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*Short Communication*

# Growth Performance of Broiler Chicken Supplemented with *Bacillus velezensis* D01Ca and *Bacillus siamensis* G01Bb Isolated from Goat and Duck Microbiota

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## ABSTRACT

The increasing global demand for sustainable agricultural practices and the quest for food security has intensified the need for alternative solutions to promote healthy growth in farm animals. One potential strategy is the use of probiotics derived from diverse sources, which remains relatively uncharted. In this context, this study aimed to assess the probiotic potentials of *Bacillus velezensis* D01Ca and *Bacillus siamensis* G01Bb, strains sourced from the gut of ducks and goats. Using two completely randomized experimental designs with 150-day-old broiler chickens, two distinct set-ups were implemented. In the first, broilers were subjected to either a control condition, a single dose of *B. velezensis* D01Ca at $2.4 \times 10^7$ cfu/ml, or its double dose. The second set-up followed a similar setup, but with *B. siamensis* G01Bb at $2.29 \times 10^7$ cfu/ml. Throughout the 42-day trial, all broilers consumed a commercial ration ad libitum and accessed water freely, with specific groups receiving the supplemented water based on the treatment. Results show that the feed intake of broilers remained unaffected by the probiotic supplementation, with no significant difference ($P \leq 0.05$). However, broilers in the supplemented groups exhibited a noticeable increase in body weight and body weight gain when compared to the control. The feed conversion ratio, crucially, was consistent across all test groups. Conclusively, our findings suggest that *B. velezensis* D01Ca and *B. siamensis* G01Bb hold promise as viable probiotics for

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broiler chickens, offering potential strides toward sustainable agricultural practices and enhanced food security.

*Keywords*: *Bacillus velezensis* D01Ca, *Bacillus siamensis* G01Bb, broiler chicken, growth performance, gut microbiota

## INTRODUCTION

In recent years, animal production and consumption levels have rapidly increased due to the demand for animal protein. According to Hosain et al. (2021), this demand also denotes the changes in food production and feeding regimens, including increased antimicrobial use (AMU) in the livestock sector. Under intensive production systems to achieve high economic efficiency, chickens predominantly use antimicrobials to avoid stress, overcrowding, and unfavorable ambient conditions, ensuring good health. According to Elliott et al. (2017), the repeated misuse of antibiotics in food-producing animals is a key factor accelerating the emergence of drug-resistant microorganisms that has become a global public health challenge. Restrictions on the use of antimicrobials at sub-therapeutic concentrations in livestock due to the growing concerns of antimicrobial resistance (AMR) have prompted poultry researchers to look for a viable alternative.

Using growth promoters such as probiotics, prebiotics, symbiotics, organic acid, and bioactive compounds is currently being studied as an alternative to antibiotics. These are proven safe and have no negative impact on the environment, and are safe for livestock production, improved growth performance, and immunity (Callaway et al., 2008; Firth et al., 2019; Markowiak & Śliżewska, 2018). Various types of probiotics are being researched in the poultry industry to improve chicken performance. Boirivant and Strober (2007) define probiotics as viable and non-pathogenic microorganisms (bacteria and yeast) that can reach the intestines in sufficient numbers to confer benefits to the host. When probiotics are consumed in sufficient quantities, they will benefit the host by assisting digestion and nutrient absorption (Liu et al., 2009). Probiotics were initially used to prevent episodic diarrhea in poultry because they lessen intestinal salmonella and *Clostridium perfringens* (Bailey et al., 2000). However, Khan et al. (2007) found that probiotics also encouraged weight gain in broiler chickens, even in the absence of diarrheal outbreaks. Khaksefidi and Ghoorchi (2006) also reported that probiotic supplementation to broiler chickens has been shown to benefit feed intake, weight gain, and feed conversion ratio (FCR). Mountzouris et al. (2010) and Shabani et al. (2012) also reported similar observations.

