# MANAGEMENT AND PRODUCTION

## Growth, carcass characteristics, meat quality, and microbial aspects of growing quail fed diets enriched with two different types of probiotics (*Bacillus toyonensis* and *Bifidobacterium bifidum*)

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**ABSTRACT** The present investigation aimed to explore the impact of dietary graded levels of 2 types of probiotic bacteria (*Bacillus toyonensis* [**BT**] and *Bifidobacterium bifidum* [**BB**]) on growth, carcass traits, meat quality, and bacteriology of growing Japanese quail reared under the cage system. One thousand three hundred sixty Japanese quail day-old chicks were randomly divided into 10 groups (8 replicates each). Birds were fed a basal diet (control, T1) and the basal diet plus 0.05, 0.075, 0.10, and 0.125% BT (T2, T3, T4, and T5, respectively), 0.10% BB (T6), and the same previous doses of BT plus 0.05% BB (T7, T8, T9, and T10, respectively). Results showed a significant ($P < 0.001$) increase in final BW and weight gain because of probiotic supplementation (except T2 for weight gain). Both feed intake and feed conversion ratio did not differ during the overall experimental period (1–42 D of age) except feed intake that was reduced in T2 and increased in T5 and T9 groups. All carcass traits studied were significantly ($P < 0.01$) affected by probiotics, and the combination between BT and BB in group T8 increased all studied parameters as compared with the other treatment groups. The quail meat color of redness $a^*$ and $L^*$ values, thiobarbituric content, cooking loss, proteolysis, and total coliform were decreased ($P < 0.001$) by probiotic treatment. In general, supplementing BT, BB, or their combination to the basal diet delayed the proliferation of pathogenic bacteria in the diet and intestine. Using BT and BB as feed supplements enhanced growth performance and meat quality of quails as well as diminished pathogenic bacteria proliferation in their diet and intestine. As per our results, we can recommend the application of T5 and T8 to T10 levels for the best performance, carcass traits, and meat quality of growing quails.

**Key words:** probiotic, quail, growth, carcass, meat quality

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## INTRODUCTION

The indiscriminate use of antibiotics in poultry farms increased the public health skepticism concerning the creation of resistant strains of pathogenic bacteria (Abd El-Moneim et al., 2020; Abd El-Moneim and Sabic, 2019) and residual contamination in poultry products (Shewita and Taha, 2018; Alagawany et al., 2019a; Soomro et al., 2019). The ban of antibiotic growth promoters from the markets of the European Union and many other countries challenged poultry producers to find suitable alternative solutions (Abdelnour et al., 2019; Alagawany et al., 2019b). Probiotics have been considered as green feed additives and promising unconventional substitutions to chemotherapy in poultry. Moreover, any animal needs to maintain specific numbers of beneficial microbiota in



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the gastrointestinal tract to ensure at all times that the animal has the proper microbial balance (Abd El-Hack et al., 2018; Alagawany et al., 2018; Arif et al., 2019). This proper balance could not be guaranteed under the natural conditions of the farm. Therefore, adding the probiotics to the bird's diet improves their utilization (Alagawany et al., 2016; Farghly et al., 2018; Taha et al., 2019) because probiotics can neutralize and stabilize the gut ecosystem, compete for the enteric pathogens for nutrients and intestinal attachment sites, inhibit pathogenic adhesion, inhibit epithelial invasion, prevent common intestinal clinical signs for example, diarrhea, promote the metabolic processes of digestion and absorption of nutrients and supply the birds with several substrates that enhance their immune response and serve as a source of metabolic energy (Estrada et al., 2001; Lodemann et al., 2008). Numerous bifidobacteria and *Bacillus* strains have been used as alternatives to chemotherapeutic agents in poultry, animals, and humans (Dankowiakowska et al., 2013; Kantas et al., 2015). Bifidobacteria, as anaerobic, non–spore-forming bacteria, produce antimicrobial proteinaceous substances such as bacteriocins (bifidin and bifidocin B) as well as lactic acid and acetic acid, which are thought to suppress the growth of several gram-positive and gram-negative bacteria in vitro (Shah and Dave, 2002; Touré et al., 2003). Bifidocin B shows antibacterial activity against some foodborne pathogens such as *Leuconostoc*, *Enterococcus*, *Bacillus*, *Listeria*, *Lactobacillus*, and *Pediococcus* spp. whereas bifidin is active against *Micrococcus flavus* and *Staphylococcus aureus* (Shah and Dave, 2002). Bifidocin B shows antibacterial activity against some foodborne pathogens such as *Bacillus cereus*, *Listeria monocytogenes*, whereas bifidin is active against *M. flavus* and *S. aureus* (Shah and Dave, 2002). *Bacillus toyonensis* (**BT**) is an aerobic nonpathogenic gram stain–positive, fermentative, and spore-forming bacterium that has been used as a probiotic in animal feed (Williams et al., 2009; Roos et al., 2018; Abdel-Moneim et al., 2020). Using the probiotic mixture combining aerobic and anaerobic bacterial strains may present synergistic improving effects on birds' production, health, and welfare. Therefore, the present study aimed to explore the impact of dietary supplementation of 2 types of probiotic bacteria (BT and *Bifidobacterium bifidum* [**BB**]) on growth performance, carcass traits, meat quality, and bacteriology of growing Japanese quail under the cage system. The selected doses of BB in this study are based on the previous published (Abd El-Moneim et al., 2020; Abdel-Moneim et al., 2020) work made by our laboratory where we found that the better dose of BB that achieved higher growth performance of birds was ranged between $10^8$ to $10^9$ CFU/kg diet, so we decided to use the dose of 5 × 108 CFU/1 mL in the present study. The hypothesis of the present study was to study the effects of gradual dietary levels of BT alone or combined with the half dose of BB in comparison with the optimal dose of BB and the control.

