

枯草芽孢桿菌發酵大豆粕產物取代大豆粕對白肉雞 生長性能、屠體及血液性狀之影響⁽¹⁾

李宗育⁽²⁾ 廖仁寶⁽³⁾ 施柏齡⁽²⁾⁽⁴⁾

收件日期：113 年 10 月 8 日；接受日期：114 年 5 月 20 日

摘 要

本研究旨在探討以枯草芽孢桿菌 TLRI 211-1 進行大豆粕之不同水分固態發酵條件，以較佳水分條件發酵後之大豆粕產物添加不同含量並取代飼糧中大豆粕，以評估對白肉雞生長性能改善情形。大豆粕添加菌液後分別調整水分含量為 50% 及 60%，進行 48 小時固態發酵，結果顯示 60% 水分組之菌數、pH 值及粗蛋白含量均略優於 50% 組，其中必需胺基酸組成亦較高，故後續以 60% 水分組之發酵大豆粕產物進行動物試驗。以 1 日齡白肉雞 160 隻，逢機分配於 4 組，分別為對照組 (0%)，發酵產物分別以 0.5% 組、1% 組及 2% 組用量取代飼糧中大豆粕，每組 40 隻，各組 4 重複，每重複 10 隻，試驗期至 35 日齡，試驗期間測定生長性能、屠體及血液性狀等。結果顯示試驗全期飼糧中含大豆粕發酵物 (The product of *Bacillus subtilis* TLRI 211-1 fermented soybean meal, FSMP) 之體增重顯著優於對照組 ($P < 0.05$)，飼料轉換率則以 1% 及 2% FSMP 組顯著高於對照組 ($P < 0.05$)；在屠體性狀方面，FSMP 之各組屠體重均顯著高於對照組；在血液生化值結果顯示，FSMP 1% 與 2% 組的血液尿素氮濃度顯著高於對照組 ($P < 0.05$)，且其尿酸濃度顯著低於對照組 ($P < 0.05$)，而 FSMP 0.5% 組其血液生化值之各項血液生化值均與對照組無顯著差異。綜上，本研究推薦以枯草芽孢桿菌大豆粕固態發酵物使用 0.5% 於飼糧中，對白肉雞之血液生化值無影響且有助提高體增重及屠體重量。

關鍵詞：白肉雞、發酵大豆粕產物、生長性能。

緒 言

生長用抗生素禁止添加於飼料中，因而益生菌、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有益 (Houshmand *et al.*, 2012)。益生菌自 1970 年起，即作為保健或促進生長飼料添加物使用 (Abd - Talib *et al.*, 2013)。芽孢桿菌屬 (*Bacillus* spp.) 之內生孢子 (endospore)，於飼料製造過程與貯藏過程、宿主胃酸與膽鹽等不良環境下具有保護菌體活力之作用 (黃, 2007; Sharp *et al.*, 1989)，因而成為優良的飼料添加用益生菌。而枯草芽孢桿菌 (*Bacillus subtilis*) 廣泛分布於自然環境中，可產生內孢子抵抗高溫、低 pH 值、膽鹽及消化酶之傷害，同時可分泌多種酵素、黏蛋白 (mucin)、胺基酸及礦物質 (許, 2003)，主要用於蛋白酶、澱粉酶及納豆的生產 (黃, 2007)。

枯草芽孢桿菌飼予白肉雞，顯示除可改善腸道菌群外 (Hosoi *et al.*, 1999)，並可緩解下痢 (Maruta *et al.*, 1996)、改善體增重及飼料轉換率 (Jiraphocakul *et al.*, 1990)。枯草芽孢桿菌 PB6 作為飼料添加物，可改善白肉雞體增重和飼料轉換率 (Teo and Tan, 2006)。以本所自篩之 *Bacillus subtilis* TLRI 211-1 菌株已證實，於飼糧中添加菌液 0.1% 可改善白肉雞生長性能及生產效率因子 (production efficiency factor, PEF) (李等, 2022)，亦可顯著提高蛋雞之飼料換蛋率，及維持儲存期之蛋品質 (Tsai *et al.*, 2023)。

固態發酵 (solid-state fermentation, SSF) 通常定義為微生物生長在較少自由水 (free water) 含量的固體基質上，基質水分含量約 40 至 80%，而基質作為微生物發酵所需之碳氮源，即是利用微生物可進行發酵生產各種有用的物質或發酵品的過程 (Singhania *et al.*, 2009)。由於固態發酵的操作簡單、發酵產率高、容易將產物濃縮以及不易產生污

(1) 農業部畜產試驗所研究報告第 2827 號。
(2) 農業部畜產試驗所動物營養組。
(3) 農業部畜產試驗所飼料作物組。
(4) 通訊作者，E-mail: borling@mail.tlri.gov.tw。

