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Original article

Productive performance, fertility and hatchability, blood indices and gut microbial load in laying quails as affected by two types of probiotic bacteria

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ABSTRACT

This study investigated two kinds of probiotic bacteria (*Bacillus toyonensis*, B1 and *Bifidobacterium bifidum*, B2) on laying Japanese quail's performance, egg quality, fertility and hatchability, blood biochemical characteristics and microbiological parameters. A total of 270 mature quails (180 females and 90 males) were distributed into ten groups in a completely randomized design at eight weeks of age. The experimental groups were as follows: T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Results revealed that egg number (EN) and egg weight (EW) were gradually increased ($P < 0.01$) as the levels of both probiotic types increased. The feed conversion ratio (FCR) was significantly ($P < 0.05$) better within the total experimental period (8–20 weeks) due to B1 alone or/with B2 supplementation. Values of yolk percentage (Y%) were statistically ($P < 0.01$) higher only at 8–20 weeks of age and T10 recorded the highest value. By increasing the level of probiotics, fertility and hatchability percentages (F% and H%) were gradually increased ($P < 0.01$ and $P < 0.05$). Creatinine (CR) level was statistically reduced in birds fed T4 diet. Also, urea-N and aspartate aminotransferase (AST) levels were reduced in treated birds. The opposite was found regarding alkaline phosphatase (ALP). Conclusively, using B1 and B2 enhanced the productive performance, some egg quality traits, fertility and hatchability, digestive enzyme activities, and reduced the harmful bacteria in the gut of laying Japanese quail. Our findings could recommend to apply T4 (basal diet + 0.10 % B1), T6 (basal diet + 0.10% B2) and T9 (basal diet + 0.10% B1 + 0.05% B2) levels for the best results.

1. Introduction

Japanese quail is widely used for several biological studies (Tsudzuki, 1994). Probiotics are mono or mixed cultures of live microorganisms which confer a health benefit on the host when administered in small amounts (Abd El-Hack et al., 2017; Abd El-Hack et al., 2020; Elbaz et al., 2021). The dietary addition of a *Lactobacillus* cultures to maize-barley hen diets improved feed conversion ratio (FCR), egg yield, egg mass and albumen quality (Abd El-Hack et al., 2018).

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The application of probiotics in poultry feed can improve the performance, nutrient digestibility and enhance immunity in birds (Abd El-Hack et al., 2020; Abdel-Moneim et al., 2020a; Saleh et al., 2021). Researchers confirmed that probiotics could maintain the performance, intestinal health and humoral immunity of birds (Soomro et al., 2019; Abdel-Moneim et al., 2020b). Probiotics maintains the beneficial microflora in the intestine, improves the resistance to enteric pathogens such as *Campylobacter* and *Salmonella* species (Abd El-Hack et al., 2020). These results in a healthy gastrointestinal tract that improves intestinal function, feed utilization, and birds' reproductive performance (Mountzouris et al., 2010; Abd El-Moneim & Sabic, 2019; Abd El-Moneim et al., 2020c). This beneficial effect could be due to the secretion of enzymes such as lipase, amylase, and protease in the intestine; which helps nutrients' digestion (Wang & Gu, 2010; Abdel-Moneim et al., 2020a).

The most usually used probiotics belonged to genera *Lactobacillus, Streptococcus, Bacillus*, *Bifidobacterium, Enterococcus, Aspergillus, Candida* and *Saccharomyces* (Gaggìa et al., 2010; Ahmed et al., 2014; Elbaz et al., 2021). Li et al. (2006) reported significant enhancements in egg yield and egg quality when 500 mg *Bacillus subtilis* culture/kg diet were added to hen diets. Abd El-Moneim & Sabic (2019) also confirmed that probiotics supplementation could increase egg productivity and improve feed efficiency. Mikulski et al. (2012) demopnsrastted that probiotics supplementation could also improve eggshell quality in laying hen. Recently, Xiang et al. (2019) reported that dietary addition of *Clostridium butyricum* might be useful for gut health, productive performance and egg quality traits of hen. Several other studies also confirmed the ability of probiotic supplementation to improve the oxidative status of birds (Fathi et al., 2018; Abd El-Moneim & Sabic, 2019; Abdel-Moneim et al., 2020a).

These beneficial microorganisms improve host health via the competition with pathogenic bacteria and the enhancement of beneficial gut microbiota in the digestive tract of broiler birds (Martínez et al., 2016; Wang et al., 2017a; Yadav & Jha, 2019).

The present study aimed to examine the impacts of gradual dietary levels of *Bacillus toyonensis,* (B1) alone or combined with *Bifidobacterium bifidum* (B2) on laying performance, egg quality, fertility, hatchability, blood biochemical parameters, antioxidant status and caecal microflora of laying Japanese quail from 8 to 20 weeks of age.

2. Materials and methods

2.1. Strains of probiotic bacteria

Two pure bacterial strains were used in the current study. These were *B. toyonensis* ATCC 55050 (B1) and *B. bifidum* ATCC 29521 (B2). These cultures were obtained from the Egyptian Culture Collection MERCIN 108 (Ain Shams University, Cairo, Egypt).

2.2. The experimental design, birds and diets

The experimental procedures were performed according to the Local Experimental Animal Care Committee. The ethical approval code was ZU-IACUC/2/F/95/2018. A total number of 270 Japanese quail birds at eight weeks of age with approximately the same average body weight were randomly distributed into ten experimental groups in a complete randomized experiment. Each group had nine replicates, each of three quails (two females + a male) in one cage. The basal diet (Table 1) was formulated as recommended by NRC (1994). The experimental groups were as follows: T1: basal diet only (control); T2, T3, T4 and T5: the basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7, T8, T9 and T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. The concentrations of B1 and B2 solutions were 5×10^8 and 6×10^8 colony forming units/ml, respectively.

Table1
Ingredients and composition of the basal diet.

Ingredients	(g/ kg)
Yellow corn	538.5
Limestone	57.0
Soybean meal (44%)	345
Di-calcium phosphate	12.0
Soybean oil	40.0
Vitamin-mineral premix[1]	3.00
Sodium chloride	3.00
DL-methionine	1.50
Calculated analysis[2] *(g/kg)*	
Metabolizable energy (MJ/kg)	12.161
Crude protein	198.4
Crude fiber	36.0
Methionine + cystine	8.00
Lysine	10.7
Phosphorus (available)	3.50
Calcium	25.8
Composition analysis (as fed)	
Moisture	122.0
Dry matter	878
Organic matter	829.4
Crude protein	207.5
Crude fiber	32.1
Ash	48.6

[1] Vitamin-mineral premix provided per kg diet: Vit. A, 4,000,000 IU; Vit.D3, 500,000 IU; Vit, E, 16.7 g., Vit.K, 0.67 g., Vit. B1, 0.67 g., Vit.B2, 2 g., Vit.B 6, 0.67 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] According to NRC (1994).

