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飼糧添加枯草芽孢桿菌 TLRI 211-1 對白肉雞生長性能、屠體性狀及血液生化值之影響 (1)

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摘　要

枯草芽孢桿菌為我國飼料管理法中可供給畜禽之飼料添加物，其具生長快速、耐酸、耐鹼、耐熱及可形成內孢子等特性。本研究以篩選自活性污泥之枯草芽孢桿菌 TLRI 211-1 (*Bacillus subtilis* TLRI 211-1) 添加於飼糧中，探討對白肉雞生長、屠體性狀及血液生化值之影響。將篩選自活性污泥之枯草芽孢桿菌 TLRI 211-1 培養後，調整菌數為 1×10^8 CFU/mL。以 1 日齡 ROSS 308 白肉雞 192 隻，分配於對照組 (0%)、添加枯草芽孢桿菌 TLRI 211-1 菌液 0.1、0.3% 或商用枯草芽孢桿菌組 0.1%，各組 4 重複 (欄)，每欄 12 隻。試驗為期 5 週。結果顯示，飼糧添加枯草芽孢桿菌 TLRI 211-1 0.1% 組之雞隻增重、生產效率及屠體重顯著高於對照組及商用菌組 ($P < 0.05$)。飼糧添加枯草芽孢桿菌處理組與對照組比較，雞隻腹脂明顯較低，但腸道重顯著較高 ($P < 0.05$)。各組間在血液生化炎症指標酵素－麩胺酸草醋酸轉胺酶、丙酮酸轉胺酶、肌酸磷酸酶及 γ- 麩胺醯轉胺酶等均無顯著差異。綜上所述，飼糧添加枯草芽孢桿菌 TLRI 211-1 菌數達 1×10^8 CFU/kg，可提高白肉雞增重及生產效率，因此可作為促進雞隻生長性能之益生菌飼料添加物。

關鍵詞：白肉雞、枯草芽孢桿菌、生長性能。

緒　言

近年來細菌抗藥性和畜產品藥物殘留問題一直受到觀注，歐盟在 2006 年已禁用促進生長用抗生素，我國近年減少飼料可添加之抗生素品項，自 2019 年起僅 9 項可使用於飼料中 (行政院農業委員會，2019)。益生菌、益生素和有機酸為應用最多的飼料添加物，其對家禽在飼料能量和蛋白質利用上有助益 (Houshmand *et al*., 2012)。益生菌如乳酸桿菌屬 (*Lactobacillus* sp.)、雙歧桿菌屬 (*Bifidobacterium* sp.) 及芽孢桿菌屬 (*Bacillus* sp.) 可作為飼料添加物或發酵菌元，菌株在動物體內可直接作用，增加和維持腸道有益菌，或分泌酵素以提高飼糧營養吸收利用，進而改善動物經濟性能 (Goldin, 1998)。

在諸多應用於家禽生產之益生菌產品中，可芽孢化之芽孢桿菌屬在飼料生產上有利於儲存及降低造粒過程中溫度的破壞，進而提供較長的儲存期限 (Cartman *et al*., 2008; Chaiyawan *et al*., 2010)。枯草芽孢桿菌 (*Bacillus subtilis*) 為我國飼料管理法中可供給畜禽之飼料添加物，其具生長快速、耐酸、耐鹼、耐熱及形成內孢子等生物特性。內孢子在經過飼料加工之擠壓造粒過程和動物胃中暴露於強酸性環境後，仍可達腸道內生長及繁殖，對恢復和維持動物腸道菌群平衡，提高免疫功能，增強動物抗病能力，促進其生長發育具有重要意義 (Rajput *et al*., 2013)。

白肉雞飼糧添加枯草芽孢桿菌可改善腸道菌群 (Fritts *et al*., 2000)、增重及飼料轉換率 (Teo and Tan, 2006)。腸道菌群中產氣莢膜梭菌數量隨著枯草芽孢桿菌 PB6 用量增加而下降 (Teo and Tan, 2004)；體外試驗顯示，枯草芽孢桿菌 PB6 對產氣莢膜梭菌具抑制作用 (Teo and Tan, 2005)，並可增加腸道中乳桿菌屬有益菌 (Teo and Tan, 2006; 2007)。