Among the species of bacteria, the genus *Bacillus* is of particular interest as a probiotic. Based on the study conducted by Elshaghabee et al. (2017) and Liu et al. (2009), *Bacillus* spp. has been found to have high stability to the surrounding atmospheric conditions such as heat, gastric conditions, and moisture. Bailey et al. (2000) added that the ability of *Bacillus* to form spores ensures their stability and viability during feed manufacturing processes, storage,

and movement through the gastrointestinal tract, implying their suitability for adoption in the poultry industry (Bernardeau et al., 2017). The study by Bailey et al. (2000) reported that *Bacillus* probiotics positively influenced the feed intake, FCR, and body weight gain (BWG) of disease-challenged broiler chickens. The same results were also reported by Adhikari et al. (2019) and Roy et al. (2015). However, according to Mingmongkolchai and Panbangred (2018), the efficacy of probiotics may vary from one study to the other due to differences in *Bacillus* probiotics composition, dosage, duration of supplementation, and strain used as well as chicken's age and health status. The current study aims to evaluate *B. velezensis* D01Ca and *B. siamensis* G01Bb isolated from the gut of ducks and goats as probiotics for broiler chickens. Specifically, to evaluate if *B. velezensis* D01Ca and *B. siamensis* G01Bb will enhance the growth performance, such as the feed intake, BWG, and FCR of the broiler chickens.

## MATERIALS AND METHODS

### Isolation, Morphological, and Enzymatic Testing

Three mature female grazing pekin ducks (4–6 months old) and one mature upgraded native female goat (12 months old) were chosen as the source of the gut. These animals were grazed freely and exposed to environmental conditions ranging from 35–45ºC, where *Bacillus* spp. are predominant, as described by Garbeva et al. (2003). The animals were slaughtered following the slaughtering method described in the Bureau of Agriculture and Fisheries Standards (BAFS) (2015, 2017) for ducks and goats. The gut was extracted, homogenized, serially diluted to $10^{-2}$, and subjected to heat shock at 85-90ºC for 10 min. The mixture was plated using trypticase soy agar (HiMedia, India) and incubated at 37ºC till the appearance of microbial colonies.

Colonies that appeared in plates were sub-cultured and modified through microscopy after a series of staining procedures. A total of 72 isolates were obtained from the guts of ducks and goats (36 bacterial isolates for each animal). Of these 72 isolates, only 30 were identified as *Bacillus* species (Table 1). Morphological identification of the isolates was based on Elliott et al. (2017). Further testing reveals that 25 of the 36 *Bacillus* species isolated from ducks were Gram-positive, with 20 endospore formers. At the same time, 31 Gram-positive and 19 endospore formers *Bacillus* species were identified from goats. The top *Bacillus* isolates were found to be all catalase positive.

Only 20 of the 31 pre-screened suspected *Bacillus* isolates passed the antibiotics and acid tolerance tests, indicating that only 20 can be tested for acid-bile tolerance (Table 2). All these strains were susceptible to ofloxacin (TM Media, India), and G01Ab was the most resistant to ofloxacin, followed by D02ha.

Twelve *Bacillus* strains from duck and goat isolates were tested for acid bile tolerance test (Figure 1).

Enzymatic activities were conducted on the top-performing *Bacillus* spp. from

duck and goat isolates. The procedure for protease was adopted from Vijayaraghavan and Vincent (2013), cellulase, lipase (Zarei et al., 2021), amylase (Abd-Elhalem et al., 2015), and chitinase (Xia et al., 2011). The summary of the enzymatic activity of the potentially viable *Bacillus* strains is presented in Figure 2. Based on the tests,

