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Original article

Effect of graded levels of dietary *Bacillus toyonensis* and *Bifidobacterium bifidum* supplementation on growth, carcass traits and ileal histomorphometry and microbiota of growing quails

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ABSTRACT

This experiment investigated the role of graded dietary levels of two probiotic strains (*Bacillus toyonensis;* BT and *Bifidobacterium bifidum;* BB) on the growth rate, carcass traits, physiological and histological aspects of growing Japanese quail. One thousand and three hundred sixty one-day-old un-sexed Japanese quail chicks were distributed randomly into ten groups. The 1st group served as a control and fed the basal diet without supplement while the 2nd, 3rd, 4th and 5th groups received the control diet supplemented with 0.05, 0.075, 0.10 and 0.125% BT, respectively. The 6th group fed the control diet plus 0.10% BB while the remaining groups (7th to 10th) received the basal diet incorporated with the previous levels of BT rich with 0.05% BB. Dietary supplementation of BT and/or BB increased body weight and gain; however, feed intake and feed conversion were not affected. Amylase activity was significantly elevated in 5th, 7th and 9th groups, while lipase activity was improved in all treatment groups except 3rd and 6th groups. Results obtained concluded that dietary supplementation of BT with or without BB is useful for performance, digestive enzyme activities, blood cholesterols, antioxidant status and ileal histomorphometry and microbiota of growing Japanese quail.

1. Introduction

The confusing use of antibiotics in poultry ranches expanded the general wellbeing suspicion related to the development of resistant pathogens (Abd El-Moneim, 2017; Abd El-Moneim et al., 2019; Abd El-Moneim and Sabic, 2019; Abdel-Moneim et al., 2019) and antibiotics residuals in meat and egg products (Chen et al., 2005; Abou-Kassem et al., 2018). Prohibiting antibiotic growth providers from European Union, followed by several other countries, has challenged poultry scientists to find appropriate alternatives. As unconventional substitutions to chemotherapy and green feed additives, probiotics are recently used in the poultry industry to maintain enteric counts of beneficial microbiota (Alagawany et al., 2018; Abd El-Hack et al., 2018; Mahrose et al., 2019), intestinal pathogens competition for nutrients and attachment sites and inhibit their *adhesion*, enhance diets utilization by improving digestive absorption process, enhance the immune response of the host and supply it with compounds that served as metabolic energy sources (Abd El-Hack et al., 2017; Abd El-Moneim, 2017; Abd El-Moneim et al., 2019; Alagawany et al., 2016; Estrada et al., 2001; Lodemann et al., 2008). Various strains of bifidobacteria, including *Bifidobacterium bifidum* (BB), have been used in poultry and animal production as well as in humans diets as alternatives to chemotherapeutic agents (Abd El-Moneim, 2017; Abd El-Moneim et al., 2019; Dankowiakowska et al., 2013; Kantas et al., 2015 press) because of its ability to produce antimicrobial

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substances for instance bacteriocins (bifidin and bifidocin B) and inhibit the multiplication of numerous gram-positive and gram-negative bacteria (Shah and Dave, 2002; Touré et al., 2003). Bifidocin B has antibacterial properties for numerous pathogens, for instance, Enterococcus, *Pediococcus spp*., Leuconostoc, Lactobacillus, and Listeria, while bifidin can inhibit counts of *Micrococcus flavus* and *Staphylococcus aureus* (Shah and Dave, 2002). *Bacillus toyonensis* (BT), as aerobic non-pathogenic gram-positive, is a spore-forming bacterium, fermentative and has been considered as probiotic supplement in animal rations (Roos et al., 2018; Abdel-Moneim et al., in press-b; Williams et al., 2009). Synergistic effects of mixtures combining aerobic and anaerobic probiotic strains might be presented by improving poultry health and production. Based on our lab's previous work either published (Abd El-Moneim et al., 2019; Abdel-Moneim et al., 2019a,b) or not we noticed that BB could promote birds growth performance at doses of 10^8 to 10^9 CFU/ kg diet. Therefore, the current study aimed to evaluate the effect of dietary gradual levels of BT, a single level of BB or mixture between BT levels and the half dose of BB on growing performance, carcass traits, physiological and histological aspects of growing quail.

2. Materials and method

The present study conducted at the Poultry Research Farm, Department of Biological Applications, Radioisotope Application Division, Nuclear Research Center, Egyptian Atomic Energy Authority (EAEA), Inshas region. The experimental procedures were performed due to the etical guidelines of the Department of Biological Applications, Nuclear Research Center, Egyptian Atomic Energy Authority (EAEA).

2.1. Animals, strains and diets

Bacillus toyonensis (BT) and *Bifidobacterium bifidum* (BB) strains have been obtained from Prof. Samir Mahgoub at the Department of Microbiology, Faculty of Agriculture, Zagazig University, Egypt. These strains were examined as probiotic bacteria. One thousand and three hundred sixty at one day of age, quail chicks were randomly divided into ten equal groups; each was sub-divided equally into eight replications. During the experimental period (1 to 42 d), control birds were fed a corn-soybean basal diet (T1) while T2, T3, T4 and T5 were fed the control diet plus 0.05, 0.075, 0.1 and 0.125% BT. Groups T6 fed the control diet supplemented with 0.10% BB. The remaining groups from T7 to T10 were fed on the basal diet incorporated with the same doses of groups T2 to T5, respectively, plus 0.05% BB. The diets (in mash form) were formulated to cover growing quail requirements as suggested by the NRC (1994). Ingredients and chemical composition of the basal diet shown in Table 1.

2.2. Management

Quail chicks of whole groups were reared throughout the trial period inappropriate conditions. Each replicate was housed in one cage ($100 \times 50 \times 60$ cm^3). Quail chicks were exposed to continuous program light up to the end of the first three days of age after that received 23 h light per day. Each brooder cage was supplied by one fluorescent lamp. All birds were kept in the same environmental, managerial status and hygienic terms and all chicks had free access to water and feed during the investigation periods. All drinkers and feeding troughs were cleaned daily.

Table 1
Composition of the basal diet and its calculated analysis.

