**Effect of Bacillus subtilis spore (GalliPro®) nutrients equivalency value on broiler chicken performance**

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**Abstract**

The experiment was conducted to evaluate the nutrients equivalency value of Bacillus subtilis spore (GalliPro®) for broiler chickens and its potential for decreasing feed nutrients concentration and cost. A total of 720 day old Ross 308 broiler chicks was allocated in 6 treatments (2 sexes×3 diets) with 6 replications for 7 weeks. Dietary treatments: main treatment (MT) was routine broiler diet added 0.2 g/kg GalliPro® (Bacillus subtilis 4×10⁹ CFU/g DSM 17299) and using nutrients equivalency of GalliPro® for feed formulation; negative control (NC) was the same as main treatment without GalliPro® (subtracted the nutrients equivalency value of GalliPro®); positive control (PC) was the same as MT diet in nutrients content but without GalliPro®. Effect of dietary treatments on body weight (BW) was not significant. However, the average BW of male and female chicks receiving negative control diet was 2.0% (68 g) lower than PC and MT groups (P>0.05). Dietary treatments had no significant effect on average daily feed intake. Feed conversion ratio of chicks receiving PC and MT diets were 2.7% better than NC chicks (P<0.01). Male chicks were superior to female in all measured traits (P<0.01). Effect of treatments on carcass characteristics was not significant. There was no interaction between factors on measured parameters. Performance of chicks receiving diet with GalliPro® compared with PC showed that GalliPro® liberated 0.4 crude protein from MT diet and consequently decreased the broiler feeding cost.

**Introduction**

A probiotic, which means _for life_ in Greek (Gibson and Fuller, 2000), has been most commonly defined as viable micro-organism which, after sufficient oral intake, causes beneficial effects for the host by modifying the intestinal microbiota (FAO, 2002). Most commonly used probiotic bacteria in poultry are Lactobacillus, Bifidobacterium, Bacillus, Streptococcus, Pedicoccus, Enterococcus, and yeast such as Saccharomyces cerevisiae. The most important advantage of a probiotic is that it has not any residue in animal production and any antibiotic resistance by consumption. Probiotic bacteria have numerous and different influences on the host. The use of probiotics which can help to improve poultry performance (Bai et al., 2013; Amerah et al., 2013; Apata, 2008; Mountzouris et al., 2007) improves intestinal microbial balance (Fuller, 1989; Mountzouris et al., 2010) and immune response (Talebi et al., 2008; Apata, 2008). Naglaa (2013) studied the effects of probiotics on turkey behaviour. Their results surprisingly demonstrated that probiotics increased the feeding frequency and period and decreased distress call and aggressive behaviours in turkey (Naglaa, 2013). Several studies reported that dietary probiotic supplementation enhanced the ileal digestibilities of some nutrient like crude protein (CP) and most amino acids (Apata, 2008; Li et al., 2008). The key factors that affected nutrient digestibility are the activities of digestive enzymes, such as α-amylase, proteases and peptidases, in addition to the intestinal microflora and the intestinal absorption of the digestion products (Li et al., 2008). The study of Collington et al. (1990) indicated that addition of either the probiotic or antibiotic in pigs’ diet had a significant effect on the development of sucrase, lactase and tripeptidase activities before weaning. The study of the allocation of enzyme activity along the small intestine showed significant differences between the proximal and distal sections associated with weaning (Collington et al., 1990). According to Cartman et al. (2008), oral inoculation of B. subtilis spores (GalliPro®) could reduce intestinal colonisation of Escherichia coli O78:K90 in chickens.