Table 1
*Morphological and biochemical characterization of* Bacillus *species*

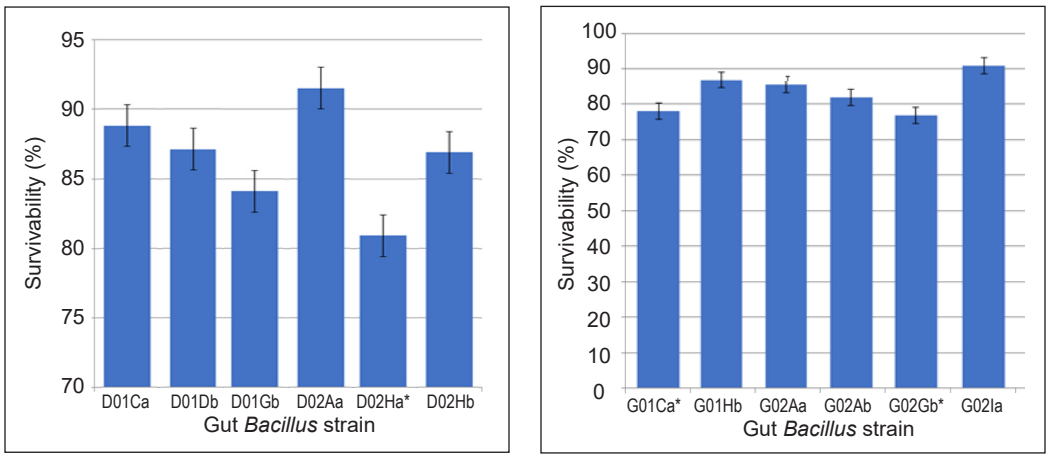
| Top performing isolates | Gram staining (+/-) | Endospore staining (+/-) | Catalase test (+/-) | Motility test (+/-) | Indole test (+/-) |
|---|---|---|---|---|---|
| D01Ca | + | + | + | + | - |
| D01Db | + | + | + | + | - |
| D01Gb | + | + | + | + | - |
| D02Aa | + | + | + | + | - |
| D02Hb | + | + | + | + | - |
| G01Bb | + | + | + | + | - |
| G01Hb | + | + | + | + | - |
| G02Aa | + | + | + | + | - |
| G02Ab | + | + | + | + | - |
| G02Ia | + | + | + | + | - |

*Note*. + = Gram positive, endospore former, catalase positive, motile, and endole positive; - = Gram negative, non-endospore former, catalase negative, and endole negative

Table 2
*Antibiotic assay of the selected* Bacillus *isolates against ofloxacin (OF5)*

| Antibiotic assay results | | | |
|---|---|---|---|
| Gut *Bacillus* isolates from duck | Average (mm) | Gut *Bacillus* isolates from goat | Average (mm) |
| D01Ca* | 23.345±3.472 | G01Ab | 26.240±1.542 |
| D01Da | 26.575±0.728 | G01Ba | 29.425±0.516 |
| D01Db* | 22.640±0.325 | G01Bb* | 21.555±0.149 |
| D01Ea* | 24.070±1.697 | G01Ca* | 21.155±0.898 |
| D01Eb* | 23.710±0.778 | G01Cb* | 22.110±0.057 |
| D01Gb* | 24.755±0.431 | G01Ga | 24.980±0.679 |
| D01Ha | 25.505±2.128 | G01Gb | 26.255±1.195 |
| D02Aa* | 23.550±0.467 | G01Ha | 25.815±0.149 |
| D02Ba* | 22.775±0.078 | G01Hb* | 24.100±0.141 |
| D02Ca* | 22.815±0.205 | G01Ia | 25.945±0.035 |
| D02Ea | 26.085±0.092 | G01Ib* | 23.725±0.347 |
| D02Ha* | 21.535±0.035 | G02Aa* | 23.065±1.138 |
| D02Hb* | 24.025±0.035 | G02Ab* | 21.145±0.757 |
| D02Ia* | 24.275±0.389 | G02Gb* | 24.685±0.05 |
| | | G02Hb | 25.880±0.085 |
| | | G02Ia* | 24.445±0.361 |
| | | G02Ib | 25.600±0.283 |

