Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

**Paper type:** Original Research

# Beneficial effect of *Bacillus coagulans* DSM 32016 on performance and productivity of broiler breeders

*Soudabeh Parhizkar, Mojtaba Zaghari* and Mahdi Zhandi*

*University of Tehran, Karaj, Department of Animal Science, University of Tehran, Karaj, Iran*

*Corresponding author,
E-mail address:
mzaghari@ut.ac.ir

*Received:* 04 Mar 2022,
*Accepted:* 21 May 2022,
*Published online:* 10 Jun 2022,


**ORCID**
Soudabeh Parhizkar
0000-0003-2886-7899
Mojtaba Zaghari
0000-0003-4440-3584
Mahdi Zhandi
0000-0003-2886-9747

**Abstract** The worldwide application of antibiotic growth promotors in the last decades in animal production at least partly contributed to the global pool of multiantibiotic-resistant bacteria causing hardly treatable and severe human but also veterinarian infectious diseases. These circumstances prompted the development of alternative strategies to replace antibiotic growth promotors without losses in animal performance. Among other feed additives, health-beneficial live microorganisms (often designated as probiotics or gut microbiota stabilizers) became promising parts of such alternative approaches. This study aimed to investigate the effects of *Bacillus coagulans* DSM 32016 ($1×10^9$ CFU/kg of diet) on the performance of broiler breeder (Arbor Acres). Broiler breeder hens (Arbor Acres; n=240) at the age of 22 weeks were randomly allocated to 1 of 2 dietary treatments with 12 replicates of 10 birds each, in a completely randomized design. Supplementation of the diet with *Bacillus coagulans* DSM 32016 increased the number of produced eggs, the number of produced settable eggs, egg yolk weight and eggshell thickness ($P≤0.05$). The probiotic supplementation reduced the mortality rate, enhanced egg hatchability, and increased the egg production-based economic profit. Thus, *Bacillus coagulans* DSM 32016 can be considered as performance-enhancing zootechnical feed additive for broiler breeders.

**Keywords:** *Bacillus coagulans*, laying performance, poultry, probiotic

## Introduction

The global population grows constantly, which results in an increased demand for food consumption and animal producs. Efficient animal production is highly dependent on the overall well-being of animals, including the intestinal health as a key factor. In the last fifty years, especially antibiotic growth promotors (AGP) have been widely used to (a) prevent a gut dysbiosis by controlling potentially harmful intestinal bacteria and (b) maintain animal performance. As a result, the unrestricted application of AGP contributed at least partly to the global pool of multiantibiotic-resistant bacteria causing hardly treatable and severe human animal infectious diseases. These circumstances spurred the ban of AGP in different countries and geographically regions such as in Chile, Turkey, European Union, South Korea, and US. Simultaneously, it drives the need for reliable and more environmentally friendly alternatives without facing a loss in animal performance. Among other circulating feed additives on the global market such as organic acids and prebiotics, live microorganism (often designated as probiotics and gut microbiota stabilizers) became promising tools and parts of AGP-replacing approaches. The variety of performance-enhancing and health-beneficial effects of such zootechnical feed additives are well documented in a broad spectrum of various scientific publications considering different animal species. For example, it is generally accepted that probiotics have the potential to improve weight gain, final body weight, feed digestion and conversion, gut microbiota composition, epithelial growth, intestinal inflammation, immune defense, and diarrheal incidence (Kabir, 2009; Gadde et al., 2017; Jha et al., 2020).

Based on their genetics, broiler breeder hens underlie similar health concerns as their offspring like obesity and lameness (Savory and Lariviere, 2000; Sandilands et al., 2006; Nielsen et al., 2011; Morrissey et al., 2014). Therefore, severe feed restriction is a routine procedure to control breeder obesity and to maximize productive and reproductive efficiency (Decuypere et al., 2010; Barzegar et al., 2021). However, due to the highly limited amount of feed that is provided only once per day, breeders experience relatively long fasting periods entailing a negative impact on health and welfare (De Jong and Guemene, 2011). Daily fee restriction, for example, can cause performance-impairing intestinal disorders such as intestinal inflammations and putrefaction, morphological changes, alterations in the intestinal microbiota composition, diarrhea, and associated food pad dermatitis (Taira et al., 2013; Awad et al., 2017; Yan et al., 2021).

*Bacillus coagulans* is a gram-positive, motile, facultative aerobe, lactic acid-producing, and spore-forming bacterium (Payot et al., 1999; Jiang et al., 2019; Zhou et al., 2020). The combination of lactic acid production and spore formation makes *Bacillus coagulans* a unique probiotic in animal nutrition. Bacterial spores are generally known to be resistant against a variety of different challenging conditions such as heat, pressure, or acidity (Zhou et al., 2010; Hung et al., 2012). Therefore, *Bacillus*-spores used in animal nutrition show not only high recovery rates after feed-pelleting processes but also withstand stomach acidity (Mingmongkolchai and Panbangred, 2018; Cao et al., 2020). Once germinated in the nutrient-rich intestine, *Bacillus coagulans* produces lactic acid which improves the gut environment and making it unfavorable for potential harmful pH-sensitive bacteria (Alakomi et al., 2000). Simultaneously, intestinal-produced lactic acid has the potential to stimulate additional health-beneficial microbes such as butyrate producers (Duncan et al., 2004; Sikora et al., 2013). In human medicine, *Bacillus coagulans* is primarily used to treat a broad range of different gastrointestinal disorders such as diarrhea, abdominal pain, digestive problems, and overgrowth of harmful bacteria (Jäger et al., 2018; Konuray and Erginkaya, 2018; Cao et al., 2020), but also immune system stimulation and improved respiratory health through *Bacillus coagulans* ingestion were reported (Baron, 2009; Anaya-Loyola et al., 2019). In animal production, these health-supportive effects (concomitant to a performance improvement and reduced mortality) were also observed in poultry nutrition when *Bacillus coagulans* was administered (Xu et al., 2017; Wu, Y. et al., 2018; Khajeh Bami et al., 2020; Zhang et al., 2021). The production of antimicrobial substances and hydrolases by *Bacillus coagulans* are additional modes of action that further support an intestinal eubiosis by directly and indirectly suppressing the growth of potentially harmful bacteria (Riazi et al., 2009; Maathuis et al., 2010; Honda et al., 2011; Abdhul et al., 2015).

