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RESEARCH ARTICLE

Effects of Probiotic *(Lactobacillus farciminis)* Supplementation in Quail *(Coturnix coturnix japonica)* Rations on Growth Performance, Blood Antioxidant Capacity and Cecal Some Short-Chain Fatty Acid Concentrations

Özlem DURNA AYDIN [1,a (*)] Gültekin YILDIZ [2,b] Oğuz MERHAN [3,c]

[1] Kafkas University, Veterinary Faculty, Animal Nutrition and Nutritional Diseases Department, TR-36040 Kars - TURKEY
[2] Ankara University, Veterinary Faculty, Animal Nutrition and Nutritional Diseases Department, TR-06110 Ankara - TURKEY
[3] Kafkas University, Veterinary Faculty, Department of Biochemistry, TR-36040 Kars - TURKEY
ORCIDs: [a] 0000-0003-4532-6795; [b] 0000-0002- 1003- 9254; [c] 0000-0002-3399-0667

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Abstract

The purpose of this study was to investigate the effect of *Lactobacillus farciminis* supplementation in quail diets on performance, blood antioxidant capacity and the concentrations of cecal short-chain fatty acid (SCFA). A total of 180 day-old quail chicks were randomly divided into 3 groups each containing 60 chicks. Each group was randomly divided into 5 subgroups each containing 12 chicks. The chicks were fed with corn, soybean meal and full-fat soybean based rations for 35 days. While the control group was fed with basal ration, the experimental groups were fed with probiotic supplementation at 0.1 g/kg and 0.3 g/kg doses, respectively. At end of the experiment, the use probiotics in quails did not affect initial LW, final LW, LWG, FI and FCR. The increase in dietary probiotic, MDA, GSH, SOD, CAT and GPx exhibited a linear response. However, ceruloplasmin, albumin, total protein and globulin were not affected by the addition of probiotic. Significant linear responses in asetic acid, isocaproic acid and SCFA were observed with the gradued level of probiotic. However, a significant quadratic response in the caproic acid was observed. Therefore, the effective dose for caproic acid was 0.1 g/kg. There were no significant differences in acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and BCFA concentrations for quails fed with different levels of probiotic. In conclusion, diets containing *Lactobacillus farciminis* in quail can be used to improve the antioxidant capacity and intestinal health.

Keywords: *Lactobacillus farciminis, Growth performance, Antioxidant capacity, Cecal short-chain fatty acid, Japanese quail*

Bıldırcın *(Coturnix coturnix japonica)* Rasyonlarına Probiyotik *(Lactobacillus farciminis)* İlavesinin Büyüme Performansı, Kan Antioksidan Kapasite ve Sekal Bazı Kısa Zincirli Yağ Asidi Konsantrasyonları Üzerine Etkileri

Öz

Bu çalışmanın amacı, bıldırcın diyetlerinde *Lactobacillus farciminis* takviyesinin performans, kan antioksidan kapasitesi ve sekal bazı kısa zincirli yağ asidi (SCFA) konsantrasyonları üzerindeki etkisini araştırmaktır. Toplam 180 günlük bıldırcın civcivleri rastgele olarak her biri 60 civciv içeren 3 gruba ayrılmıştır. Her grup rastgele olarak her biri 12 civciv içeren 5 alt gruba ayrılmıştır. Tüm civcivler 35 gün boyunca mısır, soya fasulyesi ve tam yağlı soya fasulyesine dayalı rasyonla beslenmiştir. Kontrol grubu (C) bazal rasyonla beslenirken, deney gruplarına bazal rasyona ek olarak sırasıyla 0.1 g/kg (P1) ve 0.3 g/kg (P2) dozlarında probiyotik ilavesi yapılmıştır. Deneme sonunda, bıldırcınlarda probiyotik kullanımı başlangıç canlı ağırlık, bitiş canlı ağırlık, canlı ağırlık artışı, yem tüketimi ve yemden yararlanma oranını etkilememiştir. Rasyona probiyotik ilavesi ile MDA, GSH, SOD, CAT ve GPx'teki artış lineer bir cevap vermiştir. Fakat seruloplazmin, albümin, toplam protein ve globulin probiyotik ilavesinden etkilenmemiştir. Kademeli artan probiyotik seviyesinde asetik asit, izokaproik asit ve SCFA'da önemli lineer tepkiler gözlenmiştir. Fakat, kaproik asitte önemli bir kuadratik yanıt gözlenmiştir. Kaproik asit için etkin doz 0.1 g/kg'dır. Farklı seviyelerde probiyotikle beslenen bıldırcınlar için asetik asit, propiyonik asit, alıcı asit, izobutirik asit, valerik asit, izovalerik asit ve BCFA konsantrasyonlarında önemli bir fark görülmemiştir. Sonuç olarak, bıldırcınlarda *Lactobacillus farciminis* içeren rasyonları antioksidan kapasite ve bağırsak sağlığını iyileştirmek için kullanılabilir.

