_{ij}$$

Where:
Y_{ij}: observation of each value
μ: mean of the measured trait in the population under study.
a_i: treatment effect.
e_{ij}: effect of experimental error.

RESULTS AND DISCUSSION

Hatchability and quality of one-day-old broiler chickens

Table 2 shows the effect of injection of different levels of royal jelly on the hatchability percentage and the quality of the resulting chicks. As shown in the table, the injection had no significant effect on the hatchability percentage ($P<0.05$).

However, the least amount of hatching was observed in the treatment with 0.5 mL of royal jelly and the highest percentage of hatching was observed in the treatment with 0.75 mL of royal jelly.

Although there was no significant difference from the control group, its numerical value was 0.5 mL greater than the treatment group. Table 2 shows the scores relating to the quality of the hatched chicks under the influence of the *in ovo* injection of royal jelly. The average score of chicks received 0.5 mL of royal jelly had the highest score, while the control group showed the lowest score. There was a significant difference between the control group and other examined groups.

The results showed that injection of 0.5 mL of royal jelly resulted in an increase in the quality of one-day chicken at the highest level of the results of this experiment, but the injection of 0.75 and 1 mL of royal jelly produced no significant difference.

Table 2 The effects of *in ovo* royal jelly injection on hatchability and chicks quality

Trait/treatment	Control	0.5	0.75	1
Hatchability (%)	0.77	0.75	0.78	0.77
Chick's quality (out of 100 scores)	82.61[b]	92[a]	90.16[ab]	88.6[ab]

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Weight gain

The results of the average weight gain of the broiler chickens in a breeding season for 4 weeks have been reported in Table 3. During the third and fourth weeks of the whole period, the overweight of the chicks hatched from the eggs injected with royal jelly was significantly increased as compared to the control group (P<0.01). The highest amount of overweight was related to treatment with one ml of royal jelly and the lowest amount of overweight was related to the control group throughout the whole period. The use of *in ovo* royal jelly did not have any significant impact on increasing the broiler chickens' weight during the first and second weeks. These results indicate that the royal jelly injections led to a significant increase in the broiler chickens' weight. The present experiment showed that an increase in the amount of royal jelly from 0.5 to 0.75 mL led to a decrease in the overweight and the injection of 1 mL of royal jelly resulted in an increase in the chicks' overweight during all weeks and the entire breeding period.

Feed intake

Table 4 shows the amount of feed consumed by broiler chickens during different weeks and periods of breeding. In the second and third weeks of breeding, an increase in the *in ovo* injection of royal jelly led to a decrease in the chicks' feed intake (except at the level of one milliliter royal jelly injection), so that this decrease in feed intake was statistically significant (P<0.01). An increase in the amount of royal jelly injected into eggs during the fourth week of breeding led to an increase in the chicks' feed intake (P>0.05). The control group was the in between these two groups and had no statistically significant difference with any one of these groups. During the whole period of feed intake, 0.75 mL for injection of royal jelly was the lowest and in the 1 mL group the royal jelly injection was highest, so this difference was not statistically significant (P<0.05). The control group was intermediate between the two groups and did not differ significantly between the two groups.

Feed conversion ratio

The results of comparing the means of feed conversion ratio during different weeks and periods of breeding are reported in Table 5. The results of this experiment indicate that the feed conversion ratio has been affected by experimental treatments during all weeks and periods of breeding (except for the first week).

Although feed intake declined during the first week, overweight also decreased proportionally, and therefore, the feed conversion ratio was not affected. Moreover, since there was no significant difference in the chicks' feed intake and overweight in the first week, the feed conversion ratio did not change either. The feed conversion ratio was significantly better in the chicks of royal jelly groups than those of the control group (P<0.05).

Immune system traits

The results of the cell count and antibody titers against SRBC during the breeding period are presented in Table 6. The results show that the antibody titers were not affected by royal jelly injection during the third and fourth weeks of production (P>0.05). The production of antibody titers in the third and fourth weeks was considerably higher in the experimental treatments than in the control group, although the difference was not statistically significant. The lowest amount of antibiotic titers was observed in the control group and the highest amount of it was observed in the treatments of 0.5 mL royal jelly injection. The production of antibody titers in the third and fourth weeks decreased insignificantly as a result of an increase in royal jelly levels. The production of blood cells was influenced by the different levels of royal jelly so that lymphocyte decreased significantly in the royal jelly treatments, so that the highest production of lymphocytes was observed in the control group. The production of heterophile increased significantly in the treatments containing royal jelly as compared to the control group. As a whole, the results showed that the higher levels of Royal Jelly had a greater effect on the heterophils/lymphocytes ratio than its lower levels.

