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Evaluation of the Probiotic Potential of some Native *Lactobacillus* Strains on the Laying Performance and Egg Quality Parameters of Japanese Quails

Research Article

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ABSTRACT

The objective of the present study was to evaluate the probiotic effects of different concentrations of four selected native *Lactobacillus* strains on laying performance and egg quality parameters of quails. To do this, a six-week farm trial was performed in completely randomized design with seven different probiotic treatments of four replicates each consisting of eight quails, resulting in a total of 224 quails. Four native *Lactobacillus* strains whose probiotic potentials had previously been confirmed at *in vitro* levels were cultured in a 10 liter batch fermentor and lyophilized (10^{10} CFU/g). Treatments were as follows: T1: control (basal diet without probiotics); T2: basal diet + Primalac® (454 g/ton); T3: basal diet + Protexin® (50 g/ton); and T4, T5, T6, and T7: four native strains in levels of 50, 100, 150, and 200 g/ton diets, respectively. The native strains and Protexin® could significantly improve egg production, feed conversion ratio (FCR), and egg mass during the laying period ($P<0.05$), whereas no significant effect was observed on feed intake and egg weight. The probiotic treatments had significant positive effects on the albumen height, Haugh unit, and internal quality unit (IQU) ($P<0.05$), whereas there was no difference between the treatments in terms of egg shell weight, egg yolk weight, and egg albumen weight of the quails ($P>0.05$). It can be concluded that the use of native strains (a 50 g/ton diet) improved the performance (egg production, egg mass, and FCR) and egg quality parameters (Haugh unit and IQU) of quails.

KEY WORDS egg quality traits, laying quail, native *Lactobacillus*, probiotic, production parameters.

INTRODUCTION

It is well known that the dietary inclusion of biological additives such as probiotics could benefit the bird's performance and health. It has been shown that probiotic bacteria have a strong positive effect on economic yield indexes (meat, lay intensity, and mean egg weight) (Hippenstiel *et al.* 2011; Mookiah *et al.* 2014), hen yield performance (including egg quality, egg production and FCR) (Davis and Anderson, 2002), and resistance to pathogens in laying or meat chickens (Cean *et al.* 2015; Tellez *et al.* 2012). Bacterial probiotics, such as some species of the genera *Lactobacillus*, *Streptococcus*, *Enterococcus* and *Bacillus*, and also *E. coli* have been effectively used in pigs, pre-ruminant calves, and chickens, whereas fungal probiotics such as *Saccharomyces* and *Aspergillus* have shown promising results in adult ruminants. Growth-promoting feed additives has been used extensively in the livestock industry to decrease overall production costs by influencing the digestive tract microflora and environment which, in-turn, promotes animal health and enhances the efficiency of feed conversion (Aazami *et al.* 2014; Daneshmand *et al.* 2015; Wu, 2006). Microbes that are widely used as probiotics in the poultry industry include species of the genera *Lactobacillus* and *Enterococcus* (Chen *et al.* 2012). The main postulated health benefits associated with probiotics include the improvement of microbial flora balance of the digestive system (Smug *et al.* 2014), reduction of pathogenic flora of the intestine via their antagonist action (Ramirez-Chavarin *et al.* 2013), production of different antimicrobial substances and supporting the modulation of immune response, production of digestive enzymes, and promotion of lactose tolerance (Choi *et al.* 2015). The probiotics stimulating the immune functions (Wu, 2006) and also a group of researchers showed that the broilers fed with probio-enzyme mixture had satisfactory immune response compared with control treatment (Pournazari *et al.* 2017). In poultry farms that mainly keep laying hens, breeders used to focus on enhancing the weight of eggs, egg yield, and the internal quality by, for instance, reducing the cholesterol content of their production to increase economic performance and market success (Wang *et al.* 2012). Probiotics have important positive effects on layer hens and their products (Mátéová *et al.* 2009). Among the main factors affecting the overall effectiveness of probiotics, probiotic species and/or strain composition, administration level, and the origin of the strains are considered as the key ones (Amerah *et al.* 2013; Mountzouris *et al.* 2015; Timmerman *et al.* 2004). We had isolated and characterized some potentially probiotic bacteria from Iranian native chickens and ducks (Aazami *et al.* 2014). Finally, four *Lactobacillus* strains with high probiotic potentials were selected and used in farm experiments (Aazami *et al.* 2014). According to Gardiner *et al.* (2004) and Timmerman *et al.* (2004), the functionality of a multi-strain/multi-species probiotics could be more effective than that of a mono-strain. Extensive research has been carried out to identify the effects of probiotics on growth performance in poultry. The objectives of this study were to evaluate the effects of including some native *Lactobacillus* strains on the laying performance and egg quality parameters of Japanese quails.