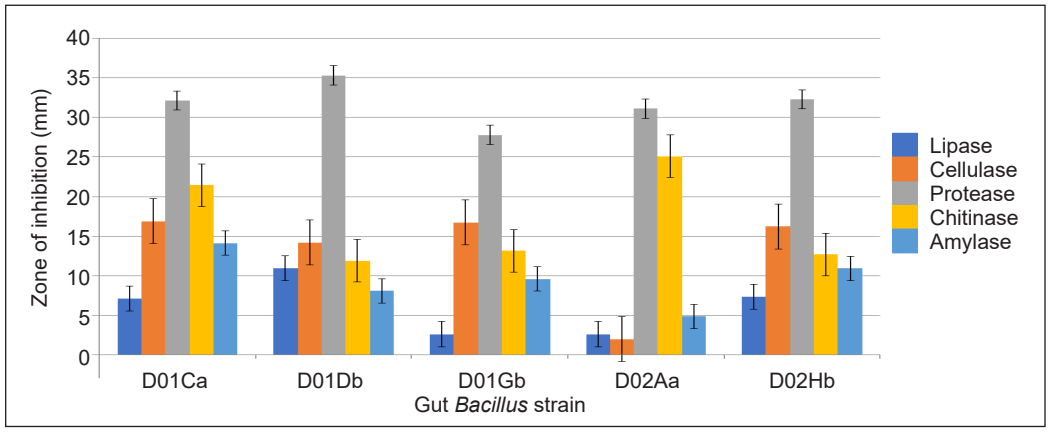
*Note*. * Denotes that the isolates were selected to continue further processes



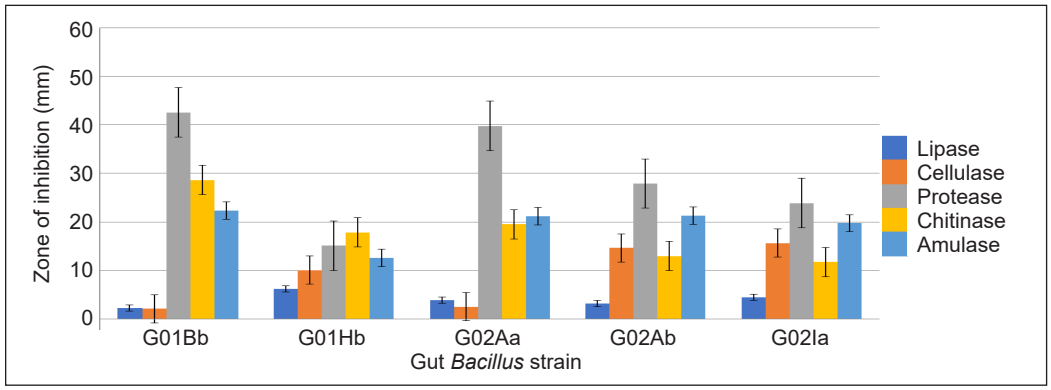

(a) (b)

*Figure 1*. Acid-bile tolerance test of the selected *Bacillus* strains that were isolated from duck (a) and goat (b), respectively

*Note*. * Denotes elimination form enzymatic assays




(a)




(b)

*Figure 2*. Enzymatic activity of the selected strain of *Bacillus* isolated from duck (a) and goat (b)

*Bacillus* strains D01Ca and G01Bb emerged as the top-performing isolates among both groups, with respective inhibitions of 18.332 and 17.938 mm.

**Molecular Identification**

Pure culture isolates were streaked on the appropriate agar and incubated at 30°C for 48 hr under aerobic conditions. The InstaGene Matrix Kit (Bio-Rad Laboratories, United Kingdom) was used for the DNA extraction according to the manufacturer's instructions. DNA purity was verified via a spectrophotometer after extraction and stored at -20°C (Olson & Morrow, 2012). Molecular identification was done in Macrogen (Korea). The sequence gathered was analyzed using Basic Local Alignment Search Tool (BLAST) software from National Center for Biotechnology Information (NCBI). Further confirmatory identification was done through the construction of a phylogenetic tree by the maximum-likelihood (ML) method and bootstrapped 10,000x for the confirmation of the identity of the isolated *Bacillus* to the nearest likely neighbor. D01Ca was confirmed and identified as *B. velezensis*; meanwhile, G01Bb diverged early from its group and was temporarily identified as *B. siamensis* G01Bb.

**Animals and Experimental Design**

One-hundred fifty (150) day-old Ross broiler chickens were randomly distributed into two experimental setups in a complete randomized design (CRD) with five replications per treatment containing five birds per replicate. SET A: T1—control (no probiotics), T2—single-dose *B. velezensis* D01Ca at $2.4 \times 10^7$ cfu/ml, T3—double-dose; SET B: T1—control (no probiotics), T2— single dose *B. siamensis* G01Bb at $2.29 \times 10^7$ cfu/ml; T3—double dose. The experiment was carried out for 42 days at the Center for Life Science Research Laboratory, Polytechnic University of the Philippines, Philippines, from February to April 2018.

