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# THE INVESTIGATION OF DIFFERENT PATTERNS OF IN-FEED OR IN-WATER PROBIOTICS ADMINISTRATION METHODS ON PERFORMANCE, SMALL INTESTINAL MORPHOLOGY AND SRBC-REACTIVE IMMUNE RESPONSES OF JAPANESE QUAIL

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Key words: Japanese quail, probiotic, administration methods, performance.

Abstract

The aim of the current study was to investigate whether continuous consumption of probiotics is advantageous over intermittent consumption. A total of 336 1-d-old Japanese quail chicks were randomly divided into seven experimental groups and administered probiotics throughout the experiment in different manners via, *A* – no probiotics administered (control group), *B* – probiotics fed continuously, *C* – probiotics fed for 2 d on & 2 d off, *D* – probiotics fed for 1 d on & 4 d off, *E* – probiotics in drinking water throughout the experiment, *F* – probiotics in drinking water for 2 d on & 2 d off, *G* – probiotics in drinking water for 1 d on & 4 d off. Administration of probiotic as feed additive significantly increased body weight gain ($P < 0.01$). Feed intake was lower ($P < 0.01$) in group F compared with other groups. The birds in groups *C*, *D* and *G* had the lowest feed conversion ratio ($P < 0.01$). In comparison with control quails, ileum length and duodenum and ileum villus was higher in probiotic-received birds ($P < 0.01$). Crypt depth was increased ($P < 0.01$) by probiotics treatments. Number of goblet cells of duodenum and ileum increased in groups *B*, *C*, *E* and *F* ($P < 0.01$). There were no significant differences in heterophil: lymphocyte ratio among the groups. Consumption of probiotics increased the blood serum total immunoglobin ($P < 0.01$), IgM ($P < 0.05$) and IgY ($P < 0.01$) levels. It was concluded that administration of probiotic either in feed or in water improved the quail's performance and immunity. Regarding advantages of administration of probiotics in drinking water this method is recommended in quail production system.

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# BADANIE WPŁYWU RÓŻNYCH METOD PODAWANIA PROBIOTYKÓW W PASZY LUB W WODZIE NA WYDAJNOŚĆ I MORFOLOGIĘ JELITA CIENKIEGO ORAZ SRBC REAKCYJNE ODPOWIEDZI ODPORNOŚCIOWEJ U PRZEPIÓREK JAPOŃSKICH

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Słowa kluczowe: przepiórka japońska, probiotyki, metody podawania probiotyków, wydajność.

Abstract

Celem pracy było sprawdzenie, czy ciągłe spożywanie probiotyków przez przepiórki japońskie jest korzystne w porównaniu z okresowym ich podawaniem. Grupę składającą się z 336 jednodniowych przepiórek japońskich podzielono losowo na siedem grup eksperymentalnych, którym w różny sposób podawano probiotyki w czasie trwania eksperymentu: *A* – brak podaży probiotyków (grupa kontrolna), *B* – probiotyki podawane w paszy stale, *C* – probiotyki podawane w paszy przez 2 dni oraz brak podaży przez 2 kolejne dni, *D* – probiotyki podawane w paszy przez 1 dzień oraz brak podaży przez 4 kolejne dni, *E* – probiotyki podawane w wodzie pitnej stale, *F* – probiotyki podawane w wodzie pitnej przez 2 dni oraz brak podaży przez kolejne 2 dni, *G* – probiotyki podawane w wodzie pitnej przez 1 dzień oraz brak podaży przez 4 kolejne dni. Stosowanie probiotyków jako dodatków paszowych znacząco wspomaga przyrosty masy ciała ($P < 0,01$). Spożycie paszy było niższe ($P < 0,01$) w grupie *F* w porównaniu z pozostałymi grupami. Ptaki z grup *C*, *D* i *G* wykazywały najniższe wskaźniki wykorzystania paszy ($P < 0,01$). W porównaniu z grupą kontrolną długości jelita biodrowego i dwunastnicy, a także kosmków w jelicie biodrowym były wyższe u ptaków, u których stosowano probiotyki ($P < 0,01$). Zastosowanie probiotyków skutkowało wzrostem głębokości krypt jelitowych ($P < 0,01$). Liczba komórek kubkowych w dwunastnicy i w jelicie biodrowym była wyższa w grupach *B*, *C*, *E* i *F* ($P < 0,01$). Nie zaobserwowano w badanych grupach znaczących różnic w stosunku heterofilii do limfocytów. Spożycie probiotyku przez przepiórki skutkowało wzrostem poziomu całościowej puli immunoglobulin ($P < 0,01$), IgM ($P < 0,05$) i IgY ($P < 0,01$) w surowicy krwi. Stosowanie u przepiórek probiotyków – zarówno w paszy, jak i wodzie pitnej – miało korzystny wpływ na wydajność i odporność ptaków. Biorąc pod uwagę zalety podawania probiotyków przepiórkom w wodzie pitnej, należy stwierdzić, że jest to zalecany system podawania u omawianego gatunku ptaków.