2.3. Management

The study was performed at the Experimental Poultry Farm of the Poultry Research Unit, Biological Application Department, Radioisotopes Applications Division, Nuclear Research Center, Egyptian Atomic Energy Authority, Inshas city, Egypt. Three birds (2 females and 1 male) were housed in individual cages and exposed to 16 h light/8 h dark cycle. A white fluorescent lamp supplied battery cages and the study was performed during the autumn season. The ambient temperature ranged from 20 to 31 °C. The experiment started at 56 days of age and lasted for 84 days.

Birds were kept under the same managerial, environmental and hygienic conditions. Birds had free access to feed and fresh water. Stainless steel nipple drinkers supplied battery cages. The sex ratio was one male: two females during all experimental periods.

2.4. Sampling and analyses

2.4.1. Laying performance

Live body weight change was determined by weighing layer quail to the nearest 0.1 g in the early morning before feeding at the start and at the end of the experiment at 56 and 140 days of age. Egg number (EN) was daily recorded per replicate in each cage during a total period of 56–140 days of age and egg weight (EW, g) was recorded to the nearest 0.01 g during the same term. Egg mass (EM, g) was obtained by multiplying EN by the average EW in each

cage per day from 56 to 140 days of age. Daily feed intake (FI, g/bird) was recorded as feed offer minus feed residue in the feeder. Feed conversion ratio (FCR, g feed/g egg) was calculated as g feed/g egg. Mortality rate (MR) was also recorded.

2.4.2. Egg quality measurements

Egg quality parameters involved egg shape index (ESI), shell thickness (ST), yolk %, albumen %, shell %, yolk index (YI) and Haugh Unite (HU) scores were estimated. Examinations were performed for eggs laid between 12:00 and 18:00 from each replicate and randomly collected at the end of the 140th days of age. Egg quality measurements were specified at the fourth week of each laying period, according to Ibrahim et al. (2020). Three eggs from each replicate were randomly taken (27 eggs/group).

A vernier caliper was used to determine the egg width and length to the nearest tenth of a millimeter. Then ESI was studied as egg width (mm)/egg length (mm) as mentioned by Abd El-Moneim & Sabic (2019). Eggs were carefully broken on a glass plate to measure the other egg quality traits. Shell thickness was determined using the instrument of Ames shell thickness Gage to the nearest 0.01 mm. The ST with shell membrane was determined at three areas on the egg (air cell, equator and sharp end). Yolks were isolated from albumen and weighed.

Albumen weight was detected by subtracting the weights of shell and yolk from the whole egg weight. Yolk, albumen and shell weights were expressed as a percentage of the whole egg weight. To determine YI, yolk diameter was measured to the nearest 0.05 mm by vernier caliper. The yolk index was calculated as the yolk height divided by yolk diameter (yolk height (mm)/yolk diameter (mm) × 100). HU scores were computed according to the following equation:

$$\mathrm{HU}(\%) = 100 \times \log\left(\mathrm{H} + 7.57 - 1.7 \times \mathrm{W}^{0.37}\right),$$

where H and W refer to albumen height and egg weight, respectively.

2.4.3. Fertility and hatchability percentages

A total number of 1080 eggs (108 eggs/treatment group) were randomly taken at 56 and 140 days of age and incubated. All eggs were set on the tray based on their treatment groups before being placed into the incubator cabinet. Eggs were set at 37.5℃ and the humidity percentage ranged from 50 to 60% in the incubator during the period from 1 until 15 days of incubation. At the same time, humidity increased to 80–90% in the hatcher and the temperature reduced by 1℃ till hatching. Temperature and ventilation were automatically adjusted. Eggs were automatically turned around once every two hours per day. The number of newly-hatched chicks and the eggs that were not hatched were counted. Unhatched eggs were broken to determine the number of non-fertilized eggs. Fertility and hatchability percentages were studied as:

$$\textit{Fertility}\ \% = (\textit{number of fertile eggs}/\textit{total eggs set}) \times 100$$

$$\textit{Hatchability}\ \% = (\textit{number of hatched chicks}/\textit{number of fertile eggs}) \times 100$$

2.4.4. Blood indices

By the end of the experiment, five birds from each treatment group were randomly chosen and slaughtered. Blood samples were centrifuged 2268 × *g* at - for 15 min. Serum samples were frozen at −80℃ until analysis. Serum concentrations of albumin (ALB), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea-N, uric acid (UA), alkaline phosphatase (ALP), creatinine (CR), glucose, triglycerides (TG), very low-density lipoprotein (VLDL), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were analyzed. According to the manufacturer's instructions, a spectrophotometer (Milton Roy Spectronic 1201, USA) with commercial kits (Spinreact Co., Santa Coloma, Spain) was used. As well, serum concentrations of thyroxine (T_4) and tri-iodothyronine (T_3) were measured using radioimmunoassay (RIA) kits. The serum contents of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione S-transferase (GST), glutathione reduced (GSH) and the activities of glutathione reductase (GSR) and glutathione peroxidase (GPx) were analyzed using commercial kits (Cell Biolabs Inc., USA).

2.4.5. Enzyme activity assay

The slaughtered birds (five/group) were immediately eviscerated to collect duodenum samples for enzymatic analysis. A homogenous duodenum digesta sample was collected by massaging the tract from both ends (Jin et al., 2000). The samples were diluted ten times, based on the sample weight with ice-cold phosphate-buffered saline (PBS, pH 7.0) and homogenized using a handheld glass homogenizer. The homogenate was then centrifuged at 5000*g* for 20 min at 4°C. The supernatants were separated and stored at 4°C till analysis. All enzymatic assays were conducted within 24 h after the extraction. Lipase, amylase, and protease activities were determined using the methods of Lowry et al. (1951), Boutwell (1962) and Coles (1986), respectively.

2.4.6. Microbiological analysis

The dietary samples (25 g of each diet) of B1, B2, and co-culture diets (B1 and B2) were subjected to microbiological analysis at the end of the experimental period. The microorganisms counts (total bacterial count, enterococci, total coliforms, and total fungi) were estimated as described by Feng et al. (2002). For microbial enumeration in the cecum, at week 20, five birds per group were chosen and slaughtered. The samples (1–2 g/bird) of fresh cecal digesta were subjected with a stream of CO_2 in bottles and immediately transferred to the laboratory for microbiological analysis.

About 25 g from the dietary samples was transferred into a stomacher bag (Sewared, London, UK), and homogenized with 225 ml of sterile saline peptone water (SPW: 1 g/l peptone, 8.5 g/l sodium chloride) for 3 min. A ten-fold serial dilution was made from each sample and used for the quantitative microbiological analysis. Serial dilutions of sterile saline peptone water with samples were prepared, and a duplicate of 1 ml samples of appropriate dilutions was poured on agar plates. Total viable bacterial count (TVC) was enumerated onto plate count agar (Merck, Darmstadt, Germany, # 1.05463) at 35 °C for 48 h. Total fungal counts (TFC) were counted on rose Bengal chloramphenicol agar (Lab M Limited, Lancashire , UK, # 36), supplemented with chloramphenicol at 25 °C for 5 days. The population of total coliform group were detected using violet red bile agar (VRB, Biolife Italiana, Milan, Italy) after 24 h of incubation at 37 °C.