染物等特性，使得固態發酵相較液態發酵更具有優勢 (Raimbault, 1998)。固態發酵過程菌株利用碳源進行培養，因此大豆粕經發酵後可降解大分子蛋白質，增加大豆粕中小分子蛋白質及可利用離胺酸含量，提高大豆粕之可溶性蛋白質含量，並改善蛋白質消化率及吸收率 (Chen *et al.*, 2009; Medeiros, 2015; Zheng *et al.*, 2017)。Kiers *et al.* (2003) 分別以 *Rhizopus microspores* Lu573 及 *Bacillus subtilis* LUB83 進行大豆粕發酵處理，餵飼保育豬而可提高其體增重、採食量及飼料轉換率。諸多研究顯示，發酵大豆粕產物 (fermented soybean meal product, FSMP) 可改善肉雞體增重及飼料轉換率 (Wang *et al.*, 2012; Li *et al.*, 2014; Soumeh *et al.*, 2019)；另有其他研究顯示，發酵大豆粕產物可調節肉雞迴腸及盲腸微生物組成、腸道型態、免疫性狀及屠體性狀 (Li *et al.*, 2014; Chachaj *et al.*, 2019; Soumeh *et al.*, 2019; Wu *et al.*, 2020)；然亦有不同的結果顯示發酵大豆粕產物不影響肉雞上市體重及體增重 (Wang *et al.*, 2012; Jazi *et al.*, 2019)。

本研究以自行篩選之枯草芽孢桿菌作為菌源，添加於不同水分大豆粕中進行固態發酵後分析發酵產物性狀，並取較佳之發酵大豆粕產物添加於飼料中，探討對白肉雞生長性能、屠體及血液性狀之影響。

材料與方法

I. 枯草芽孢桿菌及發酵大豆粕產物製備

以本所自篩之枯草芽孢桿菌 TLRI 211-1 (*Bacillus subtilis* TLRI 211-1)，將枯草芽孢桿菌菌株 TLRI 211-1 接種於 TSB 培養液 (Tryptic Soy Broth, Merck®)，並放置於 37°C 培養箱培養 24 小時後，調整菌液為 1×10^9 CFU/mL。以大豆粕作為發酵基質使總含水率為 50 及 60%。發酵基質在發酵之前先以 125°C、1.21 kg/cm² 滅菌 20 分鐘，等待冷卻。在發酵基質中加入 10% 枯草芽孢桿菌菌液 (10^9 CFU/mL)，於 37°C 下好氧發酵 48 小時，發酵完成後以 65°C 乾燥發酵原料，使水分含量至 12% 以下。發酵前後取樣，進行一般成分及胺基酸組成分析。pH 值分析：1 g 飼料與 9 mL 滅菌水震盪混合後予以靜置，而後以 pH meter (digital pH meter, Goodly, Taiwan) 直接測定 pH 值。菌數分析：取約 1 g 發酵產物與 9 mL 生理食鹽水震盪混合後序列稀釋至適當倍率厚塗盤於 TSA (tryptic Soy Agar, merck, Germany)、PDA (Potato Dextrose Agar, NEOGEN, USA) 及 MRSA (Methicillin-resistant *Staphylococcus aureus*, merck, Germany) 盤，分別培養 24、72 及 24 小時後計算其菌數。

II. 試驗動物與處理

動物試驗經過農業部畜產試驗所 (以下簡稱畜試所) 實驗動物照護及使用小組審核通過 (編號 109-34)，並於畜試所動物營養組雞舍進行。以 1 日齡 (ROSS 308 品系) 白肉雞 160 隻 (體重 42 ± 1 g)，分配於對照組 (空白組) 與處理組，處理組以 60% 水分進行固態發酵之發酵大豆粕產物分別以 0.5、1 或 2% 取代飼糧中大豆粕，每組 4 重複，每重複 10 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 3 及 4。

III. 動物試驗測定項目

試驗於雞隻 35 日齡時，每組每重複選取 2 隻雞各採血一次，自翼靜脈採血 6 mL。試驗結束時雞隻人道犧牲，秤量屠體重、肝臟、腹脂、腸道重、腺胃砂囊重，並採結直腸內容物。

(i) 生長性狀：雞隻分別於 1、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、育成率及生產效率因子 (Production efficiency factor, PEF) (Rehman *et al.*, 2017)。

$$\text{PEF} = \text{育成率} (\%) \times \text{最終活體重} (\text{kg}) / (\text{屠宰日齡} (\text{d}) \times \text{飼料轉換率}) \times 100。$$

(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：移除腹脂與內臟後之屠體重。

$$\text{屠宰率} (\text{Dressing, } \%) = (\text{屠體重} / \text{活體重}) \times 100。$$

$$\text{腹脂重} (\%) = (\text{腹脂重} / \text{活體重}) \times 100。$$

$$\text{各器官重} (\%) = (\text{各器官重} / \text{活體重}) \times 100。$$

(iv) 血液生化性狀：於試驗結束時，各組所採之 8 隻白肉雞血液樣品，公母各半靜置 1 小時後置入離心機，以 $3,000 \times g$ ，離心 10 分鐘，取血清凍存於 -20°C 備檢。以血液生化分析儀 (Hitachi 7150, Japan) 分析血清中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) 及肌酸磷酸酶 (creatinine kinase, CK) 之活性，以及葡萄糖 (glucose, Glu)、膽固醇 (cholesterol, CHOL)、三酸甘油酯 (triglycerides, TG)、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) 及尿酸 (uric acid, UA) 之濃度。

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 $P < 0.05$ 。

表 1. 不同發酵水分之大豆粕發酵物物理性狀

Table 1. Physiological characterizations of FSMP fermented with different water content