## Introduction

Commercial poultry are reared under the stress of genetic selection for high performance, therefore, exogenous opportunistic bacteria or those that inhabit in bird's gastrointestinal tract such as *E. coli* could be pathogen in specific environmental situation. Sub-therapeutic doses of antibiotics in poultry diet are used as growth promoter for controlling bacterial population in gastrointestinal tract. Concerns about undesirable side

effects of growth promoter antibiotics, such as toxicity, allergy, cancer, drug resistance and retention in food (ARSLAN 2004, CAKIR et al. 2008) resulted in a global prohibition of feed additive antibiotics. Public pressures to reduce use of antimicrobial substances and consumer's tendency to organic products have influenced the development of alternative feed additives such as probiotics (HIGGINS et al. 2008).

Probiotics are beneficial bacteria that influence the host by improving intestinal health (FULLER 1989). Probiotics have been known to exert their beneficial effects by a variety of mechanisms including: competitive exclusion, immunomodulation, decrease of pH, production of anti-microbial substances, production of some enzymes and increase villus surface area, which makes them a *multi-purpose* feed additive. Supplementation of poultry feed with probiotics (or competitive exclusions) has been developed in order to encourage a protective barrier of bacteria in their digestive tract and prevent the colonization of growth-depressing or pathogenic microorganisms (GRIMES et al. 2008). Many researchers have obtained positive significant effects of using probiotics in broiler chickens (KALBANE et al. 1992, ECKERT et al. 2010, KARIMI-TORSHIZI et al. 2010), turkey (GRIMES et al. 2008, RAHIMI et al. 2011), gees (YAMAN et al. 2006) and quail (HOMMA and SHINOHARA 2004, VRANIC et al. 2006).

Probiotics are living organisms; therefore, their proliferation in digestive tract may guarantee their presence in adequate numbers over the lifetime. Thus the continuous supplementation of probiotics might not have more beneficial effects rather than intermittent supplementation of them. To assess this hypothesis, the present experiment was designed to investigate the effects of continuous or two intermittent administration patterns of probiotics in feed or drinking water upon the performance, small intestinal morphology and SRBC-reactive immune responses of Japanese quail.

## Materials and Methods

### Animal, management and experimental groups

Three hundred and thirty six 1-d-old (unsexed) Japanese quail (*Coturnix japonica*) chicks were randomly assigned into seven experimental groups with four replicates of 12 birds each. All the groups were maintained under similar management, nutritional and environmental conditions. Birds in each experimental unit were placed in a cage (wire floor – 45 × 40 × 30 cm) furnished with an electrical bulb to provide continuous lighting and

age-appropriate supplemental heat controlled by an electrical dimmer. Temperature was maintained at 35ºC at the arrival of chicks for the initial three days and then gradually reduced 2.5ºC per week until a temperature of 22ºC was achieved. The study protocol was conducted in accordance with the Animal Care and Use Review Committee guidelines of Tarbiat Modares University, Tehran, Iran. The probiotic treated groups were offered a water dispersible probiotic (Protexin, Somerset, UK) within 24 h after hatch, continuously or intermittently either in feed or drinking water till the end of the experiment. The duration of the experiment was 35 days. The seven experimental groups were (Table 1).

