

添加枯草菌素、六肽黴素及羥四環黴素對肉雞腸道上皮 通透性、腸道菌相及細菌轉移之影響⁽¹⁾

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摘 要

本試驗探討飼糧中添加不同抗生素對肉雞腸道上皮通透性、腸道菌相及細菌轉移之影響。選取 192 隻 1 日齡愛拔益加肉雞，隨機分為對照組 (control, CON)、枯草菌素 (bacitracin) 55 ppm、六肽黴素 (nisiheptide) 2.5 ppm 及羥四環黴素 (oxytetracycline, OTC) 55 ppm 四組。每處理組 4 重複，每重複 12 隻。分別於 3 週及 6 週時，進行肉雞腸道電生理及腸道免疫能力之測定。結果顯示，添加三種抗生素皆不會影響腸道的組織電位差 (potential difference, PD)、離子電流 (short-circuit current, I_{sc}) 及電導係數 (conductance, G)。飼糧中添加 3 種抗生素，對腸道中大腸桿菌、乳酸菌及腸球菌的組成無顯著影響，但 3 種抗生素皆於 3 週齡時顯著減少腸道細菌轉移至肝臟的數量 ($P < 0.05$)，而以六肽黴素抑菌效果最好。在對照組中，0 週及 3 週細菌轉移到肝臟總菌數高於 6 週 ($P = 0.13$, 0.08)，顯示肉雞在 3 週齡之前腸道屏障功能較為脆弱。綜上所述，肉雞飼糧補充枯草菌素、六肽黴素或羥四環黴素能抑制細菌侵入，減少轉移進入體內的機會。惟飼糧中可添加抗生素種類業已限縮，應積極開發具類似抑菌功能之飼料添加物以替代抗生素。

關鍵詞：抗生素、腸道上皮通透性、腸道菌相、細菌轉移、肉雞。

緒 言

在臺灣雞隻多是以大規模、集約式方式養殖，在飼養過程中，為達疾病之治療、預防或促進生長等效益，業者大多依賴飼料中添加抗生素，以改善雞隻生產表現。於飼料中添加促進生長用之抗生素，雖可抑制家禽腸道中之微生物，但隨著使用時間的增長及細菌的選殖進化，造成近年來抗藥性問題的日益嚴重。歐盟已於 2006 年全面禁止畜禽養殖業者在動物飼料中使用促進生長用之抗生素 (Cook, 1999; Taylor, 1999)，推動無抗生素飼養已成為全球之趨勢。臺灣則從 2000 年起，逐步減少含藥物飼料添加物之使用，因此，尋找抗生素的替代品以減少對家禽生產產業對抗生素的依賴，近年來成為畜牧生產之研究重點。

腸道完整性在維持離子、營養物質和水的滲透性，以及阻絕細菌毒素和病原體的入侵方面起著重要作用 (Rescigno, 2011)。腸道完整性的喪失導致了屏障通透性的增加，此並被認為是導致代謝紊亂，炎症性腸炎和肥胖症的原因 (Connell *et al.*, 2013; Chelakkot *et al.*, 2018)。腸道中離子滲透率的變化與養分吸收的變化相關 (Dubreuil, 2017)。Na⁺ 有助於通過鈉葡萄糖連接的轉運蛋白 -1 (sodium glucose-linked transporter-1) 吸收葡萄糖 (Amat *et al.*, 1999; Daniel and Zietek, 2015)。同時，Cl⁻ 向腸腔的過度分泌會導致腹瀉 (Ahrens *et al.*, 2003)。雞隻感染 *E. coli*, *Campylobacter jejuni* 和 *Salmonella enteritidis* 會導致空腸的跨上皮離子電導率降低 (Awad *et al.*, 2012; 2014; 2015)。補充抗生素會引起腸道微生物種群的轉移，並保護小鼠小腸中緊密連接蛋白 (Knarreborg *et al.*, 2002; Lee *et al.*, 2011; Brandt *et al.*, 2017)。然而，關於抗生素對雞腸上皮的電離子遷移的影響所知之甚少。