	UFSMP	FSMP water content, %		SEM
		50	60	
pH				
Initial	—	6.32	6.33	0.04
Fermentation	—	8.12 ^b	8.18 ^a	0.01
Dry	—	6.23 ^b	6.33 ^b	0.02
<i>Bacillus</i> -like, log cfu/g				
Initial	—	6.70	6.78	0.05
Fermentation	—	8.30 ^b	8.80 ^a	0.03
Dry	—	7.51 ^b	7.65 ^a	0.04
Nutrient composition, %				
Water content	10.70	11.00	10.90	0.13
Crude protein	42.85 ^b	44.52 ^a	44.58 ^a	0.21
Ca	0.27	0.28	0.28	0.01
P	0.52	0.52	0.51	0.01

FSMP = fermented soybean meal product.

UFSMP = unfermented soybean meal.

Data are the means of 4 batches of FSMP; each batch was tested in triplicates (n = 3).

^{a, b} Means in the same row with different superscripts differ ($P < 0.05$).

表 2. 不同水分含量之大豆粕發酵物胺基酸組成

Table 2. Amino acid composition analysis of the FSMP supplement of different water content

	UFSMP	Water content, %		SEM
		50	60	
Total amino acid	43.23 ^b	45.09 ^a	45.63 ^a	0.15
Total essential amino acid	18.29 ^b	22.25 ^a	21.21 ^a	0.10
Arginine	2.93	2.84	2.86	0.01
Histidine	1.07	1.31	1.27	0.01
Isoleucine	1.90	2.32	2.23	0.01
Leucine	3.32	4.05	3.91	0.02
Lysine	2.68 ^b	3.07 ^a	2.95 ^a	0.01
Methionine	0.50 ^b	0.89 ^a	0.92 ^a	0.01
Phenylalanine	2.09 ^b	2.62 ^a	2.48 ^a	0.02
Threonine	1.63	1.84	1.81	0.01
Valine	2.17 ^b	2.86 ^a	2.78 ^a	0.01
Total nonessential amino acid	24.94	22.84	24.42	0.02
Cysteine	0.59	0.83	0.91	0.01

表 2. 不同水分含量之大豆粕發酵物胺基酸組成 (續)

Table 2. Amino acid composition analysis of the FSMP supplement of different water content (continued)

	UFSMP	Water content, %		SEM
		50	60	
Aspartic acid	4.62 ^b	5.27 ^a	5.17 ^a	0.03
Serine	1.97	1.96	2.02	0.01
Glutamic acid	7.17 ^b	8.85 ^a	8.51 ^a	0.06
Proline	2.66	2.64	2.64	0.01
Alanine	2.59	1.89	1.88	0.01
Glycine	2.09	1.80	1.81	0.01
Tyrosine	1.57	1.28	1.54	0.04

FSMP = fermented soybean meal product.

UFSMP = unfermented soybean meal product.

Data are the means of 3 batches of FSMP; each batch was tested in triplicates (n = 3).

表 3. 白肉雞 (1 - 21 日齡) 基礎飼糧組成

Table 3. The composition of experimental basal diets in 1-21 days of age of broilers

Ingredient, %	Control	FSMP, %		
		0.5	1	2
Yellow corn, grain	47.5	47.5	47.5	47.5
Soybean meal, 44% CP	38.5	38	37.5	36.5
Fish meal, 65% CP	5	5	5	5
Soybean oil	5.5	5.5	5.5	5.5
Dicalcium phosphate	1.2	1.2	1.2	1.2
Limestone, pulverized	1.2	1.2	1.2	1.2
FSMP, 44.5% CP	—	0.5	1	2
Salts	0.3	0.3	0.3	0.3
DL-Methionine	0.2	0.2	0.2	0.2
Choline chloride, 50%	0.2	0.2	0.2	0.2
Vitamin premix ¹	0.2	0.2	0.2	0.2
Mineral premix ²	0.2	0.2	0.2	0.2
Total	100	100	100	100
Calculated value				
CP, %	23	23	23	23
ME, kcal/kg	3,150	3,150	3,150	3,150
Calcium, %	0.96	0.96	0.96	0.96
Available phosphorus, %	0.48	0.48	0.48	0.48
Analyzed value, %				
Crude protein	23.12	23.20	23.22	23.18
Calcium	0.95	0.96	0.91	0.94
Total phosphorus	0.68	0.66	0.66	0.67

FSMP = fermented soybean meal product.

¹ Supplied per kilogram of diet: vitamin A, 3,000 IU; vitamin D₃, 400 IU; vitamin E, 10 IU; vitamin K₃, 1 mg; vitamin B₁, 3.6 mg; vitamin B₂, 5.4 mg; vitamin B₆, 7.0 mg; Ca-pantothenate, 20.0 mg; niacin, 70 mg; biotin, 0.3 mg; folic acid, 1.1 mg; and vitamin B₁₂, 0.02 mg.

² Supplied per kilogram of diet: Cu (CuSO₄ · 5H₂O, 25.45% Cu), 8 mg; Fe (FeSO₄ · 7H₂O, 20.09% Fe), 80 mg; Mn (MnSO₄ · H₂O, 32.49% Mn), 60 mg; Zn (ZnO, 80.35% Zn), 40 mg; and Se (Na₂SeO₃, 45.7% Se), 0.15 mg.

