

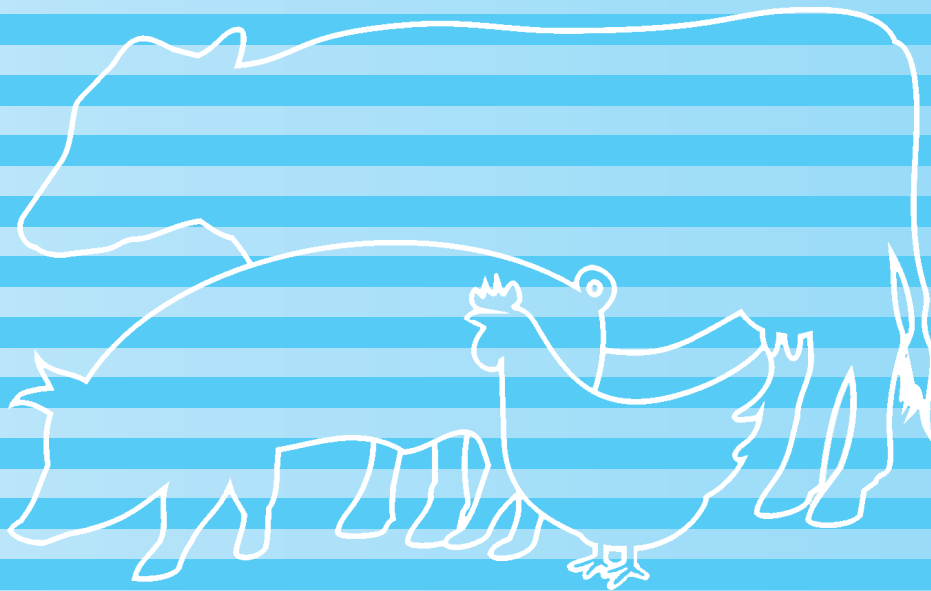
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發 行 所：行政院農業委員會畜產試驗所

地 址：臺南市新化區牧場 112 號

電 話：(06) 5911211

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杏鮑菇下腳料應用於白羅曼肉鵝飼養價值之評估⁽¹⁾

王勝德⁽²⁾⁽⁵⁾ 陳美惠⁽³⁾ 施柏齡⁽⁴⁾ 沈士怡⁽²⁾ 廖士傑⁽²⁾

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摘 要

本試驗旨在探討杏鮑菇下腳料對鵝隻生長之影響，以評估其應用價值。使用一日齡白羅曼鵝公、母各半共 96 隻供試，依性別逢機分配至 4 個處理組，每處理組 4 重複，以欄為重複，每欄 3 公 3 母共 6 隻，飼養期至 12 週齡。試驗飼糧依白羅曼鵝之飼養分期設計為等蛋白質、等能量及等粗纖維含量，分為不添加或添加 2、4 或 6% 之乾燥杏鮑菇下腳料粉等 4 組，試驗期間飼料及飲水自由採食。結果顯示，除添加 4% 組顯著較對照組降低 8 週齡鵝隻血漿三酸甘油酯濃度、亦顯著增加 9 至 12 週齡鵝隻飼料採食量外 ($P < 0.05$)，整體而言，添加乾燥杏鮑菇下腳料粉對白羅曼鵝之生長性狀、血液組成、屠體品質及品評結果均無負面影響。綜上試驗結果所述，以人工剝整杏鮑菇產生之下腳料經乾燥粉碎做成飼料原料用於飼養白羅曼肉鵝，0 至 12 週齡之飼糧添加量可達 6%，此有利於菇蕈副產物之再利用。

關鍵詞：杏鮑菇下腳料、白羅曼肉鵝、生長性狀、屠體品質。

緒 言

食用菇蕈的生理活性與營養價值吻合消費者對於健康意識的要求，栽培過程的有機生產與綠色製造亦契合環保意識及食品衛生安全 (王, 2009)，因而受到消費者認同且是國人重要的膳食來源之一。方及蔡 (2016) 指出，近 10 年臺灣菇蕈類的產量自 2006 年約 10 萬公噸增加至 2015 年約 15 萬公噸，產值自 70 億元增加至 137 億元，占我國整體蔬菜的產值由 13.7% 提升至 17.5%。惟栽培食用菇蕈所使用的木屑太空包與生產瓶，每年已超過 2 億 5 千萬包 (瓶)，所產生之農業廢棄物估計超過 120 萬公噸 (呂等, 2011)，因此菇類栽培後介質之處理或再利用成為相當重要的課題。邇來國內研究主要集中於取代部分木屑作為菇類栽培介質、製成有機堆肥或抑病介質等，至於其他應用方式如作為燃料或生質能源之原料、生態環境修復之材料；而作為禽畜飼養之飼料、酵素或多醣體萃取之來源則相對較少。

近年來探討食用菇蕈餵養畜禽期能減少抗生素的使用，以發展經濟動物的無抗生素飼養備受重視，包括蘑菇屬 (*Agaricus* sp.)、香菇 (*Lentinula edodes*)、雞油菌 (*Cantharellus cibarius*) 及側耳屬 (*Pleurotus* sp.) 等菇類最常被研究探討。Bederska-Łojewska *et al.* (2017) 指出，食用菇蕈具有改善動物生長性能、調節免疫反應、發揮組織抗氧化特性、影響腸道型態發育、改善腸道內微生物菌相等功效。栽培種洋菇 (*Agaricus bisporus*) 可誘發白肉雞之體液免疫反應 (Kavyani *et al.*, 2012)，具有促進白肉雞生長及保護其組織具有抗氧化的能力 (Giannenas *et al.*, 2010)。飼糧中添加秀珍菇 (*Pleurotus ostreatus*) 下腳料，可顯著增加白肉雞之空腸絨毛高度 (Fard *et al.*, 2014)，改善白肉雞之生長性狀 (Toghyani *et al.*, 2012; Fard *et al.*, 2014)。

2013 年國內菇類鮮重產量依序為香菇、金針菇、杏鮑菇 (*Pleurotus eryngii*)、木耳及秀珍菇 (劉等, 2016)。其中杏鮑菇產值為 20.69 億元，栽培量達 14,000 萬包，生鮮量則達 26,000 公噸。由於新鮮的杏鮑菇經採收後，需先以人工修整菇柄，修整後將之分級才會進入通路銷售，農場端因此衍生數量可觀的杏鮑菇整形下腳料。此種下腳料對以新鮮菇體為銷售模式的杏鮑菇產業而言並無經濟價值，通常廢棄移作堆肥處理。鵝是國內前三大重要的禽種，鵝肉亦屬相對高價的禽肉，飼糧粗纖維含量對飼養肉鵝而言相對重要，若飼糧纖維含量不足，鵝隻容易發生啄羽，導致傷口感染、發育受阻等生長繁殖及動物福祉等問題，本試驗目的即在評估杏鮑菇下腳料應用於肉鵝的飼養價值。

(1) 行政院農業委員會畜產試驗所研究報告第 2663 號。

(2) 行政院農業委員會畜產試驗所彰化種畜繁殖場。

(3) 財團法人食品工業發展研究所。

(4) 行政院農業委員會畜產試驗所營養組。

(5) 通訊作者，E-mail: wsd@mail.tlri.gov.tw。

材料與方法

I. 試驗動物與管理

新鮮杏鮑菇下腳料取自嘉義縣某合作農場，係該農場採收杏鮑菇後，以人工削整產生之新鮮農副產物。經收集後以 50℃ 乾燥 24 小時，共 4 批次成品粉碎製成杏鮑菇下腳料 (*Pleurotus eryngii* stump wastes, PESW)。白羅曼鵝則購自彰化縣某種鵝場，實驗動物之使用及照護經行政院農業委員會畜產試驗所彰化種畜繁殖場實驗動物照護及使用小組審核通過 (畜試彰動字第 10708 號同意書) 並據以執行。

動物試驗於非開放式全高床鵝舍內進行。供試一日齡白羅曼鵝共 96 隻，公母各半，依性別逢機分配至 4 個處理組，各處理組 4 重複，以欄為重複，每欄 3 公 3 母共 6 隻。試驗飼糧分為不添加或添加 2、4 或 6% 經粉碎之乾燥杏鮑菇下腳料，依肉鵝飼養分為育雛期 (1 日齡至 4 週齡)、生長期 (5 至 8 週齡) 及肥育期 (9 至 12 週齡) 等三階段。各階段飼糧營養標準參考 National Research Council (1994) 資料設計成等蛋白質、等能量及等粗纖維試驗飼糧，飼料及飲水均供自由取食，試驗配方列示於表 1。

表 1. 添加杏鮑菇下腳料之試驗飼糧組成

Table 1. Composition of experimental diets supplemented with *Pleurotus eryngii* stump wastes (PESW)¹

	Starter (hatched to 4 wks)				Grower (5 to 8 wks)				Finisher (9 to 12 wks)			
	Basal	2%	4%	6%	Basal	2%	4%	6%	Basal	2%	4%	6%
Ingredients												
Corn, ground	550	555	545	536	623	613	600	586	689	673	657	641
Soybean meal	312	307	307	304	247	247	250	253	175	178	181	183
Dicalcium phosphate	20	20	20	20	12	12	12	12	12	12	12	12
Limestone	10	10	10	10	6	6	6	6	6	6	6	6
Alfalfa meal	80	60	45	35	100	90	80	68	100	90	80	70
PESW powder	0	20	40	60	0	20	40	60	0	20	40	60
Soybean oil	15	15	20	22	0	0	0	3	8	11	14	18
Salt	3	3	3	3	3	3	3	3	3	3	3	3
L-lysine	2	2	2	2	1	1	1	1	1	1	1	1
DL-methionine	2	2	2	2	2	2	2	2	1	1	1	1
Vitamin premix ²	2	2	2	2	2	2	2	2	2	2	2	2
Mineral premix ³	2	2	2	2	2	2	2	2	2	2	2	2
Choline chloride, 50%	2	2	2	2	2	2	2	2	1	1	1	1
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Calculated value												
Crude protein, %	20.3	20.2	20.2	20.2	18.0	18.0	18.0	18.0	15.0	15.0	15.1	15.1
Lysine, %	1.25	1.22	1.21	1.19	1.02	1.01	1.02	1.02	0.83	0.83	0.83	0.83
SAA ⁴ , %	1.48	1.45	1.44	1.42	1.29	1.29	1.30	1.31	0.97	0.98	0.99	1.00
ME, kcal/kg	2,904	2,905	2,924	2,917	2,841	2,822	2,804	2,805	2,906	2,903	2,901	2,905
Calcium, %	1.39	1.37	1.35	1.34	1.09	1.08	1.07	1.06	1.09	1.08	1.07	1.06
Non-phytate phosphorus, %	0.57	0.58	0.58	0.58	0.42	0.42	0.42	0.43	0.40	0.40	0.41	0.41
Crude fiber, %	4.58	4.49	4.47	4.54	5.03	5.06	5.09	5.08	5.04	5.06	5.08	5.09
Analyzed value												
Dry matter, %	88.33	88.29	88.34	88.41	88.57	88.39	88.31	88.48	88.29	88.12	88.03	88.29
Crude protein, %	19.94	19.38	20.08	19.35	18.08	17.81	17.83	18.28	15.23	15.07	15.51	15.26
Cost of feed, NT\$/kg	11.53	12.22	13.10	13.92	10.85	11.62	12.41	13.27	10.64	11.52	12.39	13.29

¹ PESW: dried powder of *Pleurotus eryngii* stump wastes, cost estimated NT\$ 50 /kg.

² Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; vitamin E, 20 IU; vitamin K₃, 3 mg; vitamin B₁, 2 mg; vitamin B₂, 5 mg; vitamin B₆, 3 mg; vitamin B₁₂, 30 µg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 2 mg; and biotin, 200 µg.

³ Supplied per kilogram of diet: iron, 200 mg; copper, 30 mg; manganese, 160 mg; cobalt, 500 µg; zinc, 100 mg; Iodine, 1.7 mg; and selenium, 300 µg.

⁴ SAA: sulfur amino acids.

II. 測定項目與方法

試驗期間自 1 日齡至 12 週齡，於 4、8、12 週齡收集各欄鵝群之體重及飼料採食量供計算飼料轉換率 (Feed conversion ratio, FCR)。於 8 及 12 週齡逢機採集各欄公、母鵝各 1 隻之腳脛靜脈血樣，經離心後以 LANNER T-900 血液生化分析儀 (LANNER Biotechnology Co. Ltd., Taiwan) 配合相應套組，分析血漿中之肌酸酐 (Creatinine)、麩胺酸草醋酸轉胺酶 (Glutamic-oxaloacetic transaminase, GOT)、麩胺酸丙酮酸轉胺酶 (Glutamic pyruvic transaminase, GPT)、總膽固醇 (Total cholesterol)、三酸甘油酯 (Triglyceride) 濃度。血漿中之抗氧化力指標包括超氧歧化酶 (Superoxide dismutase, SOD)、過氧化氫酶 (Catalase, CAT)、抗氧化物 (Antioxidants, AntiOxs) 濃度，則分別使用相應套組 (Catalog No. 706002, 707002, 709001; Cayman Chemical Co., USA) 依 Lee *et al.* (2013) 所述方法測定。

於秤取 12 週齡鵝隻體重後進行 16 小時禁食，每欄取公、母鵝各 1 隻，秤其屠前體重後犧牲，紀錄屠體重與含皮層之左胸重、砂囊、肝臟、脾臟及腹脂塊等組織或器官之重量。左胸經去除皮層及可見脂肪後將胸肉橫切為二，上部供分析肌肉一般成分，下部則供品評試驗。胸肉一般成分 (水分、粗蛋白質、粗脂肪及粗灰分) 委託財團法人中央畜產會肉品檢驗中心分析。品評試驗則參照 Chang *et al.* (2016) 所述方法做部分修正，由年齡介於 40 至 60 歲之男、女各 6 位擔任品評員，採 7 級分 (1 分最差、7 分最佳) 評定胸肉之風味 (Flavor)、嫩度 (Tenderness)、咬感 (Texture)、多汁性 (Juiciness) 及總可接受性 (Acceptability)。

III. 統計分析

試驗所得數據利用 SAS 套裝軟體 (SAS, 2002) 進行統計分析。以一般線性模式程序 (General linear model procedure) 進行變方分析，當有顯著差異時，再以 LSMEANS (Least squares means) 比較四種飼糧處理平均值間之差異顯著性，顯著水準為 $P < 0.05$ 。

試驗以添加量為處理效應，白羅曼鵝生長性狀之統計分析數學模式為 $Y_{ij} = \mu + A_i + \varepsilon_{ij}$ ，式中 Y_{ij} 表示第 i 種飼糧杏鮑菇下腳料添加量處理之第 j 欄之觀測值 ($i = 0, 2, 4, 6\%$ 添加量, $j = 1$ 至 4 欄)， μ 表示觀測值之平均值， A_i 表示飼糧杏鮑菇下腳料添加量效應， ε_{ij} 為機差效應。白羅曼鵝血液性狀、屠體性狀、胸肉一般組成及官能品評之統計分析數學模式為 $Y_{ijk} = \mu + A_i + \varepsilon_{ijk}$ ，式中 Y_{ijk} 表示第 i 種飼糧杏鮑菇下腳料添加量處理之第 j 欄內第 k 隻鵝之觀測值 ($i = 0, 2, 4, 6\%$ 添加量, $j = 1$ 至 4 欄, $k = 1$ 公或 1 母)， μ 表示觀測值之平均值， A_i 代表飼糧杏鮑菇下腳料添加量效應， ε_{ijk} 為機差效應。

結果與討論

杏鮑菇子實體經收割後，須經人工修整再分級包裝出售。本試驗材料取自嘉義縣某合作農場，以人工修整產生之新鮮杏鮑菇下腳料約占其收割量的 10%，劉等 (2016) 指出，2013 年國內杏鮑菇生鮮量達 26,000 公噸，粗估我國每年產出之新鮮杏鮑菇下腳料超過 2,600 公噸。本研究分析結果顯示，新鮮之杏鮑菇下腳料水分為 77.2 g/100 g ，粗蛋白質、粗脂肪、粗灰分及粗纖維分別為 4.42 、 0.11 、 1.35 及 3.99 g/100 g (新鮮物重)。粉碎後之乾燥杏鮑菇下腳料水分為 $11.31 \pm 3.00 \text{ g/100 g}$ ，粗蛋白質、粗脂肪、粗灰分及粗纖維分別為 18.60 ± 1.29 、 0.97 ± 0.34 、 5.39 ± 0.20 及 $13.86 \pm 2.07 \text{ g/100 g}$ (乾物重, $n = 4$)。Lee *et al.* (2012) 將切除杏鮑菇子實體後而留於太空包內之殘餘菇腳加以乾燥 (65°C 乾燥 3 日) 粉碎後製成飼料添加物，分析結果顯示其乾物質 $931.0 \pm 3.0 \text{ g/kg}$ ，粗蛋白質 $86.3 \pm 0.6 \text{ g/kg}$ 、粗灰分 $56.4 \pm 0.4 \text{ g/kg}$ 、粗纖維 $133.2 \pm 2.6 \text{ g/kg}$ 、乙醚抽出物 $17.9 \pm 0.2 \text{ g/kg}$ 、鈣 $5.6 \pm 0.1 \text{ g/kg}$ 及磷 $4.7 \pm 0.1 \text{ g/kg}$ ，而其每公克乾物質所含之活性物質或功能性成分包括水溶性多醣類 165.0 mg 、 β -1-3-D-glucan 0.6 mg 、腺苷 (Adenosine) $58.9 \mu\text{g}$ 、麥角固醇 (Ergosterol) 1.28 mg 、總酚化合物 5.0 mg (Gallic acid equivalent)。本試驗所用之杏鮑菇下腳料係人工修整杏鮑菇子實體產生之下腳料，與 Lee *et al.* (2012) 所用之杏鮑菇殘餘菇腳係人工切除杏鮑菇子實體後，取自留於太空包內之菇腳體不同。此兩種杏鮑菇副產物製成之飼料添加物在粗蛋白質含量上具有明顯差異，顯示菇蕈副產物製成飼料原料，其營養組成受到菇蕈種類、來源、添加量或菇體含量多寡、栽培介質含量多寡、乾燥條件所影響 (Li and Shah, 2013; Bederska-Łojewska *et al.*, 2017)。建議應用時需適時分析杏鮑菇副產物的營養組成，再據以調整禽畜飼料配方，避免影響完全配合飼料的品質穩定性。

表 2 結果顯示，飼糧中添加杏鮑菇下腳料對 1 日齡至 4 週齡、5 至 8 週齡、9 至 12 週齡及 1 日齡至 12 週齡之白羅曼鵝體重、飼料採食量及飼料轉換率均無顯著差異，僅 4% 添加組鵝隻於 9 至 12 週齡之飼料採食量顯著高於對照組及 6% 添加組。飼糧中添加杏鮑菇下腳料對血液性狀及血漿抗氧化指標成分之影響如表 3 所示，6% 添加組鵝隻於 8 週齡血漿中之麩胺酸草醋酸轉胺酶、麩胺酸丙酮酸轉胺酶濃度顯著低於 4% 添加組，4% 添加組鵝隻之血漿三酸甘油酯濃度顯著低於對照組，2% 添加組鵝隻之血漿抗氧化物濃度顯著低於 6% 添加組，而飼糧中添加杏鮑菇下腳料則對 12 週齡白羅曼鵝血液性狀及血漿抗氧化指標成分無顯著影響。

表 2. 飼糧添加杏鮑菇下腳料對白羅曼鵝生長性狀之影響

Table 2. Effects of *Pleurotus eryngii* stump waste (PESW) in the diet on growth performances of White Roman meat-type geese

Traits	Treatment ¹				Pooled SEM
	Basal	2%	4%	6%	
1-day-old, g/bird	118.23	118.63	117.20	119.75	2.31
Body weight gain, g/b/d					
1-day-old to 4 wk	91.62	93.15	94.09	93.05	1.27
5 to 8 wk	81.04	81.67	73.52	81.70	4.19
9 to 12 wk	44.40	49.72	53.15	47.52	2.89
1-day-old to 12 wk	73.16	75.56	73.16	74.85	2.03
Feed intake, g/b/d					
1-day-old to 4 wk	149.34	153.77	151.56	149.68	1.87
5 to 8 wk	358.56	374.84	360.64	359.15	8.44
9 to 12 wk	377.23 ^b	403.17 ^{ab}	412.28 ^a	376.70 ^b	9.41
1-day-old to 12 wk	293.78	309.08	299.60	293.94	6.86
Feed conversion ratio, feed intake/body weight gain					
1-day-old to 4 wk	1.63	1.65	1.61	1.61	0.02
5 to 8 wk	4.42	4.61	5.04	4.40	0.23
9 to 12 wk	8.59	8.21	7.80	7.94	0.39
1-day-old to 12 wk	4.02	4.10	4.10	3.93	0.06
Feeding profit from 1-day-old to 12 wk, NT\$/goose					
Cost of feed eaten	269.6	304.6	323.9	332.0	
Price on sale	413.4	426.7	413.3	422.9	
Crude income	143.8	122.1	89.4	90.9	

n = 4.