Based on the consideration (a) that intestinal disorders in broiler breeders due to feed restrictions negatively impact the performance and productivity and (b) that *Bacillus coagulans* is known to improve such disorders, this study examined the potential of *Bacillus coagulans* DSM 32016 (BC*) to enhance productive and reproductive efficiency of broiler breeders by supporting overall intestinal health.

## Materials and methods

### *Location and ethical statement*

This experiment was conducted at the Experimental Farm of the Department of Animal Science, University of Tehran, Karaj, Iran (37˚47 N, 50˚55, Elevation 1312 m). All experimental procedures were ethically approved by the Department of Animal Science.

### *Broiler breeder management*

This study was conducted from August to December of 2020 under natural environmental conditions. A total of 240 broiler breeder hens (Arbor Acres) and 30 roosters were used in a feeding trial for 42 weeks. Birds were reared according to Arbor Acres management guide (Arbor-acres, 2016) until 21 weeks, and the feeding trial was performed from weeks 22 to 42 of age. The hens were randomly allocated to two groups of 12 replicates with 10 hens per replicate (1.5 × 2 m2 pens). The roosters were also divided into two groups and kept in six separate pens. Twelve roosters from each experimental group were randomly mixed with hens 6 h daily (9:30 a.m. to 3:30 p.m.). Before mixing the male and female birds, the hens were fed by the experimental diet to avoid rooster's accessibility to the hen's diet. The temperature in the layer house was 18°C to 24°C. The birds were kept under uniform management conditions throughout the experimental period with 13 h of light and 11 h of darkness program. All management points like, separating the rearing environment, instruments, changing clothes and shoes were considered during the trial to prevent the transfer of microorganisms between groups.

### *Experimental diets*

Daily feed allocations were adjusted weekly to maintain body weight gain (BWG) as recommended by the Arbor Acres breeder management recommendations (from 110 g/bird/day in week 22 to 160 g/bird/day in week 43 -

of age). The basal diet was formulated according to Arbor Acres broiler breeder recommendations (Table 1). Birds in the control group received a basal diet and the birds in the second group received the basal diet supplemented with Bacillus coagulans DSM 32016 (BC*). BC* was dosed according to manufacturer's recommendation (1×109 CFU/kg diet, Commission Implementing Regulation No 2020/1755 of 24). The amount of BC*-containing product (TechnoSpore® by Biochem, Germany) was 0.4 g per kg of breeder feed.

**Table 1.** Ingredients and nutrient composition of broiler breeder basal diet

| Ingredients | Amount (%) |
|---|---|
| Corn | 65.60 |
| Soybean meal | 20.45 |
| Wheat bran | 2.35 |
| Barley | 3.00 |
| Di-calcium phosphate | 0.76 |
| Oyster shell | 6.80 |
| Salt | 0.25 |
| Sodium bicarbonate | 0.15 |
| DL-methionine | 0.14 |
| Vitamin + mineral premix[a] | 0.50 |
| Phytase[b] | 0.005 |

| Calculated nutrient contents | Amount (%)[c] |
|---|---|
| AME (kcal/kg) [d] | 2800 |
| Crude Protein | 15.00 |
| Lys | 0.71[e] |
| Met | 0.37[e] |
| Met + Cys | 0.60[e] |
| Thr | 0.60[e] |
| Arg | 0.80[e] |
| Ile | 0.54[e] |
| Val | 0.60 |
| Ca | 3.00 |
| P[f] | 0.35 |
| Na | 0.23 |
| Cl | 0.23 |
| Linoleic acid | 1.46 |
| DCAB (MEq/kg)[g] | 182 |

[a] Vitamin + Mineral Premix provided in 5 kg/ 1000 kg of diet: Vitamins (A: $11\times10^6$ IU, $D_3$:$35\times10^5$ IU, E: $10\times10^4$ IU, $K_3$:5000 mg, $B_1$: 3000 mg, $B_2$: 12000 mg, $B_3$: 55000 mg, $B_5$: 15000 mg, $B_6$: 4000 mg, $B_9$: 2000 mg, $B_{12}$: 30 mg, $H_2$: 250 mg), Minerals (choline: $4\times10^5$ mg, Fe: 50000 mg, Mn: 120000 mg, Zn: 110000 mg, Cu: 10000 mg, Se: 300 mg, Iodine: 2000 mg).
[b] 6-Phytase with *E. Coli* origin.
[c] Unless not otherwise indicated.
[d] Apparent metabolizable energy in kilocalories per kilogram.
[e] Values are based on NIR spectroscopy and represent digestible amounts.
[f] Available phosphorus.
[g] Dietary cation-anion balance in milliequivalents per kilogram.

### *Parameters and data collection*

Body weight, mortality, and feed clean-up time

Body weight was measured weekly, and mortality numbers were recorded daily. Time (min) required for birds' consumption of the allocated daily feed, was recorded visually on weeks 25 and 38 and reported as a feed clean-up time (FCT). A timeline of the experimental design indicating the initiation of applying dietary treatment (*Bacillus coagulans* DSM 32016., $1\times10^9$ CFU/kg of diet) and times of parameter measurement are shown in Figure 1.