The additive GalliPro® is a microbial feed additive based on _B. subtilis_ spore (B. subtilis 4×10⁹ CFU/g DSM 17299). GalliPro® (a Bacillus subtilis strain) is a heat-tolerant, in-feed probiotic that has a beneficial effect on the microbial balance in the gastrointestinal tract of broilers. In the present study, it has been used as produced by Biochem Zusatzstoffe Handels-und Produktionsgesellschaft mbH (Lohne, Germany). Study of Cartman et al. (2008) has proven that spores of _B. subtilis_ are able to germinate in the chicken gastrointestinal tract. Also, they reported that _B. subtilis_ spore germination occurs rapidly in the gastrointestinal tracts of chicks, with vegetative cells outnumbering spores 20 h after spore doses were administered (Cartman et al., 2008). The manufactured company of this probiotic believes that this _B. subtilis_ strain has a particular feature, like producing peptidase enzymes. Subsequently, this company has provided nutrients equivalency values of this probiotic (Table 1). Authors did not observe any study on investigation of probiotic nutrient equivalency value on broiler performance in the literature review. Therefore, the aim of this study was to evaluate the nutrients equivalency value of _B. subtilis_ spore (GalliPro®) for broiler chicken and its potential for decreasing feed nutrients concentration and cost.

**Materials and methods**

A total of 720 1-d-old Ross 308 male and female broilers, with an initial average body weight (BW) of 48.0±8.5 g, was obtained from a commercial hatchery. Chicks were allocated in 6 treatments (2 sexes×3 diets), and each dietary treatment was offered to 6 replicates of 20 chicks. Environmental temperature in the three first days of life was 32°C and afterwards it was 31°C until the end of the first week. Then, the temperature decreased by 1°C every 4 days until reaching 22°C and this tempera-
ture was maintained until the end of the experiment. The lightening programme was 24 h light in the whole period of experiment. Completely randomised design in a factorial (2×3) arrangement was used. Factors included three dietary treatments: negative (NC) and positive control (PC) diets, and main treatment (MT). The MT was routine broiler diet added 0.2 g/kg GalliPro® (B. subtilis 4×10⁹ CFU/g DSM 17299) and using nutrients equivalency of GalliPro® for feed formulation; NC diet was the same as main treatment with no addition of GalliPro® (subtracted the nutrients equivalent value of GalliPro®); PC diet was the same as MT in nutrients content but without GalliPro®. The birds had free access to water and feed. Ingredients and composition of diets are presented in Table 2. Body weight and feed consumption were measured weekly and feed conversion ratio (FCR) was calculated. Over the entire period (1-49 day) mortality rate was evaluated. At 49 days of age, two birds with an average equivalent weight per replication were selected and slaughtered. Carcass, breast, thigh, abdominal fat and liver characteristics were measured (%).

### Table 1. Nutrient equivalency value of GalliPro®.

| Nutrient          | GalliPro® nutrient equivalency, g/kg |
|-------------------|-------------------------------------|
| CP                | 20,000                              |
| Calcium           | 370                                 |
| Amino acids (total) |                                     |
| Lys               | 1250                                |
| Met               | 695                                 |
| Thr+Cys           | 1110                                |
| Thr               | 695                                 |
| Try               | 220                                 |
| Ile               | 805                                 |
| Arg               | 1210                                |
| Leu               | 1490                                |
| Val               | 860                                 |
| Amino acids (digestible) |                                 |
| Lys               | 1125                                |
| Met               | 625                                 |
| Thr+Cys           | 1000                                |
| Thr               | 625                                 |
| Ile               | 1000                                |
| Arg               | 725                                 |
| Leu               | 1350                                |
| Val               | 775                                 |