MATERIALS AND METHODS

Strains and cultivation conditions

Previously, we had isolated and characterized some potentially probiotic bacteria from Iranian native chickens (Aazami *et al.* 2014). About 40 *Lactobacillus* isolates with high resistance to acidic gastric conditions (pH 2.5) and high concentrations of bile salts (0.5% Oxgal; Merck, Darmstadt, Germany) were selected. The 16srDNA amplification and sequencing revealed that the selected strains belonged to the species *L. crispatus*, *L. reuteri*, *L. salivarus*, *L. vaginalis*, *L. oris*, *L. agilis*, and *L. fermentum*. Then, the strains with fast auto-aggregation rates (10 to 120 min), tolerance to salinity (up to 15% NaCl), tolerance to low and high temperatures (between 4.5 and 45 ˚C), relative resistance to 16 different antibiotics used in veterinary medicine, antimicrobial activities against different pathogens, including *Pseudomonas aeroginosa*, *E. coli*, *Streptococcus mutans*, *Clostridium defficile*, *Enterococcus hirea*, *Salmonella enterica*, *Staphylococcus aureus,* and high ability for adhesion to the $CaCo_2$ epithelial cell line (up to 40 bacterial cells/cell) were selected. Finally, four *Lactobacillus* strains with high probiotic potentials were chosen and used in farm experiments. Four native *Lactobacillus* strains including OR7 (*L. crispatus*), Es7 (*L. salivarius*), OR10 (*L. crispatus*) and M4 (*L. oris*) whose probiotic potentials had previously been confirmed at *in vitro* levels were cultured in a 10-L batch fermentor (37 ˚C, pH 5.6, Bioflo 2000, New Brunswick, USA Fermentor) at microaerophilic conditions on skim milk. The samples were centrifuged at 8000g at 4 ˚C for 30 min in a Beckman Avanti J25 I centrifuge (Superma high-speed centrifuge, Tomy, Japan), and after lyophilizing (10^{10}-10^{11} CFU/g, Novalyphe-NL 500; Savant Instruments Corp., Holbrook, NY, USA), the strains were used in farm experiments. The drying situations were set at 14 pa of pressure, -25 ˚C, during 18 h. Before being used in the diet experiment, the freeze-dried culture of native probiotics was analyzed for total viable cell counts (10^{10}-10^{11} cfu/g). The viable cell count (cfu) was estimated by the standard serial dilution method on MRS agar after anaerobical incubation at 37 ˚C for 48 h (Vandeplas *et al.* 2009).