**Probiotic Preparation and Administration**

Spore solution was prepared using the Arret-Kirshbaum Agar #2 (HiMedia, India) method adapted from Arret and Kirshbaum (1959). The single and double-dose concentration of the *B. velezensis* D01Ca and *B. siamensis* G01Bb was achieved using spectrophotometry (Spectronic 20D) for OD reading. Double dosage was achieved by adding a pure spore solution containing a single dosage in microtubules until the desired OD reading was doubled. After which, the pure spore solutions were added to 1 liter of sterilized water and provided to the broiler chickens daily for 42 days, according to treatments. Water is replaced daily or as needed to avoid contamination and disease outbreaks.

**Experimental Diet**

The broiler chickens were provided commercial feeds *ad libitum* throughout the experimental trial. Feed offered, including the leftover, were recorded. The feeds used were changed periodically based on their age group, emulating the practice of

poultry farmers. For a day-old to day-14, Chick Booster Mash™ (Philippines, GMP-1) was used; Broiler Starter Crumble™ (Philippines, GMP-2) on week 4, and Broiler Finisher Crumble™ (Philippines, GMP-3) on week 6.

### Housing Preparation

An open-sided and wire mesh-sided poultry house was used. The house was cleaned and well-disinfected prior to the commencement of the experiment. A total of 15 pens were used, providing an average of 1.5 sq. ft. per bird as floor requirements. Each pen had one drinker and feeders to ensure ad libitum feeding. The temperature of the poultry house was properly monitored and maintained at 30–32ºC during brooding as recommended by Ketelaars (2005) and then decreased to 18–22ºC during the growing stage (Daghir et al., 2009).

### Data Collection and Analysis

Body weight (BW) and body weight gain (BWG) was tabulated weekly to keep track of the broiler's growth performance following the protocol from Liu et al. (2009). Daily intake of feeds collated every seven days, BWG, and FCR were calculated using the following formula:

$$BWG = BW_{Wpresent} - BW_{Wprevious}$$

$$FCR = \frac{\text{Feed intake}}{\text{Weekly weight gain}}$$

The data gathered were analyzed statistically following the analysis of variance (ANOVA) run in SPSS (version 20) with homogeneity of variance tested using Levene's test. A significant difference between treatments was analyzed using the least significant difference (LSD) at $P \leq 0.05$.

## RESULTS AND DISCUSSION

The weekly feed intake of the broiler chicken supplemented with *B. velezensis* D01Ca and *B. siamensis* G01Bb from two experimental setups is presented in Table 3. It was revealed that probiotic-treated groups from the two experimental setups had slightly elevated feed intake compared to the control groups, but based on ANOVA, these differences in feed intake were non-significant ($P \geq 0.05$).

Table 3
*Mean weekly feed intake of broiler chickens supplemented with* Bacillus velezensis *D01Ca* and B. siamensis *G01Bb (in grams)*

| Week | *Bacillus velezensis* D01ca | | | *Bacillus siamensis* G01Bb | | |
|---|---|---|---|---|---|---|
| | Control | Single dose | Double dose | Control | Single dose | Double dose |
| 1 | 130.00±1.23 | 150.00±1.02 | 155.00±1.75 | 150.00±1.07 | 180.00±0.78 | 190.00±1.67 |
| 2 | 255.00±0.95 | 248.00±2.45 | 267.00±3.12 | 280.00±1.53 | 275.00±1.23 | 286.00±2.19 |
| 3 | 380.00±1.55 | 490.00±5.12 | 450.00±3.03 | 400.00±1.65 | 480.00±2.67 | 450.00±3.09 |
| 4 | 512.00±2.03 | 580.00±4.09 | 620.00±2.16 | 565.00±4.02 | 560.00±2.55 | 545.00±3.12 |
| 5 | 720.00±1.35 | 750.00±3.13 | 740.00±2.09 | 760.00±2.33 | 800.00±4.12 | 820.00±3.54 |
| 6 | 1,150.00 ±3.21 | 1285.00±2.43 | 1,300.00±4.08 | 1,120.00±7.06 | 1,345.00±5.32 | 1,260.00±5.09 |

*Note*. Means are non-significant at $P<0.05$

During the sixth week, broiler chickens supplemented with *Bacillus* species had a slightly elevated feed intake compared to the control groups in both experimental setups. However, based on ANOVA, no significant differences ($P \geq 0.05$) were noted between the treatments and the two experimental setups.