Table 1

Continuous and intermittent patterns of probiotic administration in feed and drinking water

| Treatments | Day | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
| *A* | – | – | – | – | – | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *B* | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f | f |
| *C* | f | f | - | - | f | f | - | - | f | f | - | - | f | f | - | - | f | f | - | - | f | f | - | - | f | f | - | - | f | f | - | - | f | f | - |
| *D* | f | - | - | - | - | f | - | - | - | - | f | - | - | - | - | f | - | - | - | - | f | - | - | - | - | f | - | - | - | - | f | - | - | - | - |
| *E* | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w | w |
| *F* | w | w | - | - | w | w | - | - | w | w | - | - | w | w | - | - | w | w | - | - | w | w | - | - | w | w | - | - | w | w | - | - | w | w | - |
| *G* | w | - | - | - | - | w | - | - | - | - | w | - | - | - | - | w | - | - | - | - | w | - | - | - | - | w | - | - | - | - | w | - | - | - | - |

f – probiotics supplemented in feed
w – probiotics supplemented in drinking water
*A* – no probiotics administered (control group),
*B* – probiotics continuously in feed through out the experiment ($F_1$),
*C* – probiotics in feed in a pattern of 2 d on -2 d off ($F_2$),
*D* – probiotics in feed in a pattern of 1 d on -4 d off ($F_3$),
*E* – probiotics in drinking water continuously throughout the experiment ($W_1$),
*F* – probiotics in drinking water in a pattern of 2 d on -2 d off ($W_2$),
*G* – probiotics in drinking water in a pattern of 1 d on -4 d off ($W_3$).

The in-feed probiotics groups received 100 or 150 g ton$^{-1}$ probiotics during d 1 to 14 and d 15 to 35, respectively. The drinking water groups received half the amount of probiotics which was supplemented in feed because water intake was assumed two-fold higher than feed intake. For in-water groups, drinking water was measured every 12 hours and replaced by fresh supplemented water since viability of microorganisms might lose after 12 hours of addition in the water.

## Diets and probiotic preparation

Experimental diets were isocaloric and isonitrogenous, based on corn--soybean meal to meet or exceed NRC (1994) specifications for Japanese quail (Table 2). Each cage was equipped with a nipple drinker and a feeder. All birds had *ad libitum* access to water and feed.

Table 2

Composition of the basal diets

| Item | 1–35 d |
|---|---|
| Ingredient [%] | |
| Yellow corn | 42.32 |
| Soybean meal [44% CP] | 40.20 |
| Vegetable oil | 7.48 |
| Fish meal [65% CP] | 7.30 |
| $CaCO_3$ | 1.21 |
| Di-calcium phosphate | 0.01 |
| Sodium chloride | 0.28 |
| Mineral and vitamin premix * | 0.50 |
| DL-Methionine | 0.03 |
| Washed sand | 0.67 |
| Total | 100 |
| Calculated value** | |
| ME [kcal $kg^{-1}$] | 3130 |
| CP [%] | 25.90 |
| Lys [%] | 1.40 |
| Met + Cys [%] | 0.81 |
| Calcium [%] | 0.86 |
| Nonphytate phosphorus [%] | 0.32 |

* Supplied the following per kilogram of diet: retinyl acetate – 9,000 IU; cholecalciferol – 2,000 IU; DL-*a*-tocopheryl acetate – 12.5 IU; menadione sodium bisulfite – 1.76 mg; biotin – 0.12 mg; thiamine – 1.2 mg; riboflavin – 3.2 mg; calcium D-pantothenate – 6.4 mg; pyridoxine – 1.97 mg; nicotinic acid – 28 mg; cyanocobalamine – 0.01 mg; choline chloride – 320 mg; folic acid – 0.38 mg; $MnSO_4.H_2O$ – 60 mg; $FeSO_4$ .$7H_2O$ – 80 mg; ZnO – 51.74 mg; $CuSO_4$ .$5H_2O$ – 8 mg; Iodized NaCl – 0.8 mg; $Na_2SeO_3$ – 0.2 mg.
** Calculated from NRC (1994).

The probiotic supplement, Protexin (Protexin, Somerset, UK) used in this study contained $2 \cdot 10^9$ cfu $g^{-1}$ of *Aspergillus oryzae* PXN 68, *Lactobacillus acidophilus* PXN 35, *L. rhamnosus* PXN 54, *L. plantarum* PXN 47, *L. bulgaricus* PXN *39, Bifidobacterium bifidum* PXN 23, *Enterococcus faecium* PXN 33, *Streptococcus thermophilus* PXN 66 and *Candida pintolope-*

*sii* PXN 70. Probiotic was supplemented in feed (0.1 g $kg^{-1}$) or drinking water (0.05 g $l^{-1}$). Probiotic suspensions were prepared in sterile phosphate buffered saline directly before administration.