Birds and experimental treatments

All the experiment procedures were approved by the Animal Care and Ethics Committee of the Tarbiat Modares University and complied with the Guidelines for the Care and Use of Animals in Research. The objective of the present study was to evaluate the probiotic effects of different concentrations of four selected native *Lactobacillus* strains on the laying performance and egg quality of Japanese quails during the laying period. To do this, a six-week farm trial was performed in the format of complete randomized design through four replicates of seven different probiotic treatments, with each replicate consisting of eight quails, resulting in a total of 224 quails. In order to eliminate the effects of changes in body weight, the weight of quails was recorded in all cages, which according to the obtained weights showed that there was no significant difference between treatments. Each replicate was assigned to a clean floor pen (40×40 cm^2), and the temperature was maintained at 20-22 ˚C (Mikulski *et al.* 2012). The photoperiod was 16 h of light per day throughout the experimental period (Vatsalya and Arora, 2011). Two commercial probiotics Protexin® (2×10^9 cfu/g) and Primalac® (1×10^8 cfu/g) were used as positive control. Protexin (International Ltd, Somerest, UK) is a multistrain probiotic consists of 7 bacterial and 2 yeast strains: *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopesii*. Also commercial Primalac probiotic (Star Labs Inc., Clarksdale, MO, USA) is a multistrain probiotic with 4 bacterial strains: *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*. Treatments were as follows: T1: control (basal diet without probiotic); T2: basal diet + Primalac® (454 g/ton); T3: basal diet + Protexin® (50 g/ton); and T4, T5, T6, and T7: four native strains in the levels of 50, 100, 150, and 200 g/ton diets, respectively. The probiotic products were added to the basal diet on a weekly basis. Two feed samples, one upon mixing and the second at the end of each week, were taken and subjected to microbiological analysis to check the probiotics' viability in the feed. The basal diet was in the form of mash, and was formulated for the laying period of Japanese quails. Diets were formulated on a digestible amino acid and the quails from all treatments received a corn-soybean meal basal diet (UF-FDA, University of Georgia, 1992, Athens, GA) formulated per all the nutrients according to the recommendations by the National Research Council (NRC, 1994). The composition of the basal diet is shown in Table 1. Experimental diets were prepared every week and stored in sacks at 4 ˚C.

Laying measurements

Feed intake, egg production, egg weight, egg mass, and FCR were recorded on a cage basis every week, for hens of 8, 9, 10, 11, 12, and 13 weeks old (49-91 days old). Egg production, obtained by dividing the total eggs produced in each replicate by the number of female quails in each replicate, and the result was shown in percentage (Tůmová and Gous, 2012).

Daily feed intake per bird was calculated on a cage total feed consumption basis for the entire experimental period and for the number of days in the period. Feed conversion ratio was calculated by dividing the feed intake by egg mass (Mikulski *et al.* 2012). Egg mass was calculated by multiplying the egg production by egg weight according to (Olgun and Yıldız, 2014).

Egg quality measurements

Egg quality examination (egg yolk, albumen and shell weight percentages, Haugh unit (HU) score, height of albumen, internal quality unit, egg width, egg length, and egg shape index) was conducted at 8-13 weeks of age. To do this, four laid eggs were randomly collected from each treatment on the first day of the 8, 10 and 12th weeks of age (a total of 16 eggs per treatment) (Christaki *et al.* 2011; Mikulski *et al.* 2012).

The weights of the shell, albumen, and yolk were measured based on egg weight and expressed in percentages. Egg albumen weight was calculated by subtracting the weight of the shell and yolk from the whole quail egg weight.

Eggshells were cleaned of any adhering albumen, then dried at room temperature, and expressed as a percentage of the whole egg weight (Mikulski *et al.* 2012). Eggs were weighed individually to the nearest 0.01 g using an electronic balance (Sartorius, TE1502s, Germany). To measure the egg albumen quality, eggs were on a glass plate measurement stand and the height of albumen was determined using a spirometer (Roberts, 2004). Haugh unit was calculated according to the formula suggested by (Rodriguez *et al.* 2002).

Eq. (1) $HU\ \% = 100 \text{ Log } (H+7.57-1.7W^{0.37})$

Where:
H: stands for albumen height (mm).
W: refers to egg weight (g).

The international quality unit (IQU) (Kaur *et al.* 2007), as an alternative to HU in egg quality measurement was calculated by the following formula:

Eq. (2) $IQU = 100 \text{ Log } (H+4.18-0.8989EW^{0.6674})$

Where:
EW: stands for egg weight.

The width and length of egg were determined by a caliper to the nearest 0.05 mm, and egg shape index (width/length×100) was then calculated.