The ileal contents (10 cm anterior to the junction with the caecum and rectum) from five quails from each replicate were separately collected into the sterile tubes for microbiological examination. About 1 g of ileal digesta was added into the stomacher bag (Sewared) and homogenized with 10 ml of sterile saline peptone water. The TVC, coliform, *Escherichia coli, Bacillus toyonensis and Bifidobacterium bifidum* were determined by serial dilution before inoculation onto Petri dishes. The TVC was determined on plate count agar (Merck, 1.05463) after 48 h of incubation at 35 °C. VRB (Biolife Italiana) was used for counting coliform after 24 h of incubation at 37 °C. *E. coli* was counted on MacConkey agar (Thermo Scientific™ Oxoid, # CM0007, Kansas, USA) after 24 h at 37 °C. *Bifidobacterium* was enumerated using MRS agar (Merck, #

110660) after 24 h at 37 °C under anaerobic conditions. *Bacillus* was counted after pasteurizing the dilution at 80 °C for 15 min using nutrient agar plates (Merck, # 111471) after 24 h at 37 °C.

The basal diet treatment was supplemented with *B. toyonensis* (B1) or *B. bifidum* (B2) or their combination to reach the viable number to 1×10^8 per gram of the diet. To avoid the contamination of spore cross-contamination, the controls and the treated birds were kept in separate rooms under the same temperature and humidity conditions within the building.

2.4.7. Statistical analysis

Data were analyzed for a completely randomized design using the generalized linear model (GLM) procedures. Duncan multiple range test was used to estimate the differences among means. Statistical significance statements were based on $P < 0.05$. The standard error mean (SEM) values were reported. 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 the effect of dietary treatment, and e_{ij} is the experimental random error.

3. Results

3.1. Productive performance

A Significant ($P < 0.05$) increase was shown in body weight change of quail layers at 20 weeks of age, which fed the diet supplemented with B1 plus B2 (T8) as compared to the other groups. In comparison, T6 and T9 recorded the lowest body weight change compared to the control group (Table 2).

3.2. Mortality rate

The MR was insignificantly affected by probiotic supplementation during the experimental period of 8–20 weeks of age (Table 2).

3.3. Egg number

Values of EN statistically ($P < 0.01$) increased in quails alongside increasing the concentration of probiotics supplementation at all experimental periods. The supplementation of B1 and/or B2 increased ($P < 0.01$) EN compared to the untreated group (T1) (Table 2). The T6 group gave the highest EN value (22.13 egg/ bird). The opposite was found in the control group, which recorded the lowest EN value (Table 2).

3.4. Egg weight

Values of EW were significantly ($P < 0.05$) higher in probiotic-treated groups than in control (T1) during the total experimental period (Table 2). The best EW value (14.29 g/ egg) was recorded by the T8 group, which had a combination between B1 and B2 (Table 2).

3.5. Egg mass

Quails fed diets supplemented with probiotics had ($P < 0.01$) increased egg mass at all interval periods and the whole experimental period than the control group (Table 2). In groups T4, T6 and T9, birds gave the highest egg mass values (10.61, 11.11 and 10.70 g/bird/day, respectively) compared to the other groups (Table 2).

3.6. Feed intake and feed conversion ratio

Feed intake was not affected by probiotics addition in all groups during all experimental periods (Table 3). However, the FCR was significantly impacted ($P < 0.05$) only at the total period. It was noticeable that T6 and T9 groups recorded the best FCR ($P < 0.05$) (3.22 and 3.15 g feed/g egg, respectively) during 8–20 weeks of age compared to the other groups (Table 3).

3.7. Fertility and hatchability percentages

Fertility percentage was gradually ($P < 0.01$) increased with increasing the level of probiotics B1 or/with B2 (Table 3). The highest values of fertility percentage were observed in T5 and T10 groups (96.69% and 96.95%, respectively). On the contrary, the lowest value was recorded by the control group (88.52%). With the same trend, hatchability percentage was significantly ($P < 0.05$) increased with increasing B1 or/with B2 during 16–20 weeks and the whole period of 8–20 weeks. The best value was recorded by T5 and T10 groups compared to the other groups (Table 3).

Table 2
Body weight change, egg number, egg weight, egg mass and mortality of laying Japanese quail fed diets supplemented with *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) during the experimental periods.

Treatments	Body weight change (g)		Egg number (bird/period)				Egg weight (g)				Egg mass (g/bird)				Mortality rate (%)
	8 weeks	20 weeks	8–12 weeks	12–16 weeks	16–20 weeks	8–20 weeks	8–12 weeks	12–16 weeks	16–20 weeks	8–20 weeks	8–12 weeks	12–16 weeks	16–20 weeks	8–20 weeks	8–20 weeks
T1	239.60	307.55b	16.94e	17.94e	17.72e	17.54e	12.99	13.23d	13.26	13.24f	8.13e	8.48e	8.39e	8.29e	3.70
T2	234.01	299.70b	21.50b	22.33a	20.61b	21.48b	13.11	13.75c	13.50	13.45e	10.06cd	10.97b	9.94c	10.32bc	0.00
T3	232.95	298.87b	22.22a	20.94c	21.52a	21.56b	13.45	13.76c	13.69	13.63d	10.67b	10.30c	10.52b	10.50b	3.70
T4	238.42	297.21b	21.78b	21.92ab	20.87b	21.52b	13.39	14.10b	13.94	13.81c	10.41c	11.04b	10.56b	10.61b	0.00
T5	237.60	302.28b	20.95c	19.61c	19.14c	19.90c	13.41	14.50a	14.09	14.00b	10.03cd	10.15c	9.63d	9.95c	0.00
T6	237.03	289.95c	22.33a	22.66a	21.38a	22.13a	13.70	14.33a	14.15	14.06b	10.93a	11.60a	10.81b	11.11a	0.00
T7	238.17	307.78b	18.30d	19.54c	18.57d	18.84d	13.51	13.88c	13.62	13.67d	8.83d	9.70d	9.03de	9.20d	3.70
T8	237.35	322.45a	20.44c	21.21b	19.40c	20.35c	14.08	14.37a	14.42	14.29a	10.28c	10.88bc	9.99c	10.39bc	0.00
T9	238.71	287.64c	21.78b	20.75c	20.07b	21.53b	13.30	14.13b	14.33	13.92bc	10.34c	10.47c	11.29a	10.70b	0.00
T10	238.53	301.94b	22.08a	21.40b	21.74a	20.74c	13.44	14.12b	13.83	13.80c	10.60b	10.79bc	9.26d	10.22bc	0.00
P-value	0.988	0.038	<0.001	< 0.001	<0.001	<0.001	0.220	0.037	0.200	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.843
SEM	1.25	2.37	0.34	0.28	0.27	0.26	0.08	0.11	0.10	0.06	0.18	0.17	0.17	0.15	0.17

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean.

Table 3
Feed intake, feed conversion ratio, fertility and hatchability of laying Japanese quail fed diets supplemented with *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) during the experimental periods.