腸道中細菌藉由影響宿主腸道結構、營養吸收及免疫反應來影響宿主健康。雞隻腸道的菌相複雜，腸道中約有 90% 細菌尚未能完全分離鑑定其種別 (Apajalahti *et al.*, 2004)，在雞隻的盲腸中，每公克腸道中含有 10^{11} CFU 細菌存在，其中至少有 38 種不同形式、不同品系的細菌 (Barnes *et al.*, 1972; Barnes, 1979)。抗生素作為促進生長之飼料添

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加物使用，可藉由影響腸道中的菌相分佈來促進動物生長。Collier *et al.* (2003) 指出添加泰黴素 (tylosin) 能減少雞隻腸道中 *Clostridium perfringens* 的存在，進而減少壞死性腸炎 (necrotic enteritis)，添加經四環黴素 (oxytetracycline) 減少盲腸中腸球菌 (*Enterococcus* spp.)、大腸桿菌數量 (Fairchild *et al.*, 2005)，添加枯草菌素 (bacitracin) 也會減少迴腸大腸桿菌 (Engberg *et al.*, 2000)。然 Van Lunen (2003) 指出，具有脂多糖之葛蘭氏陰性菌，會增加腸道上皮細胞的通透性，而影響腸道屏障功能，故需要進一步確認不同的抗生素對於雞隻腸道菌相調控是否一致，是否會影響腸道屏障功能，而造成細菌轉移至宿主體腔內。因此，本試驗探討促進生長用劑量之不同類型抗生素對腸道微生物群組成的影響及其與細菌易位的關係。

材料與方法

I. 試驗動物與飼養管理

自商業孵化場購入日齡白肉雞 192 隻，平均分入四組，分別為對照組 (control, CON)、枯草菌素 (bacitracin) 添加組 (55 ppm)、六肽黴素 (nisiheptide) 添加組 (2.5 ppm)、經四環黴素 (oxytetracycline, OTC) 添加組 (55 ppm)，公母混飼，每處理組 4 重複，每重複 12 隻，試驗為期六週 (第 5 週停藥)，試驗期間抗生素添加於飼料中，飲水與飼料皆任飼，飼糧組成如表 1。雞隻於 5 月以平飼方式飼養於 5.8 m² 雞欄。畜舍平均溫度為 27.5 ± 1°C 及 68 ± 3%。試驗進行分別於 0 週 (1 日齡)、3 週 (21 日齡) 及 6 週 (42 日齡) 試驗結束時，每組犧牲 8 隻雞，採集臟器與腸道進行試驗分析，所有實驗動物程序均經國立臺灣大學實驗動物管理與使用委員會批准，並符合 96 年含藥物飼料添加物使用規範。

表 1. 飼料組成分

Table 1. Composition of basal diets

Ingredients	0 — 3 wk	3 — 6 wk
	----- % -----	
Yellow corn, grain	48.70	57.30
Soybean meal, 44%	34.88	29.64
Fish meal, 65%	5.00	2.80
Soybean oil	7.65	6.40
Dicalcium phosphate	1.10	1.38
Calcium carbonate	1.30	1.31
DL-methionine	0.30	0.32
Choline-chleride, 50%	0.07	0.05
Vitamin premix ^a	0.30	0.30
Mineral premix ^b	0.20	0.20
Salt	0.50	0.30
Total	100	100
Calculated analysis		
Crude protein, %	23.00	20.05
ME, kcal/kg	3,202	3,205
Calcium, %	1.03	1.00
Available phosphorus, %	0.46	0.45

^a Vitamin premix supplied per kilogram of diet: Vitamin A, 12,000 IU; Vitamin D₃, 3,125 ICU; Vitamin E, 37.5 IU; Vitamin K₃, 6.25 mg; Vitamin B₁, 3.75 mg; Vitamin B₂, 12.5 mg; Vitamin B₆, 10.0 mg; Ca-pantothenate, 18.8 mg; Niacin, 50 mg; Biotin, 0.06 mg; Folic acid, 1.25 mg; Vitamin B₁₂, 0.05 mg.

^b Mineral premix supplied per kilogram of diet: Cu (CuSO₄ · 5H₂O, 25.45% Cu), 6 mg; Fe (FeSO₄ · 7H₂O, 20.09% Fe), 50 mg; Mn (MnSO₄ · H₂O, 32.49% Mn), 40 mg; Zn (ZnO, 80.35% Zn), 60 mg; Se (NaSeO₃, 45.56% Se), 0.075 mg.