Laying performance, egg quality, and hatchability

The number of weekly hen-housed egg production (HHEP) and the number of weekly hen-housed settable egg production (HHSEP) were calculated at the end of each week using Equation 1 and Equation 2, respectively (Hunton, 1995). Eggs that were not broken or cracked, dirt-free, not deformed, and not excessively small were considered as settable eggs.

*Equation 1:* Number of hen-housed egg production per week.

$$HHEP = \frac{Total\ number\ of\ eggs\ produced\ per\ week}{Total\ number\ of\ hens\ at\ trial\ start}$$

*Equation 2:* Number of hen-housed settable egg production per week.

$$HHEP = \frac{Total\ number\ of\ settable\ eggs\ produced\ per\ week}{Total\ number\ of\ hens\ at\ trial\ start}$$

*Equation 3:* Total number of hen-housed egg production (See table 3).

$$THHEP = \frac{Total\ number\ of\ eggs\ produced\ during\ whole\ trial\ period}{Total\ number\ of\ hens\ at\ trial\ start}$$

The quality of collected eggs, including the egg weight, yolk weight, and eggshell thickness were assessed during the trial. Briefly, two eggs from each replicate were weighed individually and broken into a glass plate to measure the yolk weight and shell thickness.

The hatchability of eggs was determined on weeks 35 and 42 as percentage of total settable eggs. All eggs were candled on the seventh day of incubation. Eggs with unclear embryos were removed and opened for visual admission as fertile or infertile (Hajati et al., 2014).

### *Statistical analysis and economic profit*

All replicated parameters were analyzed using repeated measurement in MIXED procedure of the SAS software (SAS Institute Version 9.4, Cary, NC, USA). The overall differences between the control group and BC* group were analyzed through an independent sample *t*-test and multiple comparisons of means produced by LSD (Zou et al., 2021). Before analysis, the data were checked for normal distribution through the UNIVARIATE procedure and Shapiro–Wilk test. The results were expressed as mean values ± standard error (Means ± S.E.) and P values ≤0.05 were considered statistically significant.

The economic profit was calculated using the differences in settable egg production between control group and BC* group. As the rearing costs were similar in both groups, we only considered the BC*-containing product price as a different factor between the two groups. Labor costs, the total cost of veterinary management such as service, treatment, disinfectant, and veterinary supervision costs were considered as fixed amounts and similar in both groups. The price of the BC*-containing product in this trial was about USD 9.00 per kg. The BC* group received an average of 151 g/day of the diet containing BC* (0.4 g of BC*-containing product/kg of diet). The price of each produced eggs was about USD 0.22.

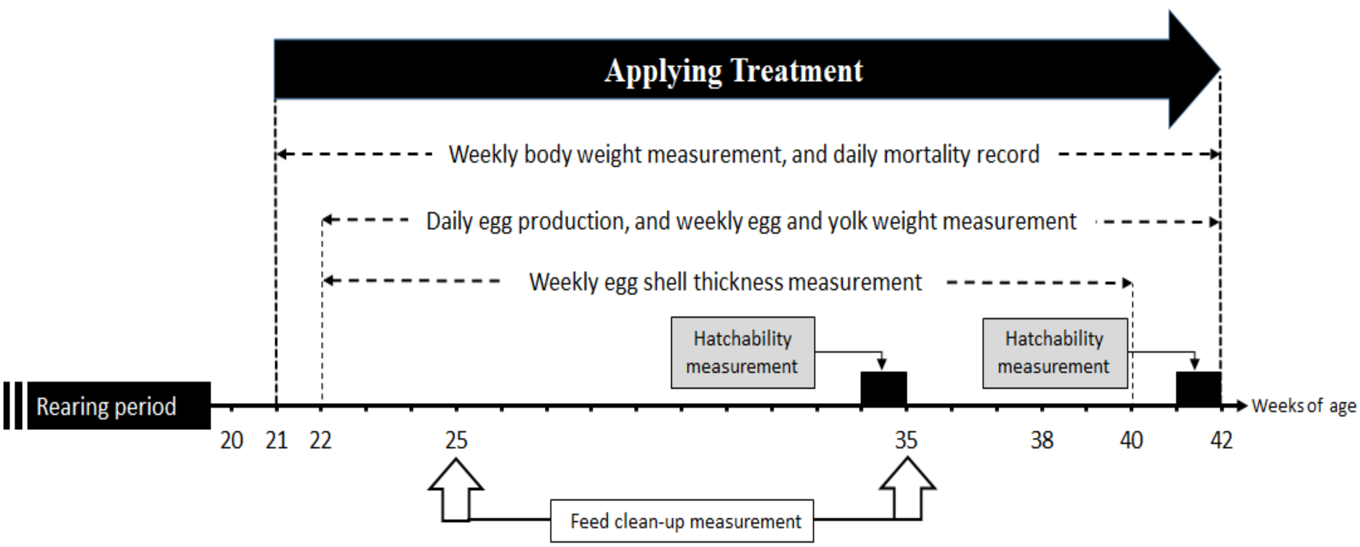


**Figure 1.** A timeline of the experimental design indicating initiation of applying dietary treatment (*Bacillus coagulans* DSM 32016, $1\times10^9$ CFU/kg of diet) and times of parameter measurement.

## Results

Although the egg weight was slightly decreased in the BC* group (-1.3 %), BC* supplementation significantly increased the average number of HHEP (+4.4%), the average number of HHSEP (+4.0%), egg yolk weight (+2.2%), and eggshell thickness (+2.8%) (Table 2). The difference in body weight observed between the two groups was not significant (Table 2). In contrast to the control group in which eight fatalities were recorded (mortality rate of 6.7 %), no deaths in the BC* group were observed (Table 3). The interactive statistical analysis of group × week indicated significant results for eggshell thickness. In contrast, this analysis did not show a significant outcome regarding the number of HHEP, the number of HHSEP, body weight, egg yolk weight, and rooster body weight. Furthermore, the analysis of total produced eggs demonstrated that BC* supplementation significantly increases the total number of HHEP (5.62 %) and HHSEP (4.71 %) (Table 3). The cleaning up time was not significantly affected by BC* supplementation.