Treatments	Feed intake (g/day/bird)				Feed conversion ratio (g feed/g egg)				Fertility (%)				Hatchability (%)			
	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks
T1	30.27	31.23	37.33	32.95	3.85	3.70	4.44	3.98a	88.57e	91.27e	85.71f	88.52e	84.48	82.09	78.60e	81.72f
T2	32.47	33.36	39.55	35.12	3.23	3.06	3.99	3.41c	94.29c	93.33d	87.91e	91.85d	83.01	84.38	80.35de	82.58e
T3	31.30	33.63	40.18	35.04	2.95	3.30	3.83	3.35c	95.71b	95.00c	92.86de	94.52bc	83.10	88.11	81.29d	84.17c
T4	33.17	34.13	39.43	35.58	3.20	3.09	3.74	3.36c	94.29c	96.56a	95.03c	95.29b	85.41	90.20	84.56bc	86.72b
T5	33.23	35.09	37.77	35.37	3.31	3.45	3.91	3.55b	96.19a	96.67a	97.21b	96.69a	85.09	93.09	88.81a	88.99a
T6	33.20	34.79	39.17	35.72	3.04	3.01	3.63	3.22d	92.82d	90.00f	94.42d	92.41c	84.91	83.16	82.18c	83.41d
T7	30.98	36.98	41.25	36.41	3.51	3.69	4.43	3.97a	95.64b	95.00c	93.21de	94.62bc	81.84	88.97	77.87f	82.56e
T8	31.54	37.02	38.50	35.02	3.07	3.40	3.86	3.37c	95.82b	95.83b	95.37c	95.68b	81.62	89.88	78.59e	83.37d
T9	33.37	30.35	37.46	33.73	3.31	2.90	3.31	3.15d	92.64d	96.67a	96.43bc	95.25b	85.51	90.89	85.27b	87.22b
T10	30.11	33.80	38.37	34.10	2.85	2.81	4.14	3.34c	96.33a	96.33a	98.21a	96.95a	85.95	91.23	89.09a	88.76a
P-value	0.952	0.96	0.66	0.35	0.07	0.11	0.09	0.041	0.010	< 0.001	< 0.001	< 0.001	0.581	0.208	0.012	0.040
SEM	0.62	0.915	0.972	0.604	0.318	0.590	0.075	0.009	1.37	1.67	1.85	1.34	1.02	1.03	2.20	3.79

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean.

Table 4
Egg shape index, shell thickness and internal egg quality traits of laying Japanese quail fed diets supplemented with *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) during the experimental periods.

Treatments	Egg shape index				Shell thickness (mm)				Yolk index				Haugh unit (%)			
	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks	8–12 weeks	12–16 weeks	12–16 weeks	8–20 weeks
T1	0.78	0.79	0.78	0.78	0.27	0.26	0.26	0.26	0.39	0.40	0.39	0.39	87.95	88.36	87.39	87.90
T2	0.81	0.79	0.78	0.79	0.28	0.25	0.26	0.26	0.40	0.39	0.42	0.41	88.36	88.95	87.88	88.40
T3	0.78	0.77	0.81	0.79	0.27	0.25	0.26	0.26	0.41	0.42	0.42	0.42	89.45	90.80	89.79	90.01
T4	0.80	0.82	0.81	0.81	0.27	0.24	0.23	0.25	0.41	0.44	0.43	0.43	90.14	90.17	89.57	89.96
T5	0.80	0.79	0.78	0.79	0.29	0.23	0.24	0.26	0.42	0.43	0.43	0.43	91.03	91.55	90.37	90.98
T6	0.80	0.81	0.76	0.79	0.28	0.23	0.27	0.26	0.41	0.40	0.40	0.40	88.79	89.42	91.05	89.75
T7	0.79	0.81	0.78	0.79	0.27	0.25	0.24	0.25	0.40	0.41	0.41	0.41	87.94	88.87	90.77	89.14
T8	0.79	0.81	0.80	0.80	0.29	0.24	0.27	0.27	0.41	0.42	0.40	0.41	89.13	89.43	90.45	89.67
T9	0.81	0.79	0.79	0.79	0.27	0.24	0.24	0.24	0.41	0.43	0.44	0.43	89.76	90.20	89.52	89.83
T10	0.81	0.80	0.81	0.80	0.28	0.25	0.25	0.26	0.41	0.42	0.43	0.42	90.44	91.54	88.94	90.31
P-value	0.594	0.665	0.399	0.529	0.287	0.923	0.245	0.556	0.247	0.610	0.415	0.387	0.385	0.278	0.347	0.517
SEM	0.01	0.02	0.01	0.01	0.003	0.004	0.004	0.002	0.01	0.03	0.02	0.01	2.08	3.11	2.17	1.93

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean.

3.8. Egg quality traits

Table 4 showed that ESI and ST were not affected by probiotic supplementation. The results of B1 showed no significant differences among all the experimental groups regarding YI and HU values during all intervals and total periods (Table 4).

Yolk % significantly ($P < 0.01$) increased only at 8–20 weeks due to probiotic supplementation in all groups compared to the control (Table 5). The highest value of yolk % was recorded by T3 (31.55%) and T10 groups (32.39%) (Table 5). At the same time, the lowest value (29.51%) was found in the control group (Table 5).

In contrast, albumen % was not influenced by B1 or B2 during all experimental periods (Table 5). Shell % was significantly ($P < 0.05$) affected by probiotics addition at the second interval period of (12–16 weeks) compared to the control (Table 5).

3.9. Blood indices

Data in Table 6 showed that CR level was significantly ($P = 0.027$) lower only in birds fed T4 diet but was not affected in the remaining groups. Urea-N and AST levels were reduced in treated birds. T5 and T6 groups recorded the highest ($P = 0.022$) levels of ALP (Table 6). On the other hand, dietary probiotic supplements did not affect TP, UA (Table 6), TC, LDL and glucose (Table 7). Moreover, TG and VLDL levels were decreased ($P = 0.018$ and 0.003, respectively) in probiotic treated groups compared to the control (Table 7).

Thyroid activity (T_3 and T_4) increased with elevating the probiotic level compared to the control group (Table 7). Antioxidant enzyme activities were remarkably enhanced by the probiotic supplements (Table 7).

Both B1 and B2 at all levels increased ($P < 0.001$) the activities of GSH, GPx, SOD, GSR, GST and CAT compared to the control (Table 8). Lipid peroxidation biomarker (MDA) was not altered except for the T6 group, which was lower ($P = 0.002$) than the control (Table 8).

3.10. Enzyme activity assay

Amylase and lipase activities were higher ($P < 0.001$) in T3, T4 and T5 groups than the other groups (Table 8). In contrast, the activity of these enzymes was not affected by the treatment with B2 alone and reduced in groups fed the combination between B1 and B2 (Table 8). Interestingly, protease activity had a different pattern from the enzymes above. It was decreased ($P < 0.001$) in

Table 5
Egg components % of laying Japanese quail fed diets supplemented with *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) during the experimental periods.