Basal Diet	Ingredients
554	Yellow maize
396	Soybean meal (44%)
7.50	Di-calcium phosphate
15.0	Limestone
3.00	Sodium chloride
3.00	Premix[1]
1.50	D. L. methionine
20.0	Palm oil
Calculated analysis[2] (g. per kg)	
220.0	Crude protein (CP)
12.186	Metabolizable Energy (MJ/kg)
39.9	Crude fiber (CF)
12.1	Lysine (L)
5.20	Methionine (Met)
8.60	Methionine + Cysteine (Met + Cys)
8.52	Calcium (Ca)
3.30	Available phosphorus (P)

[1] Premix mixture provided each kg. diet contained: Vit, A, 12,000 I.U, Vit. D3, 5,000 I.U, Vit, E, 16.7 g, Vit. K, 0.67 g, Vit. B1, 0.67 g, Vit. B2, 2 g, Vit. B6, 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).

2.3. Growth performance

Quails were individually weighed weekly to the nearest 0.10 g before receiving any feed and water in the early morning to obtain the body weight. Weight gain and feed intake were recorded weekly to calculate the feed conversion ratio as g feed/ g gain. At the group's experiment end, eight birds were randomly chosen, fasted overnight, weighed, and then slaughtered. Empty hot carcasses, liver, gizzard and heart were weighed and proportioned to pre-slaughtering weight. All of the carcass parts calculated as described by (Abd El-Moneim et al., 2019). Duodenum samples were collected to determine activities of digestive enzymes, while ileum samples were subjected to histological examination.

2.4. Enzyme activity assay

As described by Abdel-Moneim et al. (in press-b), collected duodenum samples were used to obtain a homogeny digesta sample by tract massaging from both ends. Based on samples weights, they were diluted ten times with ice-cold neutral PBS, homogenized and then centrifuged at 4500g for 15 min at 5 °C and supernatants stored at 4 °C. Protease, lipase and amylase analyses were conducted within 24 h after extraction following the methods qualified by Lowry et al. (1951), Boutwell (1962) and Coles (1986), respectively.

2.5. Blood sampling and biochemistry

Blood sampling was performed during slaughtering from eight quails per group, immediately centrifuged at 3500 g for 20 min. and then sera were frozen at −25 °C till the biochemical analysis. The concentrations of serum total protein, albumin, glucose level, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, creatinine urea-N, triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) were determined due to the manufacturer's instructions of commercial kits (Spinreact Co., Spain). Thyroxine and triiodothyronine concentrations in sera samples were measured using

radioimmunoassay (RIA) kits. Serum contents of glutathione (GSH), malondialdehyde (MDA), and activities of glutathione reductase (GSR), glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined according to the manufacturer's instructions of commercial kits (Cell Biolabs Inc., USA).

2.6. Histological observations

Ileal specimens were gathered and fixed in formalin solution 10% (pH 7.0) for 48 h, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Paraffin blocks were sliced using a microtome (Leica RM 2155, England) into five-micron slices. After section preparation, they were stained with hematoxylin and eosin for histological evaluation. Morphometric analysis was done by camera microscope AmScope® software as the following: Villus height (μm) measured from the tip to the base of villus and diameter. Villus width, Muscular thickness and submucosa layer (Crypt depth) thickness were also calculated (Seyyedin and Nazem, 2017).

2.7. Bacterial analysis

At the end of the experimental period (42 d), the ileal contents (10 cm anterior to the junction with caecum and rectum) of 3 birds in each replicate were separately collected into the sterile tubes for microbiological examination. The control birds were fed a corn-soybean basal diet (T1) while T2, T3, T4 and T5 were fed the control diet plus 0.05, 0.075, 0.1 and 0.125% BT. Groups T6 fed the control diet supplemented with 0.10% BB. The remaining groups from T7 to T10 were fed on the basal diet incorporated with the same doses of groups T2 to T5, respectively, plus 0.05% BB. The dietary samples supplemented by *B. toyonensis* (BT) probiotic bacteria (0.5, 0.75, 1.0 and 1.25 ml/kg) named T1, T2, T3 and T4, respectively. While the dietary samples supplemented by *Bifido. bifidum* (BB) probiotic bacteria (1.0 ml/kg) named T5. T6 treatment service as control. The dietary samples supplemented by co-culture from the two probiotic bacteria (0.5 BT + 0.5 BB, 0.75 BT + 0.5 BB, 1.0 BT + 0.5 BB and 1.25 BT + 0.5 BB) named T7, T8, T9 and T10, respectively. About one g of ileal digesta was added into the stomacher bag (Sewared, London, UK), and homogenized with 10 ml of sterile saline peptone water (SPW:1 g/l peptone, 8.5 g/l sodium chloride) for 3 min. The TBC, coliform, *E. coli*, *Salmonella* spp., *Bacillus toyonensis* and *Bifidobacterium bifidum* were determined by serial dilution (10^{-2} to 10^{-8}) of ileal samples before inoculation onto Petri dishes. The TBC were determined on plate count agar (Merck, 1.05463) after 48 h incubation at 30 °C. Violet Red Bile agar (VRB, Biolife, Italy) was used for counting coliform after 24 h incubation at 37 °C in anaerobic media. *E. coli* were counted on MacConkey agar (Oxoid, CM0007) after 24 h at 37 °C in anaerobic media. *Salmonella* spp. were counting onto SS agar (*Salmonella* and *Shigella* agar, Biolife, Melano, Italy). *Bifidobacterium* were counted on MRS agar after 24 h at 37 °C in anaerobic media. *Bacillus* were counted after pasteurized the dilution at 80 °C for 15 min. on nutrient agar after 24 h at 35 °C in anaerobic media All plates were examined for typical colony types and morphological characteristics associated to each culture medium. The number of bacterial group was transferred to Log number before statistical analysis.

2.8. Statistics

Collected data were analyzed by one-way analysis of variance using the general linear model (GLM) procedures of SPSS software, Version 18.0. The experiment unit was considered the cage for productive performance parameters, whereas the individual data was the experimental unit for the rest of the parameters. Tukey's multiple comparison test estimated significant differences among means at a significant level of $P < 0.05$. The statistical model used was:

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

where Y_{ij} is an observation, μ is the overall mean, T_i is effect of dietary treatment, and e_{ij} is the experimental random error.