## MATERIAL AND METHODS

### Experimental Design

The present study was conducted at the Poultry Research Unit, Biological Application Department, Radioisotopes Applications Division, Nuclear Research Center, Egyptian Atomic Energy Authority, Inshas area. The experimental procedures were performed in accordance with the guidelines of the Biological Application Department, Nuclear Research Center, Egyptian Atomic Energy Authority and procedures were approved by the Committee of the Nuclear Research Center, Egyptian Atomic Energy Authority.

### Strains and Diets

Bacterial strains of BT ATCC 55050 and BB ATCC 29521 were obtained from Egyptian Culture Collection MERCIN (Ain Shams University, Cairo, Egypt). A total of 1,360 day-old quail chicks were randomly allotted into equal 10 treatments (136 birds each), while each group was subdivided into 8 replicates each of 17 chicks during the experimental period extended from 1D to 42 D of age. Birds were fed a basal diet (control, T1) and the basal diet plus 0.5, 0.75, 1.0, and 1.25 mL BT/kg diet (T2, T3, T4, and T5, respectively), 1.0 mL BB/kg diet (T6), and the same previous doses of BT plus 0.5 mL BB/kg diet (T7, T8, T9, and T10, respectively). Concentrations of BT and BB solutions were $5 \times 10^8$ and $6 \times 10^8$ CFU/mL, respectively. The diet (in mash form) was formulated using a horizontal mixer with a capacity of 200 kg (Lucato, Limeira, Brazil) to offer the nutritional requirements of growing quail, according to the NRC (1994) recommendations. Table 1 shows the ingredients and chemical analysis of the basal diet.

**Table 1.** Composition and calculated analysis of the experimental diet.

| Ingredients (g/kg) | Basal diet |
|---|---|
| Yellow maize | 554 |
| Soybean meal (44%) | 396 |
| Dicalcium phosphate | 7.50 |
| Limestone | 15.0 |
| Sodium chloride | 3.00 |
| Vitamin–mineral premix[1] | 3.00 |
| DL-methionine | 1.50 |
| Soybean oil | 20.0 |
| Calculated analysis[2] (g/kg) | |
| CP | 220.0 |
| ME (MJ/kg) | 12.196 |
| Crude fiber | 39.9 |
| Lysine | 12.1 |
| Methionine | 5.20 |
| Methionine + Cysteine | 8.60 |
| Calcium | 8.50 |
| Available phosphorus | 3.30 |

[1]Vitamin–mineral premix provided per kg diet: vit. A, 12,000 IU; vit. D3, 5,000 IU; vit, E, 16.7 g; vit. K, 0.67 g; vit. B1, 0.67 g; vit. B2, 2 g; vit. B 6, .67 g; vit. B12, 0.004 g; nicotinic acid, 16.7 g; pantothenic acid, 6.67 g; biotin, 0.07 g; folic acid, 1.67 g; choline chloride, 400 g; Zn, 23.3 g; Mn, 10 g; Fe, 25 g; Cu,1.67 g; I, 0.25 g; Se, 0.033 g; and Mg, 133.4 g.
[2]Calculated, according to NRC (1994).

## Management

Birds in all groups were kept during the experimental period in suitable conditions. Each replicates housed in 1 battery cage (100 x 50 × 60 cm). Quail chicks were kept daily on continuous light up to the end of the first 7 D of age, after that received 22 h light per day. The brooder battery was supplied with 1 white fluorescent lamp. All birds were kept under the same hygienic, environmental, and managerial conditions. Feed was offered as ad libitum, and freshwater was supplied throughout the experimental periods. Drinkers and feeding troughs were daily cleaned.

## Collection of Data

***Growth Performance*** To obtain live BW and body weight gain (**WG**), birds were individually weighed to the nearest 0.1 g at 1, 21, and 42 D of age. Weight gain was recorded for each replicate to obtain average WG per each replicate. Feed intake (**FI**) was calculated by subtracting the period remaining feed from the presented feed for each replicate/group. Feed conversion ratio (**FCR**) was calculated as the number of g of feed required to produce 1 g of gain during a certain period. The mortality rate of Japanese quail chicks was recorded daily and calculated for each experimental period (grower, 1–21 D; finisher, 22–42 D; and overall, 1–42 D) (no mortality rate was recorded for all treatments during the finisher period, so those data are not shown).

***Carcass Traits*** At the experiment end of (42 D of age), 3 male birds from each replicate were randomly chosen around the overall mean of group, fasted overnight, weighed, and then slaughtered to complete bleeding. After plucking the feather and evisceration, the empty carcass, giblets, proventriculus, and whole intestine were weighed. The intestine length was also measured. Carcass yield was calculated as follows: Carcass yield = [(Empty carcass weight (g) + edible offal's weight (g))/Live preslaughtering weight] × 100.

***Meat Quality Traits*** After slaughtering, 3 samples from breast and thigh muscles/replicate from each treatment group were collected, kept in polyethylene clear bag under freezing conditions (−18°C), and transformed to the laboratory within 1 h. For microbial a counts, 10 g of each meat sample was treated with 90 mL physiological solution and homogenized to make dilutions (Lab Blender 400; Seward Medical) for the 60 s at room temperature. Five replications of at least 3 appropriate dilutions were enumerated (Al-Jasser, 2012).