Anahtar sözcükler: *Büyüme performansı, Lactobacillus farciminis, Japon bıldırcını, Probiyotik, Sekum kısa zincirli yağ asitleri*

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(*) Corresponding Author

Tel: +90 474 242 6836
E-mail: odurna36@gmail.com (Ö. Durna Aydın)

Introduction

With advances in animal nutrition and biotechnology, various feed additives are now used in poultry feed in order to increase healthy animal production. Antibiotics used for growth promoters have been produced by selected microorganisms and the development of natural metabolites [1]. The prolonged use of antibiotics as growth factor in poultry has prevented the growth of pathogenic microorganisms in the digestive tract of animals as well as beneficial microorganisms. The use of antibiotics in feeds has promoted development of resistance aganist pathogenic bacteria. In addition, residues in animal products become a risk to human health [2]. For these negative reasons, the use of antibiotics as a growth factor in animal feeds have been prohibited. After this ban, probiotics, prebiotics, enzymes, organic acids and some products such as essential oils are started to be used as an alternative feed additives to antibiotics [3].

The term "probiotic" is derived from Latin preposition and pro ("for" or "supported") and the Greek word biotikos means "for life". Probiotics are described as living microbial feed supplements that beneficially affect the host animal by improving intestinal microbial balance. They have strengthened the immune system by increasing the level of antibodies in the digestive system [4]. In the recent research, the use of probiotics as feed additives in Japanese quails was observed to improve feed bioavailability, health and immune status [5]. Probiotic bacteria have significant antioxidant abilities both *in vivo* and *in vitro* [6]. With the use of *Lactobacillus* spp. supplement for manipulation of secum fermentation in broiler, may increase some short chain fatty acid accumulation in the cecum and thus improve gastrointestinal system development and prevent disease [7]. Using *Lactobacillus acidophilus* and *L. casei* as probiotics in broiler rations may affect growth performance positively [8].

The aim of this study was to investigate the effect of probiotic in Japanese quail diets on growth performance, some blood parameters, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), malon-dialdehyde (MDA) and glutathione (GSH), ceruloplasmin, albumin, total protein, globulin and cecal short-chain fatty acid (SCFA) concentrations.

Material and Methods

Animals, Experimental Design and Feed

The study was carried out with the permission of the Kafkas University Animal Experiments Local Ethics Committee (Decision No: KAU-HAYDEK/2019-055) report. A total of 180 one-day-old Japanese quails *(Coturnix coturnix japonica)* were included in the study regardless of gender. All chicks were randomly divided into 3 groups each containing 60 chicks. Each group was then randomly divided into 5 subgroups each containing 12 chicks. The animals were fed with corn, soybean meal basal ration and trial continued for 35 days *(Table 1)*. All diets were determined according to NRC [9] standards. Nutrient analyses (dry matter, crude protein, crude fibre, ether extract, ash, Ca and total P) of the feed were performed according to AOAC [10]. Birds were caged in breeding cages. Each subgroup was equipped with manual feeders and automatic nipple drinkers. Water and feed were given *ad libitum*. The house temperature was monitored thermostatically throughout the study. The temperature, which was 32-35°C on the first day, was gradually lowered and maintained at 22°C for the last two weeks. Artificial light program was implemented in accordance with commercial conditions (23 h of lighting throughout the experiment per day). The experimental diets were as follows: C, basal diet (Control; without addition probiotic); P1; 0.1 g/kg probiotic and P2; 0.3 g/kg probiotic. Probiotic (Biacton+®) used in the study were supplied from a commercial company (TARIMSAN CHEMICAL A.Ş. Istanbul, Turkey). Composition of probiotic used in the study has contained *Lactobacillus farciminis* $5x10^9$ CFU/g. In our study, we determined the probiotic doses of the groups by considering the doses recommended by the commercial product.

