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The effect of *in-ovo* injection of *Lactobacilla Rhamnosus* on hatching traits and growth parameters of quails

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ABSTRACT

The aim of this study was to investigate the effect of *in-ovo* injection of *Lactobacilla Rhamnosus* (0, $16x10^3$, $32x10^3$ and $48x10^3$ cfu) as probiotic source on hatching parameters and growth performance of quails. In this, a total of 400 quail hatching eggs, were randomly distributed among 4 experimental groups. Each experimental group contained 4 replicates of 25 eggs each. *In-ovo* injections of *Lactobacilla Rhamnosus* into quail eggs did not statistically affect incubation characteristics and some tissue weights. A high amount of *Lactobacilla Rhamnosus* injection into the breeder quail eggs negatively affected live weight and live weight gain. As a result, it can be said that low or medium levels *Lactobacilla Rhamnosus* injection into quail eggs have no effect on incubation and growth parameters of quails.

1. Introduction

In-ovo injection is a method to administer substances into the egg during incubation with the objective of promoting positive effects on hatchability and post-hatch growth performance (Uni and Ferket 2004). The *in-ovo* method was first used by Sharma and Burmester (1982) for the vaccination of turkey eggs against Marek's disease.

Researchers have recently used materials such as probiotic (Maiorano et al. 2012; Majidi-Mosleh et al. 2017; Triplett et al. 2018), proteins (Ohta et al. 1999; Bhanja et al. 2014; Kermanshahi et al. 2015), carbohydrates (Ipek et al. 2004; Tako et al. 2004; Zhai et al. 2011; Salmanzadeh et al. 2012), vitamins and minerals (Bello et al. 2013; Salary et al. 2014; Oliveira et al. 2015), ascorbic acid (Elibol et al. 2001; Ipek et al. 2004; Sgavioli et al. 2015), hormones (Kocamis et al. 1999), bee products (Coşkun et al. 2014; Moghaddam et al. 2014; Aygun 2016; Bozbay et al. 2016) *in-ovo* administration.

Lactobacillus rhamnosus is a member of the family Lactobacilliaceae and has received this name because it largely ferments carbohydrates to lactic acid. The digestive system of humans and animals is the natural habitat of these bacteria as well as finding a wide spreading area in the land (Mao et al. 2016). Immunity of interested hosts with Lactobacillus is increasing day by day due to its high antimicrobial effect and its ability to withstand high acidity (Naqid et al. 2015; Sugiharto et al. 2015; Xie et al. 2015). *Lactobacillus rhamnosus* have a high resistance to acidity and bile salts (Goldin et al. 1992; Alander et al. 1999), antimicrobial effect (Toki et al. 2009), has the properties of reducing negative effects against aflatoxin B1. (Gratz et al. 2007). Chen et al. (2017) stated that the addition of *Lactobacillus rhamnosus* in the broiler rations increased the growth performance and the meat quality, improvement of digestive system microflora and consequently, *Lactobacillus rhamnosus* may be a good probiotic for poultry. It has been reported that different probiotics *in-ovo* injection has a positive effect on digestive secretions and live body weight (Maiorano et al. 2012; Pruszynska-Oszmalek et al. 2015), but the feed conversion was not affected Pruszynska-Oszmalek et al. (2015) or adversely affected (Maiorano et al. 2012).

The aim of this study is to determine the effect of *in-ovo Lactobacillus rhamnosus* quail fertile eggs injection on incubation features and growth parameters.

2. Materials and Methods

This study was carried out at the Agricultural Faculty of Selcuk University, Konya, Turkey. A total of 400 quail hatching eggs were obtained from a

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commercial farm (Konya, Turkey). As probiotic source *Lactobacillus rhamnosus* GG (Mansel pharmaceutical industry and trade inc.) microorganism were used. The physiological serum containing 50 ml of 0.9% NaCl was diluted to $16x10^3$, $32x10^3$ and $48x10^3$ cfu per egg of the probiotic source and was stirred at 500 rpm for 3 hours. A total of 400 quail hatching eggs, were randomly distributed among 4 experimental groups. Each experimental group contained 4 replicates of 25 eggs each.

The experimental groups consisted of a control group (without *in-ovo* injection) and 3 trial groups injected with $16x10^3$, $32x10^3$ and $48x10^3$ cfu of *Lactobacillus rhamnosus* GG per egg.