3. Results

3.1. Growth performance

Results in Table 2 show a significant increase in quails' body weight at all studied ages except the first wk of age by dietary treatment with BT and/or BB compared to the control. Weight gain was also increased with these dietary treatments during all experimental periods except the period from 21 to 28 d as compared to the control. Groups T9 and T10 recorded the best growth performance at the overall experimental period, followed by T5 and T6 groups (Table 3). Feed intake was increased ($p < 0.01$) in groups T4 to T8 only at the period (8–14 d) while not affected at the remaining experimental periods and the overall one (Table 4). The feed conversion ratio was also non-significantly influenced by dietary treatments during all experimental periods (Table 5). The effects of dietary BT and/or BB on the carcass traits of growing quail birds are shown in Table 6. Dressing percentage, carcass yield and relative weights of liver and heart were significantly ($p < 0.01$) influenced while the gizzard's relative weight was not affected. The highest dressing percentage and carcass yield values were recorded in the T8 group compared with the other treatment groups.

3.2. Digestive enzyme activities

Duodenal digestive enzyme activities across all treatments are presented in Table 7. Amylase activity was significantly elevated in T5, T7 and T9, while lipase activity was improved in all treatment groups except T3 and T6 compared with the control. Protease activity was insignificantly affected in all treated groups. The highest values of amylase and lipase were recorded in birds of the T5, T7 and T9 groups.

3.3. Blood biochemistry

Serum hepatic and renal function biomarkers of quails treated with BT and/or BB are shown in Table 8. Increasing the supplementation level of BT increased serum levels of total protein and albumin and decreased AST and ALT activities. Feeding on the T6 diet was not significantly affected the parameters above, while the combination between BT and BB generally, not affect these parameters except in groups T8 and T9. Serum ALP, uric acid, urea-N and creatinine values were not influenced by all treatments than the control group.

As presented in Table 9, all feeding treatments except groups T2 and T3 reduced serum levels of TG and VLDL and increased HDL level, while TC and LDL values were insignificantly affected. The hypocholesterolemic impact of feeding BT was significantly enhanced by increasing its dietary level and its combination with BB in the quail diet. Furthermore, blood glucose concentrations were insignificantly altered among experimental groups, while triiodothyronine level was increased significantly in groups fed BT levels. Thyroxin activity was also markedly elevated in the abovementioned groups and T6 group. Interestingly, the blood levels of triiodothyronine and thyroxin were not affected by the BT and BB mixture's dietary supplementation.

Table 2
Effect of *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on body weight of growing quail.

Treatments (ml/kg diet)	1 d	7 d	14 d	21 d	28 d	35 d	42 d
Control	9.37	23.23	37.79^{e}	85.65^{e}	122.05^{e}	173.50^{e}	209.83^{de}
0.50 BT	9.58	24.35	40.37^{de}	89.35^{d}	131.28^{d}	177.11^{de}	206.93^{e}
0.75 BT	9.41	24.32	43.08^{d}	90.87^{d}	130.14^{d}	180.05^{d}	213.79^{d}
1.00 BT	9.47	25.57	40.90^{de}	94.73^{c}	141.28^{cd}	175.72^{de}	225.07^{bc}
1.25 BT	9.60	26.96	54.89^{a}	105.28^{b}	149.24^{b}	196.35^{a}	241.64^{a}
1.00 BB	9.53	24.93	55.80^{a}	100.65^{bc}	135.65^{d}	189.41^{b}	226.86^{b}
0.50 BT + 0.50 BB	9.60	26.33	51.53^{b}	113.46^{a}	158.96^{a}	193.75^{ab}	223.22^{c}
0.75 BT + 0.50 BB	9.54	25.36	49.16^{b}	116.78^{a}	160.88^{a}	195.32^{a}	221.57^{c}
1.00 BT + 0.50 BB	9.59	24.85	45.50^{c}	102.72^{b}	146.19^{c}	179.09^{d}	227.67^{b}
1.25 BT + 0.50 BB	9.57	24.90	46.88^{c}	104.96^{b}	149.48^{b}	186.51^{c}	240.97^{a}
SEM	0.09	0.21	0.49	1.91	2.13	2.35	2.22
P-value	0.748	0.452	0.010	<0.001	0.007	0.013	0.009

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.

Table 3
Effect of dietary *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on weight gain of growing quail.

Treatments (ml/kg diet)	1–7 d	8–14 d	15–21 d	22–28 d	29–35 d	36–42 d	1–42 d
Control	1.98^{f}	2.08^{e}	3.12^{ef}	5.27	7.35^{a}	5.19^{cd}	4.33^{d}
0.50 BT	2.11^{e}	2.34^{de}	3.44^{e}	5.99	6.69^{b}	4.26^{de}	4.30^{d}
0.75 BT	2.13^{e}	2.68^{d}	3.41^{e}	5.61	7.13^{ab}	4.82^{d}	4.47^{cd}
1.00 BT	2.30^{c}	2.19^{de}	3.82^{d}	6.65	4.92^{d}	7.05^{b}	4.66^{bc}
1.25 BT	2.48^{a}	3.99^{b}	3.59^{d}	6.28	6.73^{b}	6.47^{c}	5.09^{a}
1.00 BB	2.20^{d}	4.41^{a}	2.99^{f}	5.00	7.68^{a}	5.35^{cd}	4.77^{b}
0.50 BT + 0.50 BB	2.39^{b}	3.60^{bc}	5.54^{b}	6.50	4.97^{d}	4.21^{de}	4.70^{bc}
0.75 BT + 0.50 BB	2.26^{c}	3.40^{c}	6.17^{a}	6.30	4.92^{d}	3.75^{e}	4.63^{bc}
1.00 BT + 0.50 BB	2.18^{d}	2.95^{cd}	4.93^{c}	6.21	4.70^{d}	6.94^{b}	4.82^{ab}
1.25 BT + 0.50 BB	2.19^{d}	3.14^{cd}	4.76^{c}	6.36	5.29^{c}	7.78^{a}	5.09^{a}
SEM	0.03	0.17	0.21	0.16	0.24	0.27	0.05
P value	0.001	0.005	0.001	0.282	0.001	0.001	0.001

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.