Hatchability and quality of one-day-old broiler chickens

According to Table 2 which compares the average of the traits related to the hatchability and quality of one-day-old broiler chickens, the results of this research are consistent with the results reported by Mousavi *et al*. (2011). These researchers showed that injection of amino acid, carbohydrates and butyric acid did not affect the hatchability percentage.

In another study, Zhai *et al*. (2008) investigated the effects of *in ovo* injection (through injection into amniotic fluid) of L-carnitine on the 17th and 18th days of incubation on hatchability. The 0.25, 0.5, 1 and 2 levels of L-carnitine solution in salt solution were used in this research.

Table 3 The effects of *in ovo* royal jelly injection into amniotic fluid on live weight gain of broiler chicks

Trait/treatment	0-7 days	7-14 days	14-21 days	21-28 days	0-28
Control	109.97	154.83	251.86^{b}	351.67^{b}	868.30^{b}
0.5	108.6	168.16	289.83^{a}	414.03^{ab}	980.63^{a}
0.75	106.42	163.13	285.81^{ab}	391.58^{ab}	946.9^{a}
1	112.23	160.67	289.33^{a}	443.39^{a}	1005.57^{a}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 The effects of *in ovo* royal jelly injection into amniotic fluid on feed intake of broiler chicks

Trait/treatment	0-7 days	7-14 days	14-21 days	21-28 days	0-28
Control	159.67	234.67^{a}	526.86^{a}	760.67	1681.30
0.5	159.83	212.16^{c}	516.03^{ab}	802.00	1689.63
0.75	155.5	220.75^{b}	491.81^{b}	783.05	1651.91
1	159.67	215.83^{bc}	536.01^{a}	807.67	1719.7

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 The effects of *in ovo* royal jelly injection into amniotic fluid on conversion ratio of the hatched chicks

Trait/treatment	0-7 days	7-14 days	14-21 days	21-28 days	0-28
Control	1.44	1.5^{a}	2.1^{a}	2.17^{a}	1.81^{a}
0.5	1.47	1.26^{b}	1.78^{b}	1.94^{ab}	1.61^{b}
0.75	1.46	1.36^{b}	1.72^{b}	2.0^{ab}	1.63^{b}
1	1.42	1.34^{b}	1.85^{b}	1.84^{b}	1.61^{b}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 6 Comparisons of the average traits related to the immune system of the hatched chicks

Trait/treatment	Heterophile to lymphocyte ratio	An antibody titer against the sheep red blood cell at 21 days	An antibody titer against the sheep red blood cell at 28 days
Control	0.32^{b}	2	2.25
0.5	0.48^{a}	3	3.25
0.75	0.46^{a}	2.75	3.25
1	0.56^{a}	2.75	3

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

The results showed that the *in ovo* injection of L-carnitine had no effect on the hatchability percentage, which is consistent with the results of the present study. Ebrahimi *et al*. (2012) investigated the effect of injection of sodium bicarbonate, vitamin E, L-carnitine and vitamin C on the hatching rate, showing that the hatchability percentage decreased by 3-5% as compared to the control group, which is inconsistent with the results of this research. Bhanja *et al*. (2007) conducted a trial, in which they injected 100 units of vitamin A, 0.5 unit of vitamin E, 50 mg of vitamin C, 100 micrograms of vitamin B_1 and 100 micrograms of vitamin B_6 in 0.5 mg of sterilized water into eggs on the fourteenth day of the incubation period. This experiment showed that the hatchability percentage was higher in the vitamin B_6 treatment than in the control group. Moreover, the quality of the one-day chickens was higher in vitamin A and C treatments than in the control group. The main reason for the difference in these tests can be due to the type of substance injected as well as the injection day. Many of the studies having been conducted on *in ovo* injection show that the injection of amino acids into the air chambers of fertilized eggs during the first week of incubation increases the amount of amino acid in the fetus, albumin, allantoic and amniotic fluids, and embryo weight on the 19th day of incubation. In addition, the injection of 0.5 mL of amino acid on the 7th day of incubation increased the chick-egg weight ratio (Ohta *et al*. 2001). Since royal jelly is a mixture of amino acids and carbohydrates, it can have a significant effect on hatchability.