**Table 2. Ingredient and nutrient content of experimental diets.**

| Feed          | Starter (0-7 days) | Grower (8-28 days) | Finisher (29-49 days) |
|---------------|--------------------|--------------------|-----------------------|
| Ingredients, g/kg |                   |                    |                       |
| GalliPro®     | 0.0                | 0.2                | 0.0                   |
| Corn          | 565.8              | 565.8              | 552.3                 |
| Soybean meal  | 330.7              | 380.7              | 382.6                 |
| Wheat         | 0.0                | 0.0                | 0.0                   |
| Corn oil      | 9.7                | 9.7                | 12                   |
| Dicalcium phosphate | 19.0                | 19.0              | 18.9                 |
| Salt          | 3.8                | 3.8                | 3.8                   |
| Oyster shell  | 10.7               | 10.7               | 10.9                 |
| Vit.-premix   | 2.5                | 2.5                | 2.5                   |
| Min.-premix   | 2.5                | 2.5                | 2.5                   |
| DL-met        | 2.9                | 2.9                | 3.0                   |
| L-lys H       | 1.6                | 1.6                | 1.5                   |
| L-thr         | 0.6                | 0.6                | 0.6                   |
| Calciumpkg    | 11.92              | 11.92              | 11.92                 |
| CP,%          | 21.3               | 21.7               | 21.7                  |
| Available P,% | 0.98               | 0.99               | 0.99                  |
| Ca,%          | 0.47               | 0.47               | 0.47                  |
| Na,%          | 0.16               | 0.16               | 0.16                  |
| Lys,%         | 1.17               | 1.20               | 1.20                  |
| Met,%         | 0.57               | 0.59               | 0.59                  |
| Met+Cys,%     | 0.86               | 0.88               | 0.88                  |
| Thr,%         | 0.76               | 0.78               | 0.78                  |
| Cost, $/kg    | 0.397              | 0.40               | 0.40                  |

**NC, negative control; MT, main treatment; PC, positive control; met, methionine; lys, lysine; thr, threonine; ME, metabolisable energy; CP: crude protein; P: phosphorus; Ca: calcium; Na: sodium; Cys, cysteine.**

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Economic analysis
The feed cost per kilogram weight gain of broiler chickens (male and female) was calculated. It was calculated by taking into consideration the cost of major feed ingredients and feed additives used at the time of study. The feed cost per kilogram weight gain was calculated by multiplying FCR by average weighted price (AWP) of diets. The feed cost per kilogram weight gain of one diet was calculated as follows:

$$\text{AWP} = (\% \text{SFI} \times \text{SDP}) + (\% \text{GFI} \times \text{GDP}) + (\% \text{FFI} \times \text{FDP})$$

where, % SFI and SDP are starter feed intake (FI) (% of whole feed intake) and starter diet price, respectively; % GFI and GDP are grower FI (% of whole feed intake) and grower diet price; % FFI and FDP are finisher FI (% of whole feed intake) and finisher diet price.

Statistical analysis
The experimental design was a completely randomised design with a factorial arrangement of treatments. The SAS 9.1 analysis programme was used for statistical analysis (SAS, 2002). Data obtained from the research were analysed by GLM procedure. Ryan-Einot-Gabriel-Welsch F multiple comparison tests used for separate means.

Results and discussion
The results of this experiment indicated that dietary treatments did not statistically affect birds’ BW and FI (Table 3). However, BW of birds that received NC diet was lower than MT and PC. Our results are in agreement with those of Li et al. (2008). On the contrary, the results of Kehlet et al. (2014) showed that GalliPro® can significantly improve live weight in broilers. The results of Bai et al. (2013) indicated that dietary addition of probiotic significantly increased BW of 21-day-old chicks and average daily FI during the starter period. Awad et al. (2009) demonstrated that the addition of probiotic or symbiotic significantly increased the villus height and its ratio to crypt depth (vh:cd) in both duodenum and ileum; increment of this ratio was related with improvement of growth performance.

Addition of GalliPro® did not have significant effect on FCR of male chicks, while it significantly improved FCR at 49 days of age in female chicks (P<0.01) (Tables 4 and 5, respectively). At the end of the experiment,
FCR in male and female chicks receiving NC diet was about 2.7% higher than that of chicks receiving MT and PC diets. This result was in agreement with outcomes of Zhang and Kim (2014), Amerah et al. (2013), Toghyani et al. (2011), and Opalinski et al. (2007). Amerah et al. (2013) reported that probiotic supplementation reduced FI and improved FCR with no effect on weight gain throughout the trial, indicating that the probiotic indeed exerted some beneficial effect on broilers. The results of Li et al. (2008) demonstrated that supplementing the broiler diet with probiotic, improved the ileal digestibilities of CP, most amino acids, dry matter, energy, calcium and phosphate in the birds. The improved performance of chickens and turkeys fed probiotics can be correlated with microstructures in the intestine where villus height and goblet cell numbers were increased, and crypt depth was decreased (Edens, 2003). Thus, probiotics improve the morphology of the intestinal tract leading to improved absorption of nutrients (Edens, 2003). Kehlet et al. (2014) reported that GalliPro® exhibits increased enzyme activity compared with many commercially available probiotics and may confer benefits of increased nutrient digestibility. So, the improvement in the FCR in this study may be due to the increased efficiency of digestion and nutrient absorption processes by the probiotic bacteria. Other studies reported no or minimal effect of probiotics on broiler performance (Mountzouris et al., 2007; Lee et al., 2010; Zhang et al., 2011).