The pH degree of meat samples was determined. Briefly, 5 g of each meat sample was blended with 45 mL of sterilized water, and the pH of the suspension was measured using a glass electrode pH meter (Zheng et al., 2014; Hussein et al., 2019). Breast and thigh meats were weighed before and after cooking at 70°C, and the percent of the cooking loss was calculated as the difference between both weights. Using the Huff-Lonergan and Lonergan (2005) method, water holding capacity (**WHC**) was estimated. Five grams of each sample was taken in a 50-mL centrifuge tube that was preweighted for all samples. The distilled water was supplied in small increments to a series of tubes under continuous stirring with a glass rod. Samples were centrifuged (4,000 r.p.m, for 10 min) after being thoroughly wetted. After centrifugation, the supernatant was isolated by decantation. Water holding capacity was calculated.

According to Liu et al. (2012), quail breast muscles were used to determine the cooking loss. The color was determined using a Hunter MiniScan EZ colorimeter (Hunter, Reston, VA). Five random locations on each side surface of the muscles were taken. Values of $L^*$, $a^*$, and $b^*$ were recorded (AMSA, 2012). Thiobarbituric (**TBA**) values were assessed in triplicate by the modified method described by Buege and Aust (1978).

***Microbial Analysis of the Dietary and Ileum Content*** About 25 g of dietary samples inclusion levels of BT, BB, and coculture diets (BT and BB) were used to determine the content of total bacterial count (**TBC**), coliforms, and total fungi (Feng et al., 2002). The contents of the ileum (5 g) of 3 birds per replicate were used to determine the content of TBC, probiotic, coliforms, and *Escherichia coli*. Cloacal (fecal) swabs were taken with a sterile cotton swab inserted 10–12 mm into the cloacal opening and gently rotated to collect a sample of the fecal material. Cecal contents were collected by opening the birds immediately after euthanasia, cutting off 1 cecum, and manually squeezing content into a sterile tube. From 1 trial, both ceca were harvested from 20 birds, and the contents of each were taken as separate samples. The swabs and cecal content were snap frozen on dry ice and transported to the laboratory for processing. The contents of the ileum (5 g) of 3 birds per replicate were used to determine the content of viable bacterial count. The analysis of viable bacterial count of the TBC, BT was done on plate count agar under aerobic condition. At the same time, BB were determined on MRS agar under anaerobic conditions. Before starting the trial, the total viable bacterial count of each bacterium was about $1.5 \times 10^9$ CFU/mL. The diet treatment was supplemented by BT or BB or their combination to reach the viable number to $1 \times 10^8$ per gram in the diet. The feed and intestinal contents were analyzed using enumeration of the viable bacterial count (microbiological spread plate method for TBC, total fungi, and BT) and (microbiological poured plate method and incubation under the anaerobic condition for coliforms BB.

The bacterial group number was converted to log number to be ready for the statistical analysis. The TBC was enumerated on plate count nutrient agar (PCA; Merck, 1.05463) by incubating at 37°C for 72 h. Yeasts and molds were enumerated using Rose Bengal Chloramphenicol Agar (36 supplemented with chloramphenicol, X009; Lab M) and incubated at 25°C for 5 D (Speak, 1984). *E. coli* was determined using Tryptone Bile Glucuronide Agar (TBX, Lab M) and incubation at 37°C for 24 h (Oxoid, 1982). Probiotic bacteria were determined using milk nutrient medium and incubation at 37°C for 72 h, as described by Nester (1978).

### Statistical Analysis

Data were subjected to ANOVA procedures appropriate for a completely randomized design using the GLM procedures of the SPSS software (SPSS, 1999). The statistical model used was

$$Y_{ij} = \mu + T_i + e_{ij}$$

where $Y_{ij}$, observed value; $\mu$, overall mean; $T_i$, treatment effect (1–10); and $e_{ij}$, random error. Differences among means were estimated by the test of Student–Newman–Keuls. The SE and mean values were reported. Statistical significance statements were based on $P < 0.05$.

## RESULTS

### Growth Performance

Results in Table 2 showed a significant ($P < 0.001$) increase in quail weight at 21 and 42 D of age because of probiotics (except T2 at 42 D) as compared with the control. The highest values of final BW were recorded in the T5 and T10 groups. Results also showed a significant ($P < 0.001$) increase in WG at the grower and the overall experimental periods (except T2 for the overall one) because of probiotics as compared with the control. However, during the finisher period, all probiotic-treated groups increased ($P < 0.001$) WG except the lowest single dose of BT (T2) and groups T7 and T8 in which WG was reduced. The highest values of WG during the finisher and overall periods were accompanied by the highest dietary single level of BT (T5), which recorded 112.93 and 208.61 g, respectively. No significant differences in mortality were recorded among the probiotic-supplemented groups and the control during all experimental periods (Table 2).

### Feed Intake and FCR

During the grower period, all treatment groups increased ($P < 0.001$) FI except the T2 and T4 groups, while during the finisher period, birds of T2, T6, T7, T8 and T10 consumed less feed ($P = 0.007$) than those of control birds (Table 3). Total FI was reduced ($P = 0.023$) in T2 and increased in T5 and T9, whereas it was not significantly affected by the remaining dietary treatments. The highest values of FI were recorded for groups T6 and T9 during the grower and finisher experimental periods, respectively. Moreover, the T5 and T9 groups recorded the highest FI during the whole period. Furthermore, as shown in Table 3, FCR was significantly improved ($P = 0.013$) in all probiotic-treated groups except T6 during the grower period, while during the finisher one, only groups T2, T4, T5, T6, and T10 improved FCR. Overall, FCR was not significantly affected by the probiotic supplement. The best FCR values were recorded in T4 and T5 groups at experimental periods of 1–21 D and 22–42 D of age, respectively.