Weight gain

The *in ovo* injection of royal jelly during the first and second weeks of breeding did not have a significant effect on the overweight of the broiler chickens. These results indicate that the royal jelly injection leads to a significant increase in the overweight of the broiler chickens. The reason for this is probably the supply of the birds' nutrient requirements during their embryonic period and early life stages by the royal jelly. Furthermore, the improved digestibility and the increased feed intake in the higher quality chicks will naturally result in more overweight so that the optimum quality of hatched chicks and favorable conditions for their growth place them in their natural growth path and increase their rate of growth.

The present experiment showed that an increase in the royal jelly level from 0.5 to 0.75 mL will reduce the overweight and the injection of 1 mL of royal jelly will increase the overweight rate over all weeks and the entire breeding period. The reason for this inconsistency can be related to stressors and the combination of the two male and female gender so that the male has a greater weight mean than the female gender. Mousavi *et al*. (2011) reported that one- day old chicks had a greater body weight in the experimental groups than in the control group ($P<0.01$). This finding is inconsistent with the findings of the present study and there is no significant difference between the control group and the treatments of other levels of royal jelly, but the treatment of one ml of royal jelly was numerically higher than the other treatments ($P>0.05$), so that the weight gain has increased with an increase in the level of royal jelly injected to the chicks (regardless of the control group). In the same article, Mousavi *et al*. (2011) stated that the separate injection of amino acid had the greatest effect on the weight of one-day- chicks. They showed that the weight of chicks produced from amino acid treatment was 4.5% greater than that of the control group. In addition, the injection of carbohydrates and butyric acid increased the weight of the hatched chicks by 3%. Their major carbohydrates include maltose, sucrose and dextrin. In another study, Graham (1992) showed that royal jelly has an average of 12% protein and is poor in terms of amino acids. On the other hand, the carbohydrates of this nutrient are mainly monosaccharides.

Therefore, the difference between the results of these studies and those of the present study can be due to the difference between the injected substances. Uni *et al*. (2005) injected a mixture of lactose, maltose and dextrin into leghorn eggs, reporting that the use of carbohydrates increases the weight of hatched chicks. In the experiment, the response of the Cobb to injecting nutrients into the eggs was greater than that of Ross, so that the weight gain of the hatched chicks was 5.6% in the Cobb and 3.7 in the Ross. It seems that there is a difference between different races in terms of response to fetal feeding. This finding is inconsistent with the results obtained in this study.

In a study conducted by Jafari *et al*. (2012), the experimental treatments included eggs without injections of any additives (group I), eggs injected with physiological serum (group II), eggs injected with bicarbonate buffer (group III) and eggs injected with royal jelly (group IV). The results of this study showed that body weight at the 28^{th} day of the incubation period in the chicks injected with royal jelly and sodium bicarbonate was greater than the body weight of the other two groups, which is in line with the results of the present study.

Feed intake

The results of the feed intake in this study are consistent with the results of the study conducted by Jafari *et al*. (2012). These researchers investigated broiler chickens produced from eggs without injections of any additives (group I), eggs injected with physiological serum (group II), eggs injected with bicarbonate buffer (group III) and eggs injected with royal jelly (group IV) from 1 to 21 days and 1 to 28 days. In the experiment, the highest feed intake was observed in the fourth group.

Feed conversion ratio

The feed conversion ratio is dependent on feed intake and overweight. Although feed intake declined during the first week, overweight also decreased proportionally, and therefore, the feed conversion ratio was not affected. In addition, since there was no significant difference in the feed intake and overweight of the broiler chickens in the first week, the feed conversion ratio did not change either. In contrast to the results of this study, Keralapurath *et al*. (2010) injected the levels of 0.5, 2 and 8 mg L-carnitine into 100 mL of commercial diluent on the 18^{th} day of incubation into the Amnion fluid inside eggs. The traits of the hatched eggs (body weight, feed intake, feed conversion ratio and mortality percentage) were not affected by the treatment injections until 46 days.