In the evaluation of the weekly body weights of the broiler chickens, a significant difference ($P \leq 0.05$) was observed between the treated groups compared to the control (Table 4). The body weight of the groups supplemented with *B. velezensis* D01Ca was heavier compared to the control during the fourth and sixth weeks of the experimental trial. The comparison between treatments revealed that a single dosage of *B. velezensis* D01Ca obtained a significant ($P \leq 0.05$) heavier body weight compared to the double doses and the control. The supplementation of *B. siamensis* G01Bb, on the other hand, also affected the body weights of the broiler chickens. Heavier body weight was also noted in broiler chickens supplemented with double doses of *B. siamensis* G01Bb compared to the single dose and the control during the fifth and the sixth weeks.

The body weight gain assessment revealed that groups supplemented with *B. velezensis* D01Ca and *B. siamensis* G01Bb obtained elevated weight gain compared to the control (Figure 3). This significant improvement in body weight gain from the treated groups ($P \leq 0.05$) was noted during the fourth week for *B. velezensis* D01Ca and during the third and fourth weeks for *B. siamensis* G01Bb. The body weight gain of broiler chickens supplemented with a single dose of *B. velezensis* D01Ca significantly obtained heavier body weight gain compared to the double dose and the control ($P \leq 0.05$) (Figure 3). The single dose and double doses of *B. siamensis* G01Bb significantly obtained heavier body weight gain during the third week of the experimental trial compared to the control ($P \leq 0.05$). However, during the fourth week of observation, a significant increase in body weight gain on the double dose of *B. siamensis* G01Bb was recorded compared to the single dose and the control ($P \leq 0.05$). Comparison between the two experimental setups revealed that the body weight gain of broiler chickens supplemented with *B.*

Table 4
*Mean weekly body weights of broiler chickens supplemented with Bacillus velezensis D01Ca* and *B. siamensis G01Bb (in grams)*

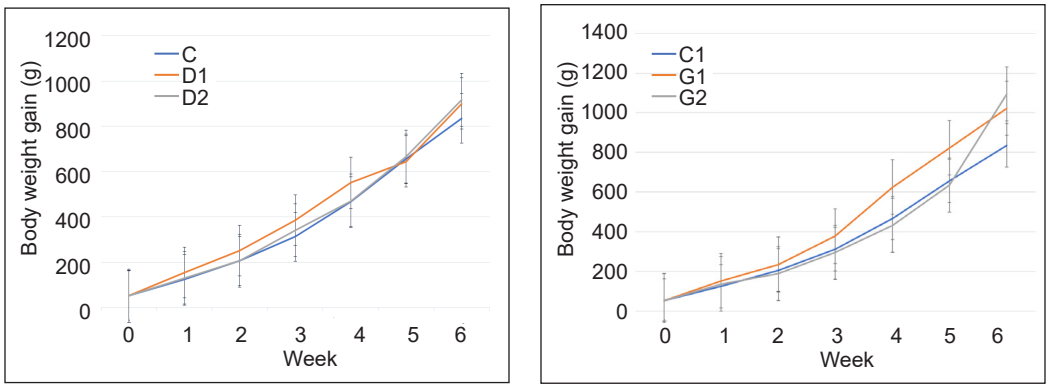
| Week | *Bacillus velezensis* D01ca | | | *Bacillus siamensis* G01Bb | | |
|---|---|---|---|---|---|---|
| | Control | Single dose | Double dose | Control | Single dose | Double dose |
| 1 | 160.00±1.23 | 185.00±1.02 | 175.00±1.75 | 175.00±1.07 | 180.00±0.78 | 190.00±1.67 |
| 2 | 365.00±0.95 | 415.00±2.45 | 450.00±3.12 | 330.00±1.53 | 445.00±1.23 | 430.00±2.19 |
| 3 | 765.00±1.55 | 825.00±5.12 | 885.00±3.03 | 725.00±1.65 | 880.00±2.67 | 795.00±3.09 |
| 4 | 1,015.00±2.03$^{b}$ | 1,295.00±4.09$^{a}$ | 1,880.00±2.16$^{a}$ | 1,995.00±4.02$^{a}$ | 1,115.00±2.55$^{a}$ | 1,212.00±3.12$^{a}$ |
| 5 | 1,280.00±1.35$^{b}$ | 1,550.00±3.13$^{a}$ | 1,375.00±2.09$^{b}$ | 1,195.00±2.33$^{c}$ | 1,365.00±4.12$^{b}$ | 1,550.00±3.54$^{a}$ |
| 6 | 1,680.00±3.21$^{b}$ | 1,890.00±2.43$^{a}$ | 1,860.00±4.08$^{a}$ | 1,545.00±7.06$^{b}$ | 1,755.00±5.32$^{b}$ | 1,840.00±5.09$^{a}$ |