Table 1. *Composition of basal diets used in experiment (%)[1]*

Feed Materials	**%**
Corn	56.35
Soyben meal	36.10
Corn gluten (CP, 60%)	4.35
Limestone	1.45
Dicalciumphosphate	1.00
DL- Methionine	0.08
L-Lysine Hydrochloride	0.07
Vitamin- mineral premix	0.40
Salt	0.20
Total	100.00
The Calculated Value	
Crude protein, %	23.00
ME (kcal/kg)	2909.33
Ca, %	0.90
Total P, %	0.59
Analysis Values	
ME (kcal/kg)	2915.25
Crude protein, %	23.11
Ca, %	1.01
Total P, %	0.49

[1] As-fed basis; [2] Vitamin-mineral premix provided per kg diet: Vit. A 8.000 IU, Vit. D_3 1.000 IU, Vit. E 20 IU, Vit. K 0.5 mg, Vit. B_1 3 mg, Vit. B_2 9 mg, Vit. B_6 7 mg, Vit. B_{12} 0.03 mg, niacin 35 mg, D-pantothenic acid 10 mg, folic acid 0.55 mg, biotin 0.18 mg, Fe 100 mg, Cu 8 mg, Zn 100 mg, Mn 120 mg, I 0.7 mg, and Se 0.3 mg

Growth Performance

In the study, live weights (LW) were recorded weekly for each subgroups. Live weight gain (LWG) was determined by the difference between following weeks. Each subgroup's feed intake (FI) was recorded weekly and used for the calculation of feed conversion ratio (FCR).

Blood Antioxidant Capacity

At the end of the experiment, blood samples were taken from wing vein of the animals to the anticoagulant (EDTA) tubes. Plasma samples were taken after centrifugation of the blood samples at 3000 rpm for 15 min and stored at -20°C until the analyses were carried out. SOD, GPx and CAT antioxidant enzyme activities in plasma were determined by ELISA device (Epoch, Biotek, USA) using commercial kits (Cayman Chemical Company, USA). Whole blood reduced GSH analysis was determined colorimetrically (Epoch, Biotek, USA) according to the method of Beutler et al.[11]. MDA in plasma was determined by the method of Yoshoiko et al.[12], ceruloplasmin by the method of Colombo and Ricterich [13], and albumin and total protein levels by a commercial test kit (Biolabo, Maizy, France). The globulin value was determined by subtraction of the albumin from the total protein [14].

Cecal Short-Chain Fatty Acid Concentrations

The cecal digesta that were obtained after sacrification of the animals was used for the determination acetic, propionic, butyric, isobutyric, valeric and caproic acid with a gas chromatography (GC) (Shimadzu GC, Shimadzu Co., Kyoto, Japan), a flame ionization detector (FID) and colons (Teknokroma; TR-151035, TRB-FFAP 30m×0.53 mm×0.50 µm). At the end of the study, the cecum content were stored at -18°C and then were dissolved at + 4°C before analysis. The contents were centrifuged at 4000 rpm for 15 min at +4°C for homogenization. The supernatant was taken into a 750 µL Eppendorf tube and mixed with 150 µL ice-cold 25% metaphosphoric acid solution. After that, the tubes were kept in ice for 30 min to ensure the collapse of proteins. Subsequently, tubes were centrifuged for 10 min at 10000 rpm at +4°C. Supernatants were analyzed using GC. The analysis was performed according to Zhang et al.[15]. Helium was used for the carrier gas and the colon temperature was programmed so that it was increased stepwise from 110°C to 180°C. Also, the FID (Flame Ionization Detector) and injector block temperature was set to 250°C. The calibration curve was drawn with the Supelco Volatile Free Acid Mix, 46975-U (10 mmol/L) as a standard curve.