### Carcass Traits

Table 4 shows the effects of probiotics on the carcass traits of growing Japanese quail at slaughter age of 42 D. Dressing percentage was elevated ($P = 0.003$) in groups T6 and T8, whereas it was reduced in T3, T4, T7, and T9. The highest dressing values were recorded in groups T8 and T6. All dietary levels of BT (except T2 and T8) reduced ($P = 0.003$) carcass yield compared with T6 and T1 groups. Giblet percentage was increased ($P = 0.002$) in groups T2, T8, and T10 and decreased in T3, T5, T6 and T7. Dietary addition of low doses of BT (T2 and T3), as well as group T8, increased ($P = 0.003$) proventriculus relative weight, whereas it was reduced in the T5, T6, and T7 groups. The intestine length was increased ($P = 0.013$) in all groups supplied by probiotics except T6, T9, and T10, whereas the relative intestine weight was not altered. In general, the combination of BT and BB in the group T8 was able to elevate ($P < 0.01$) all the parameters higher thanthe control.

### Meat Quality Traits

Results in Table 5 showed that pH of fresh quail meat increased with dietary probiotic treatment from groups

**Table 2.** BW, weight gain, and mortality of the growing Japanese quail supplemented with dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* during the experimental periods.

| | Live BW (g) | | | BW gain (g/bird/period) | | | Mortality (%) | |
|---|---|---|---|---|---|---|---|---|
| Treatments | 1 D | 21 D | 42 D | 1–21 D | 22–42 D | 1–42 D | 1–21 D | 1–42 D |
| T1 | 9.37 | 85.65$^{e}$ | 182.53$^{e}$ | 76.28$^{f}$ | 96.88$^{d}$ | 173.16$^{e}$ | 2.78 | 2.78 |
| T2 | 9.58 | 89.35$^{d}$ | 183.52$^{e}$ | 79.77$^{e}$ | 94.17$^{d}$ | 173.94$^{e}$ | 2.78 | 2.78 |
| T3 | 9.41 | 90.87$^{d}$ | 191.73$^{d}$ | 81.46$^{e}$ | 100.86$^{c}$ | 182.32$^{d}$ | 0.00 | 0.00 |
| T4 | 9.47 | 94.73$^{c}$ | 199.45$^{c}$ | 85.25$^{d}$ | 104.73$^{b}$ | 189.98$^{c}$ | 0.00 | 0.00 |
| T5 | 9.60 | 105.28$^{b}$ | 218.21$^{a}$ | 95.68$^{c}$ | 112.93$^{a}$ | 208.61$^{a}$ | 0.00 | 0.00 |
| T6 | 9.53 | 100.65$^{b,c}$ | 203.78$^{b,c}$ | 91.12$^{c,d}$ | 103.13$^{b}$ | 194.25$^{c}$ | 0.00 | 0.00 |
| T7 | 9.60 | 113.46$^{a}$ | 201.72$^{c}$ | 103.24$^{b}$ | 88.27$^{e}$ | 192.12$^{c}$ | 0.00 | 0.00 |
| T8 | 9.54 | 116.78$^{a}$ | 198.62$^{c}$ | 107.27$^{a}$ | 81.84$^{f}$ | 189.08$^{c}$ | 0.00 | 0.00 |
| T9 | 9.59 | 102.72$^{b}$ | 206.15$^{b}$ | 93.13$^{c}$ | 103.44$^{b}$ | 196.56$^{b}$ | 0.00 | 0.00 |
| T10 | 9.57 | 104.96$^{b}$ | 208.43$^{b}$ | 95.38$^{c}$ | 103.48$^{b}$ | 198.86$^{b}$ | 0.00 | 0.00 |
| *P*-value | 0.748 | <0.001 | <0.001 | <0.001 | 0.001 | <0.001 | 0.552 | 0.552 |
| SEM | 0.09 | 1.91 | 2.22 | 1.90 | 1.89 | 2.21 | 0.39 | 0.39 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

**Table 3.** Feed intake and feed conversion ratio of the growing Japanese quail supplemented with dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* during the experimental periods.

| | Feed intake (g/bird/period) | | | Feed conversion ratio (g feed/g gain) | | |
|---|---|---|---|---|---|---|
| Treatments | 1–21 D | 22–42 D | 1–42 D | 1–21 D | 22–42 D | 1–42 D |
| T1 | 182.54$^{f}$ | 421.21$^{c}$ | 603.75$^{b,c}$ | 2.39$^{a}$ | 4.36$^{c}$ | 3.49 |
| T2 | 170.49$^{g}$ | 384.28$^{e}$ | 554.77$^{d}$ | 2.14$^{c}$ | 4.10$^{d}$ | 3.23 |
| T3 | 189.75$^{e}$ | 431.15$^{b}$ | 620.90$^{b}$ | 2.33$^{b}$ | 4.29$^{c}$ | 3.41 |
| T4 | 160.23$^{h}$ | 435.03$^{b}$ | 595.26$^{c}$ | 1.92$^{e}$ | 4.15$^{d}$ | 3.13 |
| T5 | 222.60$^{b}$ | 434.00$^{b}$ | 656.60$^{a}$ | 2.33$^{b}$ | 3.71$^{f}$ | 3.08 |
| T6 | 230.65$^{a}$ | 383.69$^{e}$ | 614.34$^{b}$ | 2.43$^{a}$ | 3.73$^{f}$ | 3.16 |
| T7 | 224.02$^{b}$ | 392.21$^{d}$ | 616.23$^{b}$ | 2.16$^{c}$ | 4.44$^{b}$ | 3.21 |
| T8 | 216.02$^{c}$ | 395.81$^{d}$ | 611.83$^{b}$ | 2.02$^{d}$ | 4.67$^{a}$ | 3.24 |
| T9 | 195.39$^{d}$ | 453.93$^{a}$ | 649.32$^{a}$ | 2.10$^{c,d}$ | 4.41$^{b}$ | 3.31 |
| T10 | 197.49$^{d}$ | 409.76$^{d}$ | 607.25$^{b,c}$ | 2.08$^{c,d}$ | 3.96$^{e}$ | 3.06 |
| *P*-value | <0.001 | 0.007 | 0.023 | 0.013 | 0.043 | 0.089 |
| SEM | 4.78 | 5.46 | 6.59 | 0.04 | 0.08 | 0.03 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

T4 to T6 and decreased in T2 as compared with the control and remaining groups. The TBA values were reduced significantly ($P < 0.001$) in all treatment groups compared to the control.