## Data collection

Performance: body weight (BW) and feed intake (FI) were recorded for d 1–14 and d 15–35 then body weight gain (BWG) and feed conversion ratio (FCR) were calculated.

## Small intestinal morphometric assay

The birds were killed by severing the cervical vessels at d 35 and tissues collected accordingly. Segments (approximately two cm) taken from the midpoint of duodenum and ileum were gently flushed twice with phosphate buffer saline and were fixed in fresh 10% formalin. All samples were dehydrated, cleared, and embedded in paraffin. Sections of five µm thickness placed on glass slides were stained using eosin-haematoxylin-alcian blue and periodic acid-Schiff which manifest acidic mucin producer and neutral mucin producer goblet cells, respectively (KIERNAN 2008). Villus height, crypt depth, number of goblet cells (acidic mucin producer and neutral mucin producer) along 100 µm of villus length was determined under light microscope (Carl ZEISS standard 20, Germany). The results of the morphometric determinations were from at least ten well-oriented crypt villus structures from each chick. The measurements were done using DinoCapture software (Dino-lite, Ver. 3.3.0.0, Korea).

**Immune responses**. Two male birds per cage were immunized by intramuscular injection of 0.2 ml of sheep red blood cells (SRBC) suspension in PBS (5% v/v) on d 11. Blood samples were drawn 7 days following the SRBC injection. Anti-SRBC antibodies were tittered before and after 2-mercaptoethanol (ME) treatment to further assess the total immunoglobulin (IgT), ME sensitive (IgM) and ME resistant (IgY) titres. Antibody titres were reported as $\log_2$ of the reciprocal of the last dilution at which complete agglutination was observed (QURESHI and HAVENSTEIN 1994).

Heterophil to lymphocyte ratio (H:L): Blood smears were prepared from two male birds per cage on d 35 to obtain H:L. Specimens were stained by Wright's stain (LUCAS and JAMROZ 1961). Total of 100 white blood cells including heterophils and lymphocytes were counted differentially and the H:L ratio was calculated by dividing the total number of heterophils by the total number of lymphocytes.

## Statistical analysis

Data were analysed by one-way ANOVA using the GLM procedure of Statistical Analysis System (SAS Institute Inc). Statements of statistical significance were based on $P \leq 0.05$ or lower (STEEL and TORRIE 1980). Duncan's multiple range comparison tests were used to examine significant differences between treatment means.

## Results and Discussion

The effects of different probiotics administration methods and frequencies on BWG, FI and FCR are presented in Table 3. In d 1–14 only the group $F_1$ which received probiotics continuously in feed showed higher BWG than control ($P < 0.01$), however the different frequencies in each method (i.e. in-feed or in-water methods) were not different among the relevant method. In d 15–35 the different frequencies of each method did not show significant differences in BWG as well. In the whole period (d 1–35) supplementation of probiotics in feed resulted in higher BWG ($P < 0.01$).

Table 3

Effects of probiotic administration methods and consumption frequency on BW, BW gain and FCR of Japanese quail*