Time-resolved analysis of eggshell thickness indicated that although the difference in eggshell thickness between the two groups showed some fluctuations regarding the statistical significance, BC* supplementation led overall to constantly improved eggshell thickness during the whole trial period in comparison to the control group (Table 3). Additionally, a positive but non-significant effect of BC* supplementation on the hatchability (an average of 2.5 %) was observed compared with the control group (Figure 2).

**Table 2.** The effect of dietary BC* supplementation from weeks 22 to 42 of age on broiler breeder laying performance, body weight, and egg quality

| Parameter [1] | **Group** (Mean ± SE) [2] | | **P-value** | | |
|---|---|---|---|---|---|
| | Control | BC* | Group | Week | Group × Week |
| HHEP [eggs/week/hen] | 5.76 ± 0.08 | 6.03 ± 0.07 | <0.001 | <0.001 | 0.530 |
| HHSEP [eggs/week/hen] | 5.52 ± 0.09 | 5.75 ± 0.07 | <0.001 | <0.001 | 0.830 |
| Hen Body Weight [g] | 3240 ± 7.71 | 3218 ± 7.80 | 0.052 | <0.001 | 0.990 |
| Egg Weight [g] | 53.73 ± 0.21 | 53.03 ± 0.19 | <0.001 | <0.001 | 0.990 |
| Egg Yolk Weight [g] | 16.97 ± 0.15 | 17.35 ± 0.13 | <0.001 | <0.001 | 0.250 |
| Eggshell Thickness [mm] | 0.36 ± 0.01 | 0.37 ± 0.01 | <0.001 | <0.001 | <0.001 |
| Rooster Body Weight [g] | 3950 ± 22.75 | 3881 ± 21.98 | <0.001 | <0.001 | 0.990 |

[1] HHEP, average number of hen-housed egg production; HHSEP, average number of settable hen-housed egg production; Time-resolved data to eggshell thickness shown in Table 4; all parameters represent mean values of data collected throughout the whole trial period.
[2] Mean values ± standard error; control, control broiler breeder group; BC*, broiler breeder group fed *Bacillus coagulans* DSM 32016.

**Table 3.** The effect of dietary BC* supplementation from weeks 22 to 42 of age on total broiler breeder laying performance, feed clean-up time and total mortality

| Parameter [1] | **Group** (Mean ± SE) [2] | | **P-value** |
|---|---|---|---|
| | Control | BC* | |
| THHEP | 112.70 ± 1.94 | 119.04 ± 1.19 | 0.01 |
| THHSEP | 108.93 ± 1.46 | 114.06 ± 0.89 | 0.01 |
| FCT (week 25) | 45.17 ± 1.93 | 45.33 ± 1.16 | 0.940 |
| FCT (week 38) | 109.17 ± 3.13 | 104.17 ± 2.60 | 0.230 |
| Mortality (%) | 6.68 ± 30.30 | 00.00 ± 0.00 | 0.007 |

[1] THHEP, total hen house egg production; THHSEP, total hen house settable egg production; FCT; feed clean-up time (minutes).
[2] Mean values ± standard error; control, control broiler breeder group; BC*, broiler breeder group fed *Bacillus coagulans* DSM 32016.

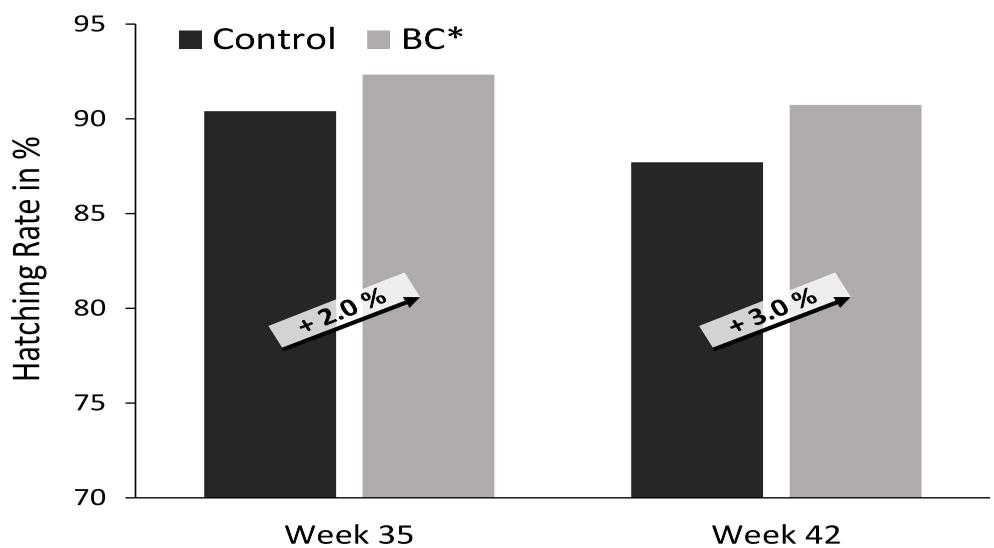


**Figure 2.** The effect of dietary supplementation of *Bacillus coagulans* on egg hatchability at weeks 35 and 42 in Control, and broiler breeder hens fed *Bacillus coagulans* DSM 32016 (BC*).