Statistical analysis

Laying performance and egg quality parameters were analyzed for differences using a ANOVA mixed model (PROC MIXED) with repeated measures using SAS/STAT software (SAS, 2003). The time (weeks) was considered as a main effect in the statistical analysis with the repeated measures analysis. The treatment comparison was statistically conducted by Tukey's test (P<0.05) using the same software.

RESULTS AND DISCUSSION

Effects of probiotics on production performance

The effect of dietary treatments on production performance (feed intake, FCR, egg production, egg mass and egg weight) during the experimental period (between 49th and 91th days of age) in the laying quails is shown in Table 2.

Table 1 Ingredient composition and nutrient content of the basal diets

Ingredients	**Basal diet (%)**
Corn grain	46.00
Soybean meal (44% CP)	38.90
vegetable oil (soybean oil)	5.10
Limestone	7.30
Dicalcium phosphate	1.20
Common salt	0.23
DL-methionine	0.17
Vitamin A	0.10
Vitamin E	0.10
Vitamin K	0.10
Vitamin D	0.10
Vitamin B	0.10
Mineral premix[1]	0.30
Vitamin premix[2]	0.30
Chemical composition[3]	
Metabolizable energy (ME) (kcal/kg)	2916
Crude protein (CP) (%)	20.07
Ca (%)	2.57
Available P (%)	0.36
Na (%)	0.18
Cl (%)	0.17
K (%)	0.91
Arg (%)	1.28
Lys (%)	1.04
Met (%)	0.46
Met + Cys (%)	0.77
Thr (%)	0.74
Trp (%)	0.27

[1] Levels per kg of diet: Mn: 88 mg; Cu: 66 mg; Fe: 8.5 mg; Zn: 88 mg and Se: 0.30 mg.
[2] Levels per kg of diet: vitamin A: 2400000 IU; vitamin D_3: 720000 IU; vitamin E: 14.4 g; vitamin K_3: 2.0 g; vitamin B_1: 0.612 g; vitamin B_2: 3 g; vitamin B_3: 4.89 g; vitamin B_6: 0.612 g; vitamin B_9: 0.5 g; Niacin: 12 g and Biotin: 2 g.
[3] Based on NRC (1994) values for feed ingredients.

Table 2 Effect of native and commercial probiotics on laying production performance of Japanese quails

Treatments	**Production performance**				
	Feed intake (g/bird/d)	Hen-day egg production (%)	FCR (g/g)	Egg mass (g/bird/d)	Egg weight (g)
Control (T_1)	32.13	80.58^{b}	3.41^{a}	9.43^{b}	11.71
Primalac® (T2)	32.04	84.68^{ab}	3.20^{b}	10.03^{ab}	11.83
Protexin® (T3)	32.39	87.05^{ab}	3.14^{bc}	10.35^{ab}	11.90
Probiotic 50 g/ton (T_4)	32.06	92.54^{a}	2.84^{d}	11.24^{a}	12.15
Probiotic 100 g/ton (T_5)	31.20	85.25^{ab}	3.11^{bc}	10.09^{ab}	11.82
Probiotic 150 g/ton (T_6)	32.45	91.96^{a}	2.91^{cd}	11.15^{a}	12.12
Probiotic 200 g/ton (T_7)	32.46	88.09^{ab}	3.04^{bc}	10.70^{a}	12.13
SEM	0.0750	0.0784	0.0755	0.0780	0.0419
Age (weeks)					
8	30.64^{d}	83.03^{b}	3.26^{a}	9.45^{c}	11.38^{d}
9	32.17^{bc}	87.47^{a}	3.16^{a}	10.23^{b}	11.68^{c}
10	31.34^{cd}	88.44^{a}	2.95^{b}	10.64^{a}	12.02^{b}
11	32.48^{ab}	89.83^{a}	2.99^{b}	10.88^{a}	12.11^{b}
12	32.49^{ab}	87.57^{a}	3.01^{b}	10.83^{a}	12.36^{a}
13	33.50^{a}	86.64^{a}	3.20^{a}	10.53^{ab}	12.15^{b}
ANOVA (P>F)					
Treatment	0.9299	0.0001	0.0001	0.0001	0.1997
Week	0.0001	0.0026	0.0001	0.0001	0.0001
Treatment × age	0.8625	0.9891	0.6683	0.9962	0.5938

FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results of this experiment indicated that the dietary inclusion of probiotics (native and commercial) had a significant effect on FCR, egg mass, and egg production during the laying period (P<0.05). Also the results for laying production performance at 8, 9, 10, 11, 12 and 13 weeks of age are presented in Table 2. The repeated measures analysis indicated a significant effect of time on egg production, egg mass, feed intake, and egg weight. The results showed that the dietary inclusion of probiotics could significantly increase egg mass and egg production and decrease FCR during the laying period, as compared to the control diet (T1). No significant effects of treatments were observed on feed intake (FI) and egg weight during the laying period (P>0.05) (Table 2). The highest and lowest egg production and egg mass were respectively shown in the diet with the native-strain probiotic 50 g/ton (T4) and the control diet during the egg-laying period. Interestingly, the egg mass and egg production in the diet with the native-strain probiotic 50 g/ton (T4) were significantly higher than those in the diet with commercial probiotics and the control diet (P<0.05). However, the difference between the diets with the native-strain probiotic 50 g/ton and those with the native-strain probiotic 150 g/ton (T6) was not significant (Table 2). The highest and lowest FCRs were respectively found in the control diet (T1) and the diet with native probiotic 50 g/ton (T4) during the laying period (between the 49^{th} and 91^{th} days of age). Interestingly, the FCRs in the diet with commercial probiotics and the control diet were significantly more than that in the native-strain probiotic 50 g/ton (T4) (P<0.05), and the difference between the diets with commercial probiotics with the control diet was significant.

Effects of probiotics on egg quality

The effect of treatments on internal (Haugh units, internal quality unit, albumen height, egg yolk percent and egg albumen percent) and external (eggs shape index, egg length, egg width and egg shell percent) egg quality at 8, 10, and 12 weeks of age is demonstrated in Tables 3 and 4, respectively. The repeated measures analysis indicated a significant effect of time on Haugh unit, internal quality unit, albumen height, egg length, egg width, egg albumen weight, egg yolk weight, but there was no significant effect of time on egg shape index and egg shell weight (Tables 3 and 4). The results indicated that the dietary inclusion of probiotics, as compared to the control diet (T1), could significantly improve Haugh unit, internal quality unit and albumen height during the laying period (P>0.05). There was no significant difference among treatments in terms of the egg shape index, egg albumen weight (%), egg yolk weight (%), egg length (mm), egg width (mm) and egg shell weight (%) during the egg-laying period (P>0.05). The highest and lowest Haugh units were found in the diet with native-strain probiotic 0.5% (T4, 92.96) and the control diet (T1, 86.82) respectively, while no significant effect was observed between the diet with native-strain probiotic 200 g/ton (T7), Primalac and the control diet (T1). The native-strain probiotic 50 g/ton (T4) had the highest amount of Haugh unit and internal quality unit compared to other treatments, while there was no difference among the native-strain probiotic 50 g/ton (T4), native-strain probiotic 100 g/ton (T5), native-strain probiotic 150 g/ton (T6), and Protexin probiotic (T3). In the cases of Haugh unit, albumen height, and internal quality unit, the diet with Primalac probiotic had no significant difference from control treatment, and a significant increase was recorded in the values of Haugh unit, albumen height, and internal quality unit in all probiotic groups as compared with the control and Primalac groups (P<0.05). The differences among native-strain probiotic 50 g/ton (T4), and native-strain probiotic 100 g/ton (T5), native-strain probiotic 150 g/ton (T6) and Protexin probiotic were not significant in terms of albumen height. Also the highest and lowest albumen height were found in the diet with native-strain probiotic 150 g/ton (T4, 5.22 mm) and the control diet (T1, 4.04 mm), respectively, and there was no statistically significant difference between the Primalac (T2) and Protexin probiotics (T3).