Treatments	Yolk (%)				Albumen (%)				Shell (%)			
	8–12 weeks	12–16 weeks	16–20 weeks	8–20 weeks	8–12 weeks	12–16 weeks	16–20 weeks	8–20 weeks	8–12 weeks	12–16 weeks	16–20 weeks	8–20 weeks
T1	30.29	28.83	29.39	29.51e	56.12	58.05	57.99	57.39	13.59	13.12d	12.62	13.11
T2	29.73	30.59	31.06	30.46d	57.11	55.92	55.00	56.01	13.16	13.49c	13.94	13.53
T3	32.59	30.22	31.84	31.55b	54.25	56.17	54.95	55.12	13.17	13.61c	13.21	13.33
T4	31.83	30.57	30.96	31.12c	55.86	54.60	55.41	55.29	12.31	14.83a	13.63	13.59
T5	31.18	31.43	30.87	31.16c	54.69	56.25	53.89	54.95	14.13	12.32f	15.24	13.89
T6	31.39	31.41	30.45	31.09c	54.84	55.83	55.12	55.26	13.77	12.76e	14.43	13.66
T7	29.31	32.51	30.04	30.62d	57.53	53.29	55.28	55.37	13.16	14.20b	14.68	14.02
T8	31.16	30.81	31.16	31.04c	55.08	55.66	54.30	55.02	13.76	13.53c	14.54	13.94
T9	31.05	31.87	31.47	31.46b	56.24	55.11	54.63	55.33	12.71	13.01d	13.90	13.21
T10	34.67	32.38	30.13	32.39a	52.94	54.90	56.85	54.90	12.38	12.72e	13.02	12.70
P-value	0.056	0.103	0.628	0.009	0.258	0.073	0.491	0.103	0.654	0.047	0.345	0.566
SEM	0.37	0.28	0.25	0.17	0.39	0.31	0.33	0.19	0.21	0.18	0.24	0.14

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean.

Table 6
Effects of dietary *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) on serum proteins, liver and renal functions of laying Japanese quails.

Treatments	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}
T1	4.02	2.34abc	332.3ab	14.72abc	322.8bcd	8.12	4.70a	0.72ab
T2	4.68	1.87c	292.0bc	16.07abc	446.8abcd	7.90	3.03bcd	0.67ab
T3	4.43	1.89c	366.8a	15.11abc	314.0cd	7.80	2.50d	0.72ab
T4	4.67	2.77a	284.4bc	19.14ab	326.5bcd	7.73	5.06a	0.47c
T5	4.99	2.38abc	217.0d	14.94abc	516.0a	8.68	4.27ab	0.65abc
T6	4.46	1.74c	247.5cd	14.44bc	547.4a	6.19	2.64cd	0.61bc
T7	4.90	2.36abc	297.3bc	11.02c	300.6d	8.30	4.26ab	0.81a
T8	4.54	2.66ab	273.7bcd	19.81a	454.9abcd	7.92	2.64cd	0.71ab
T9	4.85	1.82c	256.9cd	17.15ab	492.0abc	8.74	3.10bcd	0.57bc
T10	4.53	2.01bc	236.6cd	15.53abc	509.6ab	6.59	4.01abc	0.65abc
P-value	0.481	0.017	0.002	0.036	0.022	0.140	0.002	0.027
SEM	0.11	0.15	13.85	1.14	29.41	0.65	0.38	0.05

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. AST: aspartate aminotransferase, ALT: alanine aminotransferase and ALP: alkaline phosphatase. SEM: standard error mean.

Table 7
Effects of dietary *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) on serum lipid profile, glucose and thyroid hormones of laying Japanese quails.

Treatments	Cholesterol, mg. dl^{-1}	Triglycerides, mg. dl^{-1}	HDL- cholesterol, mg. dl^{-1}	LDL- cholesterol, mg. dl^{-1}	VLDL- cholesterol, mg. dl^{-1}	Glucose, mg. dl^{-1}	T_3, ng. ml^{-1}	T_4, µg. dl^{-1}
T1	368.2	1759.0a	28.39abc	284.1	351.8a	333.1	0.560e	4.065b
T2	363.0	1193.8ab	40.11a	159.1	238.8ab	360.6	0.814cd	7.085a
T3	292.6	1374.0ab	14.45c	53.25	274.8ab	373.2	0.878cd	7.220a
T4	265.9	850.4b	14.34c	81.49	170.1c	356.9	0.731d	5.800a
T5	302.7	1589.0a	22.00bc	79.60	317.8a	342.3	0.944cd	6.950a
T6	306.5	895.0b	23.73abc	240.8	119.0c	400.4	0.992bc	6.800a
T7	343.1	1341.9ab	34.57ab	223.4	270.4ab	396.4	1.135ab	6.535a
T8	360.1	1670.5a	26.84abc	199.1	334.1a	352.7	1.245a	6.795a
T9	301.8	1407.6ab	19.45bc	100.8	281.5ab	387.7	1.026abc	6.653a
T10	326.0	1256.7ab	30.16abc	144.5	251.3ab	347.0	0.854cd	6.955a
P-value	0.860	0.018	0.041	0.104	0.003	0.936	< 0.001	0.030
SEM	29.11	53.7	4.63	57.3	13.52	21.08	0.05	0.71

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very-low-density lipoprotein, T_3: triiodothyronine and T_4: thyroxine. SEM: standard error mean.

T3, T4 and T5 groups and increased by combining B1 and B2 (Table 8).

3.11. Microbiological parameters

Generally, supplementing the B1, B2, or their combination to basal diet reduced the proliferation of microorganisms in the diet. The TVC, total fungi and total coliform group in the basal diet supplemented with B1, B2 or (B1 + B2) were significantly different ($P < 0.001$) (Table 9).

Feeding T2 and T7 diets decreased ($P < 0.001$) all the microbial population in the basal diet. In addition, the TVC, coliforms and total fungi were higher ($P < 0.001$) in the control group. Therefore, it could be noticed that applying the beneficial bacteria to the quail diet reduced the population of bacteria and fungi in the basal diet (Table 9).

Table 8
Effects of dietary *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) on antioxidant status and digestive enzymes activities of laying Japanese quails.

Treatments	MDA, µmol. ml^{-1}	GSH, ng. ml^{-1}	GPX, ng. ml^{-1}	SOD, U. L^{-1}	GSR, ng. ml^{-1}	GST, pg. ml^{-1}	CAT, ng. ml^{-1}	AMZ, U.g-1 digesta	LPZ, U.g-1 digesta	PRZ, µmol.g-1 digesta
T1	0.574abc	0.101d	0.110c	0.084d	0.094d	0.103e	0.095c	738.5cd	12.40b	0.23c
T2	0.502bc	0.213bc	0.217ab	0.170bc	0.202bc	0.188abcd	0.193ab	927.0c	10.80bc	0.27abc
T3	0.543abc	0.262ab	0.229ab	0.205ab	0.253a	0.232a	0.240a	1421.5a	19.80a	0.15d
T4	0.419cd	0.187c	0.175b	0.151c	0.168c	0.152de	0.171b	1445.0a	20.75a	0.14d
T5	0.636ab	0.276a	0.254a	0.227a	0.260a	0.220abc	0.236a	1156.5b	17.23a	0.16d
T6	0.283d	0.214bc	0.195b	0.175bc	0.180c	0.169cd	0.184b	828.5cd	10.61bc	0.26abc
T7	0.722a	0.270a	0.257a	0.246a	0.247ab	0.227ab	0.236a	500.5e	6.72c	0.32a
T8	0.675ab	0.208bc	0.201b	0.171bc	0.203bc	0.178abcd	0.171b	664.5de	10.32bc	0.25bc
T9	0.684ab	0.192c	0.191b	0.164bc	0.183c	0.172bcd	0.179b	793.0cd	12.08c	0.25bc
T10	0.525abc	0.210bc	0.193b	0.158bc	0.175c	0.173bcd	0.181b	527.0e	8.30bc	0.31ab
P-value	0.002	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	49.42	1.37	0.02
SEM	0.03	0.02	0.01	0.02	0.01	0.02	0.01	< 0.001	< 0.001	< 0.001

