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Evaluation of Probiotic Potential of Some Native Lactobacillus Strains on the Growth Performance and Serum Biochemical Parameters of Japanese Quails (Coturnix Coturnix Japonica) during Rearing Period

■Author(s)

Siadati SA[I]
Ebrahimnezhad Y[I]
Salehi Jouzani Gh[II]
Shayegh J[III]

[I] Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran
[II] Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[III] Veterinary Medicine College, Shabestar Branch, Islamic Azad University, Iran

■Mail Address

Corresponding author e-mail address
Yahya Ebrahimnezhad
Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, East Azarbaijan, Iran.
Tel: (+98) 42425311
Email: ebrahimnezhad@iaushab.ac.ir

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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 the growth performance and serum biochemical parameters of Japanese quails. A completely randomized design (CRD) was applied, including seven probiotic treatments with four replicates of 20 quails each, totaling 560 quails. Treatments were applied for five weeks. Four native *Lactobacillus* strains were anaerobically grown in a 10-L batch fermenter and lyophilized (10^{10} CFU/g). Treatments were as follows: T1: control (basal diet); T2: commercial probiotic CP1; T3: commercial probiotic CP2; and T4, T5, T6, and T7: four native strains added at levels of 50, 100, 150, and 200 g/ton diet, respectively. The native probiotics significantly improved body weight gain (BWG) and feed conversion ratio (FCR) during the starter, finisher, and overall periods (35 days) ($p<0.05$), whereas no significant effect was observed on feed intake. The native strains significantly influenced the serum glucose, total protein, globulin, phosphorus, uric acid, low-density lipoprotein (LDL), LDL/HDL ratio, white blood cell counts (WBC), and mean corpuscular hemoglobin concentration (MCHC) of quails during the rearing period ($p<0.05$), whereas treatments had no influence ($p>0.05$) on blood cholesterol, calcium, high-density lipoprotein (HDL), or hemoglobin (HB) levels or on red blood cell counts (RBC). The cecal and small intestine samples of the quails fed the native *Lactobacillus* strains contained significantly higher *Lactobacillus* spp. and lower *E. coli* populations compared with the control diet and those supplemented with commercial probiotics. It was concluded that the use of the native *Lactobacillus* strains (150 g/ton diet) promoted the best performance of Japanese quails.

INTRODUCTION

The term probiotic is etymologically derived from the Latin preposition pro ("for" or "in support of") and the Greek word (biotic), which literally means "for life". In 1989, Fuller defined probiotics as live microbial food supplements that beneficially affect the host animal by improving the intestinal microbial balance (Fuller, 1989). There is a huge body of evidence that support the significant positive impacts of probiotics and bioactive compounds on poultry performance and health. Probiotic bacteria improve the economic indexes and resistance to pathogens of laying or meat-type chickens (Hippenstiel *et al.*, 2011; Aazami *et al.*, 2014; Cean *et al.*, 2015; Mountzouris *et al.*, 2007). Lactobacilli and Enterococci have been widely used as probiotics in the poultry industry (Kabir *et al.*, 2004; Awad *et al.*, 2009; Aazami *et al.*, 2014; Mountzouris *et al.*, 2007). The main postulated health benefits associated with probiotics include improving the gut microflora balance, stimulating the immune reaction, producing different antimicrobial substances,

modulating the immune response, producing digestive enzymes, and reducing cholesterol levels (Ramirez-Chavarin *et al.*, 2013; Smug *et al.*, 2014). Recent studies have confirmed that the addition of probiotic, symbiotic, and medicinal plant additives to feeds enhance nutrient bioavailability, health and immune status, and carcass yield and quality of Japanese quails (Yalçın *et al.*, 2000; Siriken *et al.*, 2003; Chimote *et al.*, 2009; Sharifi *et al.*, 2011; Kasmani *et al.*, 2012; Babazadeh *et al.*, 2011; Kheiri *et al.*, 2015). The main factors that affect the general probiotic effects are probiotic species, strain origin, and application levels (Mountzouris *et al.*, 2007; Amerah *et al.*, 2013).