| | BWG [g] | | | FI [g] | | | FCR | | |
|---|---|---|---|---|---|---|---|---|---|
| Days | 1–14 | 15–35 | 1–35 | 1–14 | 15–35 | 1–35 | 1–14 | 15–35 | 1–35 |
| Control | $73.88^{b}$ | $142.54^{abc}$ | $216.42^{c}$ | $110.92^{c}$ | $576.07^{a}$ | $686.99^{a}$ | $1.50^{ab}$ | $4.04^{a}$ | $3.17^{a}$ |
| $F_1$ | $80.93^{a}$ | $151.85^{a}$ | $232.79^{a}$ | $128.26^{a}$ | $548.22^{bc}$ | $676.49^{ab}$ | $1.58^{a}$ | $3.61^{bc}$ | $2.91^{bc}$ |
| $F_2$ | $79.37^{ab}$ | $149.92^{ab}$ | $229.29^{ab}$ | $115.79^{bc}$ | $536.92^{c}$ | $652.71^{bc}$ | $1.46^{ab}$ | $3.58^{c}$ | $2.85^{c}$ |
| $F_3$ | $78.59^{ab}$ | $144.43^{abc}$ | $223.03^{ab}$ | $110.42^{c}$ | $525.68^{cd}$ | $636.11^{cd}$ | $1.40^{b}$ | $3.65^{bc}$ | $2.85^{c}$ |
| $W_1$ | $73.32^{b}$ | $148.62^{abc}$ | $221.93^{c}$ | $112.41^{bc}$ | $563.93^{ab}$ | $676.35^{ab}$ | $1.54^{ab}$ | $3.80^{abc}$ | $3.05^{ab}$ |
| $W_2$ | $78.18^{ab}$ | $138.17^{c}$ | $216.35^{c}$ | $120.45^{ab}$ | $537.65^{c}$ | $658.10^{bc}$ | $1.54^{ab}$ | $3.89^{ab}$ | $3.04^{ab}$ |
| $W_3$ | $74.53^{b}$ | $139.72^{bc}$ | $214.25^{c}$ | $114.96^{bc}$ | $510.81^{d}$ | $625.77^{d}$ | $1.54^{a}$ | $3.66^{bc}$ | $2.92^{bc}$ |
| *P*-value | 0.004 | 0.006 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0107 | 0.0008 | 0.0001 |
| SEM | 0.711 | 1.265 | 1.429 | 1.333 | 4.418 | 4.495 | 0.015 | 0.038 | 0.024 |

$^{a–c}$ Means with different superscripts in the same column differ ($P < 0.05$ or lower).
BVG – body weight gain, FI – feed intake, FCR – feed conversion ratio.
Control: no probiotics administered; $F_1$ – probiotics continuously in feed throughout the experiment; $F_2$ – probiotics in feed in a pattern of 2 d on -2 d off; $F_3$ – probiotics in feed in a pattern of 1 d on -4 d off; $W_1$ – probiotics in drinking water continuously throughout the experiment; $W_2$ – probiotics in drinking water in a pattern of 2 d on -2 d off; $W_3$ – probiotics in drinking water in a pattern of 1 d on -4 d off.
* Mean represent 4 pens per treatment.

The quail supplemented via feed received less probiotics than those supplemented in drinking water, because half the amount of probiotics in feed was added to water, considering the assumption of water intake is approximately two fold of feed intake. The average water: feed ratio were 3.484, 2.443 or 2.618 for starter (d 1–14), grower (d 15–35) or total period (d 1–35), respectively, showing that a higher dose of probiotics in drinking water was required. Previously, KARIMI-TORSHIZI et al. (2010) had reported higher BWG in broiler chicken consumed probiotics through feed or drinking water than untreated control birds. In another study MASTBAUM et al. (1997) also confirmed that probiotics administration in feed or in water significantly increased live weight gain and feed conversion efficiency in broilers. The higher BWG which observed in the present study through probiotic supplementation in feed is in agreement with CHIMOTE et al. (2009) who found that supplementation of Japanese quails' feed with probiotics improved BWG. However, KARIMI-TORSHIZI et al. (2010) in broilers found out probiotic supplementation in drinking water was better than feed. Meanwhile, in the present study, despite the higher amount of probiotics consumed by probiotic drank birds, frequencies of in-water method showed lower BWG than in-feed counterparts.

Probiotics treatment in feed or in water resulted in significantly lower d 1–35 FI than un-treated control group ($P < 0.01$); however, the groups consumed probiotics in feed or in water continuously ($F_1$ and $W_1$) were not significantly different from the control. Findings of d 1–14 and d 15–35 FI looks surprising such that in the former all probiotics-consumed groups consumed more feed than control, whereas in the later, untreated control birds had the highest FI. The more feed intake observed in the probiotics-treated birds than control in the early part of the present study (d 1–14) may be due to the earlier establishment of gut microflora in those birds. Probiotic microorganisms can optimize intestinal flora and hence trigger the symbiotic effect of host animal's enzymes helping to improve the nutrients digestibility. Increased digestibility can lead to faster digest a passage along intestine resulting in more feed intake and eventually improved BWG as observed in the present study. BAI et al. (2013) found improved growth performance in the early stage (d 1–21) of broilers supplemented with a probiotics product composed of $1 \cdot 10^7$ cfu $g^{-1}$ of *Lactobacillus fermentum* and $2 \cdot 10^6$ cfu $g^{-1}$ of *Saccharomyces cerevisiae*. YEO and KIM (1997) and ZULKIFLI et al. (2000) also reported that supplementation of broilers with *Lactobacillus* improved average daily gain and feed efficiency from 1 to 21 d of age, but not from 22 to 42 d. LI et al. (2008) reported improved growth performance in d 1–21 in broiler supplemented with a probiotics mixture, and they observed no significant difference among different