The incubator was set to 37.5 °C and 55-60% humidity for the first 14 days of the incubation and 37.2 °C temperature and 75% humidity for the hatching machine. The eggs were turned 12 times at 90° per day until 14 days of incubation.

Table 1
Composition of basal diet

Ingredients	%	Chemical composition[2]	
Maize	55.90	Metabolic energy, kcal/kg	2899
Soybean meal	39.50	Crude protein, %	23.99
Vegetable oil	1.80	Calcium, %	1.150
Limestone	1.25	Total phosphorus, %	0.517
DCP	0.82	Available phosphorus, %	0.299
Salt	0.30	Lysine, %	1.306
Premix[1]	0.25	Methionine, %	0.439
DL Methionine	0.18	Methionine + cystine, %	0.940

[1]Vitamin mineral premix ration in 1 kg, vitamin A, 8.800 IU; vitamin D3, 2.200 IU; vitamin E, 11 mg; nicotinic acid, 44 mg; Cal-D-Pan, 8.8 mg; riboflavin 4.4 mg; 2.5 mg of thiamine; vitamin B12, 6.6 mg, folic acid, 1 mg; D-Biotin, 0.11 mg; choline, 220 mg; manganese, 80 mg; copper, 5 mg; iron, 60 mg; zinc, 60 mg; cobalt, 0.20 mg; iodine, 1 mg; selenium, 0.15 mg.

[2] Analyzed value as feed

Table 2
Effect of incubation parameters of in ovo probiotic injection into quail hatching eggs (%)

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	86.54	75.96	87.52	4.56	3.42	4.50
$16x10^3$ cfu	94.23	83.65	88.74	2.09	6.17	3.00
$32x10^3$ cfu	88.22	78.29	88.74	2.27	4.50	4.50
$48x10^3$ cfu	85.58	76.92	89.44	3.57	1.19	5.80
SEM	2.48	4.50	3.40	1.69	1.40	1.91
P-value	0.117	0.694	0.988	0.726	0.244	0.816

On the 14th day of incubation, after the egg shell was disinfected with 70% ethanol, a hole was opened for injection with a micromotor (Strong 210, Korea). The prepared solutions were injected into amnion with a 26-gauge plastic disposable syringe (0.20 mL). After the injection, the hole was sealed with adhesive and transferred to the hatch basket. Eggs in the control group were kept out of the same temperature and time, so that the effect of environmental conditions was tried to be minimized. After injection, the hole was closed by dropping and placed in the hatch trays as 25 eggs in each subgroup. The incubation results were calculated as follow

Hatchability of fertile eggs (%) = number of eggs hatched out/number of fertile eggs x 100

Fertility (%) = number of fertile eggs/number of total eggs produced or set x 100.

Hatchability of set eggs (%) = number of eggs hatched out/number of eggs set in the incubator x 100.

After hatching time was ended the non-hatched eggs according to the macroscopic analyze were examined by the method described by Aygun et al. (2012).

Table 3
Effect of *in-ovo* probiotic injection into quail hatching eggs on some organ weights and live weight

Group	Organ weights,%		Live weight, g					
	Liver	Yolk sac	At hatch	1. w	2. w	3. w	4. w	5. w
Control	2.66	8.49	8.50	19.40	36.03	76.06^{a}	116.77^{a}	151.33^{a}
$16x10^3$ cfu	2.41	9.33	8.37	19.09	35.63	68.98^{ab}	112.09^{a}	141.48^{b}
$32x10^3$ cfu	2.51	9.16	8.53	19.25	35.59	72.31^{a}	113.64^{a}	150.08^{a}
$48x10^3$ cfu	2.52	7.19	8.43	18.18	34.94	63.26^{b}	95.53^{b}	138.08^{b}
SEM	0.84	0.14	0.07	0.41	0.82	1.74	1.13	1.86
P-value	0.694	0.335	0.487	0.228	0.845	0.013	0.001	0.001

[a-b] within a column, means with no common superscript letters are significantly different (P<0.05)

The growth performance of each group having 15 quail chicks were taken and placed in the quail growing cages. On the first day after incubation the house temperature was adjusted to 33 °C and decrease by 3 °C each week and was stable at 20 °C during the growth period, 23 hours of light and one hour of darkness were applied. During the growing period the quail growing feed (Table 1) and water were given ad libitum. For the determination of live weight changes, chicks were weighed weekly and weight gain was determined from these weights.