In this context, exploring new strains with good probiotic properties and optimizing their concentrations in feed additives are important to achieve highly efficient application of probiotics. Our group previously isolated and characterized some potentially probiotic bacteria from Iranian native chickens (Aazami *et al.*, 2014). The selected *Lactobacillus* strains showed high resistance to acidic gastric conditions (pH 2.5) and to bile salts (0.5% Oxgall), as well as to salinity (up to 15% NaCl) and low and high temperatures (between 4.5 and 45°C). The strains presented fast auto-aggregation rate (10 to 120 min) and high ability for adhesion to the Caco-2 epithelial cell line (up to 40 bacterial cells). High antimicrobial activities against different pathogens (*Pseudomonas aeroginosa*, *E. coli*, *Streptococcus mutans*, *Clostridium difficile*, *Enterococcus hirea*, *Salmonella enterica*, *Staphylococcus aureus*) were detected *in vitro* (Aazami *et al.*, 2014). The aim of the present study was to evaluate the probiotic effects of these selected native Lactobacillus strains on the growth performance and serum biochemical parameters of Japanese quails.

MATERIALS AND METHODS

Strains and cultivation conditions

Four native Lactobacilli strains with probiotic potential, including OR7 (*L. crispatus*), Es7 (*L. salivarius*), OR10 (*L. crispatus),* and M4 (*L. oris*), were evaluated in this study (Aazami *et al.*, 2014). The strains were separately cultured in skim milk medium at 37°C and pH 5.6 for 24 h in a 10-L batch bioreactor (Bioflo 2000, New Brunswick, USA). The incubation was performed under microaerophilic conditions. The overnight cultures were centrifuged (8,000 g, 30 min, 4°C) in a high speed centrifuge (Beckman Avanti J25 I Superma, Tomy, Japan), and lyophilized (10^{10}-10^{11} CFU/g, Novalyphe-NL 500; Savant Instruments Corp., Holbrook, NY, USA) for subsequent experiments (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.

In total, 560 one-day-old Japanese quails of both sexes (equally divided) were distributed according to a completely randomized design into seven treatments with four replicates of 20 quails each. The experiment lasted five weeks. The day-old chicks were purchased from a commercial hatchery. Birds in each replicate was allocated to a floor pen (40x40 cm^2). Room temperature was maintained at 32°C during the first week and gradually decreased by 3°C weekly until 22°C, and then maintained constant until the end of the experiment (Khaksar *et al.*, 2012). The quails were subjected to continuous lighting program of 23L:1D during the entire experiment (Kermanshahi *et al.*, 2015).

Two commercial probiotics Perimalac® (1×10^8 cfu/g) (commercial product 1, CP1) and Protexin® (2×10^9 cfu/g) (commercial product 2, CP2) were used as positive controls. Protexin (International Ltd, Somerest, UK) is a multistrain probiotic consisting of seven bacterial and two yeast strains: *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium Aspergillus oryzae* and *Candida pintolopesii*. The commercial probiotic Primalac (Star Labs Inc., Clarksdale, MO, USA) is a multistrain probiotic including four bacterial strains: *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*. The native strains were used at 50, 100, 150 and 200 g/ton diet. The native strains consist of (*Lactobacillus crispatus*), ES7 (*Lactobacillus salivarius*), OR10 (*Lactobacillus crispatus*), and M4 (*Lactobacillus oris*) at 10^{10}-10^{11} CFU/g.

The following treatments were applied: T1: basal diet (control diet without probiotics), T2: basal diet + CP1 (908 g/ton in the starter diet and 454 g/ton in the finisher diet), T3: basal diet + CP2 (150 g/ton in the starter diet and 100 g/ton in finisher phase), T4: basal diet + 50 g of the native strains/ton, T5: basal diet + 100 g of the native strains/ton, T6: basal diet + 150 g of the native strains/ton, and T7: basal diet + 200 g of the native strains/ton. The diets were supplied as mash, and formulated for the starter (1 to 21 d) and

the finisher (22 to 35 d) rearing periods. The basal diets were based on corn and soybean meal basal and were formulated to supply the quails' nutrient requirements according to the recommendations of the National Research Council (NRC, 1994). The composition of the basal diet is shown in Table 1. The experimental diets were prepared every week, and stored in bags at 4°C.