II. 測定項目及方法

(i) 腸道上皮通透性變化：使用上皮細胞電生理分析系統 (ussing chamber)，參考 Ussing and Zerahn (1951) 之方

法進行分析。於 3 週及 6 週，每組取 8 隻雞，注射 urethan 使其麻醉。切開腹腔，先移除肝臟及脾臟之後，再取迴腸放置於 kreb buffer 中，將組織沿腸繫膜切開後，避開淋巴結，釘至腸道樣品室上，放置於 chamber 支架上。於 luminal 面置入 5 mL mannitol kreb buffer，於 serosal 面加入 glucose kreb buffer，pH 為 7.33 – 7.37，溫度為 41°C，氣體 95% O₂，5% CO₂。於組織穩定後，每 5 分鐘給予 1 mV 刺激，30 分鐘後，測量組織電位差 (potential difference, PD)，離子電流 (short-circuit current, I_{sc})，再利用 Ohm 式原理 ($V = I \times R$) 計算出電阻 (electric resistance, R) 和電導係數 (conductance, G)。電導係數為電阻之倒數。

- (ii) 腸道菌相變化：將雞隻肛門以 75% 酒精擦拭，去除肛門口之糞物，以無菌採樣棉棒刺激排便，收集雞隻結直腸新鮮糞便樣品，放入 50 mL 離心管中，置於冰浴中立即進行分析。取 1 g 裝有各樣品之培養管加入 10 倍稀釋之無菌磷酸鹽緩衝液，進行振盪均質，再離心取其上清液，以無菌磷酸鹽緩衝液序列進行 10 倍稀釋。取將上清稀釋液，均勻塗抹於 Chromocult® Coliform Agar (Merk, USA) 上，於 37°C 恆溫培養箱中培養 24 小時，計數糞便中大腸桿菌含量。另分別取稀釋液均勻塗抹於 bile esculin agar (Difco, USA) 及 rogosa agar (Difco, USA) 上，於 37°C 恆溫培養箱中培養 48 小時，計數糞便中腸球菌含量及乳酸菌含量，最後再以乾物質量進行校正。此方法依據 Shu *et al.* (2001) 測定之。
- (iii) 腸道細菌轉移體內現象之評估：於 0 週、3 週及 6 週，每組取 8 隻雞隻，解剖後以滅菌過之剪刀和鑷子取出脾臟及肝臟，依其重量以無菌磷酸鹽緩衝液以 1 : 10 (1 g/10 mL) 添加該緩衝液。均質機依序以 75% 酒精、兩次無菌磷酸鹽緩衝液清洗過後，分別將肝臟和脾臟以每次 10 秒、連續 3 次的方式將組織均質後，再使用以 75% 酒精擦拭過之超音波破膜機震盪 10 秒。將均質完後的組織液放入無菌臺中操作，分別各取組織液加入新鮮血液瓊脂平板 (fresh blood agar plate)，之後置於 37°C 培養箱培養 24 小時，計算細菌菌落形成單位 (colony forming unit, CFU) 數目。將每個培養基其菌落形成單位數目分別以組織重量作為標準化。
- (iv) 統計分析：實驗所獲得之資料使用 SAS 統計分析軟體 (statistical analysis system, Ver. 9.1.3 for Windows, XP)，以一般線性模式 (GLM, general linear models procedure) 進行變方分析，再以鄧肯氏新多變域測定法 (Duncan's new multiple range test)，進行平均值比較，檢測其差異之顯著性。資料皆以平均值 ± 標準偏差表示。細菌轉移試驗以曼惠特尼檢定 (Mann-Whitney test) U 檢定進行分析。