表 4. 白肉雞 (22 – 35 日齡) 基礎飼糧組成

Table 4. The composition of experimental basal diets in 22-35 days of age of broilers

Ingredient, %	Control	FSMP, %		
		0.5	1	2
Yellow corn, grain	50	50	50	50
Soybean meal, 44% CP	36	35.5	35	34
Fish meal, 65% CP	5	5	5	5
Soybean oil	5.5	5.5	5.5	5.5
Dicalcium phosphate	1.2	1.2	1.2	1.2
Limestone, pulverized	1.2	1.2	1.2	1.2
FSMP, 44.5% CP	—	0.5	1	2
Salts	0.3	0.3	0.3	0.3
DL-Methionine	0.2	0.2	0.2	0.2
Choline chloride, 50%	0.2	0.2	0.2	0.2
Vitamin premix ¹	0.2	0.2	0.2	0.2
Mineral premix ²	0.2	0.2	0.2	0.2
Total	100	100	100	100
Calculated value				
CP, %	21	21	21	21
ME, kcal/kg	3,120	3,120	3,120	3,120
Calcium, %	0.87	0.87	0.87	0.87
Available phosphorus, %	0.43	0.43	0.43	0.43
Analyzed value, %				
Crude protein	21.12	21.23	21.24	21.22
Calcium	0.89	0.88	0.89	0.90
Total phosphorus	0.63	0.65	0.63	0.64

FSMP = fermented soybean meal product.

¹ Supplied per kilogram of diet: vitamin A, 3,000 IU; vitamin D₃, 400 IU; vitamin E, 10 IU; vitamin K₃, 1 mg; vitamin B₁, 3.6 mg; vitamin B₂, 5.4 mg; vitamin B₆, 7.0 mg; Ca-pantothenate, 20.0 mg; niacin, 70 mg; biotin, 0.3 mg; folic acid, 1.1 mg; and vitamin B₁₂, 0.02 mg.

² Supplied per kilogram of diet: Cu (CuSO₄ · 5H₂O, 25.45% Cu), 8 mg; Fe (FeSO₄ · 7H₂O, 20.09% Fe), 80 mg; Mn (MnSO₄ · H₂O, 32.49% Mn), 60 mg; Zn (ZnO, 80.35% Zn), 40 mg; and Se (Na₂SeO₃, 45.7% Se), 0.15 mg.

結果與討論

I. 固態大豆粕發酵物一般成分分析

以大豆粕為基質添加 *B. subtilis* TLRI 211-1，其 50 或 60% 含水率發酵物之物理性狀如表 1 所示。枯草芽孢桿菌添加於基質中進行 48 小時發酵，以 60% 含水率組之 pH 及 *Bacillus* like 菌數顯著高於含水率 50% 組 ($P < 0.05$)，同時於發酵產物乾燥後仍顯著高於含水率 50% 組 ($P < 0.05$)。FSMP 組粗蛋白質顯著高於 UFSMP 組 ($P < 0.05$)。枯草芽孢桿菌可利用基質中碳源進行增殖，同時以基質中氮源產生代謝物 (如酵素、胜肽代謝物) (Singhania *et al.*, 2009)。經由發酵過程中分解粗蛋白質使 pH 值上升，可知 *B. subtilis* TLRI 211-1 具利用大豆粕中碳源及氮源之能力，進而增加菌數及提高粗蛋白質含量。

II. 固態大豆粕發酵物胺基酸組成

枯草芽孢桿菌固態發酵大豆粕產物胺基酸組成，如表 2 所示。以 *B. subtilis* TLRI 211-1 接種於 50、60% 含水率之大豆粕中，進行固態發酵，因試驗初始以 70% 含水率進行固態發酵，發現水分易集中於培養盤下方導致腐爛發臭，故而不做後續試驗檢測。以大豆粕經發酵後產物之總胺基酸量，在含水率 50 及 60% 培養基分別為

45.09 及 45.63%，顯著高於大豆粕之總胺基酸量 43.23% ($P < 0.05$)。固態發酵培養物之天門冬胺酸、麩胺酸、脯胺酸、苯丙胺酸、胱胺酸、纈胺酸及甲硫胺酸含量及比例顯著高於大豆粕。其意味高分子量的不可降解蛋白質，在固態發酵期間被枯草芽孢桿菌所釋放酵素分解為低分子量的蛋白質、胜肽或游離胺基酸 (Teng *et al.*, 2012; Rayaprolu *et al.*, 2013)；此與 Teng *et al.* (2012) 利用 *B. subtilis* 以大豆粕進行固態發酵後成分相近，同時表示 *B. subtilis* TLRI 211-1 菌株可分解大豆粕，並產生高菌數及代謝產物。因以含水率 60% 具顯著較高菌數 (表 1)，且粗蛋白質及總胺基酸含量 (表 2) 略優於含水率 50% 發酵組，故後續以發酵水分 60% 之大豆粕發酵產物進行動物試驗之飼料添加物。