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益生菌作為飼料添加物之應用，受到益生菌菌株、添加量、使用方式及飼養環境等因素影響 (Yang *et al*., 2009; Sen *et al*., 2012)。行政院農業委員會畜產試驗所 (以下簡稱畜試所) 自活性污泥篩選之枯草芽孢桿菌 TLRI 211-1 菌株，其飼養效果尚待雞隻生長試驗評估，本研究擬探討枯草芽孢桿菌 TLRI 211-1 菌株於飼糧中不同添加量對白肉雞生長、屠體性狀及血液生化值之影響。

材料與方法

I. 枯草芽孢桿菌菌液製備

以取自畜試所活性污泥篩選之枯草芽孢桿菌 (*Bacillus subtilis*)，經菌種鑑定、耐高溫 (50℃) 及產孢能力測試後，選取枯草芽孢桿菌菌株 TLRI 211-1 接種於 TSB 培養液 (Tryptic Soy Broth, Merck®)，並放置於 37℃培養箱培養 24 小時後，調整菌液為 1×10^8 CFU/mL。

II. 試驗動物與處理

動物試驗經過畜試所實驗動物照護及使用小組審核通過 (編號 108-35)，並於畜試所營養組雞舍進行。以 1 日齡 (ROSS 308 品系) 白肉雞 192 隻 (體重 42 ± 1 g)，分配於對照組 (空白組)、對照組飼糧添加枯草芽孢桿菌液 0.1%、0.3% 或商用枯草芽孢桿菌 0.1% 組 (益生康，The Best Nutrition Technology Co. Ltd., Taiwan, R.O.C.) (飼料菌數 1×10^8 CFU/kg)，每處理 4 重複，每重複 12 隻，公母各半，試驗至 35 日齡結束，飼料及飲水均為任飼。雞隻飼養期間分為育雛期 (0 － 21 日齡) 及生長期 (22 － 35 日齡)，營養分標準依 NRC (1994) 營養推薦調配，並依 AOAC (2000) 分析粗蛋白質、鈣及磷等含量，飼糧配方如表 1。

表 1.　試驗基礎飼糧組成

Table 1.　The composition of experimental basal diets

Ingredients, %	1 － 21 days of age	22 － 35 days of age
Yellow corn, grain	55.17	62.95
Full-fat soybean meal, CP 38%	5.75	6.87
Soybean meal, CP 43%	27.26	19.57
Fish meal, CP 60%	7.00	7.00
Soybean oil	2.00	1.00
Dicalcium phosphate	0.92	0.71
Limestone, pulverized	1.16	1.03
Salts	0.30	0.30
DL-Methionine	0.14	0.18
L-Lysine	0.10	0.19
Vitamin premix[1]	0.10	0.10
Mineral premix[2]	0.10	0.10
Total	100.00	100.00
Calculated value		
Crude protein, %	23.00	21.00
ME, kcal/kg	3,150.00	3,120.00
Calcium, %	0.96	0.87
Available phosphorus, %	0.48	0.43
Analyzed value, %		
Crude protein	23.12	21.22
Calcium	0.95	0.89
Total phosphorus	0.68	0.63

[1] Supplied per kilogram of diet: vitamin A, 3,000 IU; vitamin D_3, 400 IU; vitamin E, 10 IU; vitamin K_3, 1 mg; vitamin B_1, 3.6 mg; vitamin B_2, 5.4 mg; vitamin B_6, 7.0 mg; Ca-pantothenate, 20.0 mg; nia-cin, 70 mg; biotin, 0.3 mg; folic acid, 1.1 mg; and vitamin B_{12}, 0.02 mg.

[2] Supplied per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 80 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 60 mg; Zn (ZnO, 80.35% Zn), 40 mg; and Se (Na_2SeO_3, 45.7% Se), 0.15 mg.