levels (0.2 to 0.6%). However, discrepancies are seen in the results of probiotic supplementation. The discrepancy may be due to the differences in microbial species or strains of microorganisms used, dosage of supplementation or probiotic concentrations.

All frequencies of in-feed method as well as $W_3$ showed significantly lower 1 to 35 d FCR than control ($P < 0.01$). ARSALN and SAATCI (2004) pointed out that quails consumed probiotics in feed or in water had significantly lower FCR than untreated control quails. In agreement to our findings, they also stated that although probiotics-treated groups consumed less feed than control group, they showed higher live weight gain, leading to improved FCR. It is clear that in the present study probiotics consumption has led to lower FI concomitant with higher live weight gain resulting in improved FCR. This may be due to the fact that probiotics can optimize intestinal flora and hence trigger the symbiotic effects of host animal enzymes leading to improved nutrients digestibility. Over all, Table 3 showed that by decreasing the amount of probiotics consumed, FCR was improved. This means that likely there is no need to continuous or everyday use of probiotics in Japanese quail production.

Table 4

Effects of probiotic administration methods and consumption frequency on small intestinal morphology of Japanese quail*

| | Small intestinal length [cm] | | Villus height [µm] | | Crypt depth [µm] | | Villus height: crypt depth | |
|---|---|---|---|---|---|---|---|---|
| | *D* | *I* | *D* | *I* | *D* | *I* | *D* | *I* |
| Control | 10.12 | $22.00^{bcd}$ | $807.95^{c}$ | $233.23^{d}$ | $22.10^{d}$ | $19.76^{c}$ | 36.58 | 11.80b$^{c}$ |
| $F_1$ | 10.00 | $22.25^{bcd}$ | $840.51^{ab}$ | $263.56^{a}$ | $23.26^{ab}$ | $21.26^{ab}$ | 36.14 | $12.40^{a}$ |
| $F_2$ | 10.82 | $23.72^{abc}$ | $833.39^{ab}$ | $244.09^{c}$ | $22.93^{abc}$ | $21.06^{ab}$ | 36.35 | $11.59^{c}$ |
| $F_3$ | 10.50 | $25.12^{a}$ | $827.19^{b}$ | $240.14^{c}$ | $22.44^{dc}$ | $20.93^{ab}$ | 36.87 | $11.47^{c}$ |
| $W_1$ | 10.87 | $21.25^{dc}$ | $846.29^{a}$ | $268.53^{a}$ | $23.45^{a}$ | $21.38^{a}$ | 6.09 | $12.56^{a}$ |
| $W_2$ | 9.75 | $20.25^{d}$ | $831.80^{ab}$ | $254.30^{b}$ | $23.06^{abc}$ | $21.22^{ab}$ | 36.07 | $11.98^{b}$ |
| $W_3$ | 10.25 | $24.32^{ab}$ | $826.75^{b}$ | $242.59^{c}$ | $22.62^{bc}$ | $20.80^{b}$ | 36.54 | $11.66^{bc}$ |
| *P*-value | 0.756 | 0.010 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.399 | <.0001 |
| SEM | 0.202 | 0.432 | 2.455 | 2.352 | 0.100 | 0.106 | 0.110 | 0.079 |

$^{a–c}$Means with different superscripts in the same column differ ($P < 0.05$ or lower).
BVG – body weight gain, FI – feed intake, FCR – feed conversion ratio.
Control: no probiotics administered; $F_1$ – probiotics continuously in feed throughout the experiment; $F_2$ – probiotics in feed in a pattern of 2 d on -2 d off; $F_3$ – probiotics in feed in a pattern of 1 d on -4 d off; $W_1$ – probiotics in drinking water continuously throughout the experiment; $W_2$ – probiotics in drinking water in a pattern of 2 d on -2 d off; $W_3$ – probiotics in drinking water in a pattern of 1 d on -4 d off.
*D* – duodenum, *I* – ileum
* Mean represent 4 pens per treatment.