¹ Diet supplemented with dried powder of PESW at the level of 0, 2, 4 or 6% of feed, respectively.^{a, b} Means in the same row with different superscripts differ (P < 0.05).

表 3. 飼糧添加杏鮑菇下腳料對白羅曼鵝血液性狀之影響

Table 3. Effects of *Pleurotus eryngii* stump waste (PESW) in the diet on plasma profiles of White Roman meat-type geese

Traits	Treatment ¹				Pooled SEM
	Basal	2%	4%	6%	
Week 8					
BW ² , kg/bird	4.97	4.80	5.06	5.25	0.24
CREA ² , mg/dL	0.19	0.20	0.16	0.16	0.01
GOT ² , U/L	17.50 ^{ab}	18.38 ^{ab}	24.38 ^a	15.88 ^b	2.77
GPT ² , U/L	12.75 ^{ab}	14.38 ^{ab}	15.63 ^a	12.25 ^b	1.06
CHOL ² , mg/dL	151.13	149.13	156.03	146.13	8.19
TG ² , mg/dL	103.50 ^a	93.50 ^{ab}	82.00 ^b	87.13 ^{ab}	6.35
CAT ² , nmol/min/mL	7.09	8.76	5.50	7.02	1.56
AntiOxs ² , mM	0.48 ^{ab}	0.44 ^b	0.59 ^{ab}	0.66 ^a	0.06
SOD ² , U/mL	18.59	25.45	19.72	14.60	4.28
Week 12					
BW, kg/bird	6.07	6.01	6.30	6.51	0.33
CREA, mg/dL	0.16	0.14	0.14	0.14	0.02
GOT, U/L	16.75	14.13	12.75	13.63	1.54
GPT, U/L	13.25	12.75	12.38	12.88	0.76
CHOL, mg/dL	155.50	154.88	175.63	153.75	8.84
TG, mg/dL	146.38	161.75	137.13	145.63	16.61
CAT, nmol/min/mL	8.15	8.52	8.35	6.29	0.95
AntiOxs, mM	0.71	0.90	0.70	0.81	0.07
SOD, U/mL	15.48	16.38	15.47	22.23	4.32

n = 8.

¹ See footnote of Table 1.² BW: body weight, CREA: creatinine, GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, CHOL: total cholesterol, TG: triglyceride, CAT: catalase, AntiOxs: antioxidants, SOD: superoxide dismutase.^{a, b} Means in the same row with different superscripts differ (P < 0.05).

飼糧中添加杏鮑菇下腳料對鵝隻屠體性狀之影響如表 4 結果所示，飼糧中添加杏鮑菇下腳料至 6%，並不影響 12 週齡白羅曼鵝之活體重、屠前重、屠體重、屠宰率及砂囊、肝臟、脾臟、腹脂塊重量占屠體重百分比，僅 4% 添加組鵝隻之左胸重占屠體重百分比顯著高於 6% 添加組。另如表 5 結果，顯示飼糧中添加杏鮑菇下腳料不影響 12 週齡白羅曼鵝胸肉之水分、粗脂肪及粗蛋白質含量，惟 6% 添加組胸肉之粗灰分含量顯著低於其他兩添加組。品評試驗結果則以 4% 添加組胸肉之風味、嫩度、咬感及總接受度顯著較其他兩添加組差，惟與對照組並無顯著差異。

表 4. 飼糧添加杏鮑菇下腳料對白羅曼鵝屠體性狀之影響

Table 4. Effects of *Pleurotus eryngii* stump waste (PESW) in the diet on carcass performances of White Roman meat-type geese

Traits	Treatment ¹				Pooled SEM
	Basal	2%	4%	6%	
Pre-slaughter BW, kg/bird	5.50	5.49	5.56	5.70	0.24
Eviscerated weight, kg/bird	4.02	4.00	4.10	4.16	0.18
Carcass yield, %	73.22	72.89	73.70	72.89	0.60
Left breast ² , %	7.47 ^{ab}	7.51 ^{ab}	7.71 ^a	7.12 ^b	0.17
Gizzard ² , %	3.19	3.02	3.06	3.13	0.14
Liver ² , %	1.48	1.49	1.53	1.46	0.04
Spleen ² , %	0.06	0.05	0.05	0.05	0.01
Abdominal fat pad ² , %	3.36	3.97	4.01	3.92	0.27

n = 8.

¹ See footnote of Table 1.

² Percentage of eviscerated weight.

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

表 5. 飼糧添加杏鮑菇下腳料對白羅曼鵝胸肉一般成分及官能品評之影響

Table 5. Effect of *Pleurotus eryngii* stump waste (PESW) in the diet on proximate analysis and sensory evaluation of breast meat of White Roman meat-type geese

Traits	Treatment ¹				Pooled SEM
	Basal	2%	4%	6%	
Proximate analysis	5.50	5.49	5.56	5.70	0.24
Moisture, %	72.89	73.34	72.91	72.86	0.28
Crude ash, %	1.28 ^{ab}	1.30 ^a	1.30 ^a	1.25 ^b	0.02
Crude fat, %	3.44	3.06	3.21	3.13	0.21
Crude protein, %	22.93	22.88	22.91	22.48	0.19
Sensory evaluation	7.47 ^{ab}	7.51 ^{ab}	7.71 ^a	7.12 ^b	0.17
Flavor	4.64 ^{ab}	4.79 ^{ab}	4.39 ^b	5.04 ^a	0.19
Tenderness	4.11 ^{ab}	4.68 ^a	3.93 ^b	4.07 ^{ab}	0.24
Texture	4.27 ^{ab}	4.59 ^a	3.85 ^b	4.18 ^{ab}	0.22
Juiciness	3.92	4.07	3.54	4.11	0.22
Acceptability	4.35 ^{ab}	4.46 ^a	3.85 ^b	4.46 ^a	0.22

n = 8.

¹ See footnote of Table 1.

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

Giannenas *et al.* (2010) 試驗結果顯示，飼糧中添加 2% 栽培種洋菇可顯著改善 42 日齡母白肉雞之體重、增重及飼料效率，而添加 1 或 2% 均可顯著降低其肝臟、胸肉及腿肉中之丙二醛 (Malondialdehyde) 濃度，認為栽培種洋菇具有促進白肉雞生長及保護組織使具抗氧化的能力。Kavyani *et al.* (2012) 以栽培種洋菇粉取代抗生素作為白肉雞生長促進物，試驗飼糧分為基礎飼糧、每公斤基礎飼糧添加 4.5 mg flavophospholipol 或每公斤飼料中添加栽培種洋菇

粉 5、10、20 或 30 g；結果指出飼糧中添加栽培種洋菇粉達 3%，對誘發白肉雞體液免疫反應具有正面效果，且生長性能相關指標無負面影響。Fard *et al.* (2014) 探討秀珍菇下腳料 (Oyster mushroom waste) 對白肉雞生長性能、免疫反應及腸道型態之影響；結果顯示添加 1% 秀珍菇下腳料顯著提高 1 至 42 日齡白肉雞之飼料採食量，顯著提升其空腸絨毛高度。Toghyani *et al.* (2012) 比較添加 2% 秀珍菇下腳料或添加益生菌 (1 g A-Max®/kg) 對白肉雞生長性能、體液免疫及血液性狀之影響；結果顯示添加 2% 秀珍菇下腳料顯著提高白肉雞於 14、28 日齡之體重及 1 至 14 日齡之飼料轉換率，顯著降低其血清三酸甘油酯濃度。綜上文獻報告試驗結果顯示，白肉雞飼糧中添加 1 至 3% 栽培種洋菇或秀珍菇下腳料等食用菇菌副產物，可提升其空腸絨毛高度、降低血清三酸甘油酯濃度、提升體液免疫反應、保護組織使具抗氧化能力，改善生長性狀。Chang *et al.* (2016) 比較廢棄秀珍菇堆肥粉添加量對 5 至 12 週齡白羅曼鵝生長性能及肉質之影響，結果顯示飼糧中添加 5% 並不影響鵝隻生長，且顯著提升胸肉品評時的風味、肉色及總接受度。Lee *et al.* (2012) 探討飼糧中添加 0.5、1.0 及 2.0% 杏鮑菇腳殘餘料對白肉雞氧化狀態及其肌肉品質之影響，結果顯示可顯著提高白肉雞血清、肝臟、脾臟、胸肉及腿肉中之過氧化氫酶、超氧歧化酶濃度及顯著降低前述部位之丙二醛濃度，顯著降低胸肉及腿肉中之粗脂肪含量，結論認為杏鮑菇腳殘餘料可用為抗氧化物，具有降低脂質過氧化及改善白肉雞肉質之效果。Lee *et al.* (2015) 進一步以 *in vitro* 分析杏鮑菇腳殘餘料之抗菌能力，顯示約 100 mg 之杏鮑菇腳殘餘料萃取物即對 *S. typhimurium* E29、*E. coli* O157:H7 及 *E. coli* DH5α 具有顯著之抗菌效果，而添加杏鮑菇腳殘餘料可顯著改善肥育期白肉雞之飼料效率，結論認為杏鮑菇腳殘餘料於 *in vitro* 具有抗菌能力，作為飼料添加物可改善白肉雞之飼料效率。

本試驗評估製成之杏鮑菇下腳料用於白羅曼肉鵝飼料之添加量應可達 5% 以上，故設計為 0、2、4 及 6% 共四種處理。惟為使四處理組之飼糧條件相近，調整飼料配方主要以杏鮑菇下腳料取代苜蓿粉用量並增加大豆油用量，達到等能量、等蛋白、等粗纖維飼糧條件 (表 1)。造成白羅曼鵝於 8 週齡血漿中之總膽固醇、過氧化氫酶、超氧歧化酶等濃度及 12 週齡血漿中之總膽固醇、三酸甘油酯、過氧化氫酶、抗氧化物、超氧歧化酶等濃度 (表 3)，均未因添加杏鮑菇下腳料而有顯著下降的效果，與前述研究結果不一致，推測與本試驗採行等能量、等蛋白、等粗纖維之配方調整有關。而相較於對照組，本試驗於白羅曼肉鵝飼糧中添加杏鮑菇下腳料達 6%，並不影響其 1 日齡至 12 週齡之生長性能、血液組成、屠體品質及品評結果。雖 6% 添加組鵝隻相較於 4% 添加組有顯著較低之左胸重占屠體重百分比 (表 4) 及胸肉粗灰分含量 (表 5)，惟其胸肉之風味及總接受度均顯著較 4% 添加組佳 (表 5)。建議在綠色循環經濟及協助菇農去化廢棄下腳料的角度，白羅曼肉鵝飼糧添加量可提高至 6%。

運輸、人工處理、乾燥及粉碎為採摘後之杏鮑菇太空包飼料資源化再利用之主要成本，如以每車載運 20 公噸之運輸費用為新臺幣 8,000 元、經粗處理程序可獲得 2 公噸鮮濕杏鮑菇下腳料而鮮濕杏鮑菇下腳料水分含量為 77.2% 估算，每公斤乾燥杏鮑菇下腳料之運輸成本約為 17.54 元，另估計乾燥電費為 30 元 / 公斤、人工處理及粉碎成本為 2 元 / 公斤，則飼料原料化之乾燥杏鮑菇下腳料粉成本約為 50 元 / 公斤。除乾燥粉碎做為飼料原料外，後續亦可評估機能性成分萃取物或發酵產物之飼養效益，提高飼養端使用杏鮑菇副產物加值產品的意願。表 1 顯示各期試驗飼料價格均隨乾燥杏鮑菇下腳料添加量之增加而提高，飼養至 12 週齡之肉鵝飼料成本因而以添加組較對照組高，粗收益亦以添加組較對照組差 (表 2)。由於新鮮之杏鮑菇下腳料水分含量達 77.2%，體積膨大運輸成本高、不耐儲存易酸敗變質、含有栽培介質不利循環利用，惟杏鮑菇下腳料用於飼養肉鵝，可減少菇菌副產物遭致廢棄或浪費，透過鵝隻的採食利用將其轉換成禽肉，有助建構綠色循環農業生產體系。

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Evaluation of the feeding value of *Pleurotus eryngii* stump waste in White Roman meat-type geese ⁽¹⁾

Sheng-Der Wang ⁽²⁾⁽⁵⁾ Mei-Huei Chen ⁽³⁾ Bo-Ling Shih ⁽⁴⁾ Shih-Yi Shen ⁽²⁾ and Shih-Chieh Liao ⁽²⁾

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Abstract

The aim of this study was to evaluate the feeding value of *Pleurotus eryngii* stump waste (PESW) on growth performance and carcass characteristics in geese. A total of ninety-six hatched White Roman goslings (48 males and 48 females) were randomly allotted to 4 treatments. Each treatment had 4 pens, with 3 males and 3 females respectively in each pen. The experimental period was from hatched to 12 weeks of age. The four treatments were supplemented with 0 (control group), 2, 4 or 6% dried PESW powder, respectively, and the experimental diets were designed to be iso- crude protein, metabolizable energy, and crude fiber. All geese were fed ad libitum of feed and water. The results showed that higher feed consumption was found in the 4% PESW group than that of the control group at 9 to 12 weeks of age ($P < 0.05$). Lower concentration of plasma triglyceride at eight weeks of age was found in the treatment fed with 4% PESW, compared with the control diet ($P < 0.05$). Overall, there were no negative impacts on growth performances, plasma profiles, carcass characteristics and sensory evaluation in geese supplemented with PESW diet. Our results indicated that dried PESW powder could be used as a feed source in meat-type geese, which can be supplemented up to 6% in diet. We conclude that it is conducive to the recycling of mushroom by-products.

Key words: *Pleurotus eryngii* stump waste, Goose, Growth performance, Carcass characteristics.

(1) Contribution No. 2663 from Livestock Research Institute, Council of Agriculture, Executive Yuan.

(2) Changhua Animal Propagation Station, COA-LRI, Changhua 52149, Taiwan, R. O. C.

(3) Food Industry Research and Development Institute, Hsinchu 300, Taiwan, R. O. C.

(4) Nutrition Division, COA-LRI, Tainan 71246, Taiwan, R. O. C.

(5) Corresponding author, E-mail: wsd@mail.tlri.gov.tw.