Table 1 – Ingredient composition and nutrient content of basal diet

Ingredient	Control Diet (%)
Corn grain	43.18
Soybean meal	49.50
Soybean oil	3.10
Limestone	1.40
Di-calcium phosphate	1.20
Common salt	0.27
DL-methionine	0.25
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	
ME (Kcal/kg)	2901
CP (%)	24.16
Ca (%)	0.83
Available P (%)	0.39
Na (%)	0.18
Cl (%)	0.19
K (%)	1.11
Arg (%)	1.71
Lys (%)	1.44
Met (%)	0.60
Met + Cys (%)	1.01
Thr (%)	1.06
Trp (%)	0.39

[1]Levels per kg of diet: Mn, 88 mg; Cu, 66 mg; Fe, 8.5 mg; Zn, 88 mg; Se, 0.30 mg. [2]Levels per kg of diet: Manganese: 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 B9, 0.5 g; niacin, 12 g; Biotin, 2 g.

Growth performance parameters

Growth performance parameters, including body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated. Weight gain was determined for the starter (1 to 21 d), finisher (22 to 35d), and overall periods. FI, FCR, and BWG were calculated for the overall period of the experiment (Cengiz *et al.*, 2015).

Blood biochemical analyses

Two quails per replicate (one male and one female) were randomly selected on day 35, and sacrificed by ventral cutting of the neck. As both male and female Japanese quails were reared together, their blood samples were pooled before the biochemical and hematological analyses, which allows reducing the cost and increasing the level of automation of the analyses. Samples of approximately 1 mL of whole blood were collected from eight quails per treatment by jugular vein puncture after sacrifice. Serum globulin, total protein, glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, LDL/HDL ratio, uric acid, phosphorus, and calcium contents were analyzed using an automatic biochemical analyzer (DiaSys, Diagnostic Systems, Germany), following the instructions of the corresponding reagent kits (Pars Azmon Co., Tehran, Iran). Blood (approximately 2 mL) was collected from the same birds and placed into tubes containing EDTA-Na_2 (an anticoagulant) for subsequent determination of the mean corpuscular hemoglobin concentration (MCHC), hemoglobin (HB) level, and white blood cell (WBC) and red blood cell (RBC) counts using an automated hematology analyzer (Celltac E, MEK-7222 J/K; Nihon Kohden Co., Tokyo, Japan) within 2 h after collection.

Evaluation of the cecal and small intestine Lactobacilli and *E. coli* populations

At the end of the experiment, four birds per treatment were randomly selected and sacrificed by decapitation. The cecal and small intestine contents of each bird were transferred to a sterile glass vial for microbial enumeration. Cecal and small intestine digesta samples (1 g) were diluted at 1:10 with normal saline solution, which were then further diluted to 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9}, in normal saline solution, out of which 100 µL were plated on agar plates. In order to evaluate *Lactobacillus* spp. and *E. coli* populations, the diluted samples were seeded on MRS agar (Merck, Germany) and MacConkey agar (Merck, Germany), respectively, and incubated for 48 h at 37°C. Colony forming units (CFU) were expressed as log10 CFU per gram of cecal and small intestine content (Hashemi *et al.*, 2012).

Statistical analysis

Data were analyzed by analysis of variance using the GLM procedure of SAS/STAT software (SAS Institute, 2003) as a completely randomized experimental design with replicates as experimental units. Statistically significant differences among treatments was tested by Duncan's multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of probiotics on growth performance

The results (Tables 2 and 3) showed that dietary inclusion of probiotics (native strains and commercial products) significantly increased body weight gain (BWG) and improved feed conversion ratio (FCR) during the starter and overall periods (p<0.05) compared with the basal diet (T1). Feed intake was not influenced by the dietary treatments in none of the evaluated periods.

The highest and lowest BWG values were obtained with the native probiotic product at 150 g/ton (T6, 202.99 g) and with the control diet (T1, 176.41 g), respectively, during the overall period (Table 3). Interestingly, the BWG during the overall period of the birds fed the diet with native probiotic at 150 g/ton (T6) was significantly higher than that of those fed CP1 (188.72 g) and CP2 (188.50 g) (p<0.05). This was also observed for both the starter and finisher periods.