*Note.* $^{a,b,c}$ Mean within rows having different superscripts = Significant difference at $P \leq 0.05$

*siamensis* G01Bb significantly differs from the body weight gain of broiler chickens supplemented with *B. velezensis* D01Ca (Figure 3) ($P \leq 0.05$).

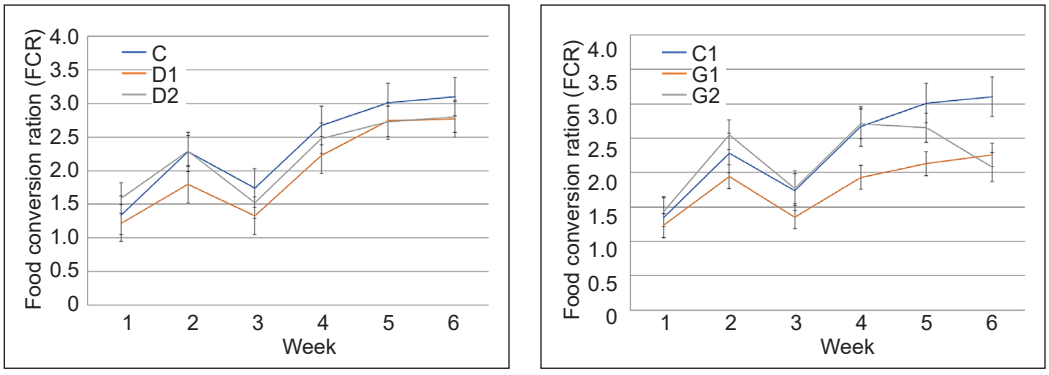
The FCR from the two experimental setups showed that treatments supplemented with both *B. velezensis* D01Ca and *B. siamensis* G01Bb obtained better FCR compared to the control, but these differences showed non-significant ($P \geq 0.05$) (Figure 4).

Though not significant, the single dose of *B. velezensis* D01Ca showed better FCR compared to the double dose. Furthermore, the single dose of *B. siamensis* G01Bb also showed better FCR compared to double those compared to the control.




(a) (b)

*Figure 3*. Average weekly weight gain of broiler chickens supplemented with *Bacillus velezensis* D01Ca (a), and *B. siamensis* G01Bb (b). Body weight gain in broiler chickens supplemented with *B. siamensis* G01Bb differed significantly from those supplemented with *B. velezensis* D01Ca ($P \leq 0.05$)
*Note*. C = Without probiotic supplementation; D1 = Single dose supplementation with *B. velezensis* D01Ca; D2 = Double dose supplementation with *B. velezensis* D01Ca; G1 = Single dose supplementation with *B. siamensis* G01Bb; G2 = Double dose supplementation with *B. siamensis* G01Bb




(a) (b)

*Figure 4*. Feed conversion ratio of broiler chickens supplemented with *Bacillus velezensis* D01Ca (a), and *B.* siamensis G01Bb (b). No significant difference at ($P<0.05$)
*Note*. C = Without probiotic supplementation; D1 = Single dose supplementation with *B. velezensis* D01Ca; D2 = Double dose supplementation with *B. velezensis* D01Ca; G1 = Single dose supplementation with *B. siamensis* G01Bb; G2 = Double dose supplementation with *B. siamensis* G01Bb