Jafari *et al*. (2012) conducted a study on the performance traits (including body weight, feed intake, and conversion ratio) and immune system traits against Newcastle disease in chicks resulting from eggs injected with royal jelly and bicarbonate buffer sodium, and showed that the conversion ratio was lower in the sodium bicarbonate treatment than in other treatments without injections of any additives (group I), eggs injected with physiological serum (group II), eggs injected with bicarbonate buffer (group III) and eggs injected with royal jelly (group IV)

Immune system traits

Since the *in ovo* injection method can be used as an effective way to improve the immune system and produce healthierchicks, many experiments have been conducted in this regard. Among the nutrients that can be used in these tests are carbohydrates, amino acids, L-carnitine, and sodium bicarbonate. The injection of such nutrients into eggs is considered prenatal nutrition, which can considerably prevent the birth of impaired chicks and increase their weight and improve their immune system (Gore and Qureshi 1997; Zhai *et al*. 2008). Jafari *et al*. (2012) investigated the immune system-related traits against newcastle disease in chickshatched from eggs injected with royal jelly and sodium bicarbonate buffer.

In this study, the antibody titer against Newcastle disease was higher in royal jelly on the 28th day than in other treatments, which is consistent with the results of this study. According to the results of this study, Gore and Qureshi (1997) reported the increased performance of white blood cells and hemorrhagic immunity in broiler chickens during the breeding period of receiving 10 units of vitamin through *in ovo* injection on the 17th day of incubation. In this study, high titers of G^3type immunoglobulin antibody were observed in response to second glomerular sheep infestation, but there was no increase in stock or spleen weight. Kreukniet *et al*. (1994) reported that the immune system manages the amount and location of pathogens in the body. Activation of the immune system occurs during growth. While the immune system is important for animal resistance to diseases, the decline in animal performance depends on the activation of the immune system. Through the balance of immune responses to optimal functional outcomes, animal production and reproduction will improve to some extent.

The first significant point in animals' immune system function is sufficient energy and protein. Energy is essential for the rapid growth of immune cells. Proteins are used in cell forming, as well as the synthesis of antibodies and other substances involved in the immune system (Singh *et al*. 2005).

Nutrition of protein and amino acids is strongly associated with immunophysiology. Understanding this association will provide a better understanding of the nutrition of protein and amino acids, which can be used to help the nutritional support of the immune system. Amino acids regulate immunity as a substrate for the development, maintenance and use of the immune system. As a result, providing amino acids in proper amounts, times and proportions is important for immunity (Brooke and Humphrey, 2010). Kidd *et al*. (2005) reported that the shortage of lysine in the poultry diet limits the synthesis of proteins such as cytokines and the frequency of lymphocytes and reduces the immune response. Feed restriction in the case of birds can have different effects on their immune system functions.

These effects vary with the number of nutrients in the diet. When chicks are fed with a diet deficient in calories and amino acids, the response of the immune system of these chicks will differ from the control chicks which have received enough diet. The increased use of amino acids, which causes deficiency in the calorie intake and the completeness of dietary amino acids, reduces the responsiveness of antibodies (Cook, 1991). Differences in the energy concentration of poultry diets will change the responses of the immune system, which is likely to affect the immune system through changes in nutritional intake.

Marsteller *et al*. (1980) reported that energy regulates the activity of immune cells, the activities of certain hormones like thyroxin, corticosteroids, the growth hormone, glucagon, and catecholamines, which will in turn influence the immune system. Moreover, differences in the levels, structure and type of dietary fatty acids affect the immune system's function by changing the structure of the cell membrane and changing the synthesis of prostaglandin (Pourreza, 1991). Calder (2001) reported that non-saturated fatty acids with multiple binds can modify the activity of the immune system when they are placed in the phospholipid membrane of lymphoid tissue cells.

CONCLUSION

If appropriate levels of royal jelly are used as *in ovo* injection, the quality of one-day broiler chickens, digestibility and feed intake efficiency in hatched chicks will improve; therefore, doing this can be beneficial if it is economical. The best performance was observed in 0.5 mL royal jelly treatment, which is due to the suitability of the food conversion factor in this experimental group as compared to other groups, as the chicks in this group had the highest weight with less food intake. As shown in this study, the increased levels of royal jelly injection improved the performance of the hatched chicks. A comparison of the yield achieved in this treatment and in other treatments shows that the increase in royal jelly levels makes no significant difference in the observed performance of the other treatments. In addition, the *in ovo* injection of royal jelly improves the humoral immune responses and the immune system in hatched eggs.