結果與討論

腸道電生理可用於評估腸道離子通透性和腸道完整性。結果顯示，添加三種抗生素對腸道的組織電位差、離子電流及電導係數皆無顯著影響 (表 2)。腸道離子電流量與腸腔內的消化吸收功能及細菌生長有關 (Castro, 1990; Yu *et al.*, 2001)。適量鈉離子的吸收有助於葡萄糖及胺基酸的吸收，而氯離子的分泌會使水分被動運輸進腸腔，以確保消化酵素作用的介質環境 (Bern *et al.*, 1989; Barrett and Dharmasathaphorn, 1991)。腸道內氯離子的分泌及鈉離子的吸收，為產生腸道離子電流淨值的主要因子。當氯離子的分泌過量，造成短暫電流；當氯離子量過高時，腸內水分也被大量排出或滯留於腸腔內，導致臨床上所見之體重降低及下痢症狀。然而當病原菌感染侵害腸道時，下痢有利於清除害菌，但過度下痢則會造成體內離子不平衡而死亡 (Castro *et al.*, 1979; Bridges and Rummel, 1986)。然添加抗生素對雞隻腸電生理基礎值的結果，經由腸道電生理上皮系統 (ussing chamber systems) 測量發現，雞隻腸道中的離子電流及組織阻力皆與對照組無顯著影響。因此，飼料中添加枯草菌素、六肽黴素和羧四環黴素不會影響正常腸道生理的離子運輸功能。

腸道微生物與宿主間存在共存關係，微生物可以競爭性的排除病原菌對宿主的干擾，但同時也與宿主競爭能量及蛋白質的吸收 (Hedde and Lindsey, 1986)，產生一些不利的代謝產物 (Alvares *et al.*, 1964)。抗生素不會促進無菌動物之生長 (Forbes and Park, 1959)，無菌雞隻的生長速率比一般常態飼養的雞隻快了 10 – 15% (Coates *et al.*, 1963)，因此，腸道微生物影響動物的生長，然抗生素對微生物具有殺死或抑制的作用，不同的抗生素，其作用機制不同，對腸道微生物影響也不同。目前，抗生素對於腸道的菌相的影響，及其對促進生長可能的機制有：1. 低劑量的抗生素會抑制部分細菌生長 (Fairchild *et al.*, 2005)，而這些細菌會與動物競爭營養份 (Buenrostro and Kratzer, 1983; Pryde *et al.*, 1999)。2. 抗生素添加會減少病原菌，降低產生嚴重疾病或腸道發炎的機會 (Collier *et al.*, 2003)。3. 腸道細菌具有競爭排除作用，抗生素的添加能消滅抑制其他細菌生長的腸內菌，此受抑制的細菌可能會生產對動物有益的營養素 (Pryde *et al.*, 1999)。4. 低劑量的抗生素能抑制可以抑制細菌製造黏附性蛋白，使細菌無法附著於腸道上，細菌隨糞便排出體外而減少 (Gaskins *et al.*, 2002)。5. 減少微生物產生抑制宿主生長的代謝物 (Yeo and Kim, 1997)。

飼料中添加抗生素，對於生病的雞隻或飼養於較髒亂的環境，會有較好的促進生長效果 (Prescott and Baggot, 1993)；於監控環境飼養下，添加泰黴素於豬隻的飼料中，對其生長促進的效果下降 (Van Lunen, 2003)，但若再給予

病原菌挑戰試驗，則促進生長的效果又出現 (CAFA, 1997)，推論抗生素促進生長，可能於控制或減少病原菌的數量所影響 (JETACAR, 1999)。Fairchild *et al.* (2005) 指出經四環黴素會減少雞隻盲腸中腸球菌屬 (*Enterococcus* spp.) 及彎曲桿菌屬 (*Campylobacter* spp.) 的數量，Lev and Forbes (1959) 指出腸道中的有害菌 *C. perfringens* 會降低雞隻生長表現，無菌雞隻接種 *Streptococcus faecalis* 也會抑制生長，抗生素會藉由抑制 *S. faecalis* 來促進雞隻生長 (Eyssen and De Somer, 1965; Eyssen and De Somer, 1967)。Knarreborg *et al.* (2002) 指出產氣莢膜梭菌 (*C. perfringens*) 受抗生素的影響最大。樂菌素 (Tylosin) 通過調節 *C. perfringens* 定植來減少壞死性腸炎。雖然補充的三種抗生素並未改變本研究的大腸桿菌、腸球菌及乳酸菌組成 (表 3)，但是否其他有害菌的改變，有待進一步的研究。