Table 4
Feed intake (g/bird/day) of Japanese quail as affected by dietary *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) during the experimental periods.

Treatments (ml/kg diet)	1–7 d	8–14 d	15–21 d	22–28 d	29–35 d	36–42 d	1–42 d
Control	4.56	7.77^{d}	13.75	17.32	20.92	21.93	14.41
0.50 BT	4.24	7.36^{de}	12.76	14.58	22.55	21.93	13.96
0.75 BT	4.39	8.26^{cd}	14.45	15.56	23.21	22.83	14.69
1.00 BT	4.84	6.81^{e}	11.24	19.14	18.89	24.12	14.23
1.25 BT	6.15	9.73^{b}	15.92	17.40	21.68	22.92	15.68
1.00 BB	6.37	10.81^{a}	15.77	13.27	21.27	20.28	14.68
0.50 BT + 0.50 BB	7.64	8.79^{c}	15.57	17.36	20.02	18.65	14.72
0.75 BT + 0.50 BB	6.70	8.59^{c}	15.56	18.31	22.10	17.57	14.89
1.00 BT + 0.50 BB	4.24	7.39^{de}	17.28	22.37	18.88	23.59	15.51
1.25 BT + 0.50 BB	4.71	7.23^{de}	16.28	20.75	16.25	21.53	14.53
SEM	0.37	0.34	0.68	0.75	0.95	0.99	0.71
P value	0.195	0.010	0.750	0.873	0.925	0.937	0.990

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.

3.4. Antioxidant status

Results presented in Table 10 revealed that the antioxidant system of Japanese quails was improved by probiotics dietary treatments, where serum CAT activity increased and MDA content reduced significantly in all treatment groups compared with those of control. Furthermore, serum GSH content and SOD activity were significantly elevated in birds of experimental groups G2, G9 and G10. However, serum GPx, GSR and GST values were insignificantly altered by dietary treatment compared with the control group.

3.5. Ileal histomorphometry

Data presented in Table 11 show significant improvement in the mucosal architecture in terms of increased ileal villus height (VH), villus width (VW), muscular thickness (MT) and VH: CD ratio, as well as decreased crypt depth (CD) in quail fed probiotic diets. Groups T5 and T6 recorded the highest VH values, VW, MT and VH: CD ratio and lowest value of CD. The mixture between BT and BB also enhanced the parameters mentioned above compared with the un-supplemented group. However, although the combination between these two probiotic strains (especially groups T9 and T10) improved ileal function, it did not exhibit a 2-fold increment impact than the individual supplementation.

3.6. Ileal bacterial content

Impacts of BT and BB on the microbiological funging of total bacterial count (TBC), total coliform count (TCC), *E. coli* and *Salmonella* count in ileal contentare are investigated in the current

Table 5
Feed conversion ratio (g feed/g gain) of Japanese quail as affected by dietary *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) during the experimental periods.

Treatments (ml/kg diet)	1–7 d	8–14 d	15–21 d	22–28 d	29–35 d	36–42 d	1–42 d
Control	2.28	4.22	4.41	3.24	2.84	3.57	3.32
0.50 BT	2.01	3.39	3.73	2.36	3.42	4.15	3.24
0.75 BT	2.06	3.09	4.21	2.72	3.24	3.93	3.28
1.00 BT	2.11	3.39	3.06	2.89	3.86	3.03	3.05
1.25 BT	2.46	2.59	4.49	2.80	3.24	3.08	3.07
1.00 BB	2.86	2.53	5.53	2.65	2.81	3.20	3.06
0.50 BT + 0.50 BB	3.19	2.63	2.84	2.68	4.02	3.57	3.13
0.75 BT + 0.50 BB	2.97	2.73	2.53	2.89	4.53	3.84	3.21
1.00 BT + 0.50 BB	1.94	2.77	3.49	3.69	4.28	3.03	3.22
1.25 BT + 0.50 BB	2.15	2.52	3.42	3.24	3.10	2.46	2.86
SEM	0.11	0.14	0.17	0.13	0.15	0.21	0.23
P value	0.302	0.333	0.079	0.919	0.455	0.464	0.986

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.

Table 6
Carcass and some digestive tract traits of Japanese quail as affected by dietary *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) at the end of the experimental periods.

Treatments (ml/kg diet)	Pre-slaughter weight	Dressing %	Liver %	Gizzard %	Heart %	Carcass yield %
Control	183.50^{e}	78.39^{b}	2.12^{cd}	2.09	0.80^{c}	83.39^{b}
0.50 BT	191.00^{d}	77.94^{b}	2.54^{a}	1.99	0.70^{f}	83.17^{b}
0.75 BT	192.50^{d}	76.10^{c}	1.99^{e}	1.89	0.91^{a}	80.90^{e}
1.00 BT	209.00^{b}	75.47^{d}	2.20^{c}	2.05	0.76^{d}	80.48^{e}
1.25 BT	219.00^{a}	77.31^{b}	2.14^{cd}	1.85	0.79^{c}	82.10^{c}
1.00 BB	204.36^{c}	79.07^{a}	1.77^{f}	1.89	0.83^{b}	83.53^{b}
0.50 BT + 0.50 BB	209.06^{b}	76.63^{c}	1.82^{f}	1.81	0.90^{a}	81.17^{e}
0.75 BT + 0.50 BB	213.21^{b}	79.78^{a}	2.40^{b}	2.03	0.73^{e}	84.94^{a}
1.00 BT + 0.50 BB	211.50^{b}	76.75^{c}	2.32^{bc}	1.77	0.87^{ab}	81.72^{d}
1.25 BT + 0.50 BB	220.03^{a}	77.46^{b}	2.45^{b}	1.91	0.84^{b}	82.65^{c}
SEM	2.25	0.29	0.05	0.08	0.03	0.75
P value	<0.001	0.003	<0.001	0.128	<0.001	0.001

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.

Table 7
Effect of dietary *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on digestive enzyme activities of growing Japanese quail.