濕紅茶渣對白羅曼肉鵝飼養價值之評估⁽¹⁾

王勝德⁽²⁾⁽⁴⁾ 簡靖華⁽³⁾ 廖士傑⁽²⁾ 沈士怡⁽²⁾

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摘 要

茶渣是茶葉生產或加工過程必然的產出物，本試驗旨在評估濕紅茶渣應用於白羅曼肉鵝之飼養價值。滿 4 週齡之公、母白羅曼鵝共 90 隻，依性別分配至 5 個飼糧處理，每處理 3 欄，每欄 3 公 3 母共 6 隻。飼糧處理依飼養分期、限飼與否及有無採食濕紅茶渣，第一組為 5－8 週齡任食飼料及 9－12 週齡任食飼料之 A5A9 供作對照組，第二組為 5－8 週齡任食飼料而 9－12 週齡限飼 20% 飼料量之 A5R9 組，第三組為 5－8 週齡任食飼料而 9－12 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食之 A5R9T9 組，第四組為 5－8 週齡限飼 20% 飼料量而 9－12 週齡任食飼料之 R5A9 組，第五組為 5－8 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食而 9－12 週齡任食飼料之 R5T5A9 組。限飼 20% 飼料量係以對照組鵝隻前 3 至 4 日飼料任食量之 80% 提供，所有試驗鵝隻於 13 週齡均採飼料任食且不提供濕紅茶渣。結果顯示，5－8 週齡 R5A9 組鵝隻之體增重低於對照組 ($P < 0.05$)，R5A9 及 R5T5A9 組鵝隻之飼料採食量均顯著低於對照組 ($P < 0.05$)，R5T5A9 組鵝隻之飼料轉換率顯著較對照組差 ($P < 0.05$)。9－12 週齡 A5R9T9 組鵝隻之飼料採食量飼料轉換率顯著低於對照組 ($P < 0.05$)。5－12 週齡或 5－13 週齡 A5R9T9 及 R5T5A9 兩組鵝隻之飼料採食量均顯著低於對照組 ($P < 0.05$)。R5T5A9 組鵝隻於 8 週齡之血清三酸甘油酯及於 12 週齡之血清總蛋白質含量均顯著低於對照組 ($P < 0.05$)，A5R9T9 組鵝隻於 12 週齡之血清總蛋白質、總膽固醇及低密度脂蛋白膽固醇含量均顯著低於對照組 ($P < 0.05$)。本試驗結論認為，白羅曼肉鵝於 5－8 週齡或 9－12 週齡減餵 20% 飼料量但額外提供濕紅茶渣任食，不影響 5－12 週齡或 5－13 週齡鵝隻之體增重及飼料轉換率而降低飼養成本。

關鍵詞：濕紅茶渣、白羅曼肉鵝、飼養價值。

緒 言

唐朝陸羽《茶經：六之飲》記載「…。茶之為飲，發乎神農氏，…」，人類採摘茶樹的葉子泡製為茶品飲用相傳源自神農氏，迄今已有數千年。茶飲品盛行全世界，為僅次於飲用水的飲品，其中以紅茶占比 78% 最高、次為綠茶 20%，烏龍茶約有 2% (Trevisanato and Kim, 2000)。以紅茶為例，Gardner *et al.* (2007) 指出人類每天喝 3 杯以上可降低冠狀動脈心臟疾病的罹患機率，每天喝 6 杯以上可改善身體的抗氧化狀況，渠等結論認為，紅茶對人類健康具有正面效果。

統計資料顯示，2018 年我國茶園種植面積 12,079 公頃，茶葉主要產自南投 (占 68.1%)、嘉義 (占 12.2%) 及桃園 (占 3.1%)，產值約 74.8 億餘元 (行政院農業委員會，2019)。另依財政部關務署資料 (海關進出口統計，2020)，我國 2019 年出口超過 1 萬公噸茶葉，主要出口至中國、美國、日本及菲律賓，其中紅茶約 0.4 萬公噸 (占 38.6%)、綠茶約 0.28 萬公噸 (占 27.1%)、含部分發酵茶之其他茶類約 0.35 萬公噸 (占 34.3%)。我國 2019 年也進口茶葉超過 3.3 萬公噸，主要來自越南、斯里蘭卡、印度及印尼，其中紅茶約 1.65 萬公噸 (占 49.5%)、綠茶約 1.02 萬公噸 (占 30.8%)、含部分發酵茶之其他茶類約 0.66 萬公噸 (占 19.7%)，單就進口茶葉量推估國內每年的茶渣 (Tea waste) 量即超過 3 萬公噸。

茶渣是茶葉生產或加工過程必然的產出物，目前的處理方式多是廢棄或當作堆肥原料。李等 (2013) 認為茶渣是良好的飼料資源，蓋因茶渣具有保健免疫作用，可提供營養物質、提高飼料品質及改善畜舍環境衛生。鵝對牧草

(1) 行政院農業委員會畜產試驗所研究報告第 2664 號。

(2) 行政院農業委員會畜產試驗所彰化種畜繁殖場。

(3) 行政院農業委員會茶業改良場魚池分場。

(4) 通訊作者，E-mail: wsd@mail.tlri.gov.tw。

中的纖維質具有部分的利用能力 (楊及林, 1975), 即使放牧於牧草地仍能生長良好, 並有良好的肉質 (Huang *et al.*, 2008)。對肉鵝而言, 適當之飼糧粗纖維含量為 6 – 9% (Hsu *et al.*, 2000)。以國內慣用的玉米大豆粕飼糧而言, 肉鵝完全配合飼料必需添加纖維質飼料原料如苜蓿、粗糠或麩皮, 或是額外供應纖維質草料如新鮮狼尾草、乾燥稻稈。若飼糧纖維含量不足容易造成鵝隻啄羽, 導致鵝隻發育受阻、傷口感染、異食癖等生長、繁殖及動物福祉問題 (Huang *et al.*, 2008)。

乾燥紅茶葉調製紅茶飲品後, 其茶渣仍存有總多酚 (Total polyphenols) 等機能性成分 (李等, 2013), 且含粗蛋白質、粗纖維、粗脂肪、礦物質等一般營養成分 (劉及梁, 2004)。關於茶渣應用於家禽飼養的效果, 相關文獻大多採用乾燥粉末 (Wu *et al.*, 2014; Rahman *et al.*, 2016)、發酵物 (Murugesan *et al.*, 2005; Xu *et al.*, 2012) 或萃取物 (Huang *et al.*, 2019) 的型態進行評估, 本試驗首次將國產紅茶渣導入肉鵝飼養, 在不耗用額外能源乾燥茶渣情況下, 評估濕紅茶渣的直接利用性, 將飲料副產物的循環再利用導入肉鵝飼養, 期以降低生產成本及跨域加值農副產物。

材料與方法

I. 試驗動物與管理

試驗使用之濕紅茶渣取自雲林縣斗六鎮某飲料加工廠, 係南投茶區大葉種乾燥紅茶葉 (*Camellia sinensis*) 於調製紅茶飲品後, 將產出的濕紅茶渣載回凍存待用。白羅曼鵝 (White Roman goose) 則購自彰化縣某種鵝場, 動物試驗飼養於非開放式全高床鵝舍內, 實驗動物之使用及照護經行政院農業委員會畜產試驗所彰化種畜繁殖場實驗動物照護及使用小組審核通過 (畜試彰動字第 10810 號同意書) 並據以進行。

II. 試驗處理

白羅曼雛鵝 100 隻飼養至 4 週齡, 選取公、母鵝各 45 隻各依性別分配至 5 個飼糧處理組, 每組 3 欄、以欄為重複, 每欄 3 公 3 母。飼糧處理依給飼條件 (任食飼料略稱 A、限飼 20% 飼料量略稱 R、額外提供濕紅茶渣任食略稱 T) 及處理階段 (5 – 8 週齡略稱 5 及 9 – 12 週齡略稱 9), 分為 A5A9 (5 – 8 週齡及 9 – 12 週齡均為任食飼料作為對照組)、A5R9 (5 – 8 週齡任食飼料, 9 – 12 週齡減餵 20% 飼料量)、A5R9T9 (5 – 8 週齡任食飼料, 9 – 12 週齡減餵 20% 飼料量但額外提供濕紅茶渣任食)、R5A9 (5 – 8 週齡減餵 20% 飼料量, 9 – 12 週齡恢復任食飼料) 及 R5T5A9 (5 – 8 週齡減餵 20% 飼料量但額外提供濕紅茶渣任食, 9 – 12 週齡恢復任食飼料) 共 5 組, 各組鵝隻於 13 週齡當週均恢復任食飼料且均不提供濕紅茶渣, 試驗設計如表 1 所示。

表 1. 濕紅茶渣對白羅曼肉鵝飼養價值之試驗設計

Table 1. Experimental design of the feeding trial with wet black tea leave residues (WBTR) for White Roman meat-type geese

Group	Dietary supplement	Age, weeks of age		
		5 to 8	9 to 12	13
A5A9 (control)	feed	<i>Ad libitum</i>	<i>Ad libitum</i>	<i>Ad libitum</i>
	WBTR	—	—	—
A5R9	feed	<i>Ad libitum</i>	-20% ¹	<i>Ad libitum</i>
	WBTR	—	—	—
A5R9T9	feed	<i>Ad libitum</i>	-20% ¹	<i>Ad libitum</i>
	WBTR	—	<i>Ad libitum</i>	—
R5A9	feed	-20% ¹	<i>Ad libitum</i>	<i>Ad libitum</i>
	WBTR	—	—	—
R5T5A9	feed	-20% ¹	<i>Ad libitum</i>	<i>Ad libitum</i>
	WBTR	<i>Ad libitum</i>	—	—

¹ The levels of feed intake were calculated by the average of the A5A9 groups for prior to 3 - 4 days duration.

試驗鵝隻之飼養分期及各分期之飼糧營養標準係參考 NRC (1994) 及國內專家學者資料 (Huang *et al.*, 2008) 設計, 試驗分期及配方列示於表 2。試驗期間為 5 – 13 週齡, 於 8、12、13 週齡收集各欄鵝群之體重及 5 – 8、9 – 12、13 週齡期間之飼料採食量, 供計算飼料轉換率 (Feed conversion ratio, FCR) 及飼糧乾物質轉換率。

並於 8 及 12 週齡分別採集每欄 1 公 1 母鵝隻之腳脛靜脈血液樣品，經離心後以 LANNER T-900 血液生化分析儀 (LANNER Biotechnology Co. Ltd., Taiwan) 配合相應套組分析鵝隻血清中之總膽固醇 (Total cholesterol)、三酸甘油酯 (Triglyceride)、麩胺酸草醋酸轉胺酶 (Glutamic-oxaloacetic transaminase, GOT)、麩胺酸丙酮酸轉胺酶 (Glutamic pyruvic transaminase, GPT)、總蛋白質 (Total protein)、高密度脂蛋白膽固醇 (High-density lipoprotein cholesterol, HDL)、低密度脂蛋白膽固醇 (Low-density lipoprotein cholesterol, LDL) 含量。濕紅茶渣樣品則先經 65°C 乾燥 48 小時，乾燥樣品再經粉碎後，取樣紅茶渣粉進行分析。其所含之水分、粗蛋白質、粗脂肪、粗纖維、酸洗纖維、中洗纖維、粗灰分、鈣、磷、碳水化合物委託行政院農業委員會畜產試驗所飼料化驗中心分析。紅茶渣之總多酚、咖啡因、游離胺基酸含量則依黃及柯 (2006) 所述紅茶化學分析方法測定之。相關成分分析值列示於表 3，係以濕紅茶渣型態表示之。

表 2. 試驗飼糧組成

Table 2. Composition of experimental diets

Ingredients	Starter (day-old to 4 wks)	Grower (5 to 8 wks)	Finisher (9 to 13 wks)
Corn, ground	61.55	64.20	61.60
Soybean meal	29.00	21.50	15.70
Alfalfa meal	—	—	11.00
Wheat bran	—	5.00	6.00
Dicalcium phosphate	1.30	1.60	1.32
Limestone	0.70	0.80	0.43
Fish meal	3.50	—	—
Molasses	3.00	3.00	—
Rice hull	—	3.00	—
Soybean oil	—	—	3.00
Salt	0.30	0.30	0.30
L-lysine	—	—	0.10
DL-methionine	0.25	0.20	0.15
Vitamin premix ¹	0.10	0.10	0.10
Mineral premix ²	0.20	0.20	0.20
Choline chloride, 50%	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated value			
Crude protein, %	19.78	15.23	14.38
Metabolizable energy, kcal/kg	2,926	2,817	2,964
Calcium, %	0.99	0.86	0.76
Non-phytate phosphorus, %	0.39	0.44	0.36
Crude fiber, %	2.93	4.45	5.40
Analyzed value			
Dry matter, %	87.95	88.43	88.92
Crude protein, %	19.61	16.20	14.22

¹ Supplied per kilogram of diet: vitamin A 10,000 IU, vitamin D₃ 2,000 IU, vitamin E 20 IU, vitamin K₃ 3 mg, vitamin B₁ 2 mg, vitamin B₂ 5 mg, vitamin B₆ 3 mg, vitamin B₁₂ 30 µg, niacin 30 mg, pantothenic acid 10 mg, folic acid 2 mg and biotin 200 µg.

² Supplied per kilogram of diet: iron 200 mg, copper 30 mg, manganese 160 mg, cobalt 500 µg, zinc 100 mg, Iodine 1.7 mg and selenium 300 µg.

表 3. 濕紅茶渣之分析值

Table 3. Nutrient Composition of wet black tea leaves residues (% , dry matter basis)

Items	
Dry matter	25.34
Crude protein	6.32
Crude fat	0.99
Crude ash	3.47
Calcium	0.51
Phosphorus	0.24
Carbohydrate	57.33
Crude fiber	11.79
Acid detergent fiber	24.42
Neutral detergent fiber	29.04
Total polyphenols	6.05
Caffeine	1.75
Free amino acids	0.50

III. 統計分析

試驗所得數據利用 SAS 套裝軟體 (SAS, 2002) 進行統計分析。以一般線性模式程序 (General linear model procedure) 進行變方分析，另以 LSMEANS (Least squares means) 比較 5 種飼糧處理平均值間之差異顯著性，顯著水準為 $P < 0.05$ 。

試驗以飼糧處理方式為處理效應，白羅曼鵝生長性狀之統計分析數學模式為 $Y_{ij} = \mu + A_i + \varepsilon_{ij}$ ，式中 Y_{ij} 表示第 i 種飼糧處理方式之第 j 欄之觀測值 ($i = A5A9, A5R9, A5R9T9, R5A9, R5T5A9$ 等 5 處理組, $j = 1$ 至 3 欄)， μ 表示觀測值之平均值， A^i 表示飼糧處理效應， ε_{ij} 為機差效應。白羅曼鵝血清性狀之統計分析數學模式為 $Y_{ijk} = \mu + A_i + \varepsilon_{ijk}$ ，式中 Y_{ijk} 表示第 i 種飼糧處理方式之第 j 欄內第 k 隻鵝之觀測值 ($i = A5A9, A5R9, A5R9T9, R5A9, R5T5A9$ 等 5 處理組, $j = 1$ 至 3 欄, $k = 1$ 公或 1 母)， μ 表示觀測值之平均值， A_i 表示飼糧處理效應， ε_{ijk} 為機差效應。

結果與討論

分析結果顯示，本試驗使用之國產大葉種濕紅茶渣乾物質含量為 25.34%，全乾基 (Dry matter) 之粗蛋白質 6.32%、粗脂肪 0.99%、粗灰分 3.47%、鈣 0.51%、磷 0.24%、碳水化合物 57.33%、粗纖維 11.79%，酸洗纖維及中洗纖維含量分別為 24.42% 及 29.04%，總多酚 6.05%、咖啡因 1.75%、游離胺基酸 0.50%。劉及梁 (2004) 分析廢棄茶渣含有粗蛋白質 17 – 19%、粗脂肪 0.5 – 1.0%、粗纖維 16 – 18%。葉等 (2014) 分析指出臺灣紅茶之兒茶素 (Catechin) 總量 26.82 mg/g、沒食子酸 (Gallic acid) 總量 2.15 mg/g，楊梅素 (Myricetin)、槲皮素 (Quercetin) 等黃酮醇及其苷類總量 4.48 mg/g，綠原酸 (Chlorogenic acid) 則未檢出。鄭等 (2015) 檢測福建省安溪縣生產之綠茶、鐵觀音、紅茶及普洱茶茶葉及其茶渣主要成分，四種茶渣的茶多酚介於 4.2 – 10.1%、粗脂肪介於 1.5 – 4.5%，紅茶渣顯著低於綠茶渣；游離胺基酸介於 1.11 – 1.37%、粗蛋白質介於 26.0 – 35.0%、粗纖維介於 13.0 – 19.0%、粗灰分介於 5.0 – 7.0%，紅茶渣與綠茶渣兩者間之差異不顯著。Konwar and Das (1990) 指出印度紅茶工廠茶渣之乾物質 90.52%、粗蛋白質 19.48%、粗脂肪 1.37%、粗灰分 7.58%、鈣 5.46%、磷 0.84%、粗纖維 11.05%。顯示茶葉經泡製為茶品後，其茶渣仍含有一般營養成分及機能性成分，但受到茶的生產型態及採摘季節 (Özyılmaz and Genç, 2019)、茶種、栽培型態及地區、泡製條件等影響。

國產大葉種乾燥紅茶葉於調製紅茶飲品後，產出之濕紅茶渣經凍存、解凍後餵飼予白羅曼肉鵝，對其生長性狀之影響列示於表 4。相較於對照組，白羅曼肉鵝於 5 – 8 週齡限飼 20% 飼料量，造成無論有、無額外提供濕紅茶渣任食 (R5T5A9 組及 R5A9 組) 均顯著降低 5 – 8 週齡鵝隻飼料採食量及合計飼料及濕紅茶渣之飼糧乾物質採食量 ($P < 0.05$)，當期之增重及飼料轉換率也顯著較對照組差 ($P < 0.05$)；顯示於 5 – 8 週齡限飼 20% 飼料量，並不利於白

羅曼鵝當期之生長性能表現。本試驗設計原擬藉由額外提供濕紅茶渣給予限飼 20% 飼料量之鵝隻可自由採食，以額外補充飼糧營養來源，惟 R5T5A9 組鵝隻對濕紅茶渣之平均隻日採食量僅 36.3g，雖飼糧乾物質轉換率與對照組無顯著差異，然其增重、飼料採食量、飼糧乾物質採食量、飼料轉換率均顯著較對照組差 ($P < 0.05$)，且與 R5A9 組無顯著差異；顯示於 5 – 8 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食，並不利於白羅曼鵝當期之生長性能表現。R5T5A9 及 R5A9 兩組均於 9 – 12 週齡恢復飼料任食且均不額外提供濕紅茶渣，而兩組鵝隻於 9 – 12 週齡之飼料採食量、飼糧乾物質採食量、增重、飼料轉換率、飼糧乾物質轉換率均較對照組佳，尤以 R5A9 組為然，推測與限飼後恢復任食之代償性生長 (Compensatory growth) 有關。相較於對照組，A5R9T9 組鵝隻係於 9 – 12 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食，其濕紅茶渣之平均隻日採食量僅 28.5g，而當期之飼料採食量、飼糧乾物質採食量、增重、飼料轉換率、飼糧乾物質轉換率均顯著較對照組差 ($P < 0.05$)。於 9 – 12 週齡僅給予限飼 20% 飼料量但不提供濕紅茶渣之 A5R9 組鵝隻，其 9 – 12 週齡飼料採食量及飼糧乾物質採食量均顯著較對照組少，而增重及飼料轉換率雖較對照組者差，惟彼此差異未達顯著水準。顯示於白羅曼鵝 9 – 12 週齡限飼 20% 飼料量，會減少 9 – 12 週齡鵝隻飼料採食量但可改善 9 – 12 週齡飼料轉換率、飼糧乾物質轉換率，而限飼 20% 飼料量但額外提供濕紅茶渣任食，仍不利於白羅曼鵝之生長性能表現。如飼料成本以 12 元 /kg 估算，濕紅茶渣成本以每車 8,000 元載運 20 公噸之運輸成本加估人工、儲藏電費共 2 元 /kg 估算，本試驗於 5 – 8 週齡限飼 20% 飼料量 (R5A9 組) 或於 9 – 12 週齡限飼 20% 飼料量 (A5R9 組)，可分別減少白羅曼肉鵝飼料成本 8.9 元 / 隻 (5.42%) 或 8.7 元 / 隻 (5.30%)；如於 5 – 8 週齡限飼 20% 飼料但額外提供濕紅茶渣任食 (R5T5A9 組) 或於 9 – 12 週齡限飼 20% 飼料但額外提供濕紅茶渣任食 (A5R9T9 組)，可分別減少飼料成本達 11.7 元 / 隻 (7.13%) 或 16.6 元 / 隻 (10.12%)。

濕紅茶渣對白羅曼肉鵝血液性狀之影響列示於表 5。相較於對照組，於白羅曼鵝 5 – 8 週齡限飼 20% 飼料量但未額外提供濕紅茶渣任食 (R5A9 組)，其 8 週齡血清中之總蛋白質及三酸甘油酯含量均顯著低於對照組 ($P < 0.05$)，惟於 9 – 12 週齡恢復任食飼料後，其 12 週齡血液性狀與對照組無顯著差異。而於白羅曼鵝 5 – 8 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食，其 8 週齡血清三酸甘油酯含量顯著低於對照組 ($P < 0.05$)，於 9 – 12 週齡恢復任食飼料後，其 12 週齡血清總蛋白質含量顯著低於對照組者 ($P < 0.05$)，血清三酸甘油酯含量則與對照組無顯著差異。而於白羅曼鵝 9 – 12 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食，其 12 週齡血清中之總蛋白質、總膽固醇及低密度脂蛋白膽固醇含量均顯著低於對照組 ($P < 0.05$)，於白羅曼鵝 9 – 12 週齡限飼 20% 飼料量但未額外提供濕紅茶渣任食，其 12 週齡血清總蛋白質含量顯著低於對照組 ($P < 0.05$)。