Table 2 – Effects of commercial and native probiotics on the performance of Japanese quails

	Starter phase (1 to 21 d)			Finisher phase (22 to 35 d)		
Treatment	FI (g)	BWG (g)	FCR	FI (g)	BWG (g)	FCR
Control (T_1)	252.83±3.99	104.66^{c}±1.41	2.41^{a}±0.036	287.55±7.46	71.75^{c}±1.12	4.01^{a}±0.084
CP1 (T_2)	248.21±4.53	112.59^{b}±2.99	2.22^{bc}±0.032	297.42±6.08	76.12^{bc}±0.66	3.91^{ab}±0.097
CP2 (T_3)	247.63±4.96	112.16^{b}±3.53	2.21^{bc}±0.027	301.75±4.76	76.33^{bc}±1.91	3.96^{ab}±0.115
NP 50g/ton (T_4)	257.78±4.19	115.95^{ab}±1.79	2.20^{b}±0.045	306.33±3.38	80.00^{ab}±1.61	3.83^{ab}±0.110
NP 100g/ton (T_5)	249.84±5.83	114.21^{ab}±1.21	2.18^{bc}±0.038	294.08±8.57	76.28^{bc}±1.22	3.85^{ab}±0.049
NP 150g/ton (T_6)	255.54±6.07	120.24^{a}±2.20	2.12^{bc}±0.029	291.95±5.17	82.75^{a}±2.09	3.53^{c}±0.065
NP 200g/ton (T_7)	250.42±3.56	119.13^{ab}±1.52	2.10^{c}±0.030	293.03±7.85	78.85^{ab}±1.56	3.71^{bc}±0.060
p-Value	0.7113	0.0017	0.0001	0.4636	0.0019	0.0131

Values are expressed as means ± standard error of the mean. $^{A-d}$ Values followed by different letters on the same row indicate significant differences (p<0.05).

FI= Feed intake, BWG= Body weight gain, FCR= Feed conversion ratio, CP = commercial probiotic, NP = native probiotic.

Table 3 – Effects of commercial and native probiotics on the performance of Japanese quails

	Overall (1 to 35d)		
Treatment	FI (g)	BWG (g)	FCR
Control (T_1)	540.39±9.66	176.41^{c}±1.20	3.06^{a}±0.039
CP1 (T_2)	545.64±9.18	188.72^{b}±3.39	2.89^{b}±0.038
CP2 (T_3)	549.45±8.10	188.50^{b}±2.26	2.91^{b}±0.010
NP 50g/ton (T_4)	564.13±3.96	195.95^{ab}±2.50	2.88^{b}±0.048
NP 100g/ton (T_5)	543.94±12.22	190.50^{b}±1.95	2.85^{b}±0.040
NP 150g/ton (T_6)	547.50±8.94	202.99^{a}±1.14	2.69^{c}±0.031
NP 200g/ton (T_7)	543.46±10.92	197.98^{a}±3.00	2.74^{c}±0.034
p-Value	0.6555	0.0001	0.0001

Values are expressed as means ± standard error of the mean. $^{A-d}$ Values followed by different letters on the same row indicate significant differences (p<0.05).

FI= Feed intake, BWG= Body weight gain, FCR= Feed conversion ratio, CP = commercial probiotic, NP = native probiotic

The FCR of the birds fed the diet with native probiotic at 150 g/ton (T6) was significantly lower than that of the T2 (CP1, 2.89), T3 (CP2, 2.91), and control treatments during the overall period, demonstrating the better efficiency of the native strains compared with the commercial products (Table 3). This pattern was also observed in the finisher period (p<0.05). However, in the starter period, the highest and the lowest FCR (p<0.05) were obtained with the control diet and the diet with native probiotic at 200 g/ton, respectively (Table 2).

These results are consistent with those of Pelicano *et al.* (2004) and Gunal *et al.* (2006) in broilers, and Cakir *et al.* (2008) and Babazadeh *et al.* (2011), who did not find any significant effect of probiotics on feed intake of Japanese quails, whereas Corrêa *et al.* (2003) and Bitterncourt *et al.* (2011) showed that dietary probiotics reduced the feed intake of broilers.