Statistical analysis

At the end of the experiment, the variance analyses were applied to all variables obtained from the trial groups (Minitab 2000), and the differences between means of the groups were determined by the Duncan test (Duncan 1955).

3. Results and Discussion

The effect of embryonic mortality on the incubation parameters of the *in-ovo* probiotic injection to breed quail eggs is shown in Table 2. No significant differences were found among the treatments groups for hatchability, fertility, and embryonic mortality. *In-ovo Lactobacillus rhamnosus* GG administration does not appear to have an adverse effect on incubation. Similar findings were reported in the previous studies. Edens et al. (1997) explained that the injection of *L. reuteri* in the turkey eggs did not influenced negatively the incubation results. Damron et al. (1981) stated that the injection of *L. acidophilus* in the turkey eggs did not influence negatively the incubation results. Triplett et al. (2018) found similar results with the injection of *L. Acidophilus* in the broiler laying hens eggs. The effects of treatments on the liver and yolk sac weights and live weight of quails at growth period are given in the Table 3. No significant differences were found between the experiment groups regarding the liver, yolk sac weights and weight at the hatch and 1st and 2nd week weights of growth period. Whereas the injection of probiotic *in-ovo* had a significant effect on the live weight of quail chicks at 3, 4 and 5 weeks of growth period .The control group at 3rd and 4th week of growth had the highest live weight value and the difference between the $48x10^3$ cfu probiotic addition group was significant. The difference between the control group which was the group with the highest live weight value and the groups $16x10^3$ and $48x10^3$ cfu injected with *in-ovo* bacteria at 5 week was statistically significant.

Table 4
Effect of *in-ovo* probiotic injection into quail hatching eggs on live weight gain

Group	0-1 w	1-2 w	2-3 w	3-4 w	4-5 w	0-5 w
Control	10.90	16.63	40.03^{a}	40.71^{a}	34.56^{ab}	142.83^{a}
$16x10^3$ cfu	10.72	16.54	33.36^{ab}	43.11^{a}	29.39^{b}	133.11^{bc}
$32x10^3$ cfu	10.72	16.34	36.72^{a}	41.33^{a}	36.44^{ab}	141.55^{ab}
$48x10^3$ cfu	9.74	16.76	28.32^{b}	32.27^{b}	42.55^{a}	129.64^{c}
SEM	0.43	0.72	1.93	1.34	1.87	1.89
P-value	0.290	0.985	0.023	0.011	0.007	0.002

$^{a-c}$ within a column, means with no common superscript letters are significantly different (P<0.05)

The effect of *in-ovo* probiotic injection on growth period live weight gain at 1st and 2nd week periods was not significant, while in other periods this trial effect became statistically significant. In the experiment 2-3, 3- 4 and 0-5 the lowest live weight gain was observed in the chicks that hatched from the eggs with the highest probiotic injections. However, the live weight gain in the last week of the growth period was again obtained in the group with the highest probiotic injection. (Maiorano et al. 2012) reported that the co-administration of *Lactococcus acidophilus* (500 cfu) and *Strepcoccus faecium* (500 cfu) at the 12th day of incubation to the breeding broiler chicken eggs increased the growth period live weight but negatively affected the feed conversion. Similarly Pruszynska-Oszmalek et al. (2015) carried a research in which the prebiotics (inulin and Bi2tos) and synbiotics (inulin +*Lactococcus lactissub sp*. lactisand Bi2tos +*Lactococcus lactissubsp.cre-moris*) were injected in-ovo into the air cell on the 12th d embryonic development, they demonstrated the beneficial effects of prebiotics and synbiotics inoculated *in-ovo*. Bi2tos and inulin given with *Lactococcus lactis ssp.* when injected in-ovo on the 12th d chicken embryo development elevated body weight at the end of the rearing period. Simultaneously, the investigated compounds significantly increased the total activity of pancreatic enzymes; amylase, lipase, and trypsin.

4. Conclusion

It was concluded that injection of *Lactobacillus rhamnosus* in the breeding quail eggs at 14 day of incubation had no negative effect on the incubation parameter. However injection at the high level of *Lactobacillus rhamnosus* had a negative effect on the growth parameters.

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