III. 動物試驗測定項目

(i) 生長性狀：雞隻分別於 0、21 及 35 日齡進行秤重，並於 21 及 35 日齡時記錄採食量及雞隻死亡率，並計算雞隻增重、飼料轉換率、育成率及生產效率 (Production efficiency factor, PEF) (Rehman *et al.*, 2017)，PEF = 育成率 (%)　最終活體重 (kg) / (屠宰日齡 (d) × 飼料轉換率) × 100。

(ii) 育成率：於試驗期間記錄雞隻死亡隻數，以計算雞隻累加育成率。

(iii) 屠體性狀：試驗結束時，各處理組取 8 隻，公母各半，人道犧牲後進行屠宰並測定屠宰率，並採取心臟、肝臟、腹脂、腺胃－砂囊及腸道 (小腸 + 大腸) 秤重，計算與體重之重量百分比。
屠體重測定：雞隻經人道犧牲，移除腹脂與內臟後之屠體重。
屠宰率 (Dressing, %) = 屠體重 / 體重 × 100。
腹脂重 (%) = 腹脂重 / 體重 × 100。
各器官重 (%) = 各器官重 / 體重 × 100。

(iv) 血液生化學性狀：於試驗結束時各組選取 8 隻白肉雞，公母各半，自雞隻翼靜脈採取血液 6 mL，靜置 1 小時後置入離心機，以 3,000 × g，離心 10 分鐘，取血清凍存於 -20℃備檢。以血液生化分析儀 (Hitachi 7150, Japan) 分析血清中之總蛋白質 (total protein, TP)、白蛋白 (albumin, ALB)、球蛋白 (globulin, GLO)、尿素氮 (blood urea nitrogen, BUN)、肌酸酐 (creatinine, CREA)、尿酸 (uric acid, UA) 及三酸甘油酯 (triglycerides, TG) 之含量，以及麩胺酸草醋酸轉胺酶 (glutamate oxaloacetate transaminase, GOT)、丙酮酸轉胺酶 (glutamic phosphate transaminase, GPT)、鹼性磷酸酶 (alkaline phosphatase, ALP)、γ- 麩胺醯轉胺酶 (γ-glutamyltransferase, γ-GT) 及肌酸磷酸酶 (creatine kinase, CK) 之活性。

IV. 統計分析

試驗所獲得之資料，利用統計分析系統 (SAS, 2002)，以一般線性模式程序 (general linear model procedure, GLM) 進行變方分析，若有顯著性，再以杜凱確實差異檢定 (Tukey honestly significant difference test) 比較各組平均值差異之顯著性，差異顯著性訂為 $P < 0.05$。

結果與討論

I. 生長性能

飼糧添加枯草芽孢桿菌對白肉雞生長性能之影響，如表 2 所示。雞隻 1 － 21 日齡，各組間飼料採食量及飼料轉換率均無顯著差異，但枯草芽孢桿菌 TLRI 211-1 添加 0.1% 組肉雞體增重顯著高於對照組 ($P < 0.05$)。雞隻 22 － 35 日齡，枯草芽孢桿菌 TLRI 211-1 添加 0.1% 及 0.3% 組，體增重及飼料轉換率顯著高於對照組 ($P < 0.05$)。試驗全期 (1 － 35 日齡) 顯示，枯草芽孢桿菌 TLRI 211-1 添加 0.1% 及 0.3% 組在增重及飼料轉換率顯著優於對照組 ($P < 0.05$)，其中枯草芽孢桿菌 TLRI 211-1 添加 0.1% 組在飼料轉換率顯著優於商用枯草芽孢桿菌 0.1% 組，在生產效率則顯著高於對照組 ($P < 0.05$)。

枯草芽孢桿菌被認為具良好益生菌生物特性 (Jeong and Kim, 2014; Latorre *et al.*, 2014)，可耐高溫和飼料加工破壞，以及低 pH 值及厭氧環境 (Nakano and Zuber, 1998; Shivaramaiah *et al.*, 2011)，以枯草芽孢桿菌添加於飼料對 35 日齡白肉雞可改善增重 (Knap *et al.*, 2011; Aliakbarpour *et al.*, 2012; Gadde *et al.*, 2017)。但也有對生長性能無影響者 (Santoso *et al.*, 1995; Jeong and Kim, 2014)，因此 Lee *et al.* (2010) 以不同枯草芽孢桿菌菌株進行白肉雞飼養試驗，發現僅有部分菌株可增加體重。本試驗添加枯草芽孢桿菌 TLRI 211-1 0.1 及 0.3% 組生長皆顯著高於對照組，並與商用菌組在組飼料轉換率及採食量無明顯差異，顯示 TLRI 211-1 菌株之添加有助於白肉雞生長。