Laying production performance

Generally, it has been shown that the efficacy for most probiotics could be found with a daily consumption of 10^8 to 10^9 microorganisms in animals (Patterson and Burkholder, 2003). However, it is extremely difficult to directly compare various studies which have used various probiotics and various administration levels. Önol *et al.* (2003) indicated that the dietary probiotic supplementation has no beneficial effect on the egg production of laying quails. Yörük *et al.* (2004) reported that the probiotic supplementation in the diet did not influence the egg production and was higher for the control group, which is in agreement with Ramasamy *et al.* (2009) who reported that the dietary probiotic supplementation had no significant effect on egg production. Balevi *et al.* (2001) observed no statistically significant differences in egg weight and egg production, which may be because of using various bacteria strains, the form of probiotics, different concentrations, and the ages of the hens.

Kurtoglu *et al.* (2004) showed that the effect of probiotics on egg production was not significant until day 60, but a considerable increase caused by dietary probiotic supplementation was observed in egg production on days 60-90 of this experiment. Patterson and Burkholder (2003) suggested that the daily consumption of probiotics could have beneficial effects in animals.

Table 3 Effect of native and commercial probiotics on internal egg quality parameters of Japanese quails

Treatments	Egg quality parameters				
	Albumen height (mm)	Haugh unit	IQU	Egg albumen weight (%)	Egg yolk weight (%)
Control (T1)	4.04[c]	86.82[c]	56.25[b]	61.25	30.58
Primalac® (T2)	4.55[bc]	89.77[bc]	61.26[ab]	59.75	31.33
Protexin® (T3)	4.98[ab]	91.49[ab]	63.53[a]	61.90	29.38
Probiotic 50 g/ton (T_4)	5.20[a]	92.96[a]	66.67[a]	60.13	31.06
Probiotic 100 g/ton (T_5)	5.19[a]	92.78[ab]	66.21[a]	60.53	30.96
Probiotic 150 g/ton (T_6)	5.22[a]	92.49[ab]	64.90[a]	61.12	30.03
Probiotic 200 g/ton (T_7)	4.36[c]	88.04[c]	56.43[b]	59.87	31.62
SEM	0.105	0.548	1.04	0.318	0.315
Age (weeks)					
8	4.29[a]	88.34[b]	58.50[b]	60.53[ab]	30.59[ab]
10	5.04[a]	91.66[a]	64.25[a]	61.91[a]	29.54[b]
12	5.06[b]	91.85[a]	63.36[a]	59.50[b]	32.00[a]
ANOVA (P>F)					
Treatment	0.0009	0.0011	0.0014	0.2973	0.2990
Week	0.0008	0.0055	0.0306	0.0137	0.0009
Treatment × age	0.1256	0.1284	0.1200	0.3942	0.3534

IQU: internal quality unit.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of native and commercial probiotics on external egg quality parameters of Japanese quails

Treatments	Egg quality parameters			
	Egg length (mm)	Egg width (mm)	Egg shape index ((width/length)×100)	Egg shell weight (%)
Control (T1)	32.58	25.71	78.92	8.41
Primalac® (T2)	32.78	25.96	79.20	8.90
Protexin® (T3)	32.86	26.03	79.27	8.70
Probiotic 50 g/ton (T4)	32.54	26.20	80.59	8.73
Probiotic 100 g/ton (T_5)	33.25	26.06	78.47	8.49
Probiotic 150 g/ton (T_6)	33.13	26.39	79.71	8.83
Probiotic 200 g/ton (T_7)	33.57	26.50	78.96	8.50
SEM	0.113	0.066	0.273	0.065
Age (weeks)				
8	32.29[c]	25.81[c]	79.98	8.85
10	32.96[b]	26.14[b]	79.35	8.54
12	33.63[a]	26.41[a]	78.59	8.57
ANOVA (P>F)				
Treatment	0.0984	0.1420	0.5493	0.3516
Week	0.0001	0.0001	0.1134	0.1135
Treatment × age	0.6025	0.3110	0.6187	0.6193

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

They have also shown that egg weight and egg production were increased by the inclusion of a probiotic in the diet. The positive effects of symbiotics and multi-strain probiotics on the egg production of Japanese quail breeders and layers were reported by previous research (Ayasan *et al*. 2006; Guclu, 2011). Manafi *et al*. (2016) showed that the laying Japanese quails fed a probiotic supplementing *B. subtilis*, significantly increase egg weight and egg production compared with the control treatment.