The quail meat color of redness $a^*$, $b^*$, and $L^*$ values were significantly ($P < 0.001$) decreased by probiotics treatment with all levels studied as compared to the control group. Samples from the T10 group had the maximum redness ($a^*$) value, with the same trend of rising of pH in the same samples. Lightness ($L^*$) values were gradually ($P < 0.001$) increased by increasing probiotics level. The T6 and T1 groups recorded the highest ($P < 0.001$) value of yellowness ($b^*$) as compared with the other groups. Table 6 showed that the worst findings regarding cooking loss, WHC, proteolysis, and TC were found in the control group. The combination between the 2 probiotic sources gave the best ($P < 0.001$) results.

## Microbiological Findings

Effects of BT and BB on the proliferation of TBC, coliform, and total fungi are shown in Table 7. Generally, supplementing BT, BB, or their combination to the basal diet delayed the proliferation of microorganisms in the diet. The TBC, total fungi, and coliform in the basal diet supplemented with BT, BB, or BT + BB were significantly different ($P < 0.001$) with level 0.5 to 0.75 $\log_{10}$ CFU/g. The basal diet supplemented with 0.5 BT or 0.5 BT+ 0.5 BB decreased ($P < 0.001$) all the microbial population in the diet. In addition, the TBC, coliforms, and total fungi were higher ($P < 0.001$) in the control group. Thus, it could be concluded that the addition of probiotic bacteria to the quail diet can delay the bacterial and fungal growth in the diet. Results in Table 8 emphasized that increasing levels of BT and BB statistically declined the intestinal coliform enumeration with approximately 0.5 to 1.0 $\log_{10}$ CFU/g and decreased the TBC population (except in T2) with ~0.5 $\log_{10}$ CFU/g without affecting the populations of probiotic bacteria. The cecal count of *E. coli* was decreased significantly ($P < 0.001$) in groups T5 and T9 and numerically nearly in all the remaining groups, as shown in Table 8. Supplementing the quail diet with BT showed strong antibacterial properties against gram-negative and gram-positive bacteria.

**Table 4.** Carcass traits (% of preslaughter weight) and digestive tract length (cm) of the growing Japanese quail supplemented with dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* at the end of experimental periods studied (42 D of age).

| Treatments | Preslaughter weight (g) | Dressing (%) | Giblets (%) | Carcass yield (%) | Intestine length (cm) | Intestine weight (g) | Poventriculus weight (g) |
|---|---|---|---|---|---|---|---|
| T1 | 183.50$^{e}$ | 78.39$^{b}$ | 5.00$^{b}$ | 83.39$^{b}$ | 68.75$^{d}$ | 5.54 | 0.43$^{d}$ |
| T2 | 191.00$^{d}$ | 77.94$^{b}$ | 5.22$^{a}$ | 83.17$^{b}$ | 75.50$^{b}$ | 5.54 | 0.52$^{a}$ |
| T3 | 192.50$^{d}$ | 76.10$^{c}$ | 4.80$^{c}$ | 80.90$^{e}$ | 71.75$^{c}$ | 5.88 | 0.46$^{c}$ |
| T4 | 209.00$^{b}$ | 75.47$^{d}$ | 5.01$^{b}$ | 80.48$^{e}$ | 81.50$^{a}$ | 6.79 | 0.43$^{d}$ |
| T5 | 219.00$^{a}$ | 77.31$^{b}$ | 4.79$^{c}$ | 82.10$^{c}$ | 72.50$^{c}$ | 5.83 | 0.38$^{e}$ |
| T6 | 204.36$^{c}$ | 79.07$^{a}$ | 4.47$^{d}$ | 83.53$^{b}$ | 69.25$^{d}$ | 5.37 | 0.33$^{f}$ |
| T7 | 209.06$^{b}$ | 76.63$^{c}$ | 4.53$^{d}$ | 81.17$^{e}$ | 79.00$^{a}$ | 5.80 | 0.37$^{e}$ |
| T8 | 213.21$^{b}$ | 79.78$^{a}$ | 5.15$^{a}$ | 84.94$^{a}$ | 73.75$^{b}$ | 6.49 | 0.49$^{b}$ |
| T9 | 211.50$^{b}$ | 76.75$^{c}$ | 4.97$^{b}$ | 81.72$^{d}$ | 70.00$^{d}$ | 5.45 | 0.41$^{d}$ |
| T10 | 220.03$^{a}$ | 77.46$^{b}$ | 5.19$^{a}$ | 82.65$^{c}$ | 69.50$^{d}$ | 5.69 | 0.42$^{d}$ |
| *P*-value | <0.001 | 0.003 | 0.002 | 0.001 | 0.013 | 0.138 | 0.003 |
| SEM | 2.25 | 0.29 | 0.05 | 0.75 | 1.19 | 0.12 | 0.01 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

**Table 5.** Meat color, pH, and TBA content of growing Japanese quails supplemented with dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* at the end of the experimental periods studied (42 D of age).