飼糧中枯草芽孢桿菌 10^6 － 10^9 CFU/kg 可促進腸道益生菌菌相發展，進而改善白肉雞生長性能 (Teo and Tan, 2007; Zhang *et al.*, 2012)；但 Lee *et al.* (2010) 指出，飼糧餵飼枯草芽孢桿菌 1.5×10^5 CFU/g 不會影響肉雞 (第 1 － 22 天) 的增重。枯草芽孢桿菌具備耐酸，並於腸道繁殖生長，以維持動物腸道菌群平衡 (Rajput *et al.*, 2013)，而不同研究間的枯草芽孢桿菌效果差異，可能與菌株之功能性不足，致使高添加量，仍未能改善雞隻生長性狀。本試驗結果顯示，畜試所枯草芽孢桿菌 TLRI 211-1，調整菌數為 1×10^8 CFU/mL，添加 0.1% 於飼糧中即可改善白肉雞生長性狀，亦即飼糧添加枯草芽孢桿菌 TLRI 211-1 菌數達 1×10^8 CFU/kg，可改善白肉雞生長性狀。

Leser *et al.* (2008) 指枯草芽孢桿菌在腸道中的競爭性黏附和免疫調節，與枯草芽孢桿菌分泌的分解酵素及其他分泌物質，有助於改善飼料轉換率和體增重，而本試驗使用枯草芽孢桿菌可改善生長性能或許與上述能力有關。

表 2. 飼糧添加枯草芽孢桿菌 TLRI 211-1 白肉雞生長性狀之影響

Table 2. Effects of dietary supplementation of *Bacillus* subtillis TLRI 211-1on growth performance of broilers

Items	Control	TLRI 211-1[1] (%)		COM[2]	SEM
		0.1	0.3		
1 to 21 d					
Weight gain, g/bird	727[b]	796[a]	746[ab]	770[ab]	6.67
Feed intake, g/bird	1,310	1,290	1,288	1,401	29.8
Feed conversion ratio, feed intake/weight gain	1.81	1.61	1.72	1.83	0.043
22 to 35 d					
Weight gain, g/bird	1,250[b]	1,478[a]	1,462[a]	1,356[ab]	17.9
Feed intake, g/bird	1,665	1,742	1,688	1,670	19.9
Feed conversion ratio, feed intake/weight gain	1.34[a]	1.18[b]	1.15[b]	1.23[ab]	0.014
1 to 35 d					
Weight gain, g/bird	1,977[b]	2,274[a]	2,208[a]	2,126[ab]	20.6
Feed intake, g/bird	2,975	3,033	2,965	3,073	25.3
Feed conversion ratio, feed intake/weight gain	1.51[a]	1.33[c]	1.34[bc]	1.45[ab]	0.012
Survival rate, %	93.7	93.7	93.7	93.7	1.6
PEF*	353[b]	458[a]	443[ab]	395[ab]	12.1

[a, b, c] Means in the same row with different superscripts differ significantly (P < 0.05).

* Production efficiency factor, PEF = (Survival rate (%) × BW (kg)) / (age (d) × feed conversion ratio) × 100.

[1] Newly isolated *Bacillus* subtillis strain-TLRI 211-1.

[2] COM = commercial *Bacillus* subtillis product.

II. 屠體性狀

飼糧添加枯草芽孢桿菌對白肉雞屠體性狀之影響列如表 3。結果顯示，飼糧中添加 TLRI 211-1 組 (0.1%、0.3%) 及商用菌組 (0.1%)，在屠體重均顯著高於對照組 (P < 0.05)。相對腸道重以枯草芽孢桿菌處理組 (TLRI 211-1 及商業菌株) 顯著高於對照組 (P < 0.05)。飼糧中添加 TLRI 211-1 0.1% 組相對腹脂重顯著低於對照組 (P < 0.05)。添加枯草芽孢桿菌對雞隻屠宰率、心臟、肝臟及腺胃－砂囊重，於各組間結果相近。

表 3. 飼糧添加枯草芽孢桿菌 TLRI 211-1 對白肉雞屠體性狀之影響

Table 3. Effects of dietary supplementation of *Bacillus* subtillis TLRI 211-1 on carcass characteristics of broilers