**Table 4.** The effect of dietary BC* supplementation from weeks 22 to 42 of age on broiler breeder eggshell thickness

| Age (week) | Group (Mean ± SE) [a] | | P- value |
|---|---|---|---|
| | Control | BC* | |
| 22 | 0.36 ± 0.004 | 0.34 ± 0.004 | <0.010 |
| 24 | 0.34 ± 0.005 | 0.35 ± 0.004 | 0.730 |
| 26 | 0.36 ± 0.005 | 0.36 ± 0.004 | 0.920 |
| 28 | 0.36 ± 0.005 | 0.36 ± 0.003 | 0.270 |
| 30 | 0.35 ± 0.003 | 0.37 ± 0.003 | <0.010 |
| 32 | 0.36 ± 0.004 | 0.38 ± 0.003 | <0.010 |
| 34 | 0.36 ± 0.005 | 0.38 ± 0.004 | <0.010 |
| 36 | 0.37 ± 0.004 | 0.39 ± 0.002 | <0.010 |
| 38 | 0.37 ± 0.004 | 0.39 ± 0.001 | <0.010 |
| 40 | 0.38 ± 0.004 | 0.39 ± 0.001 | <0.010 |

[a] Mean value ± standard error; control, control broiler breeder group; BC*, broiler breeder group fed *Bacillus coagulans* DSM 32016.

Based on the average total settable egg production (control group: 1127 eggs and BC* group: 1190 eggs), the group-related economic profit was calculated (Figure 3). The result of the economic evaluation demonstrated that the higher number of produced eggs in the BC* group not only covered the costs of the supplement, but also led to an additional study-related economical profit of at least 4.8 % (Figure 3). The slightly enhanced hatchability observed in the BC* group compared to the control group (Figure 2) further indicated a higher reproductive potential in broiler breeders fed with BC*, which may theoretically lead to an even higher economical profit.

**Total Costs of BC* supplementation (TCS):**

$$TCS = BC^*P_{Cost} \times BC^*P_{Intake} \times n_{Days} \times n_{Birds}$$

$$\text{TCS of BC* Group (\$)}: \$0.009 \times \$0.060 \times 150 \times 120 = \$9.78$$

**Group-associated ROI**

$$EP = (n_{Eggs} \times Price_{Egg}) - TCS$$

$$\text{EP Control Group (\$)}: 13094 \times \$0.22 = \$2881$$

$$\text{EP BC* Group (\$)}: (13770 \times \$0.22) - \$9.78 = \$3020$$

| | | | |
|---|---|---|---|
| $BC^*P$ | BC*-containing product | $n_{Birds}$ | number of hens in BC* group |
| $BC^*P_{Cost}$ | costs of 1 g BC*P | $n_{Egg}$ | total number of settable eggs |
| $BC^*P_{Intake}$ | consumption of BC*P/hen/day in g | $Price_{Egg}$ | price per produced egg |
| $n_{Days}$ | number of days (5 months) | $ | USD - United States Dollar |

**Figure 3:** Economic profit (EP) calculation based on average total egg production. BC*, *Bacillus coagulans* DSM 32016.

## Discussion

The results of this study indicate that the spore-forming and lactic acid- producing probiotic bacterium *Bacillus coagulans* DSM 32016 applied to animal feed ($1×10^9$ CFU/kg feed) improved the production and reproductive potential of breeder broiler hens. Although, no significant differences in body weight were recorded, *Bacillus coagulans* DSM 32016 supplementation (a) enhanced the laying performance based on an improved weekly average number and total number of produced eggs and settable eggs, (b) supported egg quality including an enhanced egg yolk weight, eggshell thickness, and hatchability, and (c) reduced the mortality rate. The positive observations in this study are reinforced by several other poultry-related studies that reported an effective enhancement of overall performance and laying performance but also immune function and gut health when *Bacillus coagulans* was added to the diet (Zhou et al., 2010; (Huang et al., 2017; Xing et al., 2020).

The beneficial effects of feeding probiotics like *Bacillus coagulans* can be driven by a broad range of different probiotic modes of action (O'Toole and Cooney, 2008). For example, *Bacillus coagulans* has the potential to improve protein digestion by producing (a) proteolytic hydrolases (Jäger et al., 2018) and (b) lactic acid which promotes the function of host proteases as well as the peristalsis of the small intestine (Giang et al., 2010; Hung et al., 2012; Ma et al., 2014). These mechanisms potentially support the overall performance and health of animals not only due to an improved feed conversion (Lei et al., 2015) but also due to an indirect action against pathogens that profit from access protein and amino acids in the digestive tract (Shojadoost et al., 2012). However, pathogen inhibition can also occur directly by the production of antimicrobial substances (Riazi et al., 2009). Likewise, lactic acid produced in the intestine shows antimicrobial properties by lowering the intestinal pH which potentially inhibits pH-sensitive harmful bacteria (Alakomi et al., 2000). Lactic acid is also a key metabolite for microbial cross-feeding and thus stimulates other health-beneficial bacteria such as butyrate-producing *Lachnospiraceae* (Sikora et al., 2013; Rivière et al., 2016). That these microbiota-modulating effects are mostly accompanied by improved overall performance (Hung et al., 2012; Lei et al., 2015; Alberoni et al., 2018; Li et al., 2019; Nour et al., 2021) further demonstrates the importance of a balanced microbiota and gut health for stable animal production. *Bacillus coagulans* is also known to stimulate the immune system which strengthens the immune defense and barrier function against a broad range of harmful bacteria such as *C. perfringens* (Xu et al., 2017; Wu, Y. et al., 2018; Guo et al., 2021). Furthermore, the microbial activity of *Bacillus coagulans* in the gut might (a) reduce oxidative stress and associated epithelial damage and (b) support the host lipid metabolism, glucose metabolism, liver function, epithelial growth, and absorption of minerals (Lee et al., 2016; Wu, T. et al., 2018; Wu, S. et al., 2018; Li et al., 2019; Xing et al., 2020; Khajeh Bami et al., 2020; Nour et al., 2021). Various *Bacillus coagulans* DSM 32016-driven benefits in this study such as increased laying performance and egg quality might have been initiated by a complex combination of all aforementioned probiotic modes of action. Independent of defining these complex mechanisms, it is scientifically accepted that *Bacillus*-based probiotics (including *Bacillus coagulans* and *Bacillus licheniformis*) can not only improve overall health and performance but also laying performance, egg quality, fertility, embryonic mortality, and hatchability (Kurtoglu et al., 2004; Mojgani et al., 2020; Nour et al., 2021). Regarding the eggshell thickness, previous research has shown that *Bacillus coagulans* supports overall absorption of calcium and phosphorus in laying hens (Huang et al., 2017), which most likely contributed to the postive effect of *Bacillus coagulans* DSM 32016 on eggshell stability in this study. Such findings were observed in other egg-producing poultry-related studies (Nour et al., 2021), and are at least partly associated to a probiotic-driven increased protein and lipid metabolisms (Saleh et al., 2017). Interestingly, although a lower dosage of *Bacillus*-based probiotics can improve hen performance and maintain egg quality, a higher dosage enhances additionally the interior protein quality of eggs (Neijat et al., 2019).