The values of small intestinal morphometric study are shown in Table 4. Duodenum length was not affected by probiotics consumption, but there were significant differences in ileum length among the experimental groups ($P < 0.01$) such that ileum was longer in the groups received fewer probiotics in feed or in water through the experiment. Administration of probiotics in feed or in water resulted in longer length of villi and deeper crypts than un-treated control quail ($P < 0.01$). AWAD et al. (2009) reported that supplementing probiotics in broiler feed caused longer duodenum and ileum villi than non-supplemented controls. Increasing the villus height introduced an increased surface area capable of greater absorption of available nutrients (CASPARY 1992). Crypts are considered as the villus factory and deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation caused by pathogens (YASON et al. 1987, PAGAN et al. 1999). The intestinal epithelial cells originate form crypts migrating along the villus surface upward to the villus tip and are extruded into the intestinal lumen within 48 to 96 h (IMONDI and BIRD 1966, POTTEN 1998). A shortening of the villi and deeper crypts may lead to poor nutrient absorption, increased secretion in gastrointestinal tract and lower performance of animal (XU et al. 2003). In contrast, increases in the villus height and villus height: crypt depth ratio is directly correlated with increased epithelial cell turnover (FAN et al. 1997), and longer villi are associated with activated cell mitosis (SAMANYA and YAMAUCHI 2002).

The number of neutral and acidic mucin producer goblet cells is shown in Table 5. In duodenum the number of neutral mucin producer goblet cells was not affected by treatments, whereas the numbers of acidic mucin producer goblet cells showed significant differences ($P < 0.05$) as $F_1$ had the highest number. In the ileum the numbers of both acidic and neutral mucin producer goblet cells showed significant differences ($P < 0.01$). In the whole period, the numbers of any kinds of goblet cells in duodenum as well as in ileum decreased as the total days of probiotic consumption decreased; i.e. the less amount of probiotics consumed the less numbers of goblet cells were observed. Goblet cells produce mucins which possess potential binding sites for both commensal and pathogenic organisms, may performing defensive role during establishment of the intestinal barrier. Formation of the mucus gel is through goblet cell secretion of polymeric mucin glycoprotein (FORSTNER and FORSTNER 1994, KLINKEN et al. 1995). These glycoproteins compete with bacteria for adhering via heterogeneous oligosaccharide chains (BELLEY et al. 1999), thereby preventing noxious agents from coming into contact with the underlying epithelial cells. Mucin provides a desirable environment for proliferation of specific

Table 5

Effects of probiotic administration methods and consumption frequency on small intestinal goblet cells of Japanese quail*

| Specification | Number of acidic mucin producer goblet cells/100 μm of villus length | | Number of neutral mucin producer goblet cells/100 μm of villus length | |
|---|---|---|---|---|
| | *D* | *I* | *D* | *I* |
| Control | $10.62^{bc}$ | $11.02^{bc}$ | 11.02 | $11.25^{bc}$ |
| $F_1$ | $11.65^{a}$ | $12.12^{a}$ | 11.67 | $11.97^{a}$ |
| $F_2$ | $11.07^{abc}$ | $11.37^{abc}$ | 11.35 | $11.62^{ab}$ |
| $F_3$ | $10.24^{c}$ | $10.92^{bc}$ | 10.90 | $11.05^{c}$ |
| $W_1$ | $11.55^{ab}$ | $11.87^{ab}$ | 11.37 | $11.67^{ab}$ |
| $W_2$ | $11.30^{ab}$ | $11.72^{abc}$ | 11.10 | $11.47^{abc}$ |
| $W_3$ | $10.17^{c}$ | $10.82^{c}$ | 10.60 | $10.92^{c}$ |
| *P*-value | 0.0131 | 0.00405 | 0.3583 | 0.0039 |
| SEM | 0.1498235 | 0.1369876 | 0.1257235 | 0.0878348 |

$^{a–c}$Means with different superscripts in the same column differ ($P < 0.05$ or lower).
BVG – body weight gain, FI – feed intake, FCR – feed conversion ratio.
Control: no probiotics administered; $F_1$ – probiotics continuously in feed throughout the experiment; $F_2$ – probiotics in feed in a pattern of 2 d on -2 d off; $F_3$ – probiotics in feed in a pattern of 1 d on -4 d off; $W_1$ – probiotics in drinking water continuously throughout the experiment; $W_2$ – probiotics in drinking water in a pattern of 2 d on -2 d off; $W_3$ – probiotics in drinking water in a pattern of 1 d on -4 d off.
* Mean represent 4 pens per treatment.

microflora due to their high carbohydrate content (DEPLANCKE and GASKINS 2001). Thus, the chemical composition of mucus is essential for establishment of the intestinal barrier.