Mohan *et al*. (1995) reported that the egg production showed a quadratic increase in chickens supplemented with 0, 100, and 150 mg probiotics (*Lactobacillus*, *Bifidobacterium*, *Aspergillus*, *Torulopsis* spp.) per kilogram diet. Chen *et al*. (2005) suggested that the increased egg production caused by dietary probiotics could be due to the elongation of small and large intestinal lengths, the repressing effects on undesirable bacteria and stimulating effects on the growth and/or activity of beneficial bacteria in the intestines and, subsequently, the increase in the absorption capacity. Previous studies reported that a probiotic such as *lactobacillus* and *B. subtilis* can have a positive effect on daily egg production and egg quality (Nahashon *et al.* 1994; Panda *et al.* 2008; Ramasamy *et al.* 2009; Zarei *et al.* 2011; Zhang *et al.* 2012). In other studies, the effects of probiotics on egg production were not observed (Davis and Anderson 2002; Mikulski *et al.* 2012). The results of the present study confirmed the probiotic potentials of the selected strains. These results are in accordance with those of previous studies, demonstrating that the presence of probiotic additives improved egg production in the laying period (Ayasan *et al.* 2006; Chen *et al.* 2005; Guclu, 2011; Mohan *et al.* 1995; Nahashon *et al.* 1994; Panda *et al.* 2008; Patterson and Burkholder, 2003; Ramasamy *et al.* 2009; Zarei *et al.* 2011; Zhang *et al.* 2012). However, these results disagree with the results of some studies which showed no significant differences in egg production between treatments with probiotics (Davis and Anderson, 2002; Kurtoglu *et al.* 2004; Mahdavi *et al.* 2005; Mikulski *et al.* 2012; Önol *et al.* 2003; Ramasamy *et al.* 2009). Nahashon *et al.* (1994) showed that dietary probiotic supplementation could decrease FCR and improve feed intake in single-comb white leghorn laying pullets. Xu *et al.* (2006) showed significant improvements in FCR and egg production when 500 mg of *B. subtilis* culture/kg was added to the diets, while excessive doses (1000 or 1500 mg/kg) did not improve the performance of layers. Goodling *et al.* (1987) concluded that diets supplemented with *Lactobacillus* fermentation products could improve the production performance of white leghorn layers. Kalavathy *et al.* (2005) observed that when layer hens were fed a probiotic mixture that included 12 *Lactobacillus* strains, egg production and FCR were significantly improved. Guclu (2011) found significant improvements in FCR in breeder Japanese quails caused by the supplementation of a combination of prebiotics and probiotics in their diet. Ayasan *et al.* (2006) observed a significant improvement in FCR in laying quails brought about by the supplementation of multi-strain probiotics (Protexin) in their diet. Yörük *et al.* (2004) reported that the probiotic supplemented in laying hens' diet (54 weeks of age) for three months decreased FCR and feed intake. Mahdavi *et al.* (2005) showed that supplementing probiotics in the diet of commercial layer hens had no positive effect on egg weight, feed intake, and FCR. The results of the present study are in agreement with those of several reports demonstrating that probiotics improved FCR (Ayasan *et al.* 2006; Guclu, 2011; Kalavathy *et al.* 2005; Nahashon *et al.* 1994; Xu *et al.* 2006; Yörük *et al.* 2004). Nevertheless, our results disagree with Mahdavi *et al.* (2005) study which showed no significant differences in feed intake and feed efficacy among treatments with probiotics. Karimi-Kivi *et al.* (2015) reported that the ostrich chickens fed with commercial probiotic treatments (Bioplas and Protexin) had higher feed intake and lower FCR than those fed the control diet (Karimi-Kivi *et al.* 2015). Also Pournazari *et al.* (2017) showed that the birds fed diets supplemented with additives had higher feed intake than those fed the control diet. Abdel-Azeem *et al.* (2005) observed that the addition of probiotics to the diet of laying quails improved egg weight and egg mass during the laying period. In contrast, the supplementation of a combination of prebiotics and probiotics (symbiotics) in the quail breeders' diet caused no improvement in egg weight in quails (Guclu, 2011). The results of the present experiment are in agreement with those reported by Abdel-Azeem *et al.* (2005). On the other hand Mahdavi *et al.* (2005) reported that the addition of probiotics to the diet of commercial layer hens had no positive effect on egg mass. The inclusion of suitable microorganisms in the diet allows for the rapid development of helpful bacteria in the intestine of the host and improves its performance (Edens, 2003). The reason for different effects of biological additives can be confounded by variations in the intestine microflora and environmental conditions. Further possible causes of variations in layers' reaction to probiotic supplementation could be the differences between strains, hybrids, ages, and nutrient compositions of diets, the plane of nutrition, and microbial population of the intestinal tract (Zarei *et al.* 2011).