III. 生長性能

以發酵大豆粕產物取代飼糧中大豆粕對白肉雞生長性能之影響，詳如表 5。飼糧中含發酵大豆粕產物不影響 1 – 21 日齡白肉雞生長性狀，但可以顯著增加 22 – 35 日齡間體增重 ($P < 0.05$)，飼料轉換率僅 2% 的 FSMP 組有顯著差異 ($P < 0.05$)。飼養全期 (1 – 35 日齡)，飼糧使用發酵大豆粕產物可顯著增加體增重，並以 1 及 2% 使用量可進一步改善飼料轉換率及 PEF (生產效率因子) ($P < 0.05$)。

本試驗以枯草芽孢桿菌 (*B. subtilis* TLRI 211-1) 添加於 60% 水分之大豆粕進行固態發酵後，其發酵物含有 7.65 log cfu/g 菌數，粗蛋白質 44.58%，同時改變發酵物胺基酸比例，尤其以總必需胺基酸、總非必需胺基酸含量增加，此與前人試驗結果一致 (Teng *et al.*, 2012)。大豆粕經發酵後可降解大分子蛋白質，及增加大豆粕中小分子蛋白質及可利用離胺酸含量，提高大豆粕之可溶性蛋白質含量，進而改善白肉雞體增重及飼料轉換率 (Wang *et al.*, 2012; Li *et al.*, 2014; Soumeh *et al.*, 2019)。而以本所自篩之 *Bacillus subtilis* TLRI 211-1 接種於大豆粕，並以 60% 水分進行固態發酵後，當使用 1% 於白肉雞飼料中即可具有促進生長之功效。

表 5. 以發酵大豆粕產物取代飼糧中大豆粕對白肉雞採食量及生長性狀之影響

Table 5. Effects of replacing soybean meal with FSMP on feed intake and growth performance of broilers

Items	Control	FSMP, %			SEM
		0.5	1	2	
1 to 21 days of age					
Weight gain, g/bird	865	879	879	867	9.8
Feed intake, g/bird	1,219	1,142	1,107	1,109	17
Feed conversion ratio, feed intake/weight gain	1.41	1.30	1.26	1.28	0.08
22 to 35 days of age					
Weight gain, g/bird	1,262 ^b	1,338 ^a	1,389 ^a	1,383 ^a	26
Feed intake, g/bird	2,082	2,234	2,224	2,143	31
Feed conversion ratio, feed intake/weight gain	1.65 ^a	1.67 ^a	1.60 ^a	1.55 ^b	0.02
1 to 35 days of age					
Weight gain, g/bird	2,128 ^b	2,217 ^a	2,268 ^a	2,250 ^a	40
Feed intake, g/bird	3,301	3,376	3,331	3,252	44
Feed conversion ratio, feed intake/weight gain	1.55 ^a	1.52 ^{ab}	1.46 ^b	1.44 ^b	0.08
Survival rate, %	97.5	100	97.5	97.5	2.9
PEF*	382 ^b	416 ^{ab}	430 ^a	432 ^a	18

FSMP = fermented soybean meal product.

^{a, b} Means in the same row with different superscripts differ ($P < 0.05$).

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

n = 4.

IV. 屠體性狀

以發酵大豆粕產物取代飼糧中大豆粕對白肉雞屠體性狀之影響，如表 6。在活體重方面，飼糧中使用枯草芽孢桿菌固態發酵產物可顯著優於對照組 ($P < 0.05$)，在屠體重亦具相同趨勢。發酵大豆粕產物使用 1% 於飼糧在屠體之相對腹脂重顯著高於對照組 ($P < 0.05$)，與其他組別間則無顯著差異 ($P > 0.05$)，雖然其他處理組相較對照組間未具顯著差異，但腹脂重則高於對照組。相對腺胃砂囊重及相對腸道重在飼糧添加枯草芽孢桿菌固態

發酵物處理均顯著低於對照組 ($P < 0.05$)，由於本試驗雞隻採禁食後屠宰，因此處理組在消化道重量與對照組相近，故而使相對消化道之重量，均顯著低於對照組 ($P < 0.05$)。故於飼糧使用枯草芽孢桿菌固態發酵產物有較佳屠體重，進而降低腸道相對重量。

表 6. 以發酵大豆粕產物取代飼糧中大豆粕對白肉雞屠體性狀之影響

Table 6. The effect of replacing soybean meal with FSMP on carcass characteristics of broilers

Items	Control	FSMP, %			SEM
		0.5	1	2	
Carcass weight, g	1,883 ^b	2,141 ^a	2,086 ^a	2,107 ^a	39.7
----- as percentage of live BW -----					
Dressing ¹	83.4	82.5	83.4	83.2	0.21
Heart	0.57	0.58	0.55	0.56	0.018
Liver	1.95	1.84	1.64	1.76	0.047
Abdominal fat	0.34 ^b	0.45 ^{ab}	0.57 ^a	0.47 ^{ab}	0.028
Proventriculus and gizzard	2.91 ^a	2.25 ^b	2.24 ^b	2.14 ^b	0.069
Intestine	3.92 ^a	3.39 ^b	3.27 ^b	3.41 ^b	0.072

FSMP = fermented soybean meal product.

^{a, b} Means in the same row with different superscripts differ ($P < 0.05$).