Findings of immune responses are shown in Table 6. There were no difference among the groups for numbers of heterophil and lymphocyte and H:L. Data analysis did not reveal any significant difference of IgT, IgM and IgY concentration in response to SRBC injection between different frequencies of probiotic administration, however continuous patterns of each method showed significantly higher concentrations than control group ($P < 0.01$).

The immune modulation property of probiotics has already been well addressed (COX and DALLOUL 2015). It is possible that commensal bacteria or their products which interact closely with cells within the chicken gut-associated lymphoid tissue play a role in the development of immune response (HAGHIGHI et al. 2005). Heterophil to lymphocyte ratio is regarded as a traditional stress indicator in birds, showing bird response to environmental stressors (DAWKINS et al. 2004). Probiotic regardless of the way of administration had no significant effect on H:L in the present study. Similar results were reported in probiotic-fed broilers raised in low and high stocking densities (CENGIZ et al. 2015).

Table 6
Effects of probiotic administration methods and consumption frequency on some SRBC-reactive immune responses*

| Specification | | Heterophil | Lymphocyte | Heterophil: lymphosyte | IgT | IgM | IgY |
|---|---|---|---|---|---|---|---|
| | | [%] | | | $Log_2$ | | |
| Control | 0.0 | 40.25 | 59.75 | 0.68 | $3.50^{c}$ | $1.33^{b}$ | $2.16^{c}$ |
| $F_1$ | 1.0 | 37.13 | 62.87 | 0.64 | $5.33^{ab}$ | $2.00^{ab}$ | $3.33^{ab}$ |
| $F_2$ | 0.5 | 35.00 | 65.00 | 0.54 | $4.42^{bc}$ | $1.67^{b}$ | $2.74^{bc}$ |
| $F_3$ | 0.2 | 34.00 | 66.00 | 0.53 | $3.79^{bc}$ | $1.54^{b}$ | $2.25^{bc}$ |
| $W_1$ | 1.0 | 38.13 | 61.87 | 0.70 | $6.67^{a}$ | $2.37^{a}$ | $4.29^{a}$ |
| $W_2$ | 0.5 | 35.50 | 64.50 | 0.58 | $4.87^{bc}$ | $1.56^{ab}$ | $3.31^{ab}$ |
| $W_3$ | 0.2 | 26.45 | 73.55 | 0.36 | $4.25^{bc}$ | $1.43^{b}$ | $2.82^{bc}$ |
| *P*-value | | 0.239 | 0.259 | 0.289 | 0.0002 | 0.0437 | 0.0037 |
| SEM | | 1.462 | 1.462 | 0.039 | 0.229 | 0.173 | 0.157 |

$^{a–c}$Means with different superscripts in the same column differ ($P < 0.05$ or lower).
BVG – body weight gain, FI – feed intake, FCR – feed conversion ratio.
Control: no probiotics administered; $F_1$ – probiotics continuously in feed throughout the experiment; $F_2$ – probiotics in feed in a pattern of 2 d on -2 d off; $F_3$ – probiotics in feed in a pattern of 1 d on -4 d off; $W_1$ – probiotics in drinking water continuously throughout the experiment; $W_2$ – probiotics in drinking water in a pattern of 2 d on -2 d off; $W_3$ – probiotics in drinking water in a pattern of 1 d on -4 d off.
IgT – immunoglobulin T; Ig M – immunoglobulin M; IgY – immunoglobulin Y.
* Mean represent 4 pens per treatment.

## Conclusions

Findings of the current study showed that administration of probiotics in feed or in water improved Japanese quail's performance. However, the study illustrated that it was not necessary to supplement Japanese quail with probiotics continuously in rearing period and then not-every-day frequencies are possible.

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