Items	Control	TLRI 211-1[1] (%)		COM[3]	SEM
		0.1	0.3		
Carcass weight, g	1,734[b]	1,980[a]	1,956[a]	1,896[a]	15.97
	------------------ as percentage of live BW --------------------				
Dressing[2]	84.7	84.5	84.7	84.3	0.14
Heart	0.64	0.64	0.66	0.64	0.008
Liver	2.63	2.48	2.63	2.67	0.043
Abdominal fat	1.10[a]	0.91[b]	0.93[ab]	0.95[ab]	0.024
Proventriculus and gizzard	3.24	3.18	3.21	3.34	0.044
Intestine	7.04[b]	7.58[a]	7.60[a]	7.62[a]	0.11

[a, b] Means in the same row with different superscripts are significantly different (P < 0.05).

[1] Newly isolated *Bacillus* subtillis strain-TLRI 211-1.

[2] Dressing = [(carcass weight / body weight] × 100.

[3] COM = commercial *Bacillus* subtillis product.

因本試驗添加 TLRI 211-1 0.1% 即可改善雞隻增重及屠體重，但對於屠宰率則無差異。此與 Molnár *et al.* (2011) 於飼料中添加枯草芽孢桿菌 2.27×10^4 CFU/g，可改善 42 日齡白肉雞屠體重結果相似。Santoso *et al.* (2001)

於白肉雞飼料添加 10 － 20 g 枯草芽孢桿菌可增加盲腸長度，並減少腹部脂肪。雞隻腹脂為家禽屠宰廢棄物，同時降低產品價值及增加處理成本，因此減少白肉雞腹脂，有助於白肉雞生產及後續加工利益。另外飼糧添加枯草芽孢桿菌可促進腸道中發展有益之高菌相環境，可改善腸道健康及絨毛性狀，促使食糜通過速率減緩，並增加絨毛吸收率 (Mahmoud *et al.*, 2017)，本試驗枯草芽孢桿菌可能因增加消化道容積，而提高腸道長度及重量，導致採食量及增重改善。

IV. 血液性狀

飼糧添加 TLRI 211-1 0.1% 組血液中鹼性磷酸酶顯著高於各組 (P < 0.05)。而在血液中總蛋白質及球蛋白濃度，則以對照組顯著高於飼糧添加 TLRI 211-1 0.1% 組及 0.3% 組 (P < 0.05) (表 4)。飼糧添加枯草芽孢桿菌均不影響白肉雞血液中麩胺酸草醋酸轉胺酶、丙酮酸轉胺酶、γ- 麩胺醯轉胺酶及肌酸磷酸酶之活性與白蛋白、尿素氮、肌酸酐、三酸甘油酯之含量 (P > 0.05)。

麩胺酸草醋酸轉胺酶、丙酮酸轉胺酶、γ- 麩胺醯轉胺酶及肌酸磷酸酶分別為肝臟及肌肉之炎症及疾病指標 (白等，1997)。枯草芽孢桿菌屬為我國飼料管理法規中可參考採用之菌種，TLRI 211-1 菌株經本研究確認不影響具身體發炎之相關指標酵素，顯示 TLRI 211-1 菌株實際餵飼予白肉雞無肝炎或毒性反應產生。

當血漿中水分改變或營養提高，將影響血中總蛋白質濃度 (白等，1997)。Mahmoud *et al.* (2017) 指白肉雞飼糧添加枯草芽孢桿菌，可增加空腸腺窩深度及迴腸粗蛋白質消化率，而改善增重及飼料效率。本試驗未記錄雞隻飲水量，但處理組之腸道重量較高，可能增加水分吸收，導致血中總蛋白質濃度較低，此在白蛋白及球蛋白濃度亦有相似趨勢。

鹼性磷酸酶是成骨細胞增殖的指標，在生長期骨轉換提高會同步增加活性 (Scholz-Ahrens *et al.*, 2016)。Guo *et al.* (2020) 以枯草芽孢桿菌 PB6 添加於白肉雞飼糧可提高雞隻後期增重、脛骨直徑及血中鹼性磷酸酶活性。本試驗 TLRI 211-1 0.1% 組可能因體重最高，而使鹼性磷酸酶活性有相似結果。