表 2. 飼糧中添加抗生素對肉雞腸道離子電流、組織電位差及電導係數之影響

Table 2. Effects of different supplemental antibiotics on the short-circuit current (Isc), transmural potential difference (PD) and electrical tissue resistance (G) across the isolated ileum mucosa of broilers at age of 3 and 6 weeks

	Control	OTC	Bacitracin	Nosiheptide
3 wk				
Isc ($\mu\text{A}/\text{cm}^2$)	11.8 ± 1.75	9.59 ± 1.26	10.6 ± 1.44	7.75 ± 1.48
G (mS/cm^2)	2.92 ± 0.54	3.54 ± 0.70	4.07 ± 1.09	4.65 ± 0.76
PD (mV)	11.6 ± 4.68	7.99 ± 2.73	11.1 ± 4.47	2.63 ± 0.64
6 wk				
Isc ($\mu\text{A}/\text{cm}^2$)	10.2 ± 2.08	12.6 ± 1.50	12.1 ± 1.09	9.03 ± 1.07
G (mS/cm^2)	5.07 ± 1.36	2.74 ± 0.57	3.76 ± 1.17	5.26 ± 1.08
PD (mV)	4.64 ± 1.83	12.4 ± 4.00	13.4 ± 5.30	6.75 ± 3.53

Data represented by means \pm standard error.

表 3. 飼糧中添加抗生素對肉雞腸道微生物組成之影響 (n = 8)

Table 3. Effects of different supplemental antibiotics on the intestinal bacterial population of broilers at age of 3 and 6 weeks

	Control	OTC	Bacitracin	Nosiheptide
3 wk				
Coliform, cfu $\times 10^7$ /g	6.49 ± 0.28^{ab}	5.89 ± 2.18^b	6.68 ± 0.43^{ab}	7.06 ± 0.47^a
Enterococcus, cfu $\times 10^7$ /g	7.43 ± 0.36	7.12 ± 1.84	7.62 ± 0.54	7.79 ± 0.47
Lactobacillus, cfu $\times 10^7$ /g	6.76 ± 0.70	6.84 ± 1.97	6.64 ± 1.89	6.03 ± 1.70
6 wk				
Coliform, cfu $\times 10^7$ /g	6.34 ± 0.78	6.3 ± 0.35	6.09 ± 0.44	5.66 ± 1.62
Enterococcus, cfu $\times 10^7$ /g	7.25 ± 0.55	7.03 ± 1.92	6.69 ± 2.55	7.79 ± 0.29
Lactobacillus, cfu $\times 10^7$ /g	7.32 ± 0.55	7.24 ± 0.84	6.78 ± 2.60	7.73 ± 0.40

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

細菌過度生長、粘膜屏障受損和免疫防禦機制會增加腸上皮的脆弱性，並增加腸道細菌轉移 (bacterial translocation) 的風險 (Conn, 1998; Magnotti and Deitch, 2005)。Awad *et al.* (2014) 指出致病性和非致病性大腸桿菌菌株改變了肉雞腸道上皮的離子通透性，並擾亂了腸道屏障功能 (Shifflett *et al.*, 2005)。補充可利斯汀 (colistin) 之抗生素可改善受到大腸桿菌攻擊的肉雞腸黏膜的完整性，並降低胃腸道中大腸桿菌和腸球菌 (enterococci)，對微生物菌群分布造成直接影響 (Torrallardona *et al.*, 2003)。Huang *et al.* (2015) 指出補充可利斯汀和北里黴素 (kitasamycin) 促進斷奶仔豬的腸道通透性降低和腸道緊連蛋白 (occludin) 表達增加。本實驗顯示無添加抗生素之對照組，0 週及 3 週細菌轉移到肝臟高於 6 週 ($P = 0.13, 0.08$)，表明肉雞在 3 週之前腸道上皮細胞屏障功能脆弱或黏膜免疫功能發展不全，而使腸道細菌經由腸繫膜血液匯流至肝臟，並在肝臟中維持活菌的型態，進而危害宿主健康。對照組雞隻在六週齡時，因免疫發展較健全或腸道屏障功能完善，肝臟細菌完全消失 (圖 1)。飼料中添加 3 種抗生素，於三週齡時細菌轉移體至肝臟相對於對照組顯著減少 (圖 2)，證明了抗生素包括枯草菌素、六肽黴素、經四環黴素能降低細菌轉移體內的情形，而其中又以六肽黴素效果最好。我們的研究結果表明，補充三種不同類型的抗生素在很大程度上降低了肝臟細菌易位的發生率，因為它可能對改善腸道屏障功能完整性有正向作用，這些反應與雞疾病感染的病理生理指標的改善有關。