Statistical Analysis

The one-way analysis of varience (ANOVA) method was used for the statistical calculations of the groups and polynomial contrast test was used to determine the dose effect of the probiotic used at different levels in the groups. Statistical differences and trend analysis were considered significant at P≤0.05. The statistical analysis was done with the SPSS software package [16].

Results

The increase in probiotic did not affect initial LW, final LW, LWG, FI and FCR. The effect of probiotic in quail rations on growth performance is presented in *Table 2*.

The increase in dietary probiotic exhibited linear response (linear, P=0.000, for all except GPx; P=0.001) with MDA, GSH, SOD, CAT and GPx. However, ceruloplasmin, albumin, total protein and globulin were not affected by the addition of probiotic. Influence of probiotic on antioxidant capacity is given in *Table 3*.

The effect of the addition of probiotic in quail rations on some cecal short-chain fatty acid concentrations (µmol/g) is given in *Table 4*. Asetic acid (P=0.021), isocaproic acid (P=0.001) and SCFA (P=0.015) were linearly affected with the gradued level of probiotic. However, a significant quadratic effect in the caproic acid (P=0.003) was observed. Therefore, the effective dose for caproic acid was 0.1 g/kg. There were no significant differences in acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and BCFA concentrations for quails fed different levels of probiotic.

Table 2. *The effect of probiotic in quail rations on growth performance*

Performance Parameters	Groups			Significance		
	Control	P1	P2			
	X±Sx	X±Sx	X±Sx	L	Q	Main Effect
Initial Live Weight, g	13.61±0.16	13.27±0.29	12.77±0.41	0.079	0.835	0.196
Final Live Weight, g	221.36±1.47	207.74±8.79	216.27±3.16	0.523	0.124	0.245
Live Weight Gain, g	207.75±1.40	194.46±9.08	203.49±3.18	0.602	0.131	0.271
Feed Intake, g	651.33±33.80	609.97±29.66	612.54±17.55	0.345	0.532	0.520
Feed Conversion Ratio	3.13±0.16	3.14±0.11	3.00±0.05	0.468	0.625	0.625

[1] The mean (x) and standard error (Sx) values of 5 replicates in each group; [2] C: Control basal ratio, P1: 0.1 g/kg probiotic added to basal ratio and P2: 0.3 g/kg probiotic added to basal ratio; [3] Polynomial contrasts: L = linear and Q = quadratic effect of supplemental probiotic

Table 3. *Influence of probiotic on antioxidant capacity*

Blood Parameters	Groups			Significance		
	Control	P1	P2			
	X±Sx	X±Sx	X±Sx	L	Q	Main Effect
MDA (µmol/L)	7.20±0.01	7.04±0.03	6.97±0.02	0.000	0.151	0.000
GSH (mg/dL)	15.26±0.10	23.68±0.87	37.12±1.54	0.000	0.068	0.000
SOD (U/mL)	22.39±1.23	29.21±1.00	37.35±1.12	0.000	0.641	0.000
CAT (nmol/min/mL)	3.36±0.04	3.28±0.02	3.16±0.01	0.000	0.616	0.002
GPx (nmol/min/mL)	33.23±0.51	37.55±1.36	39.74±0.87	0.001	0.395	0.002
Ceruloplasmin (mg/dL)	16.83±0.32	16.91±0.64	16.94±0.28	0.872	0.960	0.985
Albumin (g/dL)	2.34±0.06	2.31±0.02	2.29±0.03	0.517	0.864	0.791
Total protein (g/dL)	3.14±0.01	3.13±0.03	3.12±0.03	0.670	0.978	0.909
Globulin (g/dL)	0.79±0.06	0.82±0.04	0.82±0.02	0.716	0.859	0.918