Alkhalf *et al.* (2010) reported that a probiotic level of 0.8 g/kg feed enhanced the live performance of broilers compared with the control diet and that with 1.6 g probiotic/kg. This indicates that increasing the level of probiotics in the feed does not ensure better performance, which is in accordance with the result of the present study, as T6 (150 mg/kg) promoted better performance than T7 (200 mg/kg). Chimote *et al.* (2009) showed that significantly higher body weight gain and better feed conversion ratio in Japanese quails fed probiotics those fed a control group. Kalavathy *et al.* (2003) reported that the supplementation of probiotics in broiler diets improved their body weight gain and feed conversion ratio from 1 to 42 days of age. Yu *et al.* (2007) also showed that the supplementation of an intestinal *Lactobacillus reuteri* strain to a wheat-based diet increased body weight gain of broilers from 1 to 21 days of age. Moreover, Khaksefidi & Ghoorchi (2006) reported that the body weight gain and feed conversion ratio of broilers fed probiotics

were significantly improved compared with those fed control diets. On the other hand, Ramarao *et al.* (2004) reported that the body weight gain of broilers was not influenced by the dietary supplementation with probiotics, as opposed to findings of this experiment. The results of the present study were contrary with the findings of Ergün *et al.* (2000) and Arslan (2004), who suggested that probiotic supplementation did not influence the feed efficiency ratio or body weight gain of broilers.

The reason for the improvements in body weight gain and feed conversion ratio of the Japanese quails fed probiotics in the present experiment was probably an increase in the population of beneficial intestinal bacteria and reduction of the population of pathogenic bacteria, and consequently, better nutrient digestibility and absorption. The results of the present experiment confirmed the probiotic potential of the selected strains, and were consistent with those obtained in other studies, showing that the presence of probiotic additives enhances the performance (FCR, BWG and BW) of broilers (Ashayerizadeh *et al.*, 2009; Arslan & Saatci, 2004; Yalçın *et al.*, 2000; Sharifi *et al.*, 2011; Kasmani *et al.*, 2012; Chimote *et al.*, 2009; Babazadeh *et al.*, 2011; Kheiri *et al.*, 2015).

Effect of probiotic on blood parameters

The effects of different probiotics on serum biochemical parameters and blood parameters are shown in Tables 4, 5, and 6, respectively. The inclusion of native and commercial probiotics had a significant effect on serum glucose, total protein, globulin, phosphorus, uric acid, LDL and LDL/HDL ratio, WBC, and MCHC of Japanese quails ($p<0.05$). No differences ($p>0.05$) in cholesterol, calcium, HDL, RBC, and HB values were detected (1-35 d). The serum cholesterol results of the present study are in conflict with the findings of Panda *et al.* (2006), who reported that the probiotic *L. sporogenes* (100 mg/kg diet) significantly reduced total cholesterol levels in broilers. Dibaji *et al.* (2012) and Kalavathy *et al.* (2003) also found that feeding probiotics decreased serum LDL, but not HDL levels in broilers.

In the present experiment, feeding probiotics had no effect on serum calcium levels, in agreement with the findings of Sahin *et al.* (2008), who did not find any influence of feeding a probiotic on the serum calcium levels of quails. On the other hand, Scholz-Ahrens *et al.* (2007) explained that probiotics may increase the intestinal absorption of calcium, because the short-

Table 4 – Effects of commercial and native probiotics on selected serum biochemical parameters of Japanese quails