Treatments (ml/kg diet)	AMZ, U g^{-1} digesta	LPZ, U g^{-1} digesta	PRZ, μmol g^{-1} digesta
Control	603.7^{d}	4.053^{e}	0.294
0.50 BT	870.0^{cd}	10.85^{bcd}	0.345
0.75 BT	725.5^{d}	7.401^{de}	0.321
1.00 BT	1036.5^{bcd}	10.91^{bcd}	0.321
1.25 BT	1302.5^{bc}	18.56^{a}	0.339
1.00 BB	789.0^{cd}	8.182^{cde}	0.316
0.50 BT + 0.50 BB	1938.5^{a}	23.01^{a}	0.338
0.75 BT + 0.50 BB	1025.0^{bcd}	13.27^{b}	0.329
1.00 BT + 0.50 BB	1485.0^{ab}	12.89^{bc}	0.315
1.25 BT + 0.50 BB	1090.0^{bcd}	9.095^{bcd}	0.312
SEM	165.51	1.13	0.037
P value	<0.001	<0.001	0.993

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.
AMZ: Amylaze, LPZ: Lipase, PRZ, Protease.

study. Our results emphasized that increasing BT or BB or BT + BB levels significantly reduced the intestinal TBC, coliform *E. coli* and *Salmonella* spp. populations. The results are presented in Table 12 showed the antimicrobial activity of dietary supplemented with BT or BB or BT + BB against the TBC, coliform, *Salmonella* spp. and *E. coli* populations. Whereas increasing BT or BB or BT + BB levels in the diet decreased significantly ($P < 0.05$) of *E. coli*, *Salmonella* and coliform with approximately 0.75 to 1.5 Log_{10} CFU/g and decreased TBC (~1.0 Log_{10} CFU/g) without affecting on the populations of probiotic bacteria bacteria. Supplementation of broilers' diet with BT showed a strong anti-bacterial activity against Gram positive and Gram negative bacteria. The antibacterial activities of probiotic bacteria and bacillus such as *Bifidobacterium bifidum* are due to lactic acid contents and other compounds.

4. Discussion

The improvement in quails' body weight in the present study might be attributed to stimulating the production of certain vitamins and other active substances in a multi-species probiotic. This could inhibit the growth of the enteropathpgens in the quail gut by decreasing the pH of intestine, improving digestion, and consequently enhancing the utilization of nutrients, which positively reflected the values of body weight (Premavalli et al., 2018). Also, there was an explanation clarified by Applegate et al. (2010), who confirmed that treatment of probiotics resulted in bacterial antagonism, colonization competition and emulation for nutrients. These actions decrease toxic compounds, modulate the immune system and increase nutrient absorption and digestibility, leading to improved body weight. Enhancement in weight gain may be due to probiotics, which decreases the pathogenic bacteria and active levels of digestive enzymes, especially in T7 and T9. In the same trend (Wang and Gu, 2010) reported the improvement of weight gain might be because of the contribution of exogenous enzymes secreted by probiotics and the endogenous enzymes produced by the host. Recently, in the chicken from treatments received the probiotic in their drinking water with grading amounts of 0.05, 0.10 and 0.25 g/L, the body weight gain was significantly ($p = 0.027$) greater than in the control group not receive the probiotic, (Abramowicz et al., 2019).

On the other hand, the feed conversion ratio results were in partial agreement with (Abdel-Moneim et al., in press-b), who

Table 8
Influence of *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on serum hepatic and renal functions biomarkers of Japanese quail birds.

Treatments (ml/kg diet)	Total protein, g dl^{-1}	Albumin, g dl^{-1}	AST, U L^{-1}	ALT, U L^{-1}	ALP, U L^{-1}	Uric Acid, mg dl^{-1}	Urea-N, mg dl^{-1}	Creatinine, mg dl^{-1}
Control	3.74^{c}	1.90^{e}	227.9^{ab}	15.48^{a}	966.6	7.83	4.35	0.580^{abc}
0.50 BT	5.18^{ab}	2.87^{ab}	152.8^{de}	12.95^{ab}	1007.2	6.76	3.69	0.710^{a}
0.75 BT	5.44^{a}	2.93^{a}	145.8^{de}	16.03^{a}	983.4	8.12	3.28	0.535^{bc}
1.00 BT	4.77^{ab}	2.75^{abc}	140.5^{e}	8.09^{c}	853.1	6.67	4.48	0.430^{c}
1.25 BT	4.84^{ab}	2.66^{abcd}	155.4^{de}	8.18^{c}	864.6	8.10	4.30	0.460^{c}
1.00 BB	4.31^{bc}	2.20^{cde}	354.9^{a}	16.02^{a}	962.9	7.53	4.46	0.675^{ab}
0.50 BT + 0.50 BB	3.62^{c}	2.15^{de}	177.2^{cd}	16.08^{a}	911.9	8.62	3.69	0.565^{bc}
0.75 BT + 0.50 BB	5.15^{ab}	2.57^{abcd}	212.2^{bc}	16.66^{a}	787.2	8.21	3.79	0.520^{c}
1.00 BT + 0.50 BB	4.77^{ab}	2.32^{bcde}	178.1^{cd}	9.48^{bc}	1069.7	6.77	2.87	0.540^{bc}
1.25 BT + 0.50 BB	4.38^{bc}	1.94^{e}	197.4^{bc}	15.74^{a}	971.5	6.94	3.46	0.430^{c}
SEM	0.29	0.18	4.56	1.12	43.40	0.74	0.53	0.04
P value	0.005	0.003	< 0.001	0.001	0.703	0.370	0.396	0.003

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.
AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

Table 9
Effect of *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on the serum lipid and thyroid hormone of quail.