| Treatments | pH | TBA | Color | | |
|---|---|---|---|---|---|
| | | | a* | b* | L* |
| T1 | 6.31$^{b}$ | 1.24$^{a}$ | 19.23$^{a}$ | 7.15$^{a}$ | 43.09$^{a}$ |
| T2 | 6.02$^{c}$ | 0.20$^{b}$ | 15.33$^{b}$ | 6.63$^{b}$ | 23.53$^{f}$ |
| T3 | 6.33$^{b}$ | 0.15$^{c}$ | 8.35$^{c}$ | 6.04$^{c}$ | 40.37$^{b}$ |
| T4 | 6.83$^{a}$ | 0.15$^{c}$ | 8.22$^{c}$ | 6.73$^{b}$ | 32.12$^{d}$ |
| T5 | 6.83$^{a}$ | 0.12$^{d}$ | 7.88$^{c}$ | 6.34$^{c}$ | 23.24$^{f}$ |
| T6 | 6.71$^{a}$ | 0.13$^{d}$ | 6.23$^{d}$ | 7.34$^{a}$ | 38.33$^{c}$ |
| T7 | 6.31$^{b}$ | 0.11$^{e}$ | 8.03$^{c}$ | 6.23$^{c}$ | 30.52$^{e}$ |
| T8 | 6.43$^{b}$ | 0.11$^{e}$ | 6.35$^{d}$ | 6.18$^{c}$ | 40.18$^{b}$ |
| T9 | 6.36$^{b}$ | 0.10$^{e}$ | 5.27$^{e}$ | 6.12$^{c}$ | 40.32$^{b}$ |
| T10 | 6.37$^{b}$ | 0.11$^{e}$ | 5.12$^{e}$ | 5.03$^{d}$ | 30.35$^{e}$ |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| SEM | 0.02 | 0.01 | 0.02 | 0.05 | 0.19 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

Abbreviations: *a**, redness; *b**, yellowness; *L**, lightness; TBA, thiobarbituric.

## DISCUSSION

Increasing quail weight may be attributed to stimulating the production of certain vitamins, digestive enzymes, and other active substances in a multispecies probiotic. This can inhibit the growth of the enteropathogens in the quail gut through decreasing the pH of the intestine, improving digestion, and consequently enhancing the utilization of nutrients, which positively reflected on values of BW (Premavalli et al., 2018). The enhancement effect of probiotics may also be attributed to their ability to enhance digestive enzyme activities and reduce ammonia production (Wang and Gu, 2010; Sugiharto, 2016), increase the surface area of villi for nutrient absorption, and act as a dietary antimicrobial agent (Yazhini et al., 2018). Moreover, probiotics can produce digestive vitamins, enzymes, and antibacterial substances (e.g., bacteriocins, organic acids, hydrogen peroxide, lactoperoxidase system, lactone components, diacetyl, and acetaldehyde), reduce blood cholesterol levels, stimulate immunity, inhibit the growth of infectious bacteria, and remove carcinogens (Zubillaga et al., 2001; Mukherjee et al., 2019). A similar explanation was provided by Applegate et al. (2010), who confirmed that probiotic supplementation results in bacterial antagonism, colonization competition, and vying for nutrients. These actions reduce toxic compounds, modulate the immune system, and increase nutrient digestion and absorption, leading to improve the BW. Our findings (high values of WG in T5, T10, and T9), following Gupta et al. (2016), reported that dietary supplementation of probiotics increased WG during 2–3, 4–5, and 7–8 wk of age of the Japanese quail. The differences in WG between inoculation groups studied owing to the optimizing dose of probiotics giving high activities of BT or BT with BB colonies in the gastrointestinal tract of the growing quail. Metabolites produced by microbes may also play an important role in cellular differentiation and proliferation in the colonic mucosa by inducing apoptosis and may confer protection against colitis and colorectal cancer by modulating oncogene expression. These functions do not appear to be performed by a single species; several different species may be acting independently or in combination. Research is leading to an understanding of microbial community structure and composition dynamics concerning diet aids in establishing testable hypotheses for future research in health and beneficial microbes (Brownawell et al., 2012). Most research has been performed on the influence of beneficial intestinal bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp. on host health monitored using a cultivation approach. Cultivation-independent approaches have now become more popular, leading to the identification of new beneficial microbiota taxa and their potential functional roles in the gut as they relate to the diet.

Furthermore, Ocak et al. (2009) theorized that probiotic mixed in the feed resulted in increasing WG, which might be due to living microorganisms that help in the establishment of intestinal populations that are beneficial to the animals and antagonistic to the harmful microbes. No effects on mortality were recorded because

**Table 6.** Cooking loss, water holding capacity (WHC), proteolysis, and (TC) of meats of growing Japanese quails supplemented with dietary *Bacillus toyonensi* and *Bifidobacterium bifidum* at the end of experimental periods studied (42 D of age).

| Treatments | Cooking loss (%) | WHC (%) | Proteolysis (%) | TC (%) |
|---|---|---|---|---|
| T1 | 20.33$^{a}$ | 23.48$^{c}$ | 26.67$^{a}$ | 64.31$^{a}$ |
| T2 | 19.67$^{b}$ | 23.51$^{c}$ | 23.33$^{b}$ | 51.00$^{b}$ |
| T3 | 19.60$^{b}$ | 23.67$^{c}$ | 21.00$^{c}$ | 41.02$^{c}$ |
| T4 | 19.43$^{b}$ | 24.07$^{b}$ | 20.33$^{c}$ | 32.33$^{d}$ |
| T5 | 19.63$^{b}$ | 24.62$^{b}$ | 15.67$^{e}$ | 23.00$^{f}$ |
| T6 | 19.77$^{b}$ | 24.68$^{b}$ | 17.33$^{d}$ | 41.00$^{c}$ |
| T7 | 18.27$^{c}$ | 24.31$^{b}$ | 23.67$^{b}$ | 27.35$^{e}$ |
| T8 | 17.57$^{d}$ | 24.65$^{b}$ | 21.00$^{c}$ | 21.30$^{g}$ |
| T9 | 17.47$^{d}$ | 25.33$^{a}$ | 15.00$^{e}$ | 20.33$^{g}$ |
| T10 | 17.10$^{d}$ | 25.93$^{a}$ | 11.33$^{f}$ | 21.00$^{g}$ |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 |
| SEM | 0.04 | 0.07 | 0.63 | 0.78 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