We are not aware of studies that have evaluated the effect of *Bacillus coagulans* on broiler breeder performance. In general, the impact of probiotic feed additives on broiler breeder productivity is poorly researched. Likewise, the precise probiotic modes of action including complex microbe-microbe and host-microbe interactions that might have driven the observed performance enhancement are still less well understood. Furthermore, this work does not include microbiological or histomorphological analysis of the broiler intestines. Based on these assumptions it can only be hypothetically concluded that a generally improved overall gut health and enhanced feed conversion by *Bacillus coagulans* has caused the improvement in egg production and egg quality. However, that *Bacillus coagulans* DSM 32016 reduced mortality and enhanced overall laying performance including egg quality reinforces that such probiotic feed additives should be considered as a part of AGP-replacing approaches in animal nutrition.

## Conclusion

The collective findings of this study indicate that probiotic feed additives such as *Bacillus coagulans* DSM 32016 used in feed for laying poultry can (a) increase the laying performance including a higher number of produced eggs and produced settable eggs, (b) support egg quality including an enhanced egg yolk weight, eggshell thickness, and hatchability, and (c) reduce mortality. Thus, *Bacillus coagulans* DSM 32016 can be considered as production-, reproduction-, and profitability-enhanci--

ng zootechnical feed additive for broiler breeder farming.

## Acknowledgements

The authors thank Biochem Germany for supporting this study, and the University of Tehran for providing facilities and financial support.

## Conflict of interest statement

The authors certify that there is no conflict of interest.

## References

Abdhul, K., Ganesh, M., Shanmughapriya, S., Vanithamani, S., Kanagavel, M., Anbarasu, K., Natarajaseenivasan, K., 2015. Bacteriocinogenic potential of a probiotic strain *Bacillus coagulans* [BDU3] from Ngari. *International Journal of Biological Macromolecules* 79, 800-806.

Alakomi, H.L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., Helander, I.M., 2000. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology* 66*,* 2001-2005.

Alberoni, D., Baffoni, L., Gaggìa, F., Ryan, P.M., Murphy, K., Ross, P.R., Stanton, C., Di Gioia, D., 2018. Impact of beneficial bacteria supplementation on the gut microbiota, colony development and productivity of Apis mellifera L. *Beneficial Microbes* 9, 269-278.

Anaya-Loyola, M.A., Enciso-Moreno, J.A., López-Ramos, J.E., García-Marín, G., Álvarez, M.Y.O., Vega-García, A.M., Mosqueda, J., García-Gutiérrez, D.G., Keller, D., Pérez-Ramírez, I.F., 2019. *Bacillus coagulans* GBI-30, 6068 decreases upper respiratory and gastrointestinal tract symptoms in healthy Mexican scholar-aged children by modulating immune-related proteins. *Food Research International* 125, 108567.

Arbor-Acres. Arbor Acres plus S, parent stock nutrition specifications. Online Available at https://en.aviagen.com. 2016. [Verified 30 Dec 2021].

Awad, W.A., Hess, C., Hess, M., 2017. Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. *Toxins* 9, 60.

Baron, M., 2009. A patented strain of *Bacillus coagulans* increased immune response to viral challenge. *Postgraduate Medicine* 121, 114-118.

Barzegar, M., Zaghari, M., Zhandi, M., Sadeghi, M., 2021. Effects of zinc dosage and particle size on gut morphology, tight junctions and TNF-α expression in broiler breeder hens. *Journal of Animal Physiology and Animal Nutrition* 00, 1-11

Cao, J., Yu, Z., Liu, W., Zhao, J., Zhang, H., Zhai, Q., Chen, W., 2020. Probiotic characteristics of Bacillus coagulans and associated implications for human health and diseases. *Journal of Functional Foods* 64, 103643.

Decuypere, E., Bruggeman, V., Everaert, N., Li, Y., Boonen, R., De Tavernier, J., Janssens, S., Buys, N., 2010. The broiler breeder paradox: ethical, genetic and physiological perspectives, and suggestions for solutions. *British Poultry Science* 51, 569-579.

De Jong, I.C., Guemene, D., 2011. Major welfare issues in broiler breeders. *World's Poultry Science Journal* 67, 73-82.

Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and Environmental Microbiology* 70, 5810-5817.

Gadde, U., Kim, W.H., Oh, S.T., Lillehoj, H.S., 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews* 18, 26-45.

Giang, H.H., Viet, T.Q., Ogle, B., Lindberg, J.E., 2010. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livestock Science* 129, 95-103.

Guo, S., Xi, Y., Xia, Y., Wu, T., Zhao, D., Zhang, Z., Ding, B., 2021. Dietary Lactobacillus fermentum and Bacillus coagulans supplementation modulates intestinal immunity and microbiota of broiler chickens challenged by Clostridium perfringens. *Frontiers in Veterinary Science* 8, 483.