Treatments (ml/kg diet)	Cholesterol mg dl^{-1}	Triglycerides mg dl^{-1}	HDL- cholesterol mg.dl^{-1}	LDL- cholesterol mg dl^{-1}	VLDL- cholesterol g dl^{-1}	Glucose mg dl^{-1}	T_3, ng ml^{-1}	T_4, µg dl^{-1}
Control	233.9^{abcd}	3795.0^{a}	26.90^{c}	131.1^{abc}	75.90^{a}	353.9	0.505^{c}	3.875^{d}
0.50 BT	253.4^{abc}	3354.0^{ab}	26.17^{c}	160.2^{ab}	67.08^{ab}	271.1	0.777^{ab}	6.927^{a}
0.75 BT	281.4^{ab}	3012.0^{abc}	27.25^{c}	193.9^{a}	60.24^{abc}	276.5	0.813^{a}	6.840^{a}
1.00 BT	177.1^{d}	2466.0^{cde}	55.87^{a}	71.86^{c}	49.32^{cde}	273.7	0.773^{ab}	6.000^{ab}
1.25 BT	285.9^{ab}	2420.0^{cde}	41.10^{b}	129.7^{abc}	48.40^{cde}	233.2	0.751^{ab}	7.265^{a}
1.00 BB	290.4^{a}	1997.5^{de}	40.17^{b}	146.9^{ab}	39.95^{de}	318.9	0.601^{c}	5.695^{abc}
0.50 BT + 0.50 BB	209.3^{cd}	2691.5^{bcd}	50.07^{ab}	105.4^{bc}	53.83^{bcd}	311.4	0.556^{c}	3.905^{d}
0.75 BT + 0.50 BB	289.3^{a}	2699.3^{bcd}	39.46^{b}	149.1^{ab}	53.99^{bcd}	283.9	0.632^{bc}	5.040^{bcd}
1.00 BT + 0.50 BB	206.5^{cd}	2857.5^{bcd}	45.64^{ab}	103.7^{bc}	57.15^{bcd}	381.2	0.492^{c}	3.850^{d}
1.25 BT + 0.50 BB	215.5^{bcd}	1665.5^{e}	51.51^{ab}	130.6^{abc}	33.31^{e}	383.3	0.510^{c}	4.270^{cd}
SEM	10.52	102.30	2.89	14.35	5.60	21.92	0.04	0.55
P value	0.008	0.001	<0.001	0.020	0.001	0.060	<0.001	<0.001

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.
HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein.

Table 10
Effect of *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on antioxidant status of Japanese quail.

Treatments (ml/kg diet)	MDA, µmol ml^{-1}	GSH, ng ml^{-1}	GPX, ng ml^{-1}	SOD, qU L^{-1}	GSR, ng ml^{-1}	GST, pg ml^{-1}	CAT, ng ml^{-1}
Control	0.785^{a}	0.177^{cd}	0.184^{abc}	0.149^{c}	0.244	0.259	0.074^{d}
0.50 BT	0.578^{cd}	0.283^{ab}	0.223^{ab}	0.271^{ab}	0.393	0.312	0.257^{ab}
0.75 BT	0.585^{cd}	0.248^{abc}	0.216^{ab}	0.243^{abc}	0.314	0.390	0.219^{abc}
1.00 BT	0.558^{cd}	0.123^{d}	0.108^{c}	0.155^{c}	0.373	0.294	0.182^{bc}
1.25 BT	0.537^{d}	0.122^{d}	0.147^{bc}	0.200^{bc}	0.205	0.224	0.230^{abc}
1.00 BB	0.665^{b}	0.195^{cd}	0.248^{ab}	0.194^{bc}	0.243	0.274	0.154^{c}
0.50 BT + 0.50 BB	0.680^{b}	0.207^{bc}	0.221^{ab}	0.265^{ab}	0.365	0.305	0.154^{c}
0.75 BT + 0.50 BB	0.581^{cd}	0.235^{bc}	0.211^{ab}	0.214^{abc}	0.317	0.316	0.216^{abc}
1.00 BT + 0.50 BB	0.594^{cd}	0.316^{a}	0.265^{a}	0.284^{ab}	0.212	0.233	0.229^{abc}
1.25 BT + 0.50 BB	0.616^{bc}	0.325^{a}	0.248^{ab}	0.310^{a}	0.334	0.275	0.272^{a}
SEM	0.03	0.02	0.01	0.02	0.04	0.03	0.02
P value	<0.001	<0.001	0.047	0.025	0.121	0.255	0.001

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.
MDA: malondialdehyde, GSH: glutathione, GPx: glutathione peroxidase, SOD: superoxide dismutase, GSR: glutathione reductase, GST: glutathione S-transferase, CAT: catalase.

reported that feeding *Bacillus subtilis* spores to quails resulted in better overall FCR than the control group. Also, Panda et al. (1999) reported a non-significant effect on chicks' feed conversion rate receiving probiotics. Results of carcass traits are partly in agreement with Omar (2014), who found that liver percentage of Cobb broiler chicks increased significantly ($p < 0.05$) with group fed dietary probiotic (*L. acidophilus*) as compared to untreated ones. Contrarily, (Abd El-Moneim et al., 2019) reported insignificant alteration in carcass yield and dressing percentages in Cobb broilers *in-ovo* inoculated with different doses from BB.

The highest digestive enzyme activities were observed in birds fed the combination between Bacillus and Bifidobacteria rather than those fed Bacillus alone.

Poultry health, performance, and nutrient retention are remarkably affected by gastrointestinal enzymes, essential for nutrient digestion. In the present study, duodenal lipolytic and amylolytic activities were increased in probiotic-treated groups, while proteolytic activity was not significantly altered. These results are in agreement with the finding of Wang and Gu (2010), Jin et al. (2000) and Abdel-Moneim et al. (in press-b). They reported that

Table 11
Influence of dietary BT and BB on ileal histomorphology of growing quail.