¹ Dressing = (carcass weight / body weight) × 100.

n = 8.

V. 血液性狀

以發酵大豆粕產物取代飼糧中大豆粕對白肉雞血液性狀之影響，如表 7。以發酵大豆粕產物使用 2% 於飼糧中時雞隻血清中球蛋白含量顯著低於對照組 ($P < 0.05$)，但飼糧含發酵大豆粕產物 1 及 2% 於血清中尿素氮含量顯著高於飼糧中使用發酵大豆粕產物 0.5% 組 ($P < 0.05$)，血中尿酸含量則顯著低於對照組及 0.5% 組 ($P < 0.05$)。

表 7. 以發酵大豆粕產物取代飼糧中大豆粕對 35 日齡白肉雞血液生化值之影響

Table 7. Effects of replacing soybean meal with FSMP on serum biochemical characteristics of broilers

Items ¹	Control	0.5%	1%	2%	SEM
GOT, U/L	252	272	259	280	18.9
GPT, U/L	10.4	12.0	11.3	12.0	0.89
CK, U/L	1,786	1,651	1,445	1,364	208
ALP, U/L	1,217	1,429	1,740	1,631	110
Glu, mg/dL	263	273	274	261	3.34
CHOL, g/dL	108	104	111	101	2.83
TG, mg/dL	58.5	53.6	54.0	48.7	1.81
TP, g/dL	3.28	3.00	3.31	2.77	0.087
ALB, g/dL	1.45	1.33	1.51	1.31	0.035
GLO, g/dL	1.83 ^a	1.66 ^{ab}	1.80 ^{ab}	1.46 ^b	0.058
BUN, mg/dL	1.00 ^b	1.13 ^{ab}	1.63 ^a	1.67 ^a	0.098
CREA, mg/dL	0.015	0.011	0.020	0.012	0.01
UA, mg/dL	6.35 ^a	6.20 ^a	3.58 ^b	4.56 ^b	0.53

FSMP = fermented soybean meal product.

^{a, b} Means in the same row with different superscripts differ ($P < 0.05$).

¹ GOT = glutamate oxaloacetate transaminase; GPT = glutamic phosphate transaminase; CK = creatine kinase; ALP = alkaline phosphatase; GLU = glucose; CHOL = cholesterol; TG = triglycerides; TP = total protein; ALB = albumin; GLO = globulin; BUN = blood urea nitrogen; CREA = creatinine; UA = uric acid.

n = 8.

GOT、GPT、CK 分別為肝臟及肌肉腎臟之炎症及疾病指標 (白等, 1997)。本試驗以 *B. subtilis* TLRI 211-1 接種於大豆粕進行固態發酵後, 並添加於飼糧中對白肉雞血液中 GOT、GPT 及 CK 活性均不影響, 此與李等 (2022) 以 *B. subtilis* TLRI 211-1 直接添加於飼糧中不影響白肉雞具身體發炎之相關指標酵素, 再次證實 *B. subtilis* TLRI211-1 菌株及其發酵產物餵飼予白肉雞無肝炎或毒性反應產生情形。

尿酸、尿素氮及肌酸酐為動物腎功能評估之重要指標 (Abdelhalim *et al.*, 2020)。尿素氮是體內蛋白質代謝終端產物, 當飲食中嘌呤含量較低時, 尿酸則下降 (Ekpenyong and Daniel, 2015; Adewole *et al.*, 2021)。由於發酵產物之胺基酸組成及必需胺基酸含量顯著優於對照組, 故有較高之尿素氮含量, 而各組間尿酸含量雖符合正常值, 但以發酵大豆粕產物 1 及 2% 組較低, 可能與發酵產物中屬嘌呤先質之甘胺酸及麩胺酸比例較低如表 2, 同時可能添加發酵產物增加蛋白質及胺基酸之利用率 (Lee *et al.*, 2024), 而使血中尿酸含量較低。

結 論

以枯草芽孢桿菌 TLRI 211-1 添加含 60% 水分大豆粕進行固態發酵, 發酵物中可獲得較高菌數及總必需胺基酸含量, 同時以 60% 水分發酵之發酵物以 1% 於飼糧中並取代大豆粕, 可改善白肉雞增重、飼料轉換率及生產效率因子, 顯示以本所自篩枯草芽孢桿菌 TLRI 211-1 經 60% 水分大豆粕所得固態發酵物可作為白肉雞飼料添加物。