品種、屠宰週齡 (Fortin *et al.*, 1983; Uhlířová *et al.*, 2018)、飼糧處理 (龔等, 2008; 陳等, 2018; Arroyo *et al.*, 2012; Lui *et al.*, 2014) 影響鵝隻之生長、血液及屠體性狀。白羅曼鵝為國內最主要的鵝隻品種，雖生長相對快速，但 15 週齡時之總脂肪塊重量占體重百分比為 5.73%，華鵝則為 3.22%，說明白羅曼鵝脂肪塊蓄積能力遠大於華鵝 (陳等, 2018)。對肉鵝生產而言，脂肪含量影響消費者對鵝肉品質的評價。因此在肉鵝飼糧調配上，必需特別考量鵝隻的體脂蓄積，如蛋白質及能量對最低體脂蓄積的平衡 (Huang *et al.*, 2008)。許 (2002) 推薦肉鵝生長 (4 – 8 週齡) 及肥育 (8 週齡 – 上市) 階段的飼糧粗蛋白質及代謝能含量分別為 18.0%、2,800 kcal/kg 及 15.0%、2,850 kcal/kg，而白羅曼肉鵝的適當上市週齡為 13 週 (陳等, 2003)。鵝隻對飼料纖維具有部分消化能力 (楊及林, 1975)，粗纖維影響胺基酸、乾物質及能量的代謝及可利用性，適當的飼糧粗纖維含量除可刺激消化道的作用、清除腸道廢棄物以維持消化機能正常外，也能減少鵝隻發生啄食癖 (Huang *et al.*, 2008)。Hsu *et al.* (2000) 推薦肉鵝飼糧最適粗纖維含量為 6 – 9%，但商用飼料之粗纖維含量多數低於 6%，建議可於完全配合飼料中添加纖維質飼料原料如苜蓿、粗糠、麩皮，或是額外供應纖維質草料如狼尾草、稻稈。

本試驗依飼養分期評估國產濕紅茶渣對白羅曼肉鵝之飼養價值，結果顯示無論是 5 – 8 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食，或是 9 – 12 週齡限飼 20% 飼料量但額外提供濕紅茶渣任食，均因該階段減餵 20% 飼料量而顯著減少其 5 – 12 週齡之飼料採食量及飼糧乾物質採食量 ($P < 0.05$)。Konwar and Das (1990) 試驗結果顯示，紅茶渣含有單寧酸 6.3%、生物鹼 3.1%，但未檢出糖苷 (Glycoside) 及樹脂 (Resin)。本試驗給予鵝隻任食濕紅茶渣顯著影響其飼料採食量及飼糧乾物質採食量，推測與紅茶渣所含之生物鹼 (Alkaloid)、單寧酸 (Tannic acid) 及高量酸洗纖維 (表 2) 有關。鵝隻於 5 – 8 週齡任食濕紅茶渣，顯著降低其 8 週齡血清三酸甘油酯含量及恢復飼料任食後之 12 週齡血清總蛋白質含量，而鵝隻於 9 – 12 週齡任食紅茶渣，則顯著降低其 12 週齡血清中之總蛋白質、總膽固醇及低密度脂蛋白膽固醇含量 (表 5)。Rahman *et al.* (2016) 研究結果顯示，白肉雞飼糧中添加 1.2% 廢棄紅茶葉乾燥粉末，其體重、飼料採食量、飼料轉換率均顯著較添加 1.2% 麩皮者為佳，肝臟及胸肉中之總膽固醇含量則顯著較低。Wu *et al.* (2014) 探討烏龍茶粉的顆粒大小對肉鵝生長性狀、脂肪蓄積、鴨肉品質及抗氧化力之影響，結果顯示烏龍茶粉的粗顆粒 (357 μm) 或細粉末 (16 μm) 對肉鵝生長無顯著影響，但顯著影響其血清三酸甘油酯含量及腹脂、皮下脂肪之蓄積。本試驗評估濕紅茶渣直接餵飼對白羅曼肉鵝的飼養價值，結果顯示雖不影響其 5 – 12 週齡之增

重、飼料轉換率及飼糧乾物質轉換率，但顯著影響其血清中之總蛋白質、三酸甘油酯、總膽固醇及低密度脂蛋白膽固醇含量，與前述研究結果相近似。

表 4. 濕紅茶渣對白羅曼肉鵝生長性狀之影響

Table 4. Effects of feeding wet black tea leaves residues (WBTR) on growth performances in White Roman meat-type geese

Items	Group ¹					Pooled SEM
	A5A9	A5R9	A5R9T9	R5A9	R5T5A9	
5 to 8 weeks of age						
BWG ² , kg/pen	11.55 ^{ab}	11.51 ^{ab}	12.01 ^a	8.95 ^c	9.67 ^c	0.48
FI ² , kg/pen	39.26 ^a	38.73 ^a	38.43 ^a	33.96 ^b	32.45 ^b	0.59
WBTR ² , kg/pen	—	—	—	—	6.09	
FCR ²	3.42 ^b	3.38 ^b	3.20 ^b	3.80 ^a	3.99 ^a	0.12
DIDM ³ , kg/pen	34.71 ^a	34.25 ^a	33.98 ^a	30.03 ^b	30.01 ^b	0.52
FCR (DM) ³	3.02 ^b	2.99 ^b	2.83 ^b	3.36 ^a	3.10 ^{ab}	0.10
9 to 12 weeks of age						
BWG, kg/pen	3.87 ^{bc}	3.12 ^{cd}	2.24 ^d	5.50 ^a	4.88 ^{ab}	0.39
FI, kg/pen	33.84 ^a	30.53 ^b	24.76 ^c	35.05 ^a	33.86 ^a	1.03
WBTR, kg/pen	—	—	4.79	—	—	
FCR	8.75 ^{bc}	9.82 ^b	13.33 ^a	6.49 ^d	7.04 ^{cd}	0.60
DIDM, kg/pen	30.09 ^a	27.15 ^b	23.05 ^c	31.17 ^a	30.11 ^a	0.91
FCR (DM)	7.78 ^{bc}	8.74 ^b	10.41 ^a	5.78 ^d	6.26 ^{cd}	0.52
13 weeks of age						
BWG, kg/pen	1.00 ^{ab}	0.78 ^{ab}	0.95 ^{ab}	0.76 ^b	1.09 ^a	0.11
FI, kg/pen	8.98 ^{ab}	8.44 ^b	9.77 ^a	8.58 ^b	8.87 ^b	0.27
WBTR, kg/pen	—	—	—	—	—	
FCR	9.08	11.26	10.77	11.85	8.27	1.52
5 to 12 weeks of age						
BWG, kg/pen	15.42	14.63	14.26	14.45	14.54	0.64
FI, kg/pen	73.10 ^a	69.26 ^{ab}	63.19 ^c	69.01 ^b	66.31 ^{bc}	1.26
WBTR, kg/pen	—	—	4.79	—	6.09	
FCR	4.76	4.74	4.78	4.79	4.99	0.14
DIDM, k/pen	64.81 ^a	61.39 ^{ab}	57.03 ^c	61.20 ^b	60.11 ^{bc}	1.12
FCR (DM)	4.22	4.20	4.01	4.25	4.14	0.12
5 to 13 weeks of age						
BWG, kg/pen	16.42	15.41	15.20	15.21	15.64	0.59
FI, kg/pen	82.07 ^a	77.70 ^b	72.96 ^c	77.59 ^b	75.19 ^{bc}	1.37
WBTR, kg/pen	—	—	4.79	—	6.09	
FCR	5.01	5.05	5.12	5.11	5.20	0.12
DIDM, kg/pen	72.79 ^a	68.90 ^b	65.72 ^b	68.82 ^b	68.00 ^b	1.21
FCR (DM)	4.45	4.48	4.33	4.53	4.35	0.11
Feed cost ⁴ , 5 to 13 weeks of age						
NT\$/goose	164.1	155.4	147.5	155.2	152.4	

¹ See Table 1.

² BWG: body weight gain, FI: feed intake calculated as fed basis, WBTR: wet black tea leave residues calculated as fed basis, FCR: feed conversion ratio (feed intake calculated as fed basis /body weight gain).

³ DIDM: diet intake (feed + wet black tea residues) calculated as dry matter basis, FCR (DM): feed conversion ratio calculated as dry matter basis.

⁴ The price of feed and wet black tea leave residues was NT\$ 12/kg and 2/kg, respectively.

a, b, c, d Means with different superscripts within the same row differ significantly at P < 0.05.

表 5. 濕紅茶渣對白羅曼肉鵝血清性狀之影響

Table 5. Effects of feeding wet black tea leaves residues (WBTR) on serum profiles in White Roman meat-type geese

Items	Group ¹					Pooled SEM
	A5A9	A5R9	A5R9T9	R5T5A9	R5A9	
8 weeks of age						
GOT ² , U/L	32.00	24.33	35.50	22.17	23.00	3.96
GPT ² , U/L	11.83	11.33	11.67	7.00	10.50	1.33
TP ² , g/dL	3.88 ^a	3.60 ^{ab}	3.68 ^{ab}	3.67 ^{ab}	3.33 ^b	0.10
TG ² , mg/dL	152.2 ^a	154.2 ^a	146.5 ^{ab}	73.0 ^{bc}	67.7 ^c	18.7
CHOL ² , mg/dL	129.3	131.2	141.0	143.3	158.8	8.1
HDL ² , mg/dL	69.67	71.00	75.67	80.83	86.33	4.29
LDL ² , mg/dL	51.33	51.83	58.50	58.33	69.00	4.92
12 weeks of age						
GOT, U/L	20.67	24.00	20.83	24.50	29.33	4.00
GPT, U/L	10.50 ^{ab}	12.50 ^{ab}	6.83 ^b	11.50 ^{ab}	14.50 ^a	1.56
TP, g/dL	4.55 ^a	3.98 ^b	4.03 ^b	4.10 ^b	4.22 ^{ab}	0.10
TG, mg/dL	110.0	85.8	70.0	100.2	108.2	11.7
CHOL, mg/dL	203.5 ^a	173.8 ^{ab}	162.3 ^b	182.5 ^{ab}	209.0 ^a	8.8
HDL, mg/dL	101.2 ^{ab}	91.0 ^{ab}	87.5 ^b	92.0 ^{ab}	103.8 ^a	3.9
LDL, mg/dL	99.0 ^a	80.0 ^{ab}	71.7 ^b	84.5 ^{ab}	103.5 ^a	5.9

¹ See Table 1.² GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, TP: total protein, TG: triglyceride, CHOL: total cholesterol, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol.^{a, b} Means with different superscripts within the same row differ significantly at P < 0.05.

本試驗首次將國產紅茶渣導入肉鵝飼養，期望在不耗用額外能源將之乾燥情況下，評估濕紅茶渣的直接利用效果。結論認為，白羅曼肉鵝於 5 – 8 週齡或 9 – 12 週齡減餵 20% 飼料量但額外提供濕紅茶渣任食，雖不影響 5 – 12 週齡鵝隻之增重、飼料轉換率及飼糧乾物質轉換率而降低飼料成本。惟濕紅茶渣無法常溫貯放且極易酸敗，需先冷凍保存再予解凍方能餵飼，額外耗用能源進行凍存並不利於節省能源。又濕紅茶渣的含水率高，極易於餵飼時導致完全配合飼料的受潮及酸敗，也影響產業的實用性。未來將進一步評估國產濕紅茶渣乾燥及粉末化後應用於肉鵝的飼養價值，期能降低肉鵝飼養成本，達到農業資源多元利用之目的。

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Evaluation of the feeding value of wet black tea leave residues in White Roman meat-type geese⁽¹⁾

Sheng-Der Wang⁽²⁾⁽⁴⁾ Ching-Hua Chien⁽³⁾ Shih-Chieh Liao⁽²⁾ and Shih-Yi Shen⁽²⁾

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Abstract

Tea leave waste is the by-product from tea production or processing. The aim of this study was to evaluate the feeding value of wet black tea leave residues (WBTR) in White Roman meat-type geese. A total of ninety White Roman geese at 4 weeks of age were randomly allotted by gender to 5 groups according to feeding phase, feeding ration and WBTR supplementation. Each group was replicated in 3 pens, containing 3 ganders and 3 geese. Five groups with different rations were represented by A5A9, A5R9, A5R9T9, R5A9 and R5T5A9, respectively. The A5A9 control group represented the birds fed *ad libitum* from 5 to 8 and from 9 to 12 weeks of age. R5 or R9 represented the birds fed 80% feed provision, which was the feed intake calculated from the average of the control groups prior to 3 to 4 days between 5 to 8 or 9 to 12 weeks of age. T5 or T9 represented the birds with supplemental WBTR *ad libitum* from 5 to 8 or 9 to 12 weeks of age. Finally, all geese in each group were fed *ad libitum* without WBTR supplementation at 13 weeks of age. The results showed that lower body weight gain and feed intake, and poor feed conversion ratio (FCR) in the R5A9, R5A9 and R5T5A9, and the R5T5A9 group at 5 to 8 weeks of age, respectively ($P < 0.05$). Moreover, lower feed intake and poor FCR were found in the A5R9T9 group when compared with the A5A9 group at 9 to 12 weeks of age ($P < 0.05$). At the age between 5 and 12 weeks, feed intake of the A5R9T9 and R5T5A9 group were lower than that of the A5A9 group ($P < 0.05$). The levels of serum triglyceride at 8 weeks of age and serum total protein at 12 weeks of age of the R5T5A9 group were lower than those of the A5A9 group ($P < 0.05$). Serum total protein, total cholesterol and low-density lipoprotein cholesterol levels of the A5R9T9 group at 12 weeks of age were lower than those of the A5A9 geese group ($P < 0.05$). We concluded that body weight gain and FCR at 5 to 12 weeks of age were not affected by the 20% feed restriction with supplemental WBTR at 5 to 8 or 9 to 12 weeks of age in White Roman meat-type geese. Moreover, the feed cost can be decreased.

Key words: Wet black tea leave residues, White Roman meat-type goose, Feeding value.

(1) Contribution No. 2664 from Livestock Research Institute, Council of Agriculture, Executive Yuan.

(2) Changhua Animal Propagation Station, COA-LRI, Changhua 52149, Taiwan, R. O. C.

(3) Yuchi Branch, Tea Research and Extension Station, COA, Nantou 555009, Taiwan, R. O. C.

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

飼養密度、飛機翼及性別對白羅曼鵝羽毛重、 血液性狀及腸道組織之影響⁽¹⁾

林旻蓉⁽²⁾ 吳國欽⁽³⁾ 賈玉祥⁽⁴⁾ 張仲彰⁽⁵⁾⁽⁶⁾

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摘 要

本試驗旨在探討飼養密度、具飛機翼與否及性別對白羅曼鵝之體重、羽毛重、血液性狀及腸道組織之影響。試驗採裂區設計 (Split-plot design)，將 384 隻白羅曼鵝逢機分配於 3 種飼養密度 (高、中及低飼養密度) 處理組，每組 4 欄，每欄公與母鵝隻數各半。中及高飼養密度處理組之飼養密度分別為低密度者之 1.33 倍與 1.66 倍。以欄為主區試驗單位 (Main plot)，每欄內分為 2 性別 (公及母鵝) × 2 種鵝隻 (具飛機翼與否)，以隻為裂區試驗單位 (Subplot)。試驗期間 (0 至 14 週齡) 採自然光照，飼料與飲水均任飼。鵝隻育雛期 (0 至 4 週齡) 飼糧之粗蛋白質與代謝能含量分別為 20% 與 2,900 kcal/kg；育成期 (5 至 14 週齡) 則分別為 15% 與 2,800 kcal/kg。試驗結果顯示，3 種飼養密度均未對 8 與 14 週齡鵝隻之飛機翼發生率造成影響。低飼養密度於 14 週齡鵝隻之血清尿酸 (Uric acid, UA) 含量顯著較高密度者為高 ($P < 0.05$)，且其血清總膽固醇 (Cholesterol, CHOL) 含量較中與高密度者有較低之趨勢 ($P < 0.10$)。於 14 週齡具飛機翼鵝隻絕食 18 小時後體重有較正常翼者為重之趨勢 ($P < 0.10$)。於 14 週齡具飛機翼鵝隻之左翼羽毛重顯著較正常翼者為輕 ($P < 0.05$)；於 14 週齡公鵝之左翼羽毛重顯著較母鵝者為重 ($P < 0.001$)。於 8 週齡公鵝之血清 CHOL、高密度脂蛋白－膽固醇 (High density lipoprotein-cholesterol, HDL-C) 及低密度脂蛋白－膽固醇 (Low density lipoprotein-cholesterol, LDL-C) 均顯著較母鵝者為高，且於 14 週齡公鵝之血清 CHOL 與 HDL-C 亦均顯著較母鵝者為高。於 14 週齡具飛機翼鵝隻之十二指腸腺窩深度顯著較正常翼者為淺 ($P < 0.05$)。綜上所述，鵝隻之飼養密度會影響其血清 UA 及 CHOL 含量。具飛機翼白羅曼鵝之翼部羽毛重較輕，推測其因主翼羽末端外翻，造成羽毛磨損或折斷情形而有變輕現象。鵝隻之體重及血液性狀受性別影響，其血液性狀之差異推測與鵝隻之脂肪動員有關。

關鍵詞：飼養密度、飛機翼、性別、羽毛重、腸道組織、白羅曼鵝。

緒 言

臺灣飼養的鵝隻主要為白羅曼鵝及華鵝，其中白羅曼鵝占 97% 以上。在家禽方面，飛機翼常發生於鵝及鴨，經調查顯示，肉鵝飛機翼之發生率為 5 – 50%，華鵝之飛機翼發生率較白羅曼鵝者高 (李，2004)。目前臺灣之內鵝交易均以活體議價方式，若鵝隻飛機翼發生率高於 30% 以上，鵝販或屠宰業者購買該鵝群之價格則較低，造成養鵝戶之經濟損失。飛機翼之成因係鵝隻於生長期間，其單側或雙側翼末端之第三及第四掌骨關節處發生相疊的現象 (Grow, 1972; Mildred and Holderread, 1981)，導致主翼羽向體外側翻出，以致翅膀末端與身體之頭尾軸線約呈 45° 之偏離角度 (Francis *et al.*, 1967)，因其外貌有如飛機之雙翼，故國內業者習以「飛機翼」稱之。具飛機翼之加拿大鵝的翼部肌肉良好且無外傷或全身性病徵，上膊骨、尺骨、橈骨及近側掌骨等型態上均正常，僅於第四掌骨遠端的二分之一處起直至翼末端呈 90° 旋轉，使主翼羽翻轉向體側外伸展而致形如機翼般；推測飛機翼係因翅膀快速生長致重量增加，復因地心引力之拉力超過腕骨關節肌肉之負荷力，導致翼之末端偏向外側而垂下 (Kreeger and Walser, 1984)。