Hajati, H., Hassanabadi, A., Yansari, A.T., 2014. The effect of dietary supplementation of prebiotic and probiotic on performance, humoral immunity responses and egg hatchability in broiler breeders. *Poultry Science Journal* 2,1-13.

Honda, H., Hoyles, L., Gibson, G.R., Farmer, S., 2011. Impact of GanedenBC30 (*Bacillus coagulans* GBI-30, 6086) on population dynamics of the human gut microbiota in a continuous culture fermentation system. *International Journal of Probiotics and Prebiotics* 6, 65-72.

Huang, S., Huang, C., Zhao, L., Zhang, J., Ji, C., Ma, Q., 2017. Effects of *Bacillus coagulans* on performance, egg quality and plasma biochemical parameters of laying hens infected with Salmonella. *Chinese Journal of Animal Nutrition* 29, 4534-4541.

Hung, A.T., Lin, S.Y., Yang, T.Y., Chou, C.K., Liu, H.C., Lu, J.J., Wang, B., Chen, S.Y., Lien, T.F., 2012. Effects of *Bacillus coagulans* ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens. *Animal Production Science* 52, 874-879.

Hunton, P., 1995. Egg production, processing and marketing. In: World Poultry Science, Elsevier, Tokyo, pp. 457-480.

Jäger, R., Purpura, M., Farmer, S., Cash, H.A., Keller, D., 2018. Probiotic *Bacillus coagulans* GBI-30, 6086 improves protein absorption and utilization. *Probiotics and Antimicrobial Proteins* 10, 611-615.

Jha, R., Das, R., Oak, S., Mishra, P., 2020. Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: a systematic review. *Animals* 10, 1863.

Jiang, S., Xu, P., Tao, F., 2019. L-Lactic acid production by *Bacillus coagulans* through simultaneous saccharification and fermentation of lignocellulosic corncob residue. *Bioresource Technology Reports* 6, 131-137.

Kabir, S.M., 2009. The role of probiotics in the poultry industry. *International Journal of Molecular Sciences* 10, 3531-3546.

Khajeh Bami, M., Afsharmanesh, M., Ebrahimnejad, H., 2020. Effect of dietary *Bacillus coagulans* and different forms of zinc on performance, intestinal microbiota, carcass and meat quality of broiler chickens. *Probiotics and Antimicrobial Proteins* 12, 461-472.

Konuray, G., Erginkaya, Z., 2018. Potential use of *Bacillus coagulans* in the food industry. *Foods* 7*,* 92.

Kurtoglu, V., Kurtoglu, F., Seker, E., Coskun, B., Balevi, T., Polat, E.S., 2004. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *Food Additives and Contaminants* 21, 817-823.

Lee, Y., Yoshitsugu, R., Kikuchi, K., Joe, G.H., Tsuji, M., Nose, T., Shimizu, H., Hara, H., Minamida, K., Miwa, K., Ishizuka, S., 2016. Combination of soya pulp and *Bacillus coagulans* lilac-01 improves intestinal bile acid metabolism without impairing the effects of prebiotics in rats fed a cholic acid-supplemented diet. *British Journal of Nutrition* 116, 603-610.

Lei, X., Piao, X., Ru, Y., Zhang, H., Péron, A., Zhang, H., 2015. Effect of *Bacillus amyloliquefaciens*-based direct-fed microbial on performance, nutrient utilization, intestinal morphology and cecal microflora in broiler chickens. *Asian-Australasian Journal of Animal Sciences* 28, 239-246.

Li, C.L., Wang, J., Zhang, H.J., Wu, S.G., Hui, Q.R., Yang, C.B., Fang, R.J., Qi, G.H., 2019. Intestinal morphologic and microbiota responses to dietary *Bacillus spp*. in a broiler chicken model. *Frontiers in Physiology* 1968, 1-18.

Ma, K., Maeda, T., You, H., Shirai, Y., 2014. Open fermentative production of l-lactic acid with high optical purity by thermophilic *Bacillus coagulans* using excess sludge as nutrient. *Bioresource Technology* 151, 28-35.

Maathuis, A., Keller, D., Farmer, S., 2010. Survival and metabolic activity of the GanedenBC30 strain of *Bacillus coagulans* in a dynamic in vitro model of the stomach and small intestine. *Beneficial Microbes* 1, 31-36.

Mingmongkolchai, S., Panbangred, W., 2018. *Bacillus* probiotics: an alternative to antibiotics for livestock production. *Journal of Applied Microbiology* 124, 1334-1346.

Mojgani, N., Razmgah, N., Torshizi, M.A.K., Sanjabi, M.R., 2020. Effects of three *Bacillus specious* on hatchability, growth performance and serum biochemistry in Japanese quails fed diet contaminated with Aflatoxin B1. *Acta Scientiarum. Animal Sciences* 42, 1-8.

Morrissey, K.L., Widowski, T., Leeson, S., Sandilands, V., Arnone, A., Torrey, S., 2014. The effect of dietary alterations during rearing on growth, productivity, and behavior in broiler breeder females. *Poultry Science* 93, 285-295.

Neijat, M., Shirley, R.B., Barton, J., Thiery, P., Welsher, A., Kiarie, E., 2019. Effect of dietary supplementation of *Bacillus subtilis* DSM29784 on hen performance, egg quality indices, and apparent retention of dietary components in laying hens from 19 to 48 weeks of age. *Poultry Science* 98, 5622-5635.

Nielsen, B.L., Thodberg, K., Malmkvist, J., Steenfeldt, S., 2011. Proportion of insoluble fibre in the diet affects behaviour and hunger in broiler breeders growing at similar rates. *Animals* 5, 1247-1258.