Treatments (ml/kg diet)	VH	VW	CD	MT	VH:CD
Control	506.3^{f}	68.52^{c}	149.47^{a}	40.88^{e}	3.49^{f}
0.50 BT	560.5^{ef}	78.10^{bc}	139.47^{ab}	43.83^{de}	4.75^{f}
0.75 BT	640.6^{de}	84.29^{bc}	131.07^{abc}	48.57^{de}	5.67^{ef}
1.00 BT	729.4^{cd}	126.91^{abc}	88.64^{bcd}	52.47^{de}	8.25^{de}
1.25 BT	1197.3^{a}	204.86^{a}	62.45^{d}	117.38^{a}	19.16^{a}
1.00 BB	921.7^{b}	192.28^{a}	66.15^{d}	103.48^{ab}	13.94^{b}
0.50 BT + 0.50 BB	801.7^{bc}	104.96^{bc}	97.26^{abcd}	67.81^{cd}	8.47^{cde}
0.75 BT + 0.50 BB	748.6^{cd}	120.67^{abc}	94.85^{abcd}	64.73^{cde}	9.08^{cd}
1.00 BT + 0.50 BB	840.8^{bc}	156.45^{ab}	74.64^{cd}	78.73^{c}	11.33^{bc}
1.25 BT + 0.50 BB	817.1^{bc}	154.89^{abc}	65.92^{d}	82.94^{bc}	13.13^{b}
SEM	31.703	10.223	7.015	4.459	0.771
P value	< 0.001	0.007	0.008	< 0.001	< 0.001

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different.
SEM: standard error mean.
VH: Villus height, VW: Villus width, CD: Crypt depth, MT: Muscular thickness,.

treatment with probiotics (Bacillus or Lactobacillus) stimulated lipase and amylase activities. Contrarily, Rodjan et al. (2018) noticed insignificant changes in lipolytic, amylolytic, or proteolytic activities by administering probiotics.

Moreover, Zhang et al. (2016) reported that probiotic treatment did not affect protease activity. The current improvement in digestive enzymes' activity maybe because of the accumulative effect of exogenous enzymes secreted by probiotics and endogenous enzymes produced by the host. (Wang and Gu, 2010). Higher lipolytic, amylolytic activities improved starch and lipid digestion, which might explain the observed growth improvement in this study.

In the present study, probiotic supplementation generally, not altered serum concentrations of urea-N, uric acid and creatinine, increased total protein and albumin levels and decreased ALT and AST activities. In agreement with these results, Kasmani et al. (2012), Pourakbari et al. (2016) and Yazhini et al. (2018) noticed that probiotic treatments significantly increased serum proteins. Moreover, Kasmani et al. (2012) and Ahmed et al. (2015) are in line with our findings related to renal and hepatic function biomarkers. The inhibition exclusion mechanism could explain the elevation in serum total protein and albumin where lactic acid bacteria can improve utilization of dietary proteins via inhibiting the growth of pathogens that reduce degradation of proteins into nitrogen and improve the efficiency of dietary protein and increase nutrients absorption (Yazhini et al., 2018). Besides, the hepatoprotective effect of probiotics was reported by Rishi et al. (2009), who suggested that probiotic administration reduced hepatic translocation of pathogenic bacteria, which decrease blood transaminase levels.

Hypolipidemic effect of probiotics used in the present study agrees with earlier findings by Aluwong et al. (2013a), Pourakbari et al. (2016), Yazhini et al. (2018), Abd El-Moneim and Sabic (2019), Abdel-Moneim et al. (in press-b). The reduction impact of probiotics on blood triglycerides and cholesterols might be attributed to probiotics ability to incorporate cholesterol into their cells, hydrolyze bile salts or inhibit the rate-limiting enzyme of cholesterogenesis, hydroxymethylglutaryl-CoA (Pourakbari et al., 2016). Maintaining blood glucose level might be due to the suppressive impact of probiotic bacteria on glucagon (Aluwong et al., 2013a), which decrease blood glucose. However, serum glucose homeostasis maintained by the high absorptive capacity due to histological changes (Rodjan et al., 2018) and higher amylolytic activity noticed in this study, which increases the nutrient available to the birds. Moreover, Bacillus dietary inclusion increased serum levels of thyroxine and triiodothyronine. This rise in thyroid hormone could be interpreted by probiotics' ability to activate the thyroid-stimulating hormone-releasing hormone from the hypothalamus, thus stimulating the release of thyroid-stimulating hormone from the anterior pituitary gland (Abdel-Moneim et al., in press-b; Aluwong et al., 2013a). Furthermore, probiotics might enhance corticotrophin-releasing factor (CRF) activity, which promotes the secretion of thyrotropin and, hence, T4 secretion (Geris et al., 1996; Geris et al., 1999; Klieverik et al., 2009). Recently, Shini et al., (2020) found that feed conversion ratio probiotic of NE-H57 (*Bacillus amyloliquefaciens*) broilers was signif-

Table 12
Effect of *Bacillus toyonensis* (BT) and *Bifidobacterium bifidum* (BB) on Caecal microflora (Log_{10} CFU/g wet weight; total bacterial counts (TBC), total probiotic count (TPC), total Coliform count (TCC) *Escherichia coli* and *Salmonella* spp. in quail birds.

Treatments (ml/kg diet)	TBC	TPC	TCC	*Escherichia coli*	*Salmonella* spp.
Control	8.97^{a}	5.82^{a}	5.96^{a}	4.87^{a}	3.49^{a}
0.50 BT	8.56^{abcd}	7.52^{b}	5.53^{abc}	4.67^{ab}	2.85^{bc}
0.75 BT	8.68^{bc}	7.84^{b}	5.49^{abc}	4.37^{cd}	2.72^{bc}
1.00 BT	8.53^{bcd}	7.79^{e}	4.52^{d}	4.29^{de}	2.69^{c}
1.25 BT	8.58^{cd}	7.65^{b}	5.34^{bcd}	4.43^{bc}	2.57^{c}
1.00 BB	8.69^{ab}	7.65^{b}	5.83^{ab}	4.38^{cd}	2.59^{c}
0.50 BT + 0.50 BB	8.58^{cd}	7.88^{cd}	5.11^{acd}	4.43^{bc}	2.86^{bc}
0.75 BT + 0.50 BB	8.54^{cd}	7.69b^{c}	5.17^{bcd}	4.14^{e}	2.63^{c}
1.00 BT + 0.50 BB	8.49^{d}	7.49^{d}	4.75^{cd}	4.31^{cd}	2.49^{b}
1.25 BT + 0.50 BB	8.66^{ab}	7.68^{bc}	5.21^{bc}	4.09^{ef}	2.27^{bc}
SEM	8.58	7.46	5.59	0.05	0.07
P value	<0.001	0.008	0.024	0.012	0.041

Means in the same column within each classification bearing different letters are significantly (P 0.05) different.
SEM: standard error mean.
TBC: total bacterial counts, TPC: total probiotic count, TCC: total Coliform count.

icantly ($p < 0.001$) improved when compared to control birds (1.28 vs. 1.36, values), while there were no significant effects on BW and feed intake between control and H57 birds.