**Table 7.** Effect of dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* on total bacterial counts, coliform, and total fungi ($\log_{10}$ CFU/g) in the basal diet after 1, 3, and 6 wk.

| | Total bacterial count | | | Coliform | | | Total fungi ($\log_{10}$ CFU/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatments | 1 wk | 3 wk | 6 wk | 1 wk | 3 wk | 6 wk | 1 wk | 3 wk | 6 wk |
| T1 | $6.49^{b}$ | $6.53^{a}$ | $6.41^{b,c}$ | $4.93^{a}$ | $4.45^{a}$ | $4.65^{a}$ | $3.51^{a}$ | $4.13^{a}$ | $4.48^{a}$ |
| T2 | $6.40^{d}$ | $6.28^{f}$ | $6.11^{e}$ | $4.46^{d}$ | $4.13^{b}$ | $4.32^{c,d}$ | $2.32^{d,e}$ | $3.52^{a,b}$ | $2.56^{c}$ |
| T3 | $6.45^{c}$ | $6.45^{b,c,d}$ | $6.45^{b,c}$ | $4.49^{d}$ | $4.31^{a,b}$ | $4.54^{a,b}$ | $2.12^{e}$ | $2.88^{b,c,d}$ | $2.81^{b,c}$ |
| T4 | $6.39^{d}$ | $6.42^{d,e}$ | $6.33^{d}$ | $4.86^{a}$ | $4.31^{a,b}$ | $4.34^{c,d}$ | $2.84^{b,c,d}$ | $3.53^{a,b}$ | $2.72^{b,c}$ |
| T5 | $6.44^{c}$ | $6.44^{c,d,e}$ | $6.40^{b,c}$ | $4.55^{c,d}$ | $4.12^{b}$ | $4.20^{d,e}$ | $2.61^{d,e}$ | $3.09^{b,c,d}$ | $2.65^{c}$ |
| T6 | $6.45^{c}$ | $6.46^{b,c}$ | $6.43^{b,c}$ | $4.87^{a}$ | $3.12^{c}$ | $4.43^{b,c}$ | $2.66^{c,d,e}$ | $2.57^{d}$ | $2.49^{c}$ |
| T7 | $6.55^{a}$ | $6.47^{b}$ | $6.39^{c,d}$ | $4.86^{a}$ | $4.14^{b}$ | $4.42^{b,c}$ | $3.23^{a,b}$ | $2.69^{c,d}$ | $2.85^{b,c}$ |
| T8 | $6.55^{a}$ | $6.51^{a}$ | $6.51^{a}$ | $4.65^{b,c}$ | $4.45^{a}$ | $4.14^{e}$ | $3.33^{a,b}$ | $3.40^{a,b,c}$ | $2.62^{c}$ |
| T9 | $6.57^{a}$ | $6.45^{b,c,d}$ | $6.47^{a,b}$ | $4.81^{a}$ | $4.33^{a,b}$ | $4.31^{c,d}$ | $3.19^{a,b,c}$ | $2.85^{b,c,d}$ | $3.47^{b}$ |
| T10 | $6.47^{b,c}$ | $6.40^{e}$ | $6.42^{b,c}$ | $4.79^{a,b}$ | $4.20^{b}$ | $4.09^{e}$ | $3.37^{a,b}$ | $2.60^{c,d}$ | $3.24^{b,c}$ |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| SEM | 0.025 | 0.015 | 0.041 | 0.035 | 0.042 | 0.38 | 0.095 | 0.085 | 0.075 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

of probiotic supplementation. Similar results were obtained by Premavalli et al. (2018) and Abdel-Moneim et al. (2020) on growing the Japanese quail and by Jin et al. (1998) and Abd El-Moneim et al. (2020) on broiler chicks. The latter authors attributed the favorable impact of probiotic on bird's viability to the healthy digestive tract achieved by probiotic administration. Improvement of WG may be owing to the role of BT and BB in the competition with harmful bacteria in the digestive tract of the quail, which increased the utilization of nutrients.

Significant increases in daily FI and improvement in FCR were recorded in the grower periods of (1–21 D and 22–42 D) because of dietary supplementation of probiotics. However, the overall FCR at all experimental periods of (1–42 D) was not significantly affected by the probiotic-treated groups. On the other hand, the overall FI significantly increased, especially in the T5 and T9 groups beacuse of the high levels of BT, which may be the cause for improving the consumption of diet. These results are in line with Abdel-Moneim et al. (2020) who reported insignificant alterations in FCR in *Bacillus*-treated quails. Moreover, Manafi et al. (2018) found significant differences in overall FI among experimental treatment groups. In contrast, Ross 308 broiler chicks that consumed probiotic microbes (Microguard) at 150 g/ton of the diet had the highest FI as compared with the control. In partial agreement, Kumari et al. (2001) claimed that feeding probiotics to quails resulted in better FCR than the control group. As well, Premavalli et al. (2018) clarified that the improvement in FCR of quails fed diets supplemented with multispecies probiotic maybe belonged to the total impacts of the probiotic including the alteration of bacterial metabolism in the intestine and the maintenance of beneficial microbial population as well as good digestion and absorption of the feed.