REFERENCES

Bhanja S.K., Mandal A.B., Agarwal S.K., Majumdar S. and Bhattacharyya A. (2007). Effect of *in ovo* injection of vitamins on the chick weight and post-hatch growth performancein broiler chickens. Pp. 143-146 in Proc.16th European Symp. Poult. Nutr., Strasbourg, France.

Brooke D. and Humphrey A. (2010). The role of protein and amino acids in immunity. *Crit. Rev. Poult. Biol*. **16,** 107-111.

Calder P.C. (2001). Polyunsaturated fatty acids, inflammation and immunity. *Lipids*. **3(9),** 1007-1024.

Cook M.E. (1991). Nutrition and the immune response of the domestic fowl. *Crit. Rev. Poult. Biol*. **3,** 167-189.

Ebrahimi M.R., Jafari Ahangari Y., Zamiri M.J., Akhlaghi A. and Atashi H. (2012). Doespre-incubational *in ovo* injection of buffers or antioxidants improve the quality and hatchability of long-term stored eggs? *Poult. Sci*. **91(11),** 2970-2976.

Gore A.B. and Qureshi M.A. (1997). Enhancement of humoral andcellular immunity by vitamin E after embryonic exposure. *Poult. Sci*. **76,** 984-991.

Graham M. (1992). The Hive and the Honey Bee. Dadant and Sons Inc., Hamilton, USA.

Jafari Ahangari Y., Hashemi S.R., Akhlaghi A., Atashi H., Esmaili Z., Ghorbani M., Mehmandoyi A., Mastani R., Azadegan A. and Davoodi H. (2012). Effect of *in ovo* injection of royal jelly on post-hatch growth performance and immune response in broiler chickens challenged with Newcastle disease virus. *Iranian J. Appl. Anim. Sci*. **3(1),** 201-206.

Keralapurath M.M., Corzo A., Pulikanti R., Zhai W. and Peebles E.D. (2010). Effects of *in ovo* injection of L-carnitine on hatchability and subsequent broiler performance and slaughter yield. *Poult. Sci*. **89,** 497-1501.

Kidd M.T., McDaniel C.D., Peebles E.D., Barber S.J., Corzo A., Branton S.L. and Woodworth J.C. (2005). Breeder hen dietary L-carnitine affects progeny carcase traits. *Br. Poult. Sci*. **46,** 91-103.

Kocamis H., Yeni Y.N., Kirkpatrick-keller D.C. and Killefer J. (1999). Postnatal growth of broilers in response to *in ovo* administration of chicken growth hormone. *Poult. Sci*. **78,** 1219-1226.

Kreukniet M.B., Nieuwland M.G.B. and Vander-Zijpp A.J. (1994). Phagocytic activity of two lines of chicks divergently selected for antibody production. *Vet. Immunol. Immunophathol*. **44,** 377-387.

Marsteller F.A., Gross W.B. and Siegel P.B. (1980). Antibody production and *Escherichia coli* resistance in socially stable flock of dwarf and non-dwarfchicks. *Poult. Sci*. **59,** 1974-1948.

Mousavi N., Shivazad M., Chamani M., Lotfollahian H. and Sadeghi A.A. (2011). The effects of injection of amino acid, carbohydrate and butyric acid in incubation eggs on the intestinal morphology and performance of broiler chickens. *Iranian J. Anim. Sci*. **42(2),** 153-160.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Ohta Y., Tsushima N., Koide K., Kidd M.T. and Ishibashi T. (1999). Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poult. Sci*. **78,** 1493-1498.

Pourreza J. (1991). Scientific and Practical Principles of Poultry Production. Arkan Danesh, Isfahan, Iran.

SAS Institute. (2005). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Singh U., Devaraj S. and Jialal I. (2005). Vitamin E, oxidative stress, and inflammation. *Annu. Rev. Nutr*. **25,** 151-174.

Tona K., Bamelis F., Ketelaere B., Bruggeman V., Moraes V.M.B., Buyse J., Onagbesan O. and Decuypere E. (2003). Effects of egg storage time on spread of hatch, chick quality and chick juvenilegrowth. *Poult. Sci*. **82,** 736-741.

Zhai W., Neuman S.L., Latour M.A. and Hester P.Y. (2008). The effect of male and female supplementation of L-carnitine on reproductive traits of white leghorns. *Poult. Sci*. **87,** 1171-1181.