The present findings manifested the physiological role of probiotics to enhance Japanese quail's antioxidant system because its ability to produce certain substances captures reactive oxygen species, scavenge free radicals, and inhibit their cytotoxic activity (Lin and Yen, 1999). The findings of Aluwong et al. (2013b), Popović et al. (2015), Abudabos et al. (2016) are in close agreement with our results.

The development of ileum histomorphology was well presented in groups supplemented with BT and BB strains in the current study (Table 9). The better development of ileum and greater villus height are intestinal villi activity indicators and associated with better digestive enzyme activities and absorption of nutrients leading to better growth performance (Ebeid et al., 2019). However, large crypt depth can lead to poor nutrient absorption and low-performance sequences for the broiler chicks (Abd El-Moneim et al., 2019; Xu et al., 2003). The present study results are in agreement with (Abd El-Moneim, 2017; Abd El-Moneim et al., 2019). They reported that *in-ovo* inoculation of *Bifidobacterium longum* and *B. bifidum* was connected with increased villi height in hatched chicks. Furthermore, Olnood et al. (2015) and Abdel-Moneim et al. (2019a) reported a significant increase in villus length and villus length/crypt depth ratio in probiotic administrated groups.

Pathogenic bacteria (e.g., *Salmonella*, *Shigella*, *E.coli*, and *Listeria*) found when food chains (egg, meat, milk, etc..) contaminated with undesirable microbes and they could be a concern (U.S. FDA, 2016). Probiotic bacteria are defined as "live bacteria which when administered in adequate amounts confer a health benefit on the host" by WHO and FAO. Among the many probiotic bacteria, Lactobacillus and Bifidobacterium spp. are known as autochthonous microbiota in the human and animal intestinal tract. Intestinal mucus and epithelial cells are predominantly susceptible to the attachment of pathogenic microorganisms resulting in active proliferation and colonization. This microbial adhesion is critical for the beginning of pathogen and epithelial cell interaction (Ribet and Cossart, 2015). Consequently, avoiding bacterial adhesion onto the gastrointestinal mucosa is regarded as an efficient approach for decreasing the risk of foodborne disease (Thöle et al., 2015; Kim et al., 2015). Our finding showed that *Bacillus* or probiotic bacteria produce antimicrobial component and the main active components are acids such as lactic acid, which exhibit a high degree of anti-bacterial and anti-fungal activities. Serafini et al. (2013) observed inhibitory properties of B. bifidum against pathogenic bacteria (i.e., Escherichia coli and Cronobacter sakazakii) regarding enteric adaptation properties using epithelial intestinal cell monolayers (i.e., Caco-2 and HT-29). The lactic acid bacteria have been used in various functional foods for centuries. The major functional effects that are provided by probiotics are: (i) production of antimicrobial peptides (i.e., bacteriocins) (Underwood et al., 2012; Martinez et al., 2013; Mandal et al., 2014); (ii) assimilation of dietary fibers (Slavin, 2013); (iii) regulation of fat storage (Aronsson et al., 2010; DiBaise et al., 2012); (iv) modulation of mucosal immunity (Hardy et al., 2013); and (v) regulation of gut flora via competitive exclusion of pathogenic bacteria resulting in decreased pathogen colonization(Yu et al., 2010; Kim et al., 2014; Lim, 2014). Among the five key functional effects of probiotics, attachment of probiotic bacteria onto the mucosal surface of the gastrointestinal tract is regarded as essential for the competitive exclusion of pathogens and must occur before effective regulation of immune activities, resulting in protective function against intestinal pathogens (Lebeer et al., 2010; Van Tassell and Miller, 2011). The cell adhesion stage of probiotics onto colon cells is essential for the successful microbial colonization inside of the host's intestinal tract. This cell adhesion ability has been regarded as one of the critical screening standards for active probiotic strains (Duary et al., 2011) since adhesion is necessary to actively proliferate and provide a resistance to excretion from the intestinal tract as waste by peristalsis. Mechanisms of bacterial adhesion onto epithelial cell can be divided into: (i) non-specific adhesion when regarding physicochemical factors of outer cell surfaces; or (ii) specific adhesion when considering the expression of specific molecules onto the microbial membranes that directly attach to the binding sites of epithelial cell mucosal surfaces (Lebeer et al., 2010; Van Tassell and Miller, 2011; Duary et al., 2011). This adhesion ability is a function of hydrophobic properties, level of ions, pH, and physical morphology (Polak-Berecka et al., 2014). These factors considerably affect microbial adhesion onto intestinal tissues of the host, demonstrating the complexity of preliminary microbial adhesion onto the mucosal surface. According to Krasowska and Sigler (2014), microbial hydrophobicity plays a key role in the initial interaction with the mucosal surface and epithelial cells of the intestinal lining due to the chemical composition of the bacterial surfaces. The physicochemical characteristics of the microbial outer membrane are generally estimated by analysis of cell surface hydrophobicity. It has been proven that microorganisms that express higher hydrophobicity more effectively attach onto the colon cells compared to hydrophilic microbial strains (Van Tassell and Miller, 2011; Duary et al., 2011). High cost and complexity of in vivo models encouraged attention into the use of an in vitro system for the initial selection and screening of potentially adherent probiotic microorganisms. Microorganisms that express high adhesive activity to inanimate surfaces (e.g., hydrocarbon surface) or non-polar solvents are considered hydrophobic, and cells that express lower adhesive activity are considered hydrophilic (Duary et al., 2011; Krasowska and Sigler, 2014). Pelletier et al. (1997) reported that the existence of proteinaceous components on the microbial outer layer cause higher hydrophobicity, while hydrophilic properties are related to the existence of polysaccharides in the cell wall structure.

5. Conclusion

From the obtained results, it could be concluded that increasing the supplemental level of BT to the Japanese quail diet enhanced growth performance, digestive enzyme activities, antioxidant status, and ileal histomorphometry and reduced blood cholesterols levels of growing Japanese quail. Dietary incorporation of BT and BB mixture enhanced the improving impact of dietary BT alone.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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