External and internal egg quality traits

In other studies, no effect of dietary addition of probiotics was observed on egg quality (Balevi *et al.* 2001). Önol *et al.* (2003) reported that the diet supplemented with probiotics increased egg shell weight in laying hens. Ayasan *et al.* (2006) showed that the supplementation of probiotics in the Japanese quails' diet did not affect egg shape index and egg weight, but affected egg shell weight, during the egg-production period. Tortuero and Fernandez (1995) indicated that the inclusion of microbial additives in barley-based diets fed to laying hens enhanced egg production and egg weight and improved egg albumen. Xu *et al.* (2006) showed that the supplementation of 500 mg of *B. subtilis* culture/kg to the laying hens' feed had a significant effect on egg quality. Mikulski *et al.* (2012) found that the supplementation of probiotics in laying hens' diet improved FCR, egg weight, and eggshell quality during the early laying period. Ramasamy *et al.* (2009) reported that the inclusion of *Lactobacillus* in laying hens' diet (between 20 and 44 weeks of age) significantly increased egg weight. Ayasan *et al.* (2006) reported that the diet supplemented with Protexin probiotic increased egg shell weight in laying quails. Aghaii *et al.* (2010), showed that the supplementation of probiotics had no significant effects on Haugh units and albumen height.

Kalsum *et al.* (2012) investigate the effect of effect of probiotic containing *L. salivarius* on the laying performance and egg quality of Japanese quails and reported that *L. salivarius* supplementation did not affection egg quality parameters (egg albumen percent, haugh unit, egg yolk percent and egg shell thickness). Manafi *et al.* (2016) showed that inclusion of probiotic in laying Japanese quail diet had no significant effect on breaking strength, Haugh units, and eggshell percentages. Yalçin *et al.* (2008) reported that the addition of yeast culture had no significant effect on egg yolk weight, shape index, yolk index, and Haugh units.

The findings of the present study are in line with those of several reports demonstrating that probiotics supplemented to the birds improved egg quality (Tortuero and Fernandez, 1995; Xu *et al.* 2006). However, our results disagree with the results of some studies which reported no significant differences in egg quality among treatments with probiotics (Aghaii *et al.* 2010; Balevi *et al.* 2001; Yörük *et al.* 2004).

CONCLUSION

It could be concluded that the application of four native *Lactobacillus* strains with the concentration of 50 g/ton improved the performance (egg production, egg mass, and FCR) and egg quality parameters (Haugh unit and IQU) of Japanese quails compared to other treatments, whereas there was no difference between the treatments in terms of egg shell weight, egg yolk weight, egg albumen weight, egg weight, egg length, egg width, egg shape index and feed intake.

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