Nour, M.A., El-Hindawy, M.M., Abou-Kassem, D.E., Ashour, E.A., Abd El-Hack, M.E., Mahgoub, S., Aboelenin, S.M., Soliman, M.M., El-Tarabily, K.A., Abdel-Moneim, A.M.E., 2021. Productive performance, fertility and hatchability, blood indices and gut microbial load in laying quails as affected by two types of probiotic bacteria. *Saudi Journal of Biological Sciences* 28*,* 6544-6555.

O'Toole, P.W., Cooney, J.C., 2008. Probiotic bacteria influence the composition and function of the intestinal microbiota. *Interdisciplinary Perspectives on Infectious Diseases* 2008, 1-9.

Payot, T., Chemaly, Z., Fick, M., 1999. Lactic acid production by *Bacillus coagulans*-kinetic studies and optimization of culture medium for batch and continuous fermentations. *Enzyme and Microbial Technology* 24, 191-199.

Riazi, S., Wirawan, R.E., Badmaev, V., Chikindas, M.L., 2009. Characterization of lactosporin, a novel antimicrobial protein produced by *Bacillus coagulans* -

ATCC 7050. *Journal of Applied Microbiology* 106, 1370-1377.

Rivière, A., Selak, M., Lantin, D., Leroy, F., De Vuyst, L., 2016. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Frontiers in Microbiology* 7, 979.

Saleh, A.A., Gálik, B., Arpášová, H., Capcarová, M., Kalafová, A., Šimko, M., Juráček, M., Rolinec, M., Bíro, D., Abudabos, A.M., 2017. Synergistic effect of feeding *Aspergillus awamori* and lactic acid bacteria on performance, egg traits, egg yolk cholesterol and fatty acid profile in laying hens. *Italian Journal of Animal Science* 16, 132-139.

Sandilands, V., Tolkamp, B.J., Savory, C.J., Kyriazakis, I., 2006. Behaviour and welfare of broiler breeders fed qualitatively restricted diets during rearing: are there viable alternatives to quantitative restriction? *Applied Animal Behaviour Science* 96, 53-67.

Savory, C.J., Lariviere, J.M., 2000. Effects of qualitative and quantitative food restriction treatments on feeding motivational state and general activity level of growing broiler breeders. *Applied Animal Behaviour Science* 69, 135-147.

Shojadoost, B., Vince, A.R., Prescott, J.F., 2012. The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: a critical review. *Veterinary Research* 43, 1-12.

Sikora, A., Błaszczyk, M., Jurkowski, M., Zielenkiewicz, U., 2013. lactic acid bacteria in hydrogen-producing consortia: on purpose or by coincidence?. In: Lactic acid bacteria - R & D for food, health and livestock purposes (1st ed), Intech open., London, pp. 488-514.

Taira, K., Nagai, T., Obi, T., Takase, K., 2013. Effect of litter moisture on the development of footpad dermatitis in broiler chickens. *Journal of Veterinary Medical Science* 13, 0321.

Wu, S., Liu, Y., Duan, Y., Wang, F., Guo, F., Yan, F., Yang, X., Yang, X., 2018. Intestinal toxicity of deoxynivalenol is limited by supplementation with *Lactobacillus plantarum* JM113 and consequentially altered gut microbiota in broiler chickens. *Journal of Animal Science and Biotechnology* 9, 1-13.

Wu, T., Zhang, Y., Lv, Y., Li, P., Yi, D., Wang, L., Zhao, D., Chen, H., Gong, J., Hou, Y., 2018. Beneficial impact and molecular mechanism of *Bacillus coagulans* on piglets' intestine. *International Journal of Molecular Sciences* 19, 2084.

Wu, Y., Shao, Y., Song, B., Zhen, W., Wang, Z., Guo, Y., Shahid, M.S., Nie, W., 2018. Effects of *Bacillus coagulans* supplementation on the growth performance and gut health of broiler chickens with Clostridium perfringens-induced necrotic enteritis. *Journal of Animal Science and Biotechnology* 9, 1-14.

Xing, S.C., Mi, J.D., Chen, J.Y., Hu, J.X., Liao, X.D., 2020. Metabolic activity of *Bacillus coagulans* R11 and the health benefits of and potential pathogen inhibition by this species in the intestines of laying hens under lead exposure. *Science of The Total Environment* 709, 134507.

Xu, L., Fan, Q., Zhuang, Y., Wang, Q., Gao, Y., Wang, C., 2017. *Bacillus coagulans* enhance the immune function of the intestinal mucosa of yellow broilers. *Brazilian Journal of Poultry Science* 19, 115-122.

Yan, C., Xiao, J., Chen, D., Turner, S.P., Li, Z., Liu, H., Liu, W., Liu, J., Chen, S., Zhao, X., 2021. Feed restriction induced changes in behavior, corticosterone, and microbial programming in slow-and fast-growing chicken breeds. *Animals* 11, 141.

Zhang, B., Zhang, H., Yu, Y., Zhang, R., Wu, Y., Yue, M., Yang, C., 2021. Effects of *Bacillus Coagulans* on growth performance, antioxidant capacity, immunity function, and gut health in broilers. *Poultry Science* 100, 101-168.

Zhou, X., Wang, Y., Gu, Q. and Li, W., 2010. Effect of dietary probiotic, Bacillus coagulans, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science* 89, 588-593.

Zhou, Y., Zeng, Z., Xu, Y., Ying, J., Wang, B., Majeed, M., Majeed, S., Pande, A., Li, W., 2020. Application of *Bacillus coagulans* in animal husbandry and its underlying mechanisms. *Animals* 10, 454.

Zou, X., Jiang, S., Zhang, M., Hu, H., Wu, X., Liu, J., Jin, M., Cheng, H., 2021. Effects of *Bacillus subtilis* on production performance, bone physiological property, and hematology indexes in laying hens. *Animals* 11, 2041.