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(2) 行政院農業委員會畜產試驗所彰化種畜繁殖場。
(3) 行政院農業委員會畜產試驗所彰化種畜繁殖場退休。
(4) 行政院農業委員會畜產試驗所新竹分所退休。
(5) 行政院農業委員會畜產試驗所高雄種畜繁殖場。
(6) 通訊作者，E-mail: macawh@mail.tlri.gov.tw。

白羅曼鵝之飛機翼之傾向性遺傳率 (Liability heritability, h^2) 為 0.31，估算白羅曼鵝飛機翼品系之遺傳率為 0.258，屬於中度遺傳率之性狀 (Lin *et al.*, 2008)。白羅曼鵝飛機翼品系 (為本場選育飛機翼性狀之族群，因飛機翼發生部位有如人類之手腕，期未來可做為生醫用之研究平臺，供作肌腱與生長板發育之研究，故進行選育) 之鵝隻於 8 – 14 週齡之飛機翼程度評分與發生率均顯著較白羅曼鵝高體重品系 (為本場選育體重性狀之族群) 與白羅曼鵝商業用品系 (為本場引進民間種鵝場之族群) 者為高，其於 14 週齡之飛機翼程度評分與發生率依序為 1.90 與 54.8%、0.23 與 11.6% 以及 0.40 與 13.3%。故從白羅曼鵝飛機翼品系之鵝隻以飛機翼程度評分做為選拔指標者，可見鵝隻飛機翼性狀經選拔後，可造成外貌上之差異 (林等，2012)。

飼養密度會影響家禽飼料消耗量、飼料轉換率、屠體性能以及血液生化值的表現 (張等，2012; Şengül *et al.*, 2000; Özbey and Esen, 2007)。雞隻飼養於 10 到 16 隻/ m^2 之飼養密度下，血液中葡萄糖及膽固醇含量無顯著差異 (Škrbić *et al.*, 2009)；而當飼養密度達 16 隻/ m^2 時，雞隻步態評分有增加，但未達顯著差異。然鷓鴣飼養密度分別為 15、20 及 25 隻/ m^2 時，則其血液中總蛋白及鹼性磷酸酶 (ALP) 含量隨飼養密度提升而呈顯著性增高，然血液中總膽固醇、三酸甘油酯及葡萄糖含量則反之 (Özbey and Esen, 2007)。

本研究擬進行飼養密度、具飛機翼與否及性別對白羅曼鵝之體重、羽毛重、血液生化值及腸道組織之探討，做為未來供研究鵝飛機翼發生原因之參考依據。另外，本試驗探討有關飼養密度對白羅曼鵝飛機翼發生率之影響已刊登於 J. Anim. Sci. 期刊 (Lin *et al.*, 2016)。

材料與方法

I. 試驗動物與管理

0 – 3 週齡白羅曼鵝隻飼養於鋪設不銹鋼網之高床式育雛舍，每欄之長與寬度分別為 1.50 m 與 1.28 m，面積為 1.92 m^2 (0.58 坪)，每欄均設置飼料盤及自動飲水球各 1 個。4 – 6 週齡鵝隻飼養於水泥地面之育成舍，每欄之長與寬度分別為 12.0 m \times 1.10 m，面積為 13.2 m^2 (4 坪)，每欄亦均設置飼料槽及自動飲水球各 1 個之外，並有 2 m^2 之塑膠床面供鵝隻休憩用，以避免鵝隻接觸排泄物。7 – 14 週齡鵝隻飼養於水泥地面之育成舍，每欄之長與寬度分別為 8.70 m 與 2.30 m，面積為 20.0 m^2 (6 坪)，每欄亦均設置飼料槽及自動飲水球各 1 個之外，尚設置 1 個水浴池，水浴池之長與寬度分別為 2.90 m 與 2.30 m，面積為 6.67 m^2 (2 坪)。本試驗育雛期飼糧之粗蛋白質與代謝能含量分別為 20% 與 2,900 kcal/kg，生長期飼糧之粗蛋白質與代謝能含量分別為 15% 與 2,800 kcal/kg，其飼料配方和組成分列於表 1。試驗期自 97 年 4 月至 7 月，期間採自然光照、飼料及飲水任飼，每週清洗鵝舍兩次。

II. 試驗設計

雛鵝孵出後隨即進行泄殖腔公母鑑別，掛上腳號，並移入育雛舍。本試驗採裂區設計 (Split-plot design)，鵝舍內有 12 欄，鵝隻逢機分配於 3 飼養密度 (第 0 – 3 週飼養密度依序為 24 (低密度)、32 (中密度)、40 隻 (高密度)/1.92 m^2 ；第 4 – 6 週飼養密度依序為 24 (低密度)、32 (中密度)、40 隻 (高密度)/13.2 m^2 ；第 7 – 14 週飼養密度依序為 24 (低密度)、30 (中密度)、36 隻 (高密度)/20.0 m^2) 處理組，欄為一主區試驗單位 (Main plot)，每欄內分為 2 性別 (公及母鵝) \times 2 種鵝隻 (具飛機翼與否)，以隻為裂區試驗單位 (Subplot)，公與母鵝各使用 192 隻，合計 384 隻。本試驗之低密度組 (Low stocking density, LD) 以肉鵝正常飼養密度為主，第 0 – 3 週、第 4 – 6 週及第 7 – 14 週之飼養密度依序為 12.5、1.82 及 1.2 隻/ m^2 (以坪換算為 40、6 及 4 隻/坪)，中密度 (Middle stocking density, MD) 與高密度組 (High stocking density, HD) 則分別以低密度者之 1.33 倍與 1.66 倍為原則，設定每欄鵝隻之數量。MD 與 HD 組於第 7 – 14 週時，每欄分別自原欄位逢機選用公母鵝各 15 與 18 隻。

III. 正常翼及飛機翼發生率之定義

正常翼 (Normal wing, NW) 即鵝隻之主翼羽末端與身體之頭尾軸線緊貼綿密，呈現平滑、排列整齊及潔淨之外貌，並無角度之偏離 (即偏離角度 = 0°)。相對地，飛機翼 (Angel wing, AW) 者之主翼羽向體外側翻出，其主翼羽末端與身體之頭尾軸線之偏離角度介於 > 0° 與 $\leq 90^\circ$ 之間 (林等，2012)。

飛機翼發生率 (Incidence of angel wing, IAW) 為觀測鵝隻於 8 與 14 週齡之飛機翼發生情形，鵝隻未出現飛機翼 (即飛機翼程度評分為 0) 者，其飛機翼發生率為 0%；如出現飛機翼，則不論飛機翼嚴重程度，均視為飛機翼發生率為 100%。因此，每欄鵝隻出現飛機翼的隻數不一定相同。

表 1. 鵝隻育雛期和生長期飼料配方與組成分

Table 1. The feed formula and composition of the starter and grower periods in geese

Item	Starter period (0 – 4 wk)	Grower period (5 – 14 wk)
Ingredients (kg)		
Yellow corn	614	642.5
Soybean meal	260	215
Wheat bran	20	50
Fish meal, 65%	50	—
Molasses	30	30
Salt	3	3
Dicalcium phosphate	10	16
Limestone, pulverized	7	8
Choline chloride, 50%	1	1
DL-methionine	2.5	2
Rice bran	—	30
Vitamin premix ¹	1	1
Mineral premix ²	1.5	1.5
Total	1,000	1,000
Calculated values		
Crude protein, %	20	15
Metabolizable energy, kcal/kg	2,900	2,800
Calcium, %	0.82	0.73
Total phosphorus, %	0.67	0.64

¹ Supplied per kg of diet: vitamins A 10,000 IU, D₃ 2,000 IU, E 20 IU, B₁ 1 mg, B₂ 4.8 mg, B₆ 3 mg, B₁₂ 0.01 mg, biotin 0.2 mg, K₃ 1.5 mg, d-calcium pantothenate 10 mg, folic acid 0.5 mg and nicotinic acid 25 mg.

² Supplied per kg of diet: Cu 15.0 mg, Fe 80 mg, Zn 50 mg, Mn 80 mg, Co 0.25 mg, I 0.85 mg and Se 0.075 mg.

IV. 測定項目

(i) 血液檢測項目

鵝隻於 8 與 14 週齡時，在不考慮其是否具飛機翼情況下，每欄逢機選取公鵝 3 隻及母鵝 3 隻，共採血檢測 72 隻鵝隻，每次分別採其翼靜脈血液，經離心 (1,000 ×g, 10 分鐘) 收集其血清及血漿。樣品分析血清之麩胺酸草乙酸轉胺酶 (Glutamate oxaloacetate transaminase, GOT)、麩胺酸丙酮酸轉胺酶 (Glutamate pyruvate transaminase, GPT)、總蛋白質 (Total protein, TP)、三酸甘油酯 (Triglycerides, TG)、總膽固醇 (cholesterol, CHOL)、白蛋白 (Albumin, ALB)、球蛋白 (Globulin, GLO)、總鈣 (Calcium, Ca)、磷 (Phosphorus, P)、高密度脂蛋白－膽固醇 (High density lipoprotein-cholesterol, HDL-C)，低密度脂蛋白－膽固醇 (Low density lipoprotein-cholesterol, LDL-C) 等項目，各種血清生化值之測定以全自動血液生化分析儀 (Hitachi Autoanalyzer 7150, Hitachi, Tokyo, Japan)，輔以相關套組進行之。白血球 (White blood cell, WBC)、紅血球 (Red blood cell, RBC)、血容積比 (Hematocrit, HCT)、平均血球體積 (Mean corpuscular volume, MCV)、平均血球血色素 (Mean corpuscular hemoglobin, MCH)、平均紅血球血容積比 (Mean corpuscular hemoglobin concentration, MCHC)、血小板 (platelet, PLT)、血小板分佈寬度 (Platelet distribution width, PDW)、平均血小板體積 (Mean platelet volume, MPV)、大型血小板比例 (Platelet large cell ratio, PLCR)、血小板容積比 (Plateletcrit, PCT) 等各種血球之測定以全自動血球分析儀 (Sysmex K-4500 Automated Hematology Analyzer, Sysmex, Hyogo, Japan)，輔以相關試劑進行之。

(ii) 羽毛及腸道組織之測定

鵝隻於 14 週齡時，每個飼養密度處理組逢機選取飛機翼與正常翼鵝隻之公與母鵝各 2 隻，計 24

隻，屠宰前經 18 小時絕食，以檢測鵝隻之左翼重及左翼羽毛重；並取十二指腸、空腸、迴腸之腸道各約 10 cm，浸泡 10% 中性福馬林 24 h 以上，作為切片樣本，再以蘇木紫及伊紅 (Haematoxylin and Eosin, HE) 染色後，於光學顯微鏡下，每一切片選取十點並配合 Motic Image Plus 2.0 軟體測量絨毛高度及腺窩深度。

V. 統計分析

試驗所得數據以統計分析系統 (SAS, 2004) 進行統計分析，使用一般線性模式程序 (General linear model procedure, GLM) 進行變方分析，再以最小平方平均值法 (Least square means, LSMEANS) 計算平均值，並檢定其相互之間差異之顯著性。

本試驗以 3 種飼養密度處理為主效應，而以 2 性別 (公及母鵝) × 2 種 (具飛機翼與否鵝隻) 之 4 種處理為次效應，其統計分析之數學模式為：

$$Y_{ijklm} = \mu + D_i + \gamma_{ij} + A_k + S_l + (A \times S)_{kl} + \varepsilon_{klm}$$

式中 Y_{ijklm} ：表示第 i 個飼養密度處理、第 j 欄、第 k 個具飛機翼與否鵝隻處理、第 l 個性別處理、第 m 隻鵝之觀測值。

μ ：表示所有觀測值的平均值。

D_i ：表示第 i 飼養密度處理的固定效應， $i = \text{HD, MD, LD}$ 。

γ_{ij} ：表示以欄為試驗單位之機差，且 $\gamma_{ij} \cap N(0, \sigma^2\gamma)$ 。

A_k ：表示第 k 具飛機翼與否鵝隻處理之固定效應， $k = \text{Y, N}$ 。

S_l ：表示第 l 性別處理之固定效應， $l = \text{Male, Female}$ 。

$(A \times S)_{kl}$ ：表示 k 具飛機與否鵝隻處理與 l 性別處理之交感作用。

ε_{klm} ：表示次試驗單位 (鵝隻) 間其他未能解釋之隨機機差 (Random error)，且 $\varepsilon_{klm} \cap N(0, \sigma^2_\varepsilon)$ 。

結果與討論

本試驗之 8 與 14 週齡鵝隻體重於 3 種飼養密度間有顯著影響，由低至高飼養密度依序為 3.97、3.62 及 3.41 kg 與 5.09、4.84 及 4.56 kg，然此 3 種飼養密度均未對 8 與 14 週齡鵝隻之飛機翼發生率造成顯著影響。本試驗鵝隻之體重與張等 (2012) 之試驗結果有相同趨勢，鵝隻於 12 週齡體重依飼養密度 (1.2、1.5 及 1.8 隻/m²) 依序為 4.62、4.43 及 4.49 kg，其 3 至 12 週齡採食量亦依序為 17.1、16.9 及 16.5 kg。由上可知，當鵝隻飼養密度過高時，使鵝隻過度擁擠，造成採食量下降，易造成鵝隻體重較輕且整齊度較差，加上本試驗於夏季進行，夏季常為高溫多濕的環境，對鵝隻之生長性能影響甚大，因此體重相對較冬季者輕 (王等, 2004；張等, 2010；林等, 2014；Pope and Emmert, 2002)。

由過去觀察白羅曼鵝飛機翼發生之情形，得知其飛機翼之發生大多於生長期，從 6 週齡育成鵝即陸續出現飛機翼情形，至 13 – 14 週齡以後就很少再觀察到原本正常翼的鵝隻出現飛機翼的情形。同時，有些鵝隻於 6 – 12 週齡期間出現輕度飛機翼者，飼養至 13 – 14 週齡時，反而恢復成正常翼之外貌。推測鵝隻於 6 – 12 週齡時，主翼羽已先長齊，然副翼羽尚未完全長齊，使得其翼之展開與收回動作較不流暢，此時容易誤判鵝隻為輕度飛機翼者。此外，Pitman *et al.* (2012) 之報告亦顯示，藍臉鰐鳥的幼雛 (Masked Boobies chick) 出現飛機翼情形時，會於羽毛豐滿會飛時 (Fledging age)，恢復成正常翼之外貌。本試驗亦有一部分鵝隻於 8 週齡出現輕度飛機翼後，於 14 週齡恢復成正常翼之外貌，由 HD 組鵝隻於 8 週齡之飛機翼發生率為 42.1% 降至其於 14 週齡者之 39.4% 可知此現象。

本試驗之具飛機翼與否鵝隻 14 週齡之體重、左翼重及羽毛性狀如表 2 所示，其中絕食 18 小時後，14 週齡公鵝體重顯著較母鵝者為重 (5.41 vs. 4.64 kg, $P < 0.01$)。14 週齡具飛機翼鵝隻絕食 18 小時後之體重有較正常翼者為重之趨勢 (5.23 vs. 4.81 kg, $P < 0.1$)。14 週齡具飛機翼鵝隻之左翼羽毛重顯著較正常翼者為輕 (48.3 vs. 53.1 g, $P < 0.05$)。14 週齡公鵝之左翼羽毛重顯著較母鵝者為重 (54.3 vs. 47.2 g, $P < 0.001$)。14 週齡具飛機翼鵝隻之左翼羽毛重占左翼重之百分比顯著較正常翼者為低 (14.5 vs. 16.4%, $P < 0.001$)。由上述之結果顯示，公鵝絕食後之體重及左翼之羽毛重均較母鵝者為重，亦即鵝隻之體重及左翼羽毛重有性別間之差異，但是否與全身羽毛重有直接關係，則需再深入探討。具飛機翼鵝隻於翼羽毛重之差異，主要為其主翼羽末端外翻，造成羽毛磨損或折斷的情形所致。鵝隻羽毛重受品種、體重、性別及年齡等所影響 (Mustafa, 2008；Kozák, 2011)。Mustafa (2008) 指出，Turkish 公鵝之羽毛及羽絨重顯著較母鵝者為重 (230 vs. 196 g 及 129 vs. 104 g)，且 10 週齡鵝隻羽毛重顯著較 12 週齡者為輕 (202 vs. 223 g)。

表 2. 飛機翼及性別對 14 週齡鵝隻體重、左翼重及羽毛性狀之影響

Table 2. Effects of angel wing and sex on body weight, left wing weight and feather trait of geese at 14 weeks old

Item	Wing style		SEM ¹	Sex		SEM ²	Significance ³		
	AW	NW		M	F		W	S	W × S
BWFD at 14-wk-old, kg/bird	5.23	4.81	0.17	5.41 ^x	4.64 ^y	0.17	†	**	NS
Weight of left wing, g	334	328	9.64	350 ^x	312 ^y	9.65	NS	*	NS
Weight of de-feather left wing, g	271	265	8.03	289 ^x	248 ^y	8.03	NS	**	NS
Feather weight of left wing, g	48.3 ^b	53.1 ^a	1.25	54.3 ^x	47.2 ^y	1.25	*	***	NS
Feather weight of left wing, % of BWFD	0.09 ^b	0.11 ^a	0.003	0.10	0.10	0.003	***	NS	NS
Feather weight, % of left wing weight	14.5 ^b	16.4 ^a	0.41	15.5	15.3	0.41	***	NS	†

W: Wing style, S: Sex, W × S: The interaction of wing style with sex, AW: Angel wing, NW: Normal wing, M: Male, F: Female, BWFD: Body weight after 18-h feed-deprived.

¹ SEM: Standard error of means of wing style.

² SEM: Standard error of means of sex.

³ NS: Not significantly different; † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^{x, y} Means within the same row under sex without the same superscripts differ significantly ($P < 0.05$).

^{a, b} Means within the same row under wing style without the same superscripts differ significantly ($P < 0.05$).