	Item					
Treatment	Glucose (mg/dL)	Cholesterol (mg/dL)	Total protein (g/dL)	Globulin (g/dL)	Phosphorus (mg/dL)	Calcium (mg/dL)
Control (T_1)	292.25^{a}±6.33	203.00±2.73	3.87^{c}±0.22	2.62^{b}±0.38	5.12^{b}±0.23	9.07±0.53
CP1 (T_2)	276.00^{ab}±9.32	204.75±10.30	4.87^{ab}±0.35	3.62^{ab}±0.83	6.65^{a}±0.42	9.10±0.31
CP2 (T_3)	267.25^{b}±6.35	196.00±9.41	5.02^{ab}±0.42	3.72^{a}±0.79	6.35^{ab}±0.48	9.30±0.55
NP 50g/ton (T_4)	284.50^{ab}±7.53	195.00±3.57	4.0b^{c}±0.16	2.70^{b}±0.42	5.37^{b}±0.14	9.45±0.41
NP 100g/ton (T_5)	281.50^{ab}±4.99	202.75±3.81	4.10^{bc}±0.20	2.75^{ab}±0.45	5.55^{ab}±0.28	8.97±0.43
NP 150g/ton (T_6)	262.25^{b}±7.21	194.75±2.28	5.20^{a}±0.41	3.75^{a}±0.81	6.77^{a}±0.50	9.77±0.26
NP 200g/ton (T_7)	265.00^{b}±4.81	200.00±8.76	4.95^{ab}±0.25	3.55^{ab}±0.47	6.12^{ab}±0.43	9.62±0.23
p-Value	0.0417	0.8836	0.0244	0.0410	0.0329	0.7721

Values are expressed as means ± standard error of the mean. $^{A-d}$ Values followed by different letters on the same row indicate significant differences ($p<0.05$). CP = commercial probiotic, NP = native probiotic

Table 5 – Effects of commercial and native probiotics on selected serum biochemical parameters of Japanese quails

	Item			
Treatment	Uric acid (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	LDL/HDL
Control (T_1)	3.92^{a}±0.08	82.25±1.60	78.25^{a}±1.65	0.95^{a}±0.02
CP1 (T_2)	3.00^{b}±0.24	84.75±3.63	74.25^{ab}±6.70	0.87^{ab}±0.04
CP2 (T_3)	3.52^{ab}±0.16	84.00±2.67	67.00^{ab}±5.59	0.80^{ab}±0.08
NP 50g/ton (T_4)	3.37^{ab}±0.23	85.25±2.42	62.25^{b}±4.21	0.73^{b}±0.06
NP 100g/ton (T_5)	3.10^{b}±0.33	82.00±1.47	77.25^{a}±4.33	0.94^{a}±0.06
NP 150g/ton (T_6)	2.85^{b}±0.10	87.50±2.90	60.00^{b}±1.08	0.69^{b}±0.02
NP 200g/ton (T_7)	3.25^{b}±0.15	86.00±3.18	69.75^{ab}±4.59	0.81^{ab}±0.06
p-Value	0.0263	0.7637	0.0475	0.0292

$^{A-d}$ Values are Means ± S.E.M with different letters on the same row implies significant differences ($p<0.05$).

HDL=High density lipoprotein, LDL=Low density lipoprotein

Table 6 – Effects of commercial and native probiotics on hematological parameters of Japanese quails

	Item			
Treatment	RBC ($\times10^6$ /µL)	WBC ($\times10^3$ µL)	MCHC (%)	Hb (g/dL)
Control (T_1)	2.74±0.07	21.50^{b}±1.35	35.79^{b}±0.51	16.65±1.73
CP1 (T_2)	2.78±0.07	24.12^{ab}±1.15	36.30^{ab}±0.33	16.35±0.68
CP2 (T_3)	2.86±0.11	25.80^{a}±1.50	36.52^{ab}±0.32	16.80±1.51
NP 50g/ton (T_4)	2.71±0.11	25.17^{a}±1.01	37.75^{a}±0.40	16.47±0.84
NP 100g/ton (T_5)	2.66±0.15	23.45^{ab}±0.86	36.07^{b}±0.45	16.17±0.55
NP 150g/ton (T_6)	2.81±0.06	26.30^{a}±1.46	37.92^{a}±0.60	16.72±1.28
NP 200g/ton (T_7)	2.88±0.16	26.92^{a}±1.27	36.67^{ab}±0.75	17.02±1.64
p-Value	0.8207	0.0490	0.0477	0.9993

Values are expressed as means ± standard error of the mean. [A-d] Values followed by different letters on the same row indicate significant differences ($p<0.05$).