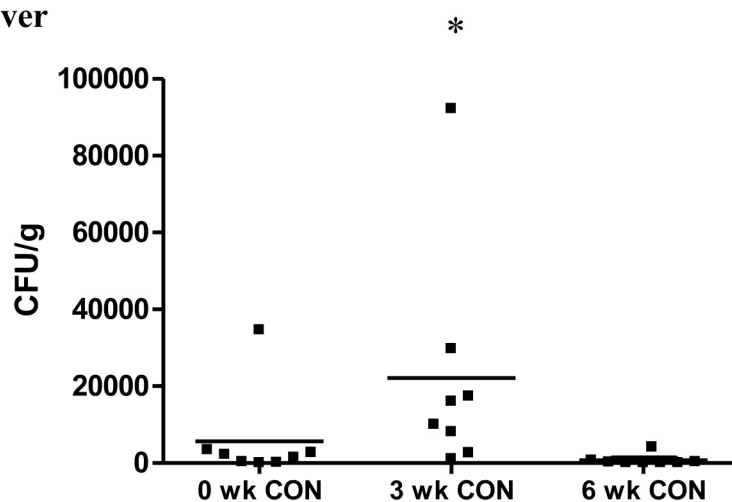
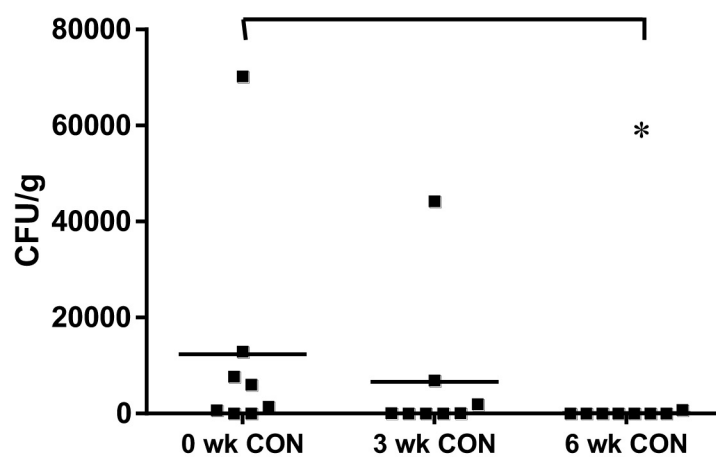
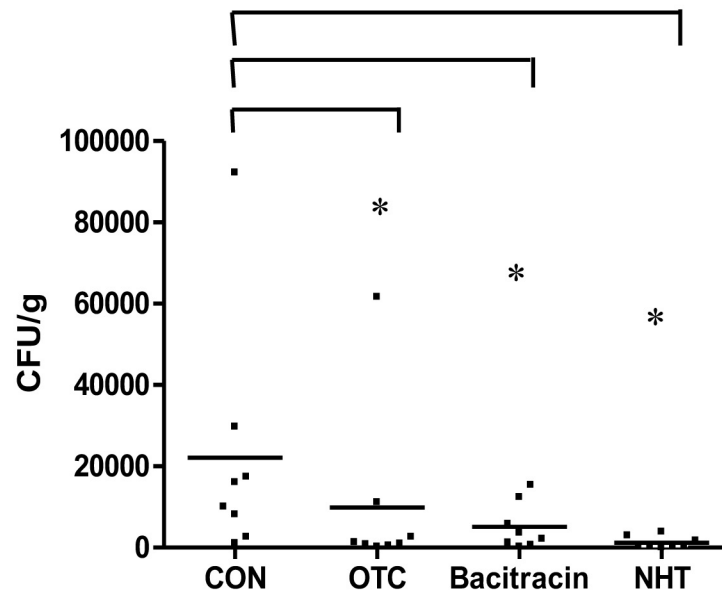
(A) Liver**(B) Spleen**

圖 1. 肉雞於 0、3 及 6 週對照組腸道細菌轉移至肝臟及脾臟之情形。(A) 肝臟 (B) 脾臟。

Fig. 1. Enteric bacterial translocation to the internal organs of control broiler chickens at age of 0, 3, and 6 weeks. Homogenates of (A) liver (B) spleen were used for bacterial culturing. (n = 8) * p < 0.05.