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean. MDA: malondialdehyde, GSH: glutathione reduced, GPx: glutathione peroxidase, SOD: superoxide dismutase, GSR: glutathione reductase, GST: glutathione S-transferase, and catalase (CAT), AMZ: Amylase, LPZ: lipase, PRZ: protease.

Table 9
Effect of dietary *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) on total bacterial counts, coliform and total fungi (Log_{10} colony forming units/g) in the basal diet at 4, 8 and 12 weeks.

Treatments (ml/ kg diet)	Total bacterial count			Total coliforms			Total fungi		
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks
T1	6.47b	6.54a	6.41bc	4.92a	4.43a	4.66a	3.52a	4.13a	4.49a
T2	6.39d	6.27f	6.12e	4.44c	4.11b	4.40b	2.32de	3.52ab	2.56c
T3	6.43c	6.45cd	6.45bc	4.46c	4.29ab	4.59a	2.13e	2.88bcd	2.81bc
T4	6.37d	6.42de	6.33d	4.86a	4.26ab	4.34bc	2.85bcd	3.54ab	2.72bc
T5	6.42c	6.44cde	6.41bc	4.54bc	4.13b	4.21cd	2.61de	3.09bcd	2.65c
T6	6.43c	6.47cd	6.44bc	4.86a	3.30c	4.43b	2.66cde	2.57d	2.50c
T7	6.52a	6.48bc	6.40c	4.85a	4.15b	4.42b	3.23ab	2.69cd	2.86bc
T8	6.53a	6.51ab	6.51a	4.62b	4.43a	4.14d	3.34ab	3.40abc	2.63c
T9	6.55a	6.45cd	6.47ab	4.80a	4.30ab	4.31bc	3.19abc	2.85bcd	3.47b
T10	6.43c	6.41e	6.42bc	4.78a	4.23ab	4.09d	3.37ab	2.61cd	3.25bc
SEM	0.011	0.013	0.020	0.034	0.060	0.034	0.096	0.110	0.124
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean.

Results in Table 10 emphasized that increasing B1 and/or B2 levels statistically reduced the intestinal coliforms enumeration with approximately 0.5 to 1.0 Log_{10} colony forming units/g and decreased TVC population (except in T2) with ~ 0.5 Log_{10} colony forming units/g without affecting the populations of probiotic bacteria (Table 10). *E. coli* count in cecum significantly decreased ($P < 0.001$) in T5 and T9 groups (Table 10).

4. Discussion

Our results assured an improvement in growth performance in birds fed probiotic-enriched diets. In agreement, Ayyat et al. (2018) found that animals fed diets supplemented with selenium, baker's yeast, probiotic (Bactocell) and mannan oligosaccharides had higher ($P < 0.001$) values of live body weight compared to the con-

Table 10
Effect of dietary *Bacillus toyonensis* (B1) and *Bifidobacterium bifidum* (B2) on caecal microflora (Log_{10} colony forming units/g wet weight; total viable bacterial count (TVC), probiotic bacteria, coliforms and *Escherichia coli*) of laying quail hens.

Treatments (ml/kg diet)	TVC	Probiotics	Total coliforms	*E. coli*
T1	8.80a	7.85	6.97a	5.94ab
T2	8.70ab	7.57	6.50abcd	6.19a
T3	8.59bcd	7.04	6.62abc	5.61abc
T4	8.64bcd	7.67	6.54abcd	5.58abc
T5	8.56cd	7.53	6.07d	4.64d
T6	8.53de	7.19	6.66ab	5.16bcd
T7	8.54de	7.51	6.23bcd	5.23bcd
T8	8.54de	7.44	6.44bcd	5.19bcd
T9	8.43e	7.48	6.11cd	4.75cd
T10	8.67bc	7.64	6.46abcd	5.21bcd
SEM	0.022	0.065	0.063	0.111
P-value	< 0.001	0.228	0.019	0.013

T1: basal diet only (control); T2-T5, basal diet plus 0.05, 0.075, 0.10 and 0.125% B1, respectively; T6: basal diet plus 0.10% B2; T7-T10: basal diet plus 0.05, 0.075, 0.10 and 0.125% B1 plus 0.05% B2, respectively. Values with the same letter within a column are not significantly ($P > 0.05$) different according to Duncan multiple range test. SEM: standard error mean. TVC: Total viable bacterial count.

trol group. On the other hand, Zhang et al. (2013) and Abou-Kassem et al. (2021) reported that adding 10^{10} colony forming units kg^{-1} probiotic to diets improved body weight gain. However, Naseem & King (2020) added different species of *Lactobacillus* (*L. rhamnosus*, *L. paracasei* and *L. plantarum*) to the drinking water of laying hens for eight weeks and found no impacts on body weight change.

Moreover, Hossain et al. (2015) reported no effects on body weight of broilers fed diets enriched with 0.10% of tri-strain probiotics (*C. butyricum, Lactobacillus acidophilus* and *B.* subtilis). They also reported that the level of 0.2% linearly increased ($P < 0.05$) body weight in comparison to the control treatment. In addition, Nosrati et al. (2017) postulated that enriching the drinking water with *Bifidobacterium thermophilum*, *Lactobacillus casei*, Enterococcus faecium and *L. acidophilus* (1×10^8 colony forming units/g) did not affect body weight of broilers.

Probiotic bacteria produce digestive enzymes, vitamins, and antibacterial substances such as hydrogen peroxide, bacteriocins, lactoperoxidase system, organic acids, lactones components, and acetaldehydes which inhibits the growth of the pathogenic bacteria and boost immunity (Mukherjee et al., 2019; Abd El-Moneim et al., 2020c). Applegate et al. (2010) demonstrated that dietary probiotic inclusion caused colonization competition and bacterial antagonism. This response reduces the toxic substances, enhances the immune system, and increases digestion of nutrients and absorption, which finally improves body weight (Abdel-Moneim et al., 2020b).

For mortality, our results were in agreement with Abou-Kassem et al. (2021). They found that the mortality rate of Japanese quails was not affected by probiotic supplementation (B1 or/with B2) compared to the control. Another beneficial explanation for mortality rate was observed when some *Bacillus* species were administered in adequate percentage and established positive impacts on performance and health. In addition the populations of pathogenic bacteria such as *Clostridium* and *Salmonella* in commercial poultry folks were also reduced (Elbaz et al., 2021).