## DISCUSSION

These findings indicated that supplementation with *B. velezensis* D01Ca and *B. siamensis* G01Bb improved broiler chicken performance, as evidenced by increased body weight and weight gain. The non-significant results observed from the broilers' feed intake in the two experimental setups is an indication that the supplementations of *B. velezensis* D01Ca and *B. siamensis* G01Bb either in a single dose or double doses maintain the feed intake of broiler chickens. This result contradicts the previous reports of Nunes et al. (2012) and Zulkifli et al. (2000), who observed increased feed intake in broiler chickens supplemented with *Bacillus* spp.

In the current study, supplementation of *B. velezensis* D01Ca and *B. siamensis* G01Bb resulted in increased body weight and weight gain compared to the control group. This result indicates that *B. velezensis* D01Ca and *B. siamensis* G01Bb survived and resisted instability inside the broiler chicken's gut, modulating better nutrient absorption and enhancing body weight and weight gain while maintaining feed intake. Guo et al. (2010) reiterated the importance of the survivability and instability of probiotics inside the intestine as it prevents pathogenic bacteria adhesion that leads to enhanced nutrient utilization and absorption. Khaksefidi and Ghoorchi (2006) similarly noted an improved weight gain with the supplementation of 50 mg/kg probiotics compared to the control. Recently, Liu et al. (2009) reported improved body weight and weight gain in broiler chickens supplemented with *Bacillus licheniformis* in drinking water. Several authors also reported the positive effect of supplementing probiotics on the body weight gain of broiler chickens (Awad et al., 2009; Timmerman et al., 2006; Zulkifli et al., 2000). Moreover, enhanced body weight and weight gain are noticeable in the single dose of *B. velezensis* D01Ca compared to the double dosage. At the same time, the double dose of *B. siamensis* G01Bb had elevated body weights and gain during the fourth and fifth weeks compared to the single dose. This variation could be attributed to the action of probiotics inside the GIT. Probiotic actions and effects inside the intestinal tract are affected by numerous factors such as strain type, probiotic doses, feed, and hygienic conditions (Patterson & Burkholder, 2003).

Though insignificant, better FCR was noticeable in treated groups compared to the control. Supplementation of *Bacillus* spp. has been reported to reduce *C. perfringens* (Jayaraman et al., 2013; Jeong & Kim, 2014; Teo & Tan, 2005), Enterobacteriaceae (Jeong & Kim, 2014), and *Campylobacter* (Guyard-Nicodème et al., 2016). The exclusion of these microorganisms inside the GIT of the broiler chicken promotes better health and absorption of essential nutrients from the feed (Ray et al., 2012). Aside from pathogenic exclusion, *Bacillus* spp. are known to produce different antioxidants (Latorre et al., 2016) and antimicrobials (Urdaci et al., 2004), such as bacteriocins and high amounts of peptides and polyketides. In the research study of Bailey et al. (2000), they observed that

*Bacillus* probiotics improved the FCR of broiler chickens. Kabir (2005) and Khan et al. (2007) also reported similar observations. Recently, Lin et al. (2017) and Zhang et al. (2011) well-documented the improvement of the performance of broiler chicken supplemented with *Bacillus* probiotics.

## CONCLUSION

*Bacillus velezensis* D01Ca and *B. siamensis* G01Bb, either in single or double doses, did not affect broiler chicken feed intake. Moreover, the single and double doses of *B. velezensis* D01Ca and *B. siamensis* G01Bb improve broiler chicken body weights and weight gain during the fourth and sixth weeks of experimental trials. Therefore, both *B. velezensis* D01Ca and *B. siamensis* G01Bb can be safely used in broiler production as probiotics ensuring better performance during the finishing stage.

## RECOMMENDATION

Further studies *in vivo* must be conducted to assess the efficacy of *B. velezensis* D01Ca and *B. siamensis* G01Bb as probiotic supplements for broiler chickens and other poultry animals, emphasizing dosage to the different health conditions challenges such as diarrhea, light stress, and heat stress.

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