(A) Liver



(B) Spleen

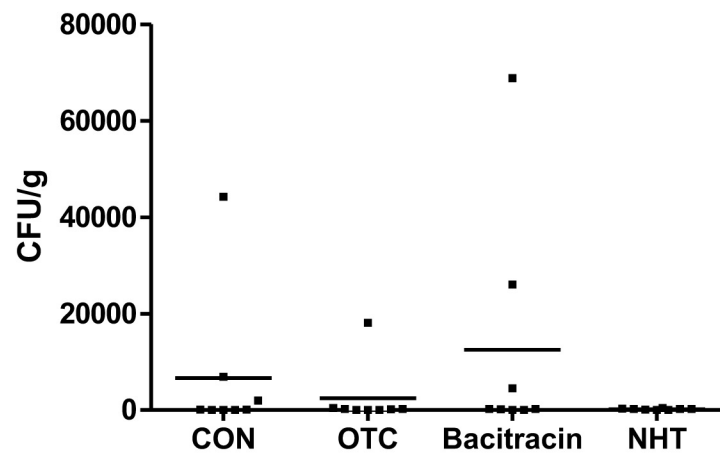


圖 2. 飼糧中添加抗生素對 3 週齡雞隻腸道細菌轉移至肝臟及脾臟之影響。(A) 肝臟 (B) 脾臟。

Fig. 2. Effects of different supplemental antibiotics on enteric bacterial translocation to the internal organs of broiler chickens at age of 3 weeks. Homogenates of (A) liver (B) spleen were used for bacterial culturing. (n = 8). * $p < 0.05$.

結 論

綜上所述，肉雞飼糧添加枯草菌素、六肽黴素及經四環黴素，可能藉由改善肉雞早期腸道屏障功能，降低肝臟細菌易位的發生率，減少細菌感染的機會。由於這三種促生長抗生素在本研究之後已被禁用，因此，應積極開發具有類似抗菌功能的飼料添加劑，以取代促進生長用抗生素之使用。

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Effects of bacitracin, nisin and oxytetracycline on intestinal epithelial permeability, intestinal flora and bacterial translocation in broilers ⁽¹⁾

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Abstract

This study aimed to investigate the effect of adding supplemental antibiotics to intestinal bacterial composition, bacterial translocation to organs and intestinal permeability. One hundred and ninety-two broilers were randomly allocated to 4 treatments with 4 replicates of 12 birds each. The 4 treatments were: control (CON), dietary supplementation with bacitracin (55 ppm), nisin (2.5 ppm), and oxytetracycline (55 ppm). The intestinal bacterial composition, bacterial translocation to organs, and intestinal permeability were measured at 3-wks and 6-wks of age. Results showed that adding the three antibiotics did not affect the potential difference (PD), short-circuit current (Isc) and conductance of intestines, nor did it have any significant impact on the bacterial composition in Coliform, *Enterococcus*, and Lactobacilli. However, all supplemental antibiotics decreased total bacteria count in the liver of birds at age of 3 wks ($P < 0.05$), while nisin had the best bacteriostatic effect. In the control group, the total bacteria transferred to the liver at 0 and 3 wks was higher than that at 6 wks ($P = 0.13, 0.08$), suggesting that intestinal barrier function was vulnerable in broilers during younger ages. Our findings indicated that supplementation of the 3 different types of antibiotics reduced the incidence of bacterial invasion and translocation into the liver to a great extent due to their beneficial effects on the improvement of intestinal barrier integrity. Because these three growth-promoting antibiotics have been banned after this study, feed additives with similar antibacterial functions should be developed to replace antibiotics.

Key words: Antibiotics, Intestinal epithelial permeability, Intestinal flora, Bacterial translocation, Broilers.

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