[1] The mean (x) and standard error (Sx) values of 5 replicates in each group; [2] C: Control basal ratio, P1: 0.1 g/kg probiotic added to basal ratio and P2: 0.3 g/kg probiotic added to basal ratio; [3] Polynomial contrasts: L = linear and Q = quadratic effect of supplemental probiotic

Table 4. *The effect of the addition of probiotic in quail rations on cecal some short-chain fatty acid concentrations (µmol/g)*

SCFA Parameters	Groups			Significance		
	Control	P1	P2			
	X±Sx	X±Sx	X±Sx	L	Q	Main Effect
Acetic acid	32.73±5.57	51.99±7.76	56.01±4.98	0.021	0.338	0.047
Propionic acid	7.57±2.63	12.30±3.34	9.22±2.68	0.696	0.294	0.525
Isobutyric acid	0.42±0.04	0.52±0.11	0.44±0.06	0.881	0.369	0.650
Butyric acid	4.98±0.92	6.06±1.12	6.32±1.83	0.498	0.810	0.765
Valeric acid	0.80±0.13	0.99±0.15	0.93±0.00	0.486	0.437	0.574
Isovaleric acid	1.91±0.06	1.98±0.21	2.03±0.00	0.545	0.970	0.825
Caproic acid	0.15±0.00	0.21±0.01	0.16±0.00	0.434	0.003	0.008
Isocaproic acid	1.62±0.01	1.69±0.02	1.73±0.00	0.001	0.419	0.002
BCFA	3.13±0.20	3.49±0.28	3.39±0.11	0.408	0.397	0.492
Total SCFA	48.43±8.03	73.85±5.91	74.95±5.73	0.015	0.161	0.025

[1] The mean (x) and standard error (Sx) values of 5 replicates in each group; [2] C: Control basal ratio, P1: 0.1 g/kg probiotic added to basal ratio and P2: 0.3 g/kg probiotic added to basal ratio; [3] Polynomial contrasts: L = linear and Q = quadratic effect of supplemental probiotic

Discussion

Probiotics modulate the intestinal microbiota by reducing the number of pathogenic microorganisms, increasing the number of beneficial bacteria and nutrient absorption [17]. With the use of *Lactobacillus acidophilus* and *Bacillus subtilis* in laying hens, there is an improvement in performance, an increase in antibody production, and a decrease in blood triglyceride and cholesterol levels [18]. The addition of *Lactobacillus plantarum* in broiler chickens caused an increase in body weight, feed consumption and feed conversion rate, a decrease in the number of fecal coliforms and an increase in the number of fecal *Lactobacillus* [19]. In our study, it was observed that the use of probiotics in quails did not affect performance parameters. However, high doses are affected more than low doses, numerically. There have been many studies that are compatible with our study [20]. In the study of Huang et al.[21] they reported that the use of *Bacillus subtilis* did not affect growth performance. Another study reported that while the LWG value increased in the use of probiotics in quail diets, the FI value was not affected [18]. On the other hand, in the study of Hosseini et al.[22], they reported that the use of probiotics positively affected performance. In a study using *Lactobacillus* strains at doses of 50, 100, 150, and 200 g/ton in quail diets, the results showed that probiotic supplementation improved LWG values [23]. Jazi et al.[24], reported that feeding quails with *Bacillus subtilis* improved growth performance. In another study that used probiotics in quail with doses of 100, 150, 200 and 250 mg/kg, improved growth performance and increased feed efficiency [25]. The difference in among the results obtained

in these studies can be explained by the dose, quality, and chemical composition of the probiotic and the difference in maintenance and feeding conditions.