RBC = red blood cells, WBC = white blood cells, MCHC = mean cell hemoglobin concentration, Hb = hemoglobin, CP = commercial probiotic, NP = native probiotic

chain fatty acids produced by some probiotic bacteria reduce gastrointestinal pH, thereby increasing calcium solubility and presumably, calcium absorption.

Significant reductions were recorded in serum glucose values in the probiotic groups (native probiotic at 150 and 200 g/ton, and CP2) compared with the control group ($p<0.05$), but no differences between commercial and native probiotics were detected ($p>0.05$). The reduction of serum glucose levels in quails fed probiotics in the present study are in agreement with the findings of Al-Kassie *et al.* (2008) and Arslan & Saatci (2004) in broilers and quails respectively, whereas Abd-El-Rahman *et al.* (2012) reported that feeding probiotics increased serum glucose levels in broilers. These differences serum glucose levels among studies may be ascribed to dietary ingredients, nutrient composition, and probiotic effectiveness.

The Japanese quails fed native probiotic strains at 150 g/ton presented higher serum total protein level compared with those fed the commercial probiotics, which were not statistically different. In addition, the highest blood globulin level was obtained in the treatments containing native probiotic strains (150 g/ton) and the commercial probiotic CP2 compared with native probiotic strains (50 g/ton) and the control group. The results of the present study are in agreement with the findings of Arslan & Saatci (2004) who showed that a probiotic-supplemented diet fed to Japanese quails increased serum total protein levels. However, Yalçın *et al.* (2000), Djouvinov *et al.* (2005), Sahin *et al.* (2008), and Alkhalf *et al.* (2010) did not detect any influence of dietary probiotic supplementation on total protein and globulin levels in the serum of quails, broilers, quails, and ducks, respectively. The higher serum total protein levels detected in the birds fed probiotics, except for those fed the native probiotic strains at 50 g/ton, compared with those fed the control diet in the present study may be due to the better protein digestion promoted by probiotics (El-Faham *et al.*, 2014).

The highest and lowest phosphorus contents were observed in the quails fed the native probiotic strains at 150 g/ton and those of the control group, respectively. Significant increases in serum phosphors values were recorded in the native probiotic strains (100, 150 and 200 g/ton) and commercial probiotics (CP1 and CP2) compared with the native probiotic strain 50 g/ton and control group ($p<0.05$). These findings are comparable with the results of Hosseini *et al.* (2013), who found higher serum phosphorus levels in broilers fed a probiotic-supplemented diet compared with those fed a control diet. Eizaguirre *et al.* (2002) reported that probiotics reduced intestinal pH in humans, improving the absorption of minerals by enhancing their solubility.

Serum uric acid levels were significantly lower in the quails fed the native probiotic at 100, 150 and 200 g/ton and the CP1 compared with the control diet, but those fed the diets containing CP2 and the native probiotic at 50g/ton presented intermediate levels, not statistically different from the other treatments. El-Faham *et al.* (2014) also reported lower uric acid levels in broilers fed a probiotic than those fed a control diet, and suggested that probiotics may improve kidney function and protein metabolism, and consequently, increase nitrogen utilization. Moreover, certain probiotic strains utilize urea, uric acid, and creatinine and other toxins as nutrients for growth (Salim *et al.*, 2011). The lower uric acid levels detected in the quails fed probiotics are consistent with the findings of Newaj-Fyzul *et al.* (2007) and Tonekabon (2013) in rainbow trout. The observed reduction in serum uric acid level with the diets containing probiotics in our experiment may be due to better kidney function and

protein metabolism and consequently, better nitrogen utilization.