Variations in efficacy of probiotics can be due to the difference in microbial species or micro-organism strains used, or to the additive preparation methods (Jin et al., 1998). Male chicks were superior to female in all measured traits (P<0.01). The results indicated that GalliPro® nutrients did not affect carcass characteristics (Table 6). These results are in agreement with Pelcano et al. (2003, 2005) who reported that, carcass and meat quality did not show alteration when the control group was compared to birds fed probiotics, except for leg yield improvement in the latter. Also, study of Toghyani et al. (2011) indicated that carcass yield and relative organ weights were not influenced by dietary probiotic and prebiotic. In contrast, the study of Willis et al. (2007) showed that fat pad relative weights percentage for males and females was affected by treatments and it was lower in birds fed probiotic diet with mushroom extract water in comparison with control diet. Some other factors such as bird species, sex, age, environmental condition, and quantity of added probiotic could affect the effects of probiotics supplementation. There was no interaction between factors on measured parameters (P>0.05). Mortality rate was 4% in all treatments and was not affected by dietary treatments (P>0.05). Similarly, Harrington et al. (2014), Kehlet et al. (2014), and Willis et al. (2007) reported that mortality was not affected by the broiler probiotic diet or mushroom extract water additive.

As shown in Table 2, the CP of NC diet was lower than PC (0.4 point), and CP of MT diet which was formulated using CP equivalency value of GalliPro® (one kg of GalliPro® is equivalent to 20 kg protein). Therefore, the recommended dosage of GalliPro® – 200 g/1000 kg feed – was the same as in PC diet. However, final BW of birds fed NC diet was 2.0% lower and their FCR was 2.7% higher than the birds fed PC diet. Moreover, the performance of birds fed MT diet was similar to the birds of PC diet. Therefore, with regard to performance of chicks fed diet containing GalliPro® in comparison with PC, it seems that GalliPro® liberates 0.4 CP from MT diet. Harrington et al. (2014) demonstrated that the supplementation of feed with GalliPro® can compensate bird performance when diets are reduced in digestible protein by 1.51% with a concurrent improvement in litter quality, most likely by improving diet digestibility.

![Figure 1](https://example.com/fig1.png)  
**Figure 1.** Comparison of average weighted price (US $) of experimental diets. NC, negative control; MT, main treatment; PC, positive control.

![Figure 2](https://example.com/fig2.png)  
**Figure 2.** The feed cost per kilogram weight gain of broiler chickens (US $). NC, negative control; MT, main treatment; PC, positive control.
According to obtained data, the share of total FI from starter, grower and finisher diets were about 2.04, 32.22 and 65.74%, respectively (data not show). Based on these data, the weighted price of each diet was calculated (Figure 1). According to Figure 1, price of PC and MT diets is higher than NC diet; on the other hand, Figure 2 shows that feed cost per kilogram weight gain of MT diet is lower than NC and PC (P<0.05). Therefore, it is concluded that the supplementation of GalliPro® in diet is able to reduce not only protein and amino acids requirements but also the feed cost per kilogram weight gain.

Conclusions

Since GalliPro® could liberate 0.4 CP from MT diet in comparison with PC, the use of this additive led to a reduction in protein and amino acids requirement, and consequently, the feed cost per kilogram weight gain could be reduced.

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