雞隻飼養密度對其血液中葡萄糖含量及膽固醇含量均無顯著差異 (Dozier III *et al.*, 2006; Škrbić *et al.*, 2009)。鵝飼養密度為 15、20 及 25 隻/m² 時，隨飼養密度增加，其血液總蛋白質及鹼性磷酸酶含量亦呈顯著性增加，然血液 CHOL、TG、GLU 及尿素 (Urea) 等之含量則反之 (Özbey and Esen, 2007)，但另有研究報告則指出白肉雞之飼養密度為 17.5 隻/m²，其血清 CHOL 含量顯著較 11.9 隻/m² 者為高 (Onbaşilar *et al.*, 2008)。肉雞採食高蛋白質飼糧會造成血漿 UA 含量增加 (Machin *et al.*, 2004)，家禽採食過量蛋白質時，其體內多餘之氮會藉由 UA 方式排出。

本試驗血液生化值檢測結果如表 3 所示，LD 組 14 週齡鵝隻之血清 UA 含量顯著較 HD 者為高 (2.91 vs. 2.27 mg/dL)；14 週齡 MD 組鵝隻之血清 TG 含量顯著較 LD 者為高 (190 vs. 140 mg/dL)；HD 及 MD 鵝隻 14 週齡之血清 CHOL 含量較 LD 者有較高之趨勢 (160 及 159 vs. 146 mg/dL)；LD 鵝隻之血液中 WBC 數值顯著較 HD 者為高 (219 vs. 196 10³/uL)。14 週齡鵝隻飼養密度愈高，血清 CHOL 含量則愈高，然血液 WBC 含量則較低，推測由於鵝隻飼養密度較高，可能造成鵝隻抵抗力較差。至於血清 CHOL 含量雖與 Onbaşilar *et al.* (2008) 有相似結果，但與 Thaxton *et al.* (2006) 指出白肉雞的飼養密度於 20 至 55kg 體重/m²，並不會改變血清 GLU 與 CHOL 含量之結果相反。因此，Onbaşilar *et al.* (2008) 推測這些差異可能由遺傳和環境因素造成，尤其是試驗族群規模大小，使用較小的圍欄可能會增加飼養密度對白肉雞的負面影響。本試驗隨鵝隻飼養密度愈高，其血清 UA 含量則呈愈低之現象。血清 UA 含量是一種評估動物是否為較佳飼養密度的指標，當家禽飼養密度過高時，雖為任飼，其飼料採食量仍較飼養密度低者為少，即採食的蛋白質量相對變少，導致代謝成嘌呤 (Purine) 減少，則家禽血清 UA 含量較低。

肝臟具有多種酵素，如 GOT、GPT 及 LDH 等，當肝臟受到損傷時則會釋出至血液 (Kaplan *et al.*, 2003)，可做為了解當時動物生理上之變化。駝鳥飼高蛋白質飼糧，發現其伴隨血漿尿酸含量亦增加 (Polat *et al.*, 2003)；當有疾病發生時，則引起血漿中肝臟酵素、TP 及 UA 含量增加 (Yüksek *et al.*, 2002; Komnenou *et al.*, 2003)。分析年輕中國鵝血清蛋白質發現，7 – 49 日齡鵝隻之血清總蛋白質、白蛋白及球蛋白含量間無顯著差異，然 49 日齡鵝隻之白蛋白與球蛋白比值顯著較 21 日齡者為低 (0.42 vs. 0.76) (Elizabeth *et al.*, 2008)。分析白羅曼鵝於 3 – 24 週齡期間之血液成分結果顯示，白羅曼公鵝於 12 週齡之血漿葡萄糖濃度顯著較其他週齡者為高 (邱等, 1998)。11 週齡雜交肉鵝之血漿 CHOL 及 HDL 含量均顯著較 6 週齡者為高 (Áprily, 2009)。白色庫德鵝血清之脂肪、CHOL 及脂肪酸 (C16:0、C18:1 及 C18:2) 含量受品種及性別影響 (Rosinski *et al.*, 1999)。母鵝開產至第 16 週期間之血清 TG 濃度為 300 – 1,200 mg/dL，顯著較休產時者高 (王等, 2002)。上述資料顯示鵝隻年齡與性別間之血液成分具有差異性存在。本試驗資料如表 4 所示，顯示 8 週齡具飛機翼與否鵝隻之血液生理值間無顯著性差異。8 週齡公鵝之血清 CHOL、HDL-C 及 LDL-C 含量均顯著較母鵝者為高，分別為 175 與 156 mg/dL、72.2 與 62.7 mg/dL 及 74.4 與 63.3 mg/dL；但 8 週齡公鵝之血清 BUN 含量有較低趨勢 (3.29 vs. 3.53 mg/dL)。如表 5 所示，14 週齡具有飛機翼與否鵝隻之血清生理值無顯著差異。14 週齡公鵝之血清 CHOL 及 HDL-C 含量均顯著較母鵝者為高 (163 vs. 149 mg/dL; 68.7 vs. 59.4 mg/dL)。膽固醇是動物體內許多荷爾蒙之基本結構，但血液中過多的膽固醇易引起心臟血管方面的疾病，由飼料攝取之膽固醇，經血流重新在肝臟合成，所以肝臟是體內膽固醇的最大來源。因此，血清 CHOL 含量可用來評估脂肪的代謝狀

態，特別是針對冠狀動脈疾病的高危險群，且其成分中之 HDL-C 被認為是好的 CHOL，可防止血管阻塞。依上述資料顯示，鵝隻外觀上具飛機翼與否並不影響其血清生理值，公鵝血清 CHOL 及 HDL-C 含量較母鵝者為高，可能係鵝隻性別影響脂肪代謝作用之故。

表 3. 飼養密度對 14 週齡鵝隻血液生化值之影響

Table 3. Effects of stocking density on blood biochemical parameters of geese at 14 weeks old

Item	Stocking Density			SEM
	HD	MD	LD	
GOT	68.0	60.1	50.3	9.58
GPT	25.5	20.5	18.5	4.09
TP	4.81	4.52	4.32	0.151
ALB	1.96	1.90	1.87	0.047
GLU	145	149	152	9.30
BUN	5.92	6.05	5.72	0.246
UA	2.27 ^b	2.46 ^{ab}	2.91 ^a	0.131
CRE	0.20	0.22	0.21	0.011
TG	164 ^{ab}	190 ^a	140 ^b	7.81
CHOL	160 ^x	159 ^x	146 ^y	4.02
HDL-C	65.2	64.6	62.1	3.58
LDL-C	54.7	50.8	49.6	2.39
WBC	196 ^b	212 ^{ab}	219 ^a	4.79
RBC	1.68	1.69	1.69	0.066
HCT	29.0	28.0	28.3	0.63
MCV	165	166	167	2.00
MCH	59.7	62.1	63.1	1.49
MCHC	36.2	37.4	37.9	1.01
PLT	9.01	11.9	10.2	0.671
PDW	6.09	7.16	7.48	0.407
MPV	7.76	7.72	7.54	0.147
PLCR	17.0	16.9	15.8	0.81

LD: Low stocking density, MD: Middle stocking density, HD: High stocking density, SEM: Standard error of means of stocking density.

GOT: Glutamate oxaloacetate transaminase (u/L), GPT: Glutamate pyruvate transaminase (u/L), TP: Total protein (g/dL), ALB: Albumin (g/dL), GLU: Glucose (mg/dL), BUN: Blood Urea Nitrogen (mg/dL), UA: Uric acid (mg/dL), CRE: Creatine (mg/dL), TG: Triglycerides (mg/dL), CHOL: Cholesterol (mg/dL), HDL-C: High density lipoprotein-cholesterol (mg/dL), LDL-C: Low density lipoprotein-cholesterol (mg/dL), WBC: White blood cell ($10^3/\mu\text{L}$), RBC: Red blood cell ($10^6/\text{ul}$), HCT: Hematocrit (%), MCV: Mean corpuscular volume (fL), MCH: Mean corpuscular hemoglobin (pg), MCHC: Mean corpuscular hemoglobin concentration (g/dL), PLT: Platelet ($10^3/\text{uL}$), PDW: Platelet distribution width (%), MPV: Mean platelet volume (fL), PLCR: Platelet large cell ratio.

^{a, b} Means within the same row without the same superscripts differ significantly ($P < 0.05$).

^{x, y} Means within the same row without the same superscripts tend to differ ($P < 0.10$).

於 4 週齡鵝隻之血紅素及血球容積範圍分別為 9.68 – 10.33 mg/dL 及 32.5 – 33.75% (林等, 1997)。白羅曼鵝公鵝及母鵝之血紅素濃度分別為 11.3 – 15.2 g/dL 及 12.0 – 16.1 g/dL，並隨週齡之增加而濃度亦漸增，直至 18 週齡時達最高，隨後則降低；以品種為例，12 及 24 週齡時，白羅曼鵝公鵝之血紅素濃度顯著較白色中國鵝者為高，然母鵝則反之 (邱等, 1998)。野雉血液中紅血球數、血紅素、血容積比、總蛋白質及白蛋白含量亦隨年齡而增加 (Kececi and Col, 2011)。1.5 – 2 歲齡蘇丹土公雞之紅血球數、血紅素含量及 MCV 均與母雞者差異顯著 (Elagib and

Ahmed, 2011)。本試驗結果如表 4 所示，8 週齡具飛機翼鵝隻之血液 HCT (公與母鵝之平均值) 較正常翼者有較高之趨勢 (34.0 vs. 32.6%)，而 8 週齡公鵝之血液 MPV (具飛機翼與正常翼鵝隻之平均值) 顯著較母鵝者為高 (7.95 vs. 7.36 fL)，其公鵝之血液 PLCR (具飛機翼與正常翼鵝隻之平均值) 則較母鵝者有較高之趨勢 (17.7 vs. 15.2)。另如表 5 所示，14 週齡具飛機翼鵝隻，血液 PLT (公與母鵝之平均值) 及 PDW (公與母鵝之平均值) 均較正常翼者有較低之趨勢 (9.38 vs. $12.0 \times 10^3/\mu\text{L}$ 及 6.59 vs. 7.35%)，而 14 週齡公鵝之血液 PDW (具飛機翼與正常翼鵝隻之平均值) 顯著較母鵝者有較低之趨勢 (6.63 vs. 7.32%)，其公鵝之血液 MCHC (具飛機翼與正常翼鵝隻之平均值) 顯著較母鵝者為低 (36.2 vs. 37.8 g/dL)。亦即白羅曼鵝 8 週齡之血液 MPV 與 14 週齡之血液 MCHC 均受性別之影響。

表 4. 飛機翼與性別對鵝隻 8 週齡血液生化值之影響

Table 4. Effects of angel wing and sex on blood biochemical parameters of geese at 8 weeks old

	Wing style					Significance ²		
Item	AW		NW		SEM ¹	W	S	W × S
	Male	Female	Male	Female				
GOT	47.7	47.1	44.6	47.6	3.47	NS	NS	NS
GPT	14.3	14.3	11.7	14.9	1.41	NS	NS	NS
TP	5.59	5.69	5.84	5.37	0.162	NS	NS	†
ALB	2.20	2.29	2.20	2.13	0.054	NS	NS	NS
GLU	88.0 ^a	55.2 ^b	73.9 ^{ab}	86.1 ^a	6.98	NS	NS	**
BUN	3.16 ^b	3.76 ^a	3.42 ^{ab}	3.30 ^b	0.140	NS	†	*
UA	3.35	3.40	3.02	3.19	0.190	NS	NS	NS
CRE	0.23	0.26	0.27	0.24	0.016	NS	NS	†
TG	131	140	131	129	5.93	NS	NS	NS
CHOL	178	157	173	154	5.85	NS	**	NS
HDL-C	73.2	61.2	71.3	64.3	3.35	NS	**	NS
LDL-C	76.2	64.3	72.6	62.4	3.79	NS	**	NS
VITC	8.61	8.11	8.82	7.46	0.907	NS	NS	NS
WBC	252	250	250	251	1.99	NS	NS	NS
RBC	2.00	1.93	1.90	1.90	0.039	NS	NS	NS
HCT	34.5	33.5	32.8	32.4	0.749	†	NS	NS
MCV	173	173	173	171	1.42	NS	NS	NS
MCH	64.0	65.0	64.5	67.1	1.22	NS	NS	NS
MCHC	37.1	37.6	37.4	39.4	0.81	NS	NS	NS
PLT	7.65	8.56	10.4	9.27	1.086	NS	NS	NS
PDW	6.24	6.85	7.07	6.43	0.48	NS	NS	NS
MPV	8.13	7.39	7.78	7.33	0.21	NS	*	NS
PLCR	19.1	15.4	16.4	15.0	1.12	NS	†	NS

W: Wing style, S: Sex, W × S: The interaction of wing style with sex, AW: Angel wing, NW: Normal wing.

¹ SEM: Standard error of means. ² NS: Not significantly different or $P > 0.10$; † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

GOT: Glutamate oxaloacetate transaminase (u/L), GPT: Glutamate pyruvate transaminase (u/L), TP: Total protein (g/dL), ALB: Albumin (g/dL), GLU: Glucose (mg/dL), BUN: Blood Urea Nitrogen (mg/dL), UA: Uric acid (mg/dL), CRE: Creatine (mg/dL), TG: Triglycerides (mg/dL), CHOL: Cholesterol (mg/dL), HDL-C: High density lipoprotein-cholesterol (mg/dL), LDL-C: Low density lipoprotein-cholesterol (mg/dL), WBC: White blood cell ($10^3/\mu\text{L}$), RBC: Red blood cell ($10^6/\mu\text{L}$), HCT: Hematocrit (%), MCV: Mean corpuscular volume (fL), MCH: Mean corpuscular hemoglobin (pg), MCHC: Mean corpuscular hemoglobin concentration (g/dL), PLT: Platelet ($10^3/\mu\text{L}$), PDW: Platelet distribution width (%), MPV: Mean platelet volume (fL), PLCR: Platelet large cell ratio.

^{a, b} Means within the same row without the same superscripts differ significantly ($P < 0.05$).

表 5. 飛機翼與性別對 14 週齡鵝隻血液生化值之影響

Table 5. Effects of angel wing and sex on blood biochemical parameters of geese at 14 weeks old

Item	Wing style				SEM ¹	Significance ²		
	AW		NW			W	S	W × S
	Male	Female	Male	Female				
GOT	51.5	60.9	57.9	74.6	12.4	NS	NS	NS
GPT	19.0	21.3	18.3	36.5	8.23	NS	NS	NS
TP	4.48	4.47	4.50	5.04	0.27	NS	NS	NS
ALB	1.85	1.92	1.87	2.10	0.10	NS	NS	NS
GLU	157	138	150	147	10.0	NS	NS	NS
BUN	6.11	5.89	5.78	6.24	0.28	NS	NS	NS
UA	2.80	2.50	2.38	2.51	0.19	NS	NS	NS
CRE	0.19	0.21	0.21	0.22	0.01	NS	NS	NS
TG	167	158	155	174	10.8	NS	NS	NS
CHOL	163	155	162	143	5.44	NS	*	NS
HDL-C	67.4 ^x	63.2 ^{xy}	70.0 ^x	55.7 ^y	3.02	NS	**	†
LDL-C	54.2	52.8	54.5	46.6	3.25	NS	NS	NS
WBC	190	218	210	213	12.1	NS	NS	NS
RBC	1.54	1.68	1.71	1.71	0.11	NS	NS	NS
HCT	29.6	27.5	28.2	28.3	1.07	NS	NS	NS
MCV	170 ^a	164 ^b	164 ^b	166 ^{ab}	1.80	NS	NS	*
MCH	59.9	62.6	61.2	62.1	1.20	NS	NS	NS
MCHC	35.2 ^y	38.2 ^x	37.2 ^{xy}	37.5 ^x	0.75	NS	*	†
PLT	9.26	9.50	11.6	12.4	1.51	†	NS	NS
PDW	6.21	6.97	7.05	7.66	0.37	†	†	NS
MPV	7.42	7.78	7.53	7.59	0.20	NS	NS	NS
PLCR	14.7	17.1	15.5	16.8	1.22	NS	NS	NS

W: Wing style, S: Sex, W × S: The interaction of wing style with sex, AW: Angel wing, NW: Normal wing.

¹ SEM: Standard error of means. ² NS: Not significantly different or $P > 0.10$; † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

GOT: Glutamate oxaloacetate transaminase (u/L), GPT: Glutamate pyruvate transaminase (u/L), TP: Total protein (g/dL), ALB: Albumin (g/dL), GLU: Glucose (mg/dL), BUN: Blood Urea Nitrogen (mg/dL), UA: Uric acid (mg/dL), CRE: Creatine (mg/dL), TG: Triglycerides (mg/dL), CHOL: Cholesterol (mg/dL), HDL-C: High density lipoprotein-cholesterol (mg/dL), LDL-C: Low density lipoprotein-cholesterol (mg/dL), WBC: White blood cell ($10^3/\mu\text{L}$), RBC: Red blood cell ($10^6/\mu\text{L}$), HCT: Hematocrit (%), MCV: Mean corpuscular volume (fL), MCH: Mean corpuscular hemoglobin (pg), MCHC: Mean corpuscular hemoglobin concentration (g/dL), PLT: Platelet ($10^3/\mu\text{L}$), PDW: Platelet distribution width (%), MPV: Mean platelet volume (fL), PLCR: Platelet large cell ratio.

^{a, b} Means within the same row without the same superscripts differ significantly ($P < 0.05$).

^{x, y} Means within the same row without the same superscripts tend to differ ($P < 0.10$).

於 1 – 70 日齡揚州鵝之十二指腸、空腸及迴腸之絨毛高度及腺窩深度隨日齡增加而增加，70 日齡小腸各段之絨毛高度及腺窩深度範圍分別為 846 – 1,003 μm 及 220 – 235 μm (Liu *et al.*, 2010)。增加絨毛高度可增加絨毛表面積，以利營養分吸收 (Caspary, 1992)。火雞之小腸腺窩深度越深，表示當絨毛受到病菌侵襲後，絨毛組織周轉 (turnover) 較為快速 (Yason *et al.*, 1987)。許多外在因素使動物小腸絨毛上皮細胞脫落速度增快及絨毛萎縮，例如微生物感染或轉換成固態飼料等。小腸絨毛上皮細胞是由腺窩細胞分化而來，當其脫落會刺激腺窩細胞增生使腺窩深度增加。本試驗結果顯示，14 週齡具飛機翼鵝隻之十二指腸腺窩深度顯著較正常翼者為淺 (193 vs. 244 μm , $P < 0.05$)，表示具飛機翼鵝隻之十二指腸絨毛上皮細胞脫落速度並未較正常翼者快，其餘腸道組織，兩者間差異不大 (如表 6 所示)，表示具飛機翼鵝隻之腸道組織相較於正常翼者，並無腸道受損現象。

表 6. 飛機翼與性別對 14 週齡鵝隻腸道組織之影響

Table 6. Effects of angel wing and sex on intestinal tissue of geese at 14 weeks old

Item	Wing style		SEM ¹	Sex		SEM ²	Significance ³		
	AW	NW		Male	Female		W	S	W × S
Duodenum									
Villus height, μm	944	857	74.5	974	828	74.9	NS	NS	NS
Crypt depth, μm	193 ^b	244 ^a	15.6	215	221	15.7	*	NS	NS
Jejunum									
Villus height, μm	721	756	55.3	692	786	55.3	NS	NS	NS
Crypt depth, μm	205	189	17.9	194	201	17.9	NS	NS	NS
Ileum									
Villus height, μm	822	863	46.8	794	891	46.8	NS	NS	NS
Crypt depth, μm	206	249	18.1	235	220	18.1	NS	NS	NS

W: Wing style, S: Sex, W × S: The interaction of wing style with sex, AW: Angel wing, NW: Normal wing.