參考文獻

- 白火城、黃森源、林仁壽。1997。家畜臨床血液生化學。立宇出版社, 臺南市。
- 李宗育、廖仁寶、王嘉惠、施柏齡、范耕榛、林義福。2022。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。畜產研究 54 : 292-299。
- 黃忠村。2007。食品微生物。臺灣復文圖書有限公司。臺南市。
- 許元勳。2003。現代科技的新寵兒—神奇納豆菌。生物產業 14 : 53-59。
- Abdelhalim, M. A. K., H. A. Qaid, Y. H. Al-Mohy, and M. M. Ghannam. 2020. The protective roles of vitamin E and α -lipoic acid against nephrotoxicity, lipid peroxidation, and inflammatory damage induced by gold nanoparticles. *Int. J. Nanomed.* 15: 729-734. doi: 10.2147/ijn.s192740.
- Abd-Talib, N., S. H. Mohd-Setapar, A. K. Khamis, L. Nian-Yian, and R. Aziz. 2013. Survival of encapsulated probiotics through spray drying and non-refrigerated storage for animal feeds application. *Agric. Sci.* 04: 78-83. doi: 10.4236/as.2013.45b015.
- Adewole, D. I., S. Oladokun, and E. Santin. 2021. Effect of organic acids-essential oils blend and oat fiber combination on broiler chicken growth performance, blood parameters, and intestinal health. *Anim. Nutri.* 7: 1039-1051. doi: 10.1016/j.aninu.2021.02.001.
- Association of Official Agricultural Chemists. 2000. Official methods of analysis. 14th ed. Washington, D. C. Association of Official Analytical Chemists.
- Chachaj, R., I. Sembratowicz, M. Krauze, and K. Ognik. 2019. The effect of partial replacement of soybean meal with fermented soybean meal on chicken performance and immune status. *J. Anim. Feed Sci.* 28: 263-271.
- Chen, K. L., W. L. Kho, S. H. You, R. H. Yeh, S. W. Tang, and C. W. Hsieh. 2009. Effects of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* mixed fermented feed on the enhanced growth performance of broilers. *Poult. Sci.* 88: 309-315.
- Ekpenyong, C. E., and N. Daniel. 2015. Roles of diets and dietary factors in the pathogenesis, management and prevention of abnormal serum uric acid levels. *Phar. Nutr.* 3: 29-45. doi: 10.1016/j.phanu.2014.12.001.
- Houshmand, M., K. Azhar, I. Zulkifli, M. H. Bejo, and A. Kamyab. 2012. Effects of nonantibiotic feed additives on performance, immunity and intestinal morphology of broilers fed different levels of protein. *S. Afr. J. Anim. Sci.* 42: 22-32.
- Hosoi, T., A. Ametani, K. Kiuchi, and S. Kaminogawa. 1999. Changes in fecal microflora induced by intubation of mice with *Bacillus subtilis* (natto) spores are dependent upon dietary components. *Can. J. Microbiol.* 45: 59-66.
- Jazi, V., H. Mohebodini, A. Ashayerizadeh, A. Shabani, and R. Barekatin. 2019. Fermented soybean meal ameliorates

- Salmonella Typhimurium* infection in young broiler chickens. *Poult. Sci.* 98: 5648-5660. doi: 10.3382/ps/pez338.
- Jiraphocakul, S., T. W. Sullivan, and K. M. Shahani. 1990. Influence of a dried *Bacillus subtilis* culture and antibiotics on performance and intestinal microflora in turkeys. *Poult. Sci.* 69: 1966-1973.
- Kiers, J. L., J. C. Meijer, M. J. R. Nout, F. M. Rombouts, M. J. A. Nabuurs, and J. van der Meulen. 2003. Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. *J. Appl. Microb.* 95: 545-552.
- Lee, T. Y., Y. S. Lee, C. P. Wu, K. W. Chan, and K. L. Chen. 2024. *Bacillus amyloliquefaciens* CU33 fermented feather-soybean meal product improves the crude protein digestibility, diarrhea status, and growth performance of goat kids. *Animals.* 14: 2809.
- Li, C. Y., J. J. Lu, C. P. Wu, and T. F. Lien. 2014. Effects of probiotics and bremelain fermented soybean meal replacing fish meal on growth performance, nutrient retention and carcass traits of broilers. *Livest. Sci.* 163:94-101. doi: 10.1016/j.livsci.2014.02.005.
- Maruta, K., H. Miyazaki, S. Masuda, M. Takahashi, T. Marubashi, Y. Tadano, and H. Takahashi. 1996. Exclusion of intestinal pathogens by continuous feeding with *Bacillus subtilis* C-3102 and its influence on the intestinal microflora in broilers. *Anim. Sci. Technol.* 67: 273-280.
- Medeiros, S. 2015. Improving the nutritional value of soybean meal through fermentation using newly isolated bacteria. MS Thesis. University of Guelph, Guelph.
- National Research Council. 1994. Nutrient Requirement of Poultry. 9th ed. National Academy Press, Washington, D. C., USA.
- Raimbault, M. 1998. General and microbiological aspects of solid substrate fermentation. *Electron. J. Biotech.* 1: 26-27. doi: 10.4067/S0717-34581998000300007.
- Rayaprolu, S. J., N. S. Hettiarachchy, P. Y. Chen, A. Kannan, and A. Mauromostakos. 2013. Peptides derived from high oleic acid soybean meals inhibit colon, liver and lung cancer cell growth. *Food Res. Intern.* 50: 282-288.
- Rehman, Z. ur, N. Chand, and R. U. Khan. 2017. The effect of vitamin E, l-carnitine, and ginger on production traits, immune response, and antioxidant status in two broiler strains exposed to chronic heat stress. *Environ. Sci. Pollut. Res.* 24: 26851-26857.
- SAS. 2002. SAS User's Guide. Statistical Institute, Inc., Cary, N.C.
- Sharp, R. J., M. D. Scawen, and T. Atkinson. 1989. Fermentation and downstream processing of *Bacillus*. Pages 255-292 in *Bacillus*. C. R. Harwood, ed. Plenum Press, New York, NY.
- Singhania, R. R., A. K. Patel, C. R. Soccol, and A. Pandey. 2009. Recent advances in solid-state fermentation. *Biochem Eng J.* 44: 13-18. doi: 10.1016/j.bej.2008.10.019.
- Soumei, E. A., H. Mohebodini, M. Toghyani, A. Shabani, A. Ashayerizadeh, and V. Jazi. 2019. Synergistic effects of fermented soybean meal and mannan-oligosaccharide on growth performance, digestive functions, and hepatic gene expression in broiler chickens. *Poult. Sci.* 98: 6797-6807. doi: 10.3382/ps/pez409.
- Teng, D., M. Gao, Y. Yang, B. Liu, Z. Tian, and J. Wang. 2012. Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae*. *Biocatal. Agric. Biotechnol.* 1: 32-38.
- Teo, A. Y. and H. M. Tan. 2006. Effect of *Bacillus subtilis* PB6 (CloSTAT) on broilers infected with a pathogenic strain of *Escherichia coli*. *J. Appl. Poult. Res.* 15: 229-235.
- Tsai, M. Y., B. L. Shih, R. B. Liaw, W. T. Chen, T. Y. Lee, H. W. Hung, K. H. Hung, and Y. F. Lin. 2023. Effect of dietary supplementation of *Bacillus subtilis* TLRI 211-1 on laying performance, egg quality and blood characteristics of Leghorn layers. *Anim. Biosci.* 36: 609-618.
- Wang, L. C., C. Wen, Z. Y. Jiang, and Y. M. Zhou. 2012. Evaluation of the partial replacement of high-protein feedstuff with fermented soybean meal in broiler diets. *J. Appl. Poult. Res.* 21: 849-855. doi: 10.3382/japr.2012-00563.
- Wu, P., M. K. Golly, Y. Guo, H. Ma, R. He, X. Luo, S. Luo, C. Zhang, L. Zhang, and J. Zhu. 2020. Effect of partial replacement of soybean meal with high-temperature fermented soybean meal in antibiotic-growth-promoter-free diets on growth performance, organ weights, serum indexes, intestinal flora and histomorphology of broiler chickens. *Anim. Feed Sci. Tech.* 269: 114616. doi: 10.1016/j.anifeedsci.2020.114616.
- Zheng, L., D. Li, Z. L. Li, L. N. Kang, Y. Y. Jiang, X. Y. Liu, Y. P. Chi, Y. Q. Li, and J. H. Wang. 2017. Effects of *Bacillus* fermentation on the protein microstructure and anti-nutritional factors of soybean meal. *Lett. Appl. Microb.* 65: 520-526.