表 4.　飼糧添加枯草芽孢桿菌 TLRI 211-1 對白肉雞血液生化值之影響

Table 4.　Effects of dietary supplementation of *Bacillus* subtillis TLRI 211-1 on serum biochemical characteristicsof broilers

Items[1]	Control	TLRI 211-1[2] (%)		COM[3]	SEM
		0.1	0.3		
GOT, U/L	240	256	225	241	7.8
GPT, U/L	2.19	2.25	2.00	2.13	0.077
ALP, U/L	10,286[b]	13,140[a]	10,917[b]	9,299[b]	282
γ-GT, U/L	21.4	19.6	20.4	23.9	0.89
TP, g/dL	3.59[a]	3.01[b]	2.88[b]	3.40[ab]	0.11
ALB, g/dL	1.73	1.65	1.51	1.66	0.04
GLO, g/dL	1.86[a]	1.45[b]	1.36[b]	1.74[ab]	0.077
CREA, mg/dL	0.15	0.14	0.13	0.16	0.008
BUN, mg/dL	0.42	0.45	0.38	0.28	0.08
UA, mg/dL	4.47	4.50	4.20	4.79	0.23
TG, mg/dL	44.7	35.9	35.0	42.0	2.51
CK, U/L	5,234	4,181	4,661	4,813	322

[a, b] Means in the same row with different superscripts are significantly different (P < 0.05) .

[1] GOT = glutamate oxaloacetate transaminase; GPT = glutamic phosphate transaminase; ALP = alkaline phosphatase; γ-GT = γ-glutamyl transferase; TP = total protein; ALB = albumin; GLO = globulin; BUN = blood urea nitrogen; CREA = creatinine; UA = uric acid; TG = triglycerides; CK = creatine kinase.

[2] Newly isolated *Bacillus* subtillis strain-TLRI 211.

[3] COM = commercial *Bacillus* subtillis product.

結　　論

飼糧添加本所自篩枯草芽孢桿菌 TLRI 211-1 菌液 0.1%，可明顯提高雞隻體增重、飼料轉換率及生產效率，並

與商用枯草芽孢桿菌產品飼養效果相近。顯示本所自篩枯草芽孢桿菌 TLRI 211-1 為具市場潛力之益生菌。

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Effects of dietary supplementation of *Bacillus subtilis* TLRI 211-1 on growth performance, carcass, and blood characteristics of broilers (1)

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Abstract

Bacillus subtilis is a feed additive that can be supplied to livestock and poultry according to the Taiwan Feed Control Act law. *B. subtilis* features characteristics of rapid growth, acid resistance, alkali resistance, heat resistance, and endospore formation. In this study, *B. subtilis* TLRI 211-1, isolated from activated sludge, was added to the diet to explore the effects on the growth performance, carcass, and blood characteristics of broilers. After culture, the number of *B. subtilis* TLRI 211-1 was adjusted to 1×10^8 CFU/mL. A total of 192 day-old broilers were allocated to four groups, including: control group (0%), *B. subtilis* TLRI 211-1 solution 0.1%, 0.3% supplementation groups, and commercial *B. subtilis* 0.1% supplementation group. Each group had 4 replicates (pens) and each replicate had 12 birds. The experiment was conducted for 5 weeks.During the experimental period, growth performance, carcass and blood characteristics were measured. The results showed that the weight gain, production efficiency and slaughter weight of the feed supplemented with TLRI 211-1 bacterial solution 0.1% were significantly higher than those of the control group and the commercial bacteria group ($P < 0.05$). The abdominal fat of birds fed with *B. subtilis* was significantly lower, but the intestinal weight was significantly higher than those of the control group ($P < 0.05$). There was no significant difference in the blood biochemical inflammation index enzymes - GOT, GPT, CK, and γ-GT among all groups. In summary, dietary supplementation of 1×10^8 CFU/kg *B. subtilis* TLRI 211-1 can increase the weight gain and production efficiency of white broilers. Thus, *B. subtilis* TLRI 211-1 strain can be used as a probiotic feed additive to promote chicken growth performance.

Key words: Broiler, *Bacillus subtilis*, Growth traits.

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