¹ SEM: Standard error of means of wing style.

² SEM: Standard error of means of sex.

³ NS: Not significantly different or $P > 0.10$; * $P < 0.05$.

^{a, b} Means within the same row under wing style without the same superscripts differ significantly ($P < 0.05$).

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Effects of stocking density, angel wing and sex on feather weight, blood biochemical parameters and intestinal tissue in White Roman geese ⁽¹⁾

Min-Jung Lin ⁽²⁾ Kwo-Chin Wu ⁽³⁾ Yu-Shine Jea ⁽⁴⁾ and Shen-Chang Chang ^{(5) (6)}

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Abstract

This study was to investigate the effects of stocking density, angel wing (AW) and sex on body weight, feather weight, intestinal tissue and blood biochemical parameters in White Roman geese. The medium and high stocking density treatment groups were 1.33 and 1.66 times the low density group respectively. The experiment was conducted under a split-plot design which consisted of three stock densities randomly assigned in 12 pens and a total of 384 geese were collected. There were 4 pens in each group, and the numbers of male and female geese in each pen were equal. The pen was the main plot and the bird was the subplot. Each pen was divided into 2 genders (male and female geese) \times 2 species of geese (whether with angel wing or not). In the experiment, natural light was used, and both feed and drinking water were provided ad libitum. The results showed that there were no influences between the 3 stocking densities in the incidence of AW of geese. The birds with AW tended to have a higher body weight after eighteen hours feed-deprived at 14 weeks old, than those with normal wing (NW) ($P < 0.10$). The birds with AW had a significantly lighter feather weight on left wing at 14 weeks old, than those in NW ($P < 0.05$). The ganders had a significantly lighter feather weight in the left wing at 14 weeks old than those in female geese ($P < 0.001$).

Low stocking density of White Roman geese had a significantly higher uric acid (UA) contents of serum at 14 week old than those in high stocking density ($P < 0.05$), and tended to have a lower cholesterol (CHOL) contents of serum than those in medium and high stocking density ($P < 0.10$). The ganders had a significantly higher CHOL, high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) contents of serum at 8 week old than those in female geese; and the ganders also had a significantly higher CHOL and HDL-C contents of serum at 14 weeks old than those in the female geese. The birds with AW had a significantly shallower crypt depth of duodenum at 14 weeks old than those with NW ($P < 0.05$). In conclusion, stocking density of White Roman geese could affect UA and CHOL of serum. The birds with AW had a lighter feather weight on wings than those with NW. The body weight and blood biochemical parameter of White Roman geese were affected by sex.

Key words: Angel wing, Feather, Intestinal tissue, Sex, Stocking density, White Roman geese.

(1) Contribution No. 2665 from Livestock Research Institute, Council of Agriculture, Executive Yuan.

(2) Changhua Animal Propagation Station, COA-LRI, Changhua 52149, Taiwan, R. O. C.

(3) Retired from Changhua Animal Propagation Station, COA-LRI, Changhua 52149, Taiwan, R. O. C.

(4) Retired from Hsinchu Branch, COA-LRI, Miaoli 36841, Taiwan, R. O. C.

(5) Kaohsiung Animal Propagation Station, COA-LRI, Pingtung 91247, Taiwan, R. O. C.

(6) Corresponding author, E-mail: macawh@mail.tlri.gov.tw.

Immunocastration of boars with GnRH-rPE conjugated antigen ⁽¹⁾

Jen-Wen Shiau ⁽²⁾⁽⁸⁾ Jenn-Fa Liou ⁽³⁾ Lih- Ren Chen ⁽³⁾ Szu-Han Wang ⁽⁴⁾
Tswen- Keit Tang ⁽⁵⁾ Chein Tai ⁽⁶⁾ and Huey-Lang Yang ⁽⁷⁾

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Abstract

Gonadotropin-releasing hormone (GnRH) is a self-antigen and a small molecule that usually could not elicit immune response. To generate an antibody against endogenous GnRH for immunocastration, it is necessary to link GnRH to immuno-carriers for increased immunogenicity. *Pseudomonas aeruginosa* is an opportunistic pathogen often causing infection in domestic animals. The toxicity of *Pseudomonas* is mainly caused by exotoxin A which is an adenosine diphosphate (ADP)-ribosyl transferase. A truncated nontoxic *Pseudomonas aeruginosa* exotoxin A (rPE) modified by gene deletion was conjugated chemically with GnRH, and the GnRH-rPE conjugate was used as an immunogen to immunize boars. The results showed that boars immunized with GnRH-rPE conjugated successfully and induced the generation of anti-GnRH and anti-PE antibodies. The immune response against GnRH resulted in the atrophy of various male reproduction related organs, in conjunction with a significantly decreased serum testosterone concentration to a level similar to castrated boars, which persisted until the end of the study (29 weeks) when boars were ready for release to the market. These results demonstrated that GnRH-rPE conjugates can be used to produce a potential castration vaccine for domestic animals.

Key words: Immunocastration, Gonadotropin-releasing hormone (GnRH), *Pseudomonas aeruginosa* (PE), Boars.

(1) Contribution No. 2666 from Livestock Research Institute, Council of Agriculture, Executive Yuan.

(2) Breeding and Genetics Division, COA-LRI, Tainan 71246, Taiwan, R. O. C.

(3) Physiology Division, COA-LRI, Tainan 71246, Taiwan, R. O. C.

(4) Hsinchu Branch, COA-LRI, Miaoli 36841, Taiwan, R. O. C.

(5) Department of Nursing, National Quemoy University, Kinmen 89250, Taiwan R. O. C.

(6) Department of Business Administration, Kun Shan University, Tainan 71003, Taiwan R. O. C.

(7) Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan 70143, Taiwan R. O. C.

(8) Corresponding author, E-mail: jwshiau@mail.tlri.gov.tw.

Introduction

Although non-castrated boars have a better growth efficiency, leaner carcasses than barrows, improved feed conversion and feed efficiency, and thus reduced production costs, the majority of male piglets are still surgically castrated to reduce aggression and sexual behaviors (Rydmher *et al.*, 2010). Besides, the most relevant motivation for castration is the need to reduce the boar taint in the meat of male pigs (Brunius *et al.*, 2011). Testosterone has been suggested to be the cause of boar taint (Bonneau *et al.*, 1992). The surgical castration not only contradicts present attitudes of animal welfare, but also causes infections, stress and growth retardation, reduced carcass quality, and even the loss of animals. To circumvent the disadvantages of surgical castration, the development of a more efficient and suitable method to eliminate boar taint is critical (Brunius *et al.*, 2011).

GnRH, an evolutionarily conserved decapeptide, is produced in the hypothalamus and released to the capillary blood within the median eminence, and eventually binds to the gonadotropes in pituitary. The binding of GnRH to the gonadotropes induces the synthesis and release of LH and FSH from the pituitary (Schally *et al.*, 1973; Braden *et al.*, 1989). In male animals, LH regulates testosterone synthesis and FSH is necessary for the initiation and maintenance of spermatogenesis. Testosterone is needed for spermatogenesis and the development of secondary sexual characteristics including male behaviours such as territorial marking, mounting, and aggressiveness (Mitjana *et al.*, 2020). In female animals, both LH and FSH are required to stimulate the ovarian activities leading to ovulation and successful pregnancy.

Pseudomonas aeruginosa is an opportunistic pathogen, which often causes infection in domestic animals that have their skin barrier breached. The toxicity of *Pseudomonas aeruginosa* is caused primarily by the exotoxin A (PE), an ADP-ribosyl transferase (Hwang *et al.*, 1987). A truncated and modified form of PE has been produced and validated as a more potent immunotoxin (Qaiser *et al.*, 2018). In addition, the *Pseudomonas* exotoxin has been suggested as an excellent biological adjuvant (Fattom *et al.*, 1995); we have also observed that the injection of recombinant detoxicated PE protein (rPE) as a immunostimulatory agent in the vaccine could elicit sufficient immune responses to protect mice from the intoxication by a high dosage of native *Pseudomonas* exotoxin (Shiau *et al.*, 2001).

Since GnRH is a hapten and a self- antigen, GnRH or GnRH analogues must be linked to a suitable carrier molecule in order to obtain immunogenicity for antibody production (Ladd *et al.*, 1988). It has been demonstrated that the active immunization against GnRH could be used as an alternative castration in addition to a surgical method. Immunological suppression of gonadotropin and androgen depletion results in the impairment of reproductive functions in males. The utilization of GnRH as an antigen for immuno-suppression of reproduction has been successfully demonstrated by using analogues conjugating to large molecules such as ovalbumin or tetanus toxin (Talwar and Raghupathy, 1989). The administration of GnRH-carrier induces anti-GnRH antibodies prevents gonadotropin release, and in turn decreases testicular size and inhibits both spermatogenesis and gametogenesis leading to infertility (Mitjana *et al.*, 2020). Anti-GnRH vaccines have been developed for the treatment of hormone-dependent malignancies and non-surgical castration in animals (Adams *et al.*, 1993; Thompson, 2000; Noya *et al.*, 2020). Accordingly, the aim of this study was to immunize the domestic boars with GnRH-rPE conjugate for its immuno-suppression effects on the sex gland repression and potential as a castration vaccine.

Materials and Methods

I. Bacterial strains and plasmid

Escherichia coli strain HB101 was used as the host for the experiments and BL21 (DE3) was used as the host for the expression of recombinant PE. In the pJH4 plasmid construct (Hwang *et al.*, 1987), the structural gene of PE was inserted on a T7 promoter. The PE gene with a deletion in the C-terminal coding for the 533 to 613 amino acid was inserted downstream to a T7 promoter, ribosome binding site and its accompanying ATG initiation codon. Consequently, the production of PE could be induced upon the addition of Isopropyl β -D-1-thiogalactopyranoside (IPTG) to drive the expression of T7 RNA polymerase gene that was conferred on the *E. coli* BL 21 (DE3) chromosome under the control of a *lac* promoter.

To produce the recombinant “detoxicated” PE toxin protein, BL21 (DE3) cells carrying the pJH4 plasmid were

grown at 37°C to an $OD_{650} = 0.3$. IPTG was added at the final concentration of 1 mM to induce T7 RNA polymerase and the incubation was continued for 2 hrs. Subsequently, the PE gene was turned off by the synthesis of T7 RNA polymerase. The proportion of protein produced by pJH4 induced with IPTG could reach as high as 20% of total *E. coli* protein (Fig. 1). PE protein was then isolated as an inclusion body and solubilized with the refolding kit (Novagen).

II. Conjugation of GnRH to PE

The rPE protein synthesized by *E. coli* was used as a carrier protein for chemical conjugation. A modified GnRH decapeptide with lysine substitutions on amino acid position six (D-Lys⁶-LH-RH) (Sigma) was conjugated to purified rPE at 1:5 (W/W) by chemical method using the amine-carboxylic conjugation kit (Pierce). The success of conjugation was determined by the molecular weight shift and by the Western blot using anti-GnRH and anti-PE antibody on 13% SDS-PAGE gel, respectively (Fig. 2). The GnRH-rPE conjugate was then used as an immunogen emulsified in the Complete Freund Adjuvant (CFA, Gibco) and Incomplete Freund Adjuvant (IFA, Gibco).

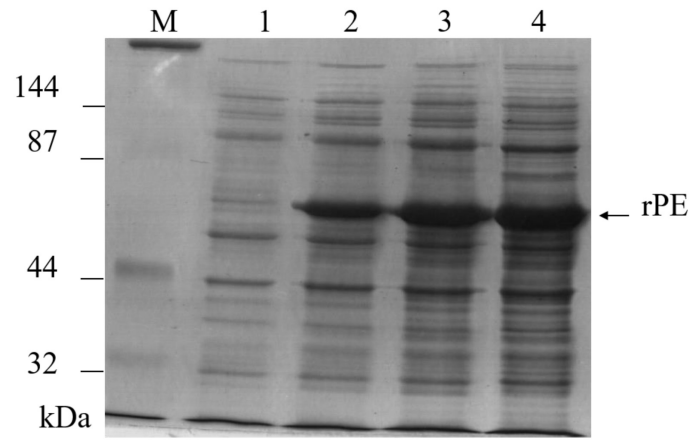


Fig. 1. The expression of rPE protein in *Escherichia coli* strain BL21 (DE3) after IPTG induction at 1 to 4 hrs. (lane 1-4). Lane M indicates the molecular weight marker.

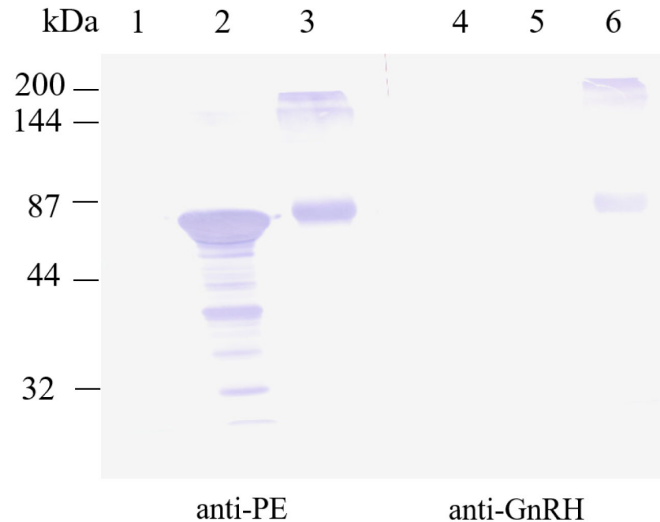


Fig. 2. Identification of the rPE and GnRH-rPE conjugate by Western blotting with antibodies to PE (lanes 1 - 3) and GnRH (lanes 4 - 6), respectively. Lanes 1 and 4 were negative controls. Lanes 2 and 5 were rPE proteins. Lanes 3 and 6 were GnRH-rPE conjugates.

III. Vaccination and serum sample collection

Sixteen crossbred (Landrace × Yorkshire × Duroc) boars were randomly divided into two groups. Boars in the control group were mock vaccinated with 1 mL PBS in CFA at 9 weeks old, and were boosted with 1 mL PBS in IFA at 17 weeks old. Boars of the vaccinated group were injected subcutaneously with 1 mg of GnRH-rPE conjugate dissolved in 1 mL of PBS and emulsified with equal volume of CFA. Booster dose in IFA with the same vaccination schedule was

performed as those of the control group.

Blood samples were collected every week from the jugular vein for determination of serum testosterone concentrations and the titer of antibodies against GnRH and PE until the end of 29 weeks of age. Sera were obtained by centrifugation separation ($1,300 \times g$ for 10 min) and stored at -20°C before assay.

IV. Serum testosterone assays

The concentrations of serum testosterone were measured by radioimmunoassays (RIAs) according to the method described by Li *et al.* (1987).

V. Detection of Antibody to GnRH and PE

Antibody titers to GnRH and PE in boars immunized against GnRH-rPE were evaluated by ELISA. Each well of the microtiter plates (Nunc 467466) was coated with $100 \mu\text{L}$ rPE protein conjugated with keyhole limpet hemocyanin (KLH) at a concentration of $5 \mu\text{g/mL}$ in 0.1 mL of 0.05 M carbonate bicarbonate coating buffer ($\text{pH } 9.6$) for overnight at 4°C . Thereafter, the plates were washed with PBS/T (0.5% Tween-20 in PBS, v/v), and blocked with a 0.5% (w/v) bovine serum albumin (BSA) solution in PBS overnight and then stored at 4°C .

The plates were washed twice with PBS/T before use. Each serum sample was diluted 1,000 and 4,000 folds, then added to a GnRH-KLH and a rPE-KLH coated well, and incubated at room temperature for two hours. The plates were washed four times with PBS/T to remove the serum sample and incubated with the goat-anti-mouse IgG (Sigma, diluted 1:10,000 in PBS) for two hrs at room temperature. Plates were washed four times with PBS/T before adding the alkaline phosphatase. Reaction was performed by incubating at 37°C for 30 min. After the addition of chromogens, the reaction was terminated by the addition of 3 N NaOH and was assayed in a microtiter plate reader (Molecular Device) to measure the absorption at 405 nm .

VI. Sex gland weights and preparations

All boars were slaughtered at the end of 29 week of age. Testes and accessory reproductive organs (epididymis, prostate seminal vesicles, and bulbo-urethral glands) were dissected, weighted, and immediately fixed in Bouin's solution (HT10132, Sigma -Aldrich) for the subsequent histological examination. Fixed tissues were embedded in the paraplast, sectioned into $5 \mu\text{m}$ slices, mounted individually on microscope slides, and stained with H & E for the histological evaluation under a light microscopy.

VII. Statistical analysis

Differences between groups were tested by one-way analysis of variance, and pairwise multiple comparisons were tested with the Chi-square method. The level of significance was $P < 0.05$.

Results

I. Antibody titer and testosterone concentration

Boars were immunized against GnRH by injections at weeks 9 and 17. All the GnRH-rPE immunized boars produced antibodies against GnRH (A) and PE (B) as evidenced by the serum antibody titers after two vaccinations (Fig. 3). The serum antibody titers of the GnRH-rPE immunized boars dropped slowly after booster immunization. No antibody titer against GnRH and PE was detected in the serum of control boars that were mock immunized injected with PBS/CFA (Fig. 3). This high antibody titer also coincided with the decrease of serum testosterone concentrations to a non-detectable level (Fig. 4), indicating a success of neutralization of endogenous GnRH. The mean of serum testosterone concentration decreased to $< 0.1 \text{ ng/mL}$ in GnRH-rPE immunized boars by week 17 and consistently remained at lower levels than that of the boars in the control group until the end of trial (Fig. 4).

II. The development of reproductive organs after immunization

GnRH-rPE immunized boars exhibited smaller sex glands as shown in Table 1 and in Fig. 5. The weight of testes, epididymis, seminal vesicles, prostate glands, and bulbo-urethral glands were significantly decreased, with a range from 90.8% for bulbo-urethral glands to that of 71.4% for seminal vesicles (Table 1). The size and weight of testis of immunized animals were significantly less than those of the control bars. Thus, the normal development of the

reproductive system in GnRH-rPE treated boars was blocked by active immunization against GnRH.