Effects of replacing feed soybean meal with soybean meal fermented by *Bacillus subtilis* TLRI 211-1 on growth performance, carcass, and blood characteristics of broilers ⁽¹⁾

Tsung-Yu Lee ⁽²⁾ Ren-Bao Liaw ⁽³⁾ and Bor-Ling Shih ⁽¹⁾⁽⁴⁾

Received: Oct. 8, 2024; Accepted: May 20, 2025

Abstract

This study aimed to explore the effects of solid-state fermentation of soybean meal inoculation with *Bacillus subtilis* TLRI 211-1 under different moisture conditions. The study then evaluated the impact of using the fermented soybean meal product (FAMP), with optimal moisture content, at different levels to the diet on the growth performance of broiler chickens. The moisture content of the soybean meal was adjusted to 50%, and 60% after inoculation, followed by 48 hours of solid-state fermentation. The results showed that the 60% moisture group had superior bacterial count, pH value, and crude protein content compared to the other groups, with higher levels of essential amino acids. Therefore, the fermented soybean meal product from the 60% moisture group was selected for subsequent animal trials. In the animal trial, 160 one-day-old broiler chickens were evenly distributed into four groups: a control group (0%), and three experimental groups replacing feed soybean meal with 0.5%, 1%, and 2% of the FSMP in the diet, with each group consisting of 40 chickens, divided into four replicates of 10 chickens each. The trial period lasted until the chickens were 35 days old, during which growth performance, carcass traits, and blood parameters were measured. The results indicated that body weight gain was significantly higher in all groups fed with the FSMP compared to the control group ($P < 0.05$), and with feed efficiency were significantly improved in the 1% and 2% FSMP groups ($P < 0.05$). Additionally, there was a significant increase in blood urea nitrogen and a decrease in uric acid levels ($P < 0.05$). In conclusion, this study recommends using 1% of *Bacillus subtilis* FSMP in the diet, as it is beneficial for improving the growth performance of broiler chickens.

Key words: Broilers, Fermented soybean products, Growth performance.

(1) Contribution No. 2827 from Taiwan Livestock Research Institute (TLRI), Ministry of Agriculture (MOA).

(2) Animal Nutrition Division, MOA-LRI, No. 112, Farm Rd, Hsinhua, 71246, Tainan, Taiwan, R. O. C.

(3) Forage Crops Division, MOA-LRI, No. 112, Farm Rd, Hsinhua, 71246, Tainan, Taiwan, R. O. C.

(4) Corresponding author, E-mail: borling@mail.tlri.gov.tw.