Studied carcass traits were greatly varied among singular dietary probiotics addition and their combination. Carcass yield was not affected by the single dose of BB and decreased by the graded doses of BT, whereas their combination in the T8 group gave the best results. Our results agree with those of the study by Abd El-Moneim et al. (2020) who found no statistical differences in carcass traits in bifidobacteria-administrated groups. Furthermore, De-Souza et al. (2018) noticed that dietary supplementation of the probiotic mixture (*Lactobacillus acidophilus*, *Bacillus subtilis*, BB, and *Enterococcus faecium*) did not affect carcass traits of broilers at 42 D of age. Regarding the effect of BT or

**Table 8.** Effect of dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* on caecal microflora ($\log_{10}$ CFU/g wet weight; total bacterial counts (TBC), probiotic bacteria, coliforms, and *Escherichia coli*) of quail birds.

| Treatments | TBC (~0.5 $\log_{10}$ CFU/g) | Probiotic | Coliforms | *E. coli* |
|---|---|---|---|---|
| T1 | $8.77^{a}$ | 7.82 | $6.96^{a}$ | $5.93^{a,b}$ |
| T2 | $8.65^{a,b}$ | 7.55 | $6.47^{b}$ | $6.13^{a}$ |
| T3 | $8.53^{b,c,d}$ | 7.02 | $6.59^{b}$ | $5.53^{a,b,c}$ |
| T4 | $8.63^{b,c}$ | 7.64 | $6.49^{b}$ | $5.49^{a,b,c}$ |
| T5 | $8.55^{b,c,d}$ | 7.49 | $5.62^{e}$ | $4.52^{d}$ |
| T6 | $8.51^{c,d}$ | 7.15 | $6.60^{b}$ | $5.04^{b,c,d}$ |
| T7 | $8.51^{c,d}$ | 7.48 | $6.12^{c,d}$ | $5.11^{b,c,d}$ |
| T8 | $8.50^{c,d}$ | 7.39 | $6.34^{b,c}$ | $5.17^{b,c,d}$ |
| T9 | $8.46^{d}$ | 7.42 | $5.93^{d}$ | $4.75^{c,d}$ |
| T10 | $8.65^{a,b}$ | 7.61 | $6.32^{b,c}$ | $5.21^{b,c,d}$ |
| *P*-value | <0.001 | 0.542 | <0.001 | <0.001 |
| SEM | 0.024 | 0.042 | 0.021 | 0.052 |

Means in the same column within each classification bearing different letters are significantly ($P < 0.05$) different.

BB on quail meat quality, a significant increase in the pH of meat collected from the T4, T5, and T6 groups and WHC in all treated groups (except T2 and T3 groups) was observed, while TBA values, the color score of quail meat, cooking loss, proteolysis and TC were reduced in all the probiotic- enriched groups (except T6 for $b^*$). Administration of probiotics in poultry diets might influence the pH of meat depending on the specifics of the experimental design and the type of microorganisms (Popova, 2007). Ivanovic et al. (2012) reported that dietary inclusion of 0.05% *Streptococcus faecium* reduced meat cut pH, whereas administration of *B. cereus* increased their pH value. Zheng et al. (2014) observed higher pH of chicken breast meat accompanied by lower drip loss and cooking loss in groups treated with *E. faecium*. Meat color (measured by redness [$a^*$], yellowness [$b^*$], and lightness [$L^*$]) is important for consumers' perception of the freshness and quality of meat. Haščík et al. (2015a) mentioned that redness in breast muscle was increased, whereas the values of $L^*$ and $b^*$ for broiler breast meat were not altered because of probiotic (*Lactobacillus fermentum*) supplementation to drinking water. However, Haščík et al. (2015b) found a significant increase in redness and yellowness of thigh and increase in the lightness of breast and thigh cuts in birds fed probiotics alone or in combination with pollen.

Studies showed that probiotic bacteria produce antimicrobial components including acetic and lactic acid, carbon dioxide, hydrogen peroxide, diacetyl, and bacteriocins or bacteriocin-like substances, which exhibit a high degree of antibacterial and antifungal activities. Serafini et al. (2013) reported an inhibitory activity of BB against pathogenic such as *Cronobacter sakazakii* and *E. coli*. Our findings revealed that increasing levels of BT or BB levels delayed the proliferation of microorganisms in the diet and depressed the intestinal coliform, TBC, and *E. coli* populations without affecting the populations of probiotic bacteria. Supplementing the quail diet with BT showed strong antibacterial properties against gram-negative and gram-positive bacteria. The main functional impacts of BT and BB are producing antimicrobial substances such as bacteriocins (Mandal et al., 2014), dietary fibers assimilation (Slavin, 2013), fat storage regulation (Di-Baise et al., 2012), mucosal immunity modulation (Hardy et al., 2013), and regulating gut flora through the competitive insularity of pathogenic bacteria, which decreases the colonization of pathogens (Yu et al., 2011). Our results are in line with those of Abd El-Moneim et al. (2020) who reported that ileal enumeration of TBC and total coliform were reduced, while the count of lactic acid bacteria was increased in broilers treated with BB. Therefore, other investigations reported similar impacts of certain probiotics on modifying and fortifying the composition of the intestinal microbiota of chickens by increasing beneficial microorganisms and suppressing pathogenic microorganisms (Mountzouris et al., 2010).

From the results mentioned previously, a conclusion could be drawn that supplementing BT or BB to a growing Japanese quail diet was found to be beneficial for improving growth performance and meat quality traits and modifing the microbial populations in both thecaecum and diets. As per our results, we can recommend the application of T5 and T8 to T10 levels for the best performance, carcass traits, and meat quality of growing quails.

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## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.04.019.

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