The maximum total protein (5.20 g/dL), globulin (3.75 g/dL), phosphorous (6.77 mg/dL), and MCHC (37.92%) as well as minimum serum glucose (264 mg/dL), uric acid (2.75 mg/dL), LDL (60 mg/dL), and LDL/HDL (0.69) were observed in T6 (1.5 % native strains). The birds fed the native probiotic strains at 150 g/ton (T6) had the highest MCHC compared to the other treatments, and there was no statistically significant difference between the commercial and native probiotics. The highest and lowest white blood cell counts was observed in native probiotic strains (200 g/ton) and control treatment, respectively, and difference between the CP1 and CP2 with native probiotics was not significant. These results are consistent with those a previous study that showed that the dietary addition of a probiotic did not affect the blood constituents, including hemoglobin concentrations, of ducklings (Djouvinov *et al.*, 2005). Thongsong & Chavananikul (2008) demonstrated that probiotics significantly increased red blood cell counts, mean hemoglobin concentration, and mean corpuscular hemoglobin concentration of broilers. The white blood cell counts obtained in the present study are in agreement with the findings of Zare *et al.* (2007) and Fathi (2013), who obtained significantly higher WBC counts in broilers fed probiotics than in those fed a control diet. The manipulation of intestinal microbiota via the utilization of probiotics influences the development of the immune response. The mechanisms that mediate the effects of probiotics on the immune system are not known. However, it was shown that probiotics stimulate several subsets of immune system cells to produce cytokines, which in turn play an important role in the regulation of the immune response (Kabir *et al.*, 2009). Haller *et al.* (2000) observed that gut microflora imbalance activated the intestinal mucosal immune system, causing an inflammatory reaction in the gut. The results of the present study suggest gut microflora imbalance and its effects on immune stimulation may increase the number of white blood cells. In the present study, the quails fed the diets containing probiotics has WBC compared with the control group. This could be due to their increased serum globulin level. Globulin serves as precursors of immunoglobulins (antibodies). The immune system of birds is composed of various cells and soluble elements (proteins) that need work together to create a protective immune response (Okuneye *et al.,* 2016).

Effect of probiotics on gut microbial population

As is shown in Table 7, all the probiotics fed in this study influenced (p<0.05) both *Lactobacillus* bacteria and *E. coli* populations in the cecal and small intestine content samples. The largest *Lactobacillus* bacterial population was observed in the cecal and small intestine contents of T6 birds (fed 150 g/ton native probiotics). Interestingly, the lowest *E. coli* populations were observed in the treatments containing different concentrations of the native probiotics (50, 150 and 200 g/ton), followed by T5 and commercial probiotics (CP1 and CP2). These results are in accordance with those of Kizerwetter-Swida & Binek (2009) who indicated that the *Lactobacillus salivarius* decreased the number of *Clostridium perfringens* and *Salmonella Enteritidis* in the group of chickens treated with *Lactobacillus*. Yu *et al.* (2007) evaluated the effects of probiotic *Lactobacillus reuteri* strain *Pg4* on the performance and intestinal characteristics of broilers, and reported that the *Lactobacillus* populations in the ileum, crop, and cecum of the broilers fed probiotics were higher than those in the control group. Other studies have also shown the potential of probiotics to populate the gut microflora of broiler chickens with beneficial bacteria and to supress the growth potentially pathogenic bacteria (Vicente *et al.*, 2008).

Table 7 – Effects of commercial and native probiotics on viable cell counts of microflora in the cecal and small intestine contents of Japanese quails

	Counts	
Treatment	*Lactobacillus* spp.	*E. coli*
Control (T_1)	2.14^{c}±0.495	1.36^{a}±0.039
CP1 (T_2)	5.42^{b}±0.428	1.28^{ab}±0.046
CP2 (T_3)	7.54^{a}±0.666	1.30^{ab}±0.039
NP 50g/ton (T_4)	7.83^{a}±0.789	1.14^{b}±0.058
NP 100g/ton (T_5)	5.26^{b}±0.400	1.27^{ab}±0.050
NP 150g/ton (T_6)	8.35^{a}±0.386	1.15^{b}±0.066
NP 200g/ton (T_7)	3.99^{b}±0.496	1.19^{b}±0.045
p-Value	0.0001	0.0355

Values are expressed as means ± standard error of the mean. $^{A-d}$ Values followed by different letters on the same row indicate significant differences (p<0.05). CP = commercial probiotic, NP = native probiotic.

CONCLUSION

It is concluded that the supplementation of the combination of four native *Lactobacillus* strains at a concentration of 150 g/ton feed effectively enhanced the growth performance (BWG and FCR) and blood biochemical parameters of Japanese quails during the starter, finisher, and overall rearing periods. The results show that the mixture of four *Lactobacillus* strains has a probiotic potential in Japanese quails.

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