Probiotics have their own antioxidant enzymatic systems [26]. The culture supernatant, intact cells, and intracellular cell-free extracts of *Bifidobacterium animalis* were found to clear up hydroxyl radicals and superoxide anion as *in vitro* by improving the antioxidase activities [27]. In our study, MDA, GSH SOD, CAT and GPx values in blood plasma were linearly affected by the addition of probiotic in quail rations. Ceruloplasmin, albumin, total protein and globulin values in blood plasma among groups were not affected by the addition of probiotic in quail diets. There have been many studies that are compatible with our study [24]. In a study in pigs, the addition of *Lactobacillus fermentum* increased serum SOD, GPx and hepatic CAT and muscle SOD [28]. In another study, the use of probiotics at different doses increased in GPx activity in chicks [29]. Abdel-Moneim et al.[18] reported that serum total protein, albumin GSH, and CAT increased while MDA decreased in due to probiotics added to Japanese quail rations. In addition, probiotics were observed to promote antioxidant enzymatic activities (eg, SOD, GPx and total antioxidant status of host [30]. On the other hand, Jazi et al.[24] reported that SOD and GSH-Px activities, and MDA content in breast muscle, were not affected by the addition of *Bacilius subtilis* in quail diets. These results are consistent with our findings except for MDA. The obtained results may be influenced by dose and content differences of the probiotics used in the study and environmental conditions such as housing.

Probiotics, that can colonize the intestinal tract, have been shown to have effects on metabolic diseases such as obesity and diabetes by modulating the intestinal micro-organisms [31]. SCFA is formed as a result of bacterial fermentation in the cecum which is needded for metabolism of the intestinal epithelial cells. They stimulate cell growth and differentiation in the intestine, improving intestinal integrity, as well as reducing the digestive tract pH and preventing the growth of pathogenic micro-organisms [32]. There are a limited number of studies that have focused on the use of the probiotic on the examination of the parameters related to the concentration of SCFA in the cecum, while there is no study investigating this parameter in quail. In our study, asetic acid, isocaproic acid and SCFA were linearly affected while caproic acid were quadratically affected by the addition of probiotic in quail rations. There were no significant differences in acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and BCFA concentrations for quails fed different amounts of probiotic. Morever, there are many studies that are compatible with our study. Researchers have reported a correlation between the secum microflora composition and the SCFA concentration [7]. Increased SCFA concentrations have been shown to have beneficial effects on energy, metabolism, microflora and immune responses [33]. Weng et al.[34] reported that in the study of correlation of variables on metabolic and microbiota with diet in case of intestinal inflammation, the value of isocaproic acid decreased. Decrease in isocaproic acid value shows that acidity in the intestine is impaired and pathogenic microorganisms are active in inflammation. In the light of these studies, the increase in secum short chain fatty acids can be interpreted as having a positive effect on gut health. In a study in pigs revealed that the usage of lactulose in the diet increased the SCFA concentration in the large intestine [35]. On the other hand, cecal fermentation was influenced by the use of probiotic in broiler diets in a study. Morever, the addition of *Bacillus licheniformis* was decreased in cecal concentrations of propionic, butyric, n-butyric and n-valeric acids [36]. The difference in among the results obtained can be explained with the dose, quality, and chemical composition of the probiotic and maintenance and feeding conditions.

In conclusion, increased dietary addition of probiotics did not affect performance. MDA, GSH, SOD, CAT and GPx exhibited a linear response. However, ceruloplasmin, albumin, total protein and globulin were not affected by the addition of probiotic. Significant linear responses in asetic acid, isocaproic acid and SCFA were observed with the gradued level of probiotic. However, a significant quadratic response in the caproic acid was observed.

Therefore, the effective dose for caproic acid was 0.1 g/kg. There were no significant differences in acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and BCFA concentrations for quails fed with different levels of probiotic. Therefore, blood antioxidant capacity and cecal SCFA results; it has been effective in protecting quail oxidative stress and improving intestinal health.

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Author Contributions

ÖDA conceived and supervised the study. GY, ÖDA and OM collected and analyzed data. ÖDA wrote the first draft of manuscript. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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