Similar to our results, Abd El-Moneim & Sabic (2019) found that probiotic addition to laying quail diets increased EW and EN compared to the control. Zhang et al. (2012) also found that the group of layers fed a composition of heat-inactivated *B.* subtilis and *Lactobacillus salivarius* recorded a significant ($P < 0.05$) increase in daily egg production.

Furthermore, Kurtoglu et al. (2004) showed that egg production increased after supplementing a combination of *B.* subtilis (3.2×10^9/g probiotic) and *B. licheniformis* (3.2×10^9 /g probiotic) to 27 weeks-old layers for the following 90 days. In line, Ramasamy et al. (2009) detected a statistical ($P < 0.05$) improvement in egg weight of layers fed diets enriched with *Lactobacillus* cultures during 20–44 weeks of age. Moreover, Jha et al. (2020) showed that adding probiotics to laying hen diets improved laying rate, raised daily feed intake and increased nitrogen and calcium retentions. On the other hand, Balevi et al. (2001) reported no differences in EM or EW due to dietary supplementation of various bacterial strains. Manafi et al. (2016) reported that the addition of probiotics such as *B.* subtilis (0.10%) to laying quail diets did not ($P > 0.05$) affect EM or EW as compared to the control. Naseem & King (2020) stated that *Lactobacillus* spp. administration in drinking water did not impact egg production of laying hens. This improvement may be due to reducing the proliferation of pathogenic bacteria, gut environmental changes, good intestinal microbial balance, increasing nutrient utilization, and improved activities of digestive enzymes (Naseem & King, 2020).

For FI results, our findings were in agreements with those of Abdel-Moneim et al. (2020b) who found that the dietary addition of strains of *Bifidobacteria* did not affect FI of birds compared to the control. In broilers, other investigators revealed that adding *E. faecium, L. casei*, *L. acidophilus* and *B. thermophilum* to drinking water (1×10^8 colony forming units/g) did not affect FI (Hossain et al., 2015). Similarly, Zhang et al. (2013) reported no significant difference in FI of broilers fed diet supplemented with *B.* subtilis (0 or 10^5 colony forming units/kg). In harmony, Manafi et al. (2016) reported that probiotics such as *B.* subtilis (0.10%) did not affect FI of laying Japanese quails.

Our current study showed an improvement in FCR of laying quails during the total period. In agreement, Zhang et al. (2013) found that laying hens fed a composition of heat-inactivated of *L. salivarius* and *B.* subtilis showed highly significant ($P < 0.05$) improvement in FCR. Manafi et al. (2016) reported that FCR was significantly ($P < 0.05$) better in the bacitracin and *B.* subtilis groups than the control group. However, Hossain et al. (2015) found no significant impacts on FCR of broilers fed diets supplemented with 0.10% of tri-strain probiotics, TSP (*B.* subtilis, *C. butyricum* and *L. acidophilus*). They also found that the level of 0.20% improved ($P < 0.05$) FCR compared to the control.

Many investigations reported that from 8 to 52 weeks of age, the fertility (F%) and hatchability (H%) of Japanese quail fertile eggs ranged from 48.00 to 94.00% to 40.00–70.34%, respectively (Seker et al., 2004). Our results were in accordance with those of Mojgani et al. (2020) who reported that quails fed probiotics (10^8 colony forming units/ml *B. megaterium*) significantly ($P < 0.05$) improved H% by about 12% and reduced embryonic mortality by about 10% compared with the control. Furthermore, Beck et al. (2019) observed that the *Bifidobacterium* animalis treatment significantly reduced the percentage of piped eggs compared to the control. This indicates the possibility of injecting *B.* animalis into the amnion of an embryo at the 18th of embryonic development with a potential to improve hatching performance. On the contrary, Ayasan (2013) found that dietary supplementation of commercial probiotics (protexin) with levels of 0.05 and 0.10%/kg diet did not affect F% and H% from the fertile eggs of Japanese quail layers compared to the control.

The present study showed increases only in yolk and shell percentages due to B1 or/with B2 supplementation. These results were in agreement with Ayasan et al. (2006) who observed higher eggshell weight with probiotic treatment in laying Japanese quail. Furthermore, egg shell weight significantly ($P < 0.05$) excelled that of the control, being 1.47 and 1.54 g for birds that received 10 g probiotic/200 L drinking water and 10 g probiotic/100 kg of feed, respectively (Lokapirnasari et al., 2019).

Nahashon et al. (1994) found that the decrease in pH in the gastrointestinal tract of White Leghorn laying pullets due to dietary inclusion of probiotics increased calcium retention which improved the eggshell quality. In line with our results, Manafi et al. (2016) reported that eggshell thickness and HU were not affected by the addition of *B.* subtilis to quail diets. A recent study by Jha et al. (2020) investigated the effect of commercial multi-strain probiotics on production performance and egg quality characteristics of laying hens. The results showed increases in some parameters related to egg production, such as egg weight and size, albumin and yolk weight, eggshell thickness and strength compared to the control. On the other hand, Saksrithai et al. (2019) concluded that a combination of probiotics (*Lactobacillus rhamnosus, L. plantarum,* and *L. paracasei*) totaling 1×10^{12} colony forming units/kg feed did not affect egg quality criteria of White Leghorn W-36 laying hens.

Dietary supplementation of B1 and/or B2 in our study did not alter serum levels of TP, ALB, ALT and UA. At the same time, urea-N and AST levels were reduced in the treated birds. These results were consistent with the previous reports of Hashemzadeh et al. (2013), Alimohamadi et al. (2014), Karimi-Kivi et al. (2015), Fathi et al. (2018), Abd El-Moneim & Sabic (2019) and Abd El-Moneim et al. (2020c). It has been reported that

plasma TP can be considered an indicator for the overall condition of an organism and any potential alterations could happen to it when affected by internal or external factors (Abd El-Moneim, 2017; Abd El-Moneim et al., 2020c).

Blood TP also plays a vital role in homeostasis maintenance by maintaining the colloid osmotic pressure, transporting minerals, enzymes, hormones and assuring glucose through gluconeogenesis (Fathi et al., 2018). Furthermore, the reduction in urea-N and AST levels could be attributed to probiotics' hepatic protection role that decreases the transmission of pathogenic bacteria to hepatic tissues, and reducing or maintaining transaminases serum levels (Rishi et al., 2009; Abdel-Moneim et al., 2020b).

The present findings in our study revealed a significant reduction in serum TC and LDL and a significant decrease in TG and VLDL levels in birds treated with 1 ml/kg B1 or B2. This potential hypolipidemic impact of B1 and B2 was in line with the findings of Aluwong et al. (2013a), Pourakbari et al. (2016), Yazhini et al. (2018) and Abdel-Moneim et al. (2020a). The effect of probiotics on blood cholesterols may be due to their ability to retardate the synthesis of cholesterol via the inhibition of the rate-limiting enzyme of cholesterogenesis, hydroxymethylglutaryl-CoA (Hajjaj et al., 2005), hydrolyze bile salts (Klaver & Van der Meer, 1993), incorporate cholesterol into their cells (Tortuero and Fernández, 1995) or convert cholesterol into coprostanol in the gut which directly excreted in feces (Ooi & Liong, 2010).

Our findings were in agreement with those of Yazhini et al. (2018), who found that the mean plasma HDL cholesterol level was significantly ($P < 0.05$) higher in the group of birds received *Lactobacillus lactis* + B2 in comparison to all the other treatment groups. They reported that supplementing probiotics to broilers favorably altered the lipoprotein metabolism with more pronounced reduction in TC and LDL cholesterol and increased HDL cholesterol concentration.

In the present study, maintaining serum glucose concentration might be due to the presence of two opposing effects of probiotics. The first was the suppressive effect on glucagons (Aluwong et al., 2013a; Abdel-Moneim et al., 2020a), which reduces blood glucose, and the second was the elevation in the absorptive capacity of glucose due to the improvement in histological architecture (Zhang et al., 2016; Rodjan et al., 2018; Abdel-Moneim et al., 2020b). Furthermore, the increase in T_3 and T_4 levels may be explained by the enhancement effect of probiotics on the corticotrophin-releasing factor (CRF) activity, which stimulates the secretion of thyrotropin hence, T_4 secretion (Geris et al., 1999; Klieverik et al., 2009). Probiotics, also might enhance the thyroid-stimulating hormone-releasing hormone (TSH-RH) activity, which stimulates the release of TSH from the anterior pituitary (Aluwong et al., 2013a; Abdel-Moneim et al., 2020b).

Our findings revealed an enhancement effect of probiotics in the antioxidant defense system of laying Japanese quails. This response could be attributed to the ability of probiotics to stimulate producing certain factors that captures reactive oxygen species, chelates free radicals and inhibits their cytotoxic activity (Lin & Yen, 1999; Abdel-Moneim et al., 2020a). Wang et al. (2017b) reported that probiotics augment the activities of antioxidant enzymes such as SOD and GPx which promotes avian antioxidant defense system. Moreover, probiotics' antioxidant enzymatic system may play a central role in promoting the antioxidant status of the host (Abdel-Moneim et al., 2020a). Our present results were in consistent with those obtained by several investigators such as Aluwong et al. (2013b), Popović et al. (2015) and Abudabos et al. (2016).

Improving the intestinal digestive enzyme activities enhances nutrient digestion, which increases the number of nutrients available for absorption, promoting poultry health and performance. Interestingly, the elevation in duodenal amylase and lipase activities in the present study was observed in the groups treated with B1 levels but was not noticed when B1 was added with B2, while protease activity was inversely affected. These findings may indicate that not all probiotic strains exert the same impact on the host cells. In addition, the relationship between probiotic strains and the mechanisms of their interaction when added together, whether in the diet or within the host body, has not been specifically described and needs further investigation.

The current results were in consistent with earlier studies which revealed that the treatment with *Bacillus* or *Lactobacillus* strains increased the amylolytic and lipolytic activities (Jin et al., 2000; Wang & Gu, 2010; Abdel-Moneim et al., 2020a). However, others reported insignificant impact of probiotics on amylolytic, lipolytic, or proteolytic activities (Zhi-gang et al., 2014; Palamidi et al., 2016; Zhang et al., 2016; Rodjan et al., 2018). The exogenous enzymes secreted by probiotic microorganisms contributes to nutrient digestion along with the host's endogenous enzymes (Pugsley & Schwartz, 1985; Bedford & Schulze, 1998; Wang & Gu, 2010; Abdel-Moneim et al., 2020a) which might be considered as an explanation for the augmentation in digestive enzymes activities and enhancement in egg productivity observed in the present study.

The TVC, total fungi and total coliforms in the basal diet supplemented with B1 or B2 or B1 + B2 were varied ($P < 0.05$) among dietary treatments. Feeding T2 and T7 diets significantly decreased ($P < 0.05$) all the microbial populations. Also, the TVC, coliforms and total fungi significantly increased ($P < 0.05$) in the control. On the other hand, supplementing the B1 or B2 or their combinations to the basal diet reduced the proliferation of microorganisms at 4, 8 and 12 weeks of age. Thus, the addition of probiotic bacteria or the combination of each bacterium reduced the bacterial and fungal growth in poultry products.

Studies showed that probiotic bacteria produces antimicrobial components such as lactic acid, which exhibits a high degree of antibacterial and antifungal activities. Serafini et al. (2013) observed inhibitory properties of *B. bifidum* against pathogenic bacteria (i.e., *E. coli* and *Cronobacter sakazakii*) regarding enteric adaptation properties using epithelial intestinal cell monolayer (i.e., Caco-2 and HT-29).

Our results also emphasized that increasing B1 or B2 or B1 + B2 levels significantly reduced the intestinal populations of TVC, coliforms and *E. coli*. Probiotic microorganisms are live microorganisms that give the host many health benefits. *Lactobacillus* and *Bifidobacterium* spp. are known as autochthonous microbiota in the human and animal intestinal tract through the many probiotic strains. At the same time, increasing B1 or B2 or B1 + B2 levels in the diet significantly decreased ($P < 0.05$) *E. coli* and coliforms with approximately 0.5 to 1.0 Log_{10} colony forming units/g. It decreased TVC (~0.5 Log_{10} colony forming units/g) without affecting the populations of probiotic bacteria.

Supplementing broilers diets with B1 showed a strong antibacterial activity against Gram-positive and Gram-negative bacteria. The major functional effects provided by probiotics are (i) the production of antimicrobial peptides (i.e., bacteriocins) (Underwood et al., 2012; Martinez et al., 2013; Mandal et al., 2014); (ii) the assimilation of dietary fibers (Slavin, 2013); (iii) the regulation of fat storage (Aronsson et al., 2010; DiBaise et al., 2012); (iv) the modulation of mucosal immunity (Hardy et al., 2013); and (v) the regulation of gut flora via competitive exclusion of pathogenic bacteria resulting in decreased pathogen colonization (Yu et al., 2011; Kim et al., 2014).

Among the five key functional effects of probiotics, the 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 the host's intestinal tract. This cell adhesion ability has been regarded as one of the critical screening standards for active probiotic strains (Jha et al., 2020). Our findings are in agreement with that of Abou-Kassem et al. (2021) who revealed that increasing levels of B1 or B2 reduced microorganisms proliferation. Also, supplementing the quail diet with B1 showed a strong antibacterial activity against Gram-positive and Gram-negative bacteria.

5. Conclusion

According to our findings, it could be concluded that dietary supplementation of graded probiotics levels (B1 or/with B2) to laying quail diets was beneficial in enhancing the productive performance, especially, EW, EN, EM and FCR. Improvements in some egg quality traits, fertility and hatchability percentages could also be achieved by the dietary addition of B1 or B2. Furthermore, the antioxidant enzyme levels, digestive enzyme activities and the microbial measurements were remarkably enhanced by the probiotic supplements. It could be recommended to apply T6 (basal diet + 0.10% B2), T9 (basal diet + 0.10% B1 + 0.05% B2) and T4 (basal diet + 0.10 % B1) levels for the best results from 8 to 20 weeks of age.

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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