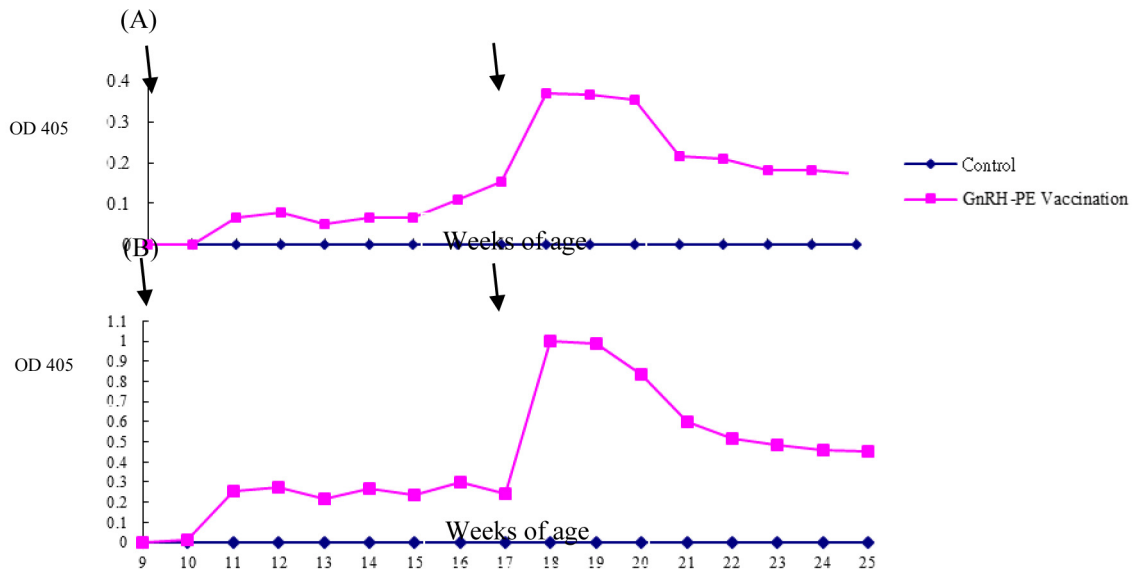


Fig. 3. Detection of GnRH and PE-reactive antibodies in the GnRH-rPE vaccinated and control boars by ELISA. Changes of anti-GnRH (A) and anti-PE (B) antibody titers at 1: 1,000 (A) and 1: 4,000 (B), respectively. Arrows indicate the time of primary and booster immunization.

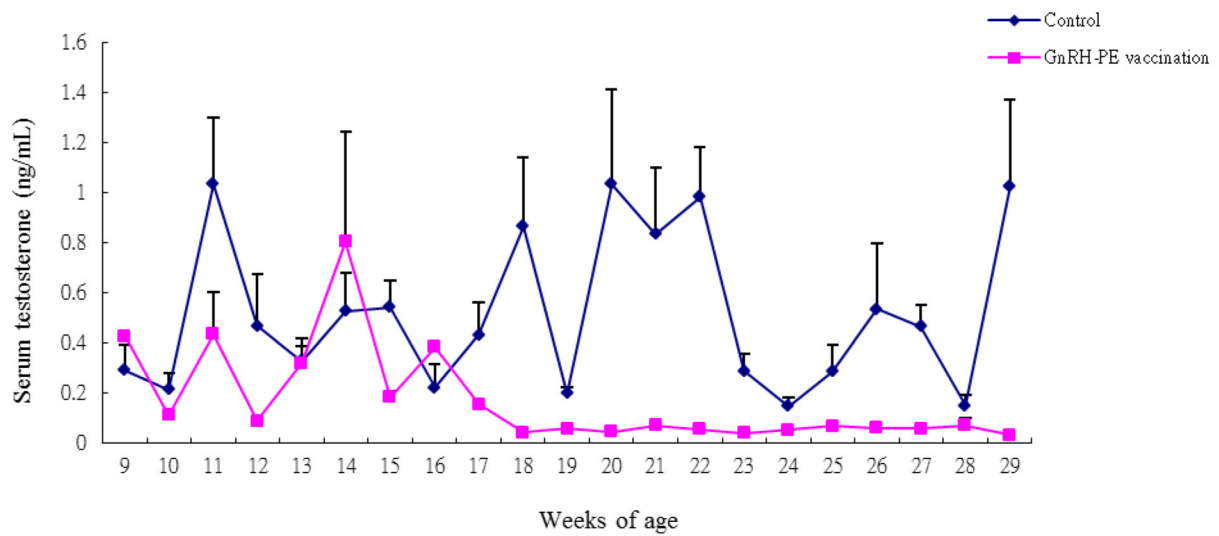


Fig. 4. Changes of serum testosterone concentration (ng/mL) in GnRH-rPE immunized and control boars. All animals were vaccinated at the age of 9 and 17 weeks. Values represent means \pm S.E of animals in the treatment group and in the control group. The intra- and inter-assay coefficients of variation (CV) of testosterone was 4.0% and 4.7%, respectively.



Fig. 5. Testes of the control (right) and GnRH-rPE immunized boars (left) at 29 week of age.

Table 1. The comparison of weight of testes, epididymis, seminal vesicles, prostate glands, and bulbo-urethral glands between the GnRH-rPE vaccinated and the control LYD boars at 29 week of age

	Mean \pm SE Weight (g) of reproductive organs		
	Vaccinated	Control	% of suppression
Number of pigs	8	8	
Testes	53.3 \pm 27.5 ^a	345.3 \pm 90.0 ^b	84.6
Epididymis	7.2 \pm 8.7 ^a	145.0 \pm 50.3 ^b	81.2
Seminal vesicles	3.2 \pm 1.5 ^a	75.8 \pm 27.1 ^b	71.4
Prostate glands	1.9 \pm 1.1	11.2 \pm 9.5	83.1
Bulbo-urethral glands	10.6 \pm 5.8 ^a	115.2 \pm 34.4 ^b	90.8

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

III. Histologic analysis

Histologic evaluations with the cross-sections of testis showed remarkable differences between the control and GnRH-rPE immunized boars (Fig. 6). In the control boars, the interstitial tissue was completely filled with distinct Leydig cells, and seminiferous tubules showed various stages of cycle of the seminiferous epithelium. The completion of spermatogenesis was evidenced by the presence of elongated spermatids in the lumen.

In contrast, as indicated by histologic observations, GnRH-rPE immunized boars showed a thickened basement membrane and reduced diameter in the seminiferous tubules. Although some pachytene spermatocytes were present in the seminiferous tubules of GnRH-rPE immunized boars, the spermatocytes appeared to undergo degenerating. Besides, motile semen samples could not be obtained from any of the GnRH-rPE immunized boars. There were no elongated spermatids present in the lumen. The nuclei of the Sertoli cells had a shrunk morphology and their cytoplasm were vacuolated while the Leydig cells had an atrophic morphology. Accordingly, the spermatogenesis was impaired in the testis of GnRH-rPE immunized boars. These findings suggest the potential of GnRH-rPE immunization as an alternative method to castration.

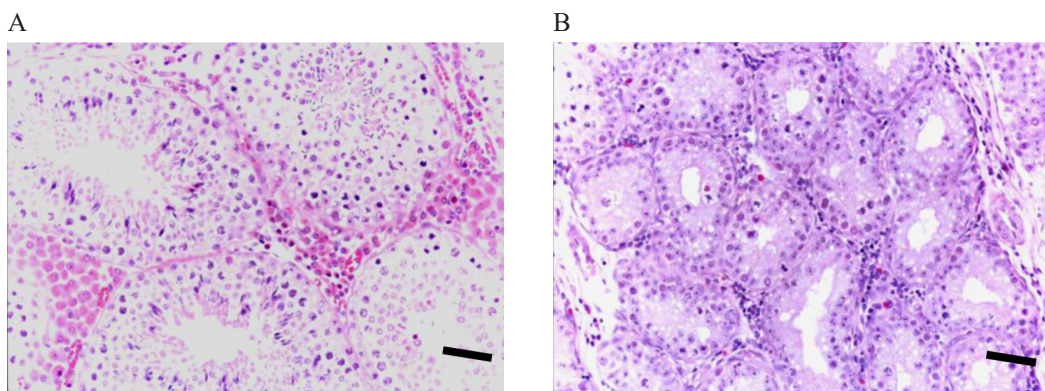


Fig. 6. Histological analysis (cross section) of testis from the control boars and the GnRH-rPE immunized LYD boars at 29 weeks of age. (A) Control boars, the completion of spermatogenesis was observed by the presence of elongated spermatids in the lumen. (B) GnRH-rPE immunized boars are absent with elongated spermatids in all tubules. The diameter of the seminiferous tubules was remarkably different between the control and immunized boars. Magnifications $\times 200$. Scale bars = 100 μ m.

Discussion

Active and passive immunization against the hypothalamic decapeptide GnRH have been proven as a potential tool for practical, on-farm applications in reproductive performance control as well as a very powerful tool for studying basic aspects of the hypothalamic-pituitary-gonadal axis (Thompson, 2000; Squires *et al.*, 2020). Reasons for such immunocastration include improvement of meat and carcass characteristics for cattle, sheep, goats, and swine; improvement on feed efficiency

relative to castrates in those species; reduction in male aggressive behaviors; reduction in male-associated odors in goats and swine; and fertility neutralization in pet species. Although application as a fertility control agent in men is unlikely, there were renewed interests in active immunization against GnRH for treating prostate cancers and related sex hormone-dependent disorders (Thompson, 2000)

GnRH is of particular importance, if completely neutralized, the fertility axis can be rendered totally ineffective (Goericke-Pesch, 2017). Since GnRH itself is too small for sufficient immunogenicity to induce antibodies against itself, a unique approach to fool the animal's immune system to recognize endogenous GnRH as foreign molecule can be achieved. GnRH has to be conjugated to large, non-self carrier molecules to become immunogenic. Commonly used carrier proteins include KLH (Goubau *et al.*, 1989), bovine serum albumin (BSA) (Fraser and Gunn, 1973), ovalbumin (Hoskinson *et al.*, 1990), glutaraldehyde (Hotzel *et al.*, 1997), toxoids (diphtheria, tetanus, or cholera) (Ladd *et al.*, 1990; Stevens, 1993), which in conjunction with an appropriate adjuvant can produce an effective immune response (Stevens, 1993). A commercial GnRH vaccine based on the GnRH-ovalbumin was available for ruminants (Adams *et al.*, 1993; Thompson, 2000; Karakuş *et al.*, 2013). In this study we used PE as the carrier protein to investigate the effect of GnRH-rPE on antibody production and testosterone levels, together with the efficacy of the conjugate in gonadal atrophy and fertility impairment. The results showed that the GnRH-rPE conjugate did induce specific antibodies against GnRH by vaccination. We also found that vaccination at 9 week of age, which is the early stage of the porcine lifespan, significantly inhibited the development of reproductive organs such as testis, epididymis, seminal vesicles, prostate glands and bulbo-urethral glands. Most important, the function of spermatogenesis was completely eliminated, and the synthesis of testosterone was also dramatically prevented. These effects indicated the immunoneutralization of GnRH by the induction of sufficient amounts of anti-GnRH antibody that was able to block GnRH in the transmitting path between the hypothalamus and pituitary.

In fact, novel uses of bacterial toxins as immune carrier, such as tetanus (Davis *et al.*, 1987), diphtheria (Allison and Gregoriadis, 1974), cholera (Peirre *et al.*, 1992) have been demonstrated that conjugation of a weak immunogen such as GnRH to bacterial toxins was a very promising approach. PE toxin is a single chain toxin with three major structural domains that inhibit protein synthesis in eukaryotic cells by catalysing ADP ribosylation of elongation factor 2. In addition, the induction of anti-PE antibody had a protective effect on mice against intoxication of the native *Pseudomonas* exotoxin A. Although we did not perform the exotoxin A intoxication experiment in boars, the vaccination of GnRH-rPE conjugate is very likely might provide the similar additional advantage to protect farm animals against *Pseudomonas* infection.

Adjuvants are commonly used in vaccine preparations to increase the immunogenicity of antigens (Charemtantanakul, 2020). The classic choice of adjuvant is FCA, an oil emulsion containing killed *Mycobacterium* components. The most successful GnRH immunization protocols have included the use of FCA. In contrast to FCA, boars immunized with alumina gel did not produce significant antibody titers (Donald and Thompson, 2000). However, FCA is not useful from a commercial standpoint due to its interference in tuberculin testing and the formation of undesirable lesions at the sites of injection in many species (Ferro and Stimson, 1998). Meloen *et al.* (1994) reported that the use of CFA/IFA in the castration vaccine formula had no systemic or adverse effects at the injection site in any of the treated animals. However, the animals treated with Freund's adjuvant in this study developed ulceration and abscesses at the site of injection.

The question remains of how GnRH is neutralized. Theoretically, free peptides could slowly diffuse out of the adjuvant and act as an agonist for GnRH, thus down-regulating its receptors on the pituitary and switching off reproductive functions. This mechanism would lead to an early disappearance of testosterone in serum. A second mechanism could be the induction of immune responses to GnRH-producing cells in the hypothalamic area and causing damage to GnRH-producing neurons. An additional mechanism could be the antibody directly reacted with GnRH when the GnRH molecules passed through the blood from the hypothalamic GnRH-producing cells to interact with the receptors on the pituitary (Donald and Thompson, 2000; Xu *et al.*, 2018).

Although the precise mechanism is unknown, our results showed the prospects for the development of a new generation, reliable immunomodulation vaccine that can be applied to endocrine and immune regulatory processes. This will be of importance to modulate the dysfunction in endocrine or immune regulation. The advantages of the anti-fertility vaccine against GnRH are the absence of need for the surgical removal of the testes and cost-effectiveness. The other aspect is to prevent prostatic and testicular carcinomas that frequently occur in aged male dogs and cats.

In most studies done with active production of antibodies against GnRH, endocrine changes as well as morphological

changes of gonads have been reported (Sad *et al.*, 1993). Active immunization against GnRH led to the suppression of testicular functions including steroidogenesis and spermatogenesis in boars. Serum testosterone levels closely followed the testicle size. The testicle size is directly proportional to its function. The role of testosterone in the qualitative and quantitative maintenance of both spermatogenesis and testicular morphologic integrity has been established.

Active immunization against GnRH is potentially useful as a nonsurgical method for the fertility regulation of boars. These results and several earlier reports of studies in laboratory animals indicated that this immunological birth control strategy is safe and the induced infertility is reversible (Donald and Thompson, 2000).

In conclusion, the results suggested that immunocastration is a potential tool for management in the pig industry. The results suggest a potential application using immunocastration as a substitute for surgical castration in the pig industry.

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應用激性腺素釋放素—重組綠膿桿外毒素接合抗原 進行 LYD 公豬之免疫去勢⁽¹⁾

蕭振文⁽²⁾⁽⁸⁾ 劉振發⁽³⁾ 陳立人⁽³⁾ 王思涵⁽⁴⁾ 唐存愷⁽⁵⁾ 戴謙⁽⁶⁾ 楊惠郎⁽⁷⁾

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摘 要

激性腺素釋放素 (Gonadotropin-releasing hormone, GnRH) 是一種小分子自體抗原，通常不會誘發免疫反應。本研究將 GnRH 聯結免疫攜帶蛋白以產生對抗 GnRH 之抗體。綠膿桿菌是一種機會型病原菌，其毒性主要源自外毒素 A，以基因剔除技術去除毒性之綠膿桿菌外毒素 (*Pseudomonas* exotoxin, PE) 可作為攜帶蛋白質，應用化學法接合 GnRH 成為重組型 GnRH-rPE，可供為疫苗進行公豬之免疫去勢。本研究於 LYD 公豬第 9 與 17 週齡時注射免疫去勢疫苗，並於第 29 週齡試驗結束時犧牲公豬，進行組織學與血清學等檢測。研究結果顯示，接種重組型 GnRH-rPE 去勢疫苗的公豬成功誘發分別對抗 GnRH 及 PE 的抗體，並導致公豬之相關生殖器官萎縮，血清中睪固酮含量亦降低至如同外科法去勢公豬的水準，並持續至 29 週齡試驗結束準備上市階段。本研究結果證明，重組型 GnRH-rPE 具有潛力開發成為家畜用之免疫去勢疫苗。。

關鍵詞：免疫去勢、激性腺素釋放素、綠膿桿菌、公豬。

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(2) 行政院農業委員會畜產試驗所遺傳育種組。

(3) 行政院農業委員會畜產試驗所生理組。

(4) 行政院農業委員會畜產試驗所新竹分所。

(5) 國立金門大學護理學系。

(6) 崑山科技大學企業管理學系。

(7) 國立成功大學生物科技與產業科學系。

(8) 通訊作者，E-mail: jwshiau@mail.tlri.gov.tw。

飼糧中添加香椿粉對肉鵝生長、免疫及屠體品質之影響⁽¹⁾

蕭智彰⁽²⁾⁽³⁾ 練慶儀⁽²⁾

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摘 要

本研究旨在探討飼糧中添加香椿粉對肉鵝生長、免疫及屠體品質之影響。試驗使用 8 週齡肉鵝 24 隻，逢機分配至對照組及額外添加 1% 香椿粉組，以欄為單位，每欄 4 隻，公母各半，3 重複。試驗期間，鵝隻每 2 週秤重 1 次，每 4 週採血 1 次，於 16 週齡進行肉鵝屠宰並分析胸肉品質。結果顯示，添加香椿粉對肉鵝生長、血液生化值及胸肉之 pH、L、a、b 值皆無顯著影響，惟顯著提升 16 週齡肉鵝血清 G 型免疫球蛋白濃度及胸肉多元不飽和脂肪酸比例，且感官品評亦有較佳之風味、嫩度及總可接受性 ($P < 0.05$)。綜上所述，飼糧中添加香椿粉對肉鵝生長雖無顯著改善效果，惟可提升其免疫力且使其肉質有較佳風味，可供特色化鵝隻生產使用。

關鍵詞：香椿、生長、G 型免疫球蛋白、脂肪酸。

緒 言

鵝係草食性且耐粗食之水禽，且為國內重要之禽肉來源，依據行政院農業委員會農業統計年報 (2019)，108 年度肉鵝屠宰量為 276 萬隻，主要品種為白羅曼鵝，市占率為 97.0%，其餘 3% 則是華鵝。而鵝肉係為一種高蛋白、低脂肪及低膽固醇的肉類，於食品與健康保健日益重視的今日，其消費量有逐年增加的趨勢 (Liu *et al.*, 2014)。隨著食品、飼料安全及動物福祉等問題持續受到關注，許多研究已在覓尋抗生素替代品 (Yang *et al.*, 2014)。而當今家禽生產者在不使用抗生素預防性投藥的情況下，面臨著疾病與保健等眾多挑戰。香椿 (*Toona sinensis*) 為多年生落葉性喬木植物，是一種著名的中草藥，具有廣泛的生物學功能如抗氧化活性及降血糖 (Liu *et al.*, 2014)，且在中醫學上具有解熱及保健等功效。在西醫研究上，香椿具有抗癌細胞效果、改善血中胰島素分泌量、舒緩疼痛、誘發血小板凝集反應等作用 (許及翁, 2010)。鵝肉在貯存過程中因其脂肪酸大多屬不飽和脂肪酸，易有酸化情形，且不同品種動物體內脂肪酸及胺基酸組成不同 (Cameron and Enser, 1991; Fisher *et al.*, 2000)，故如何預防氧化情形發生就非常重要。林等 (2014) 研究指出，飼糧添加 0.1 或 0.2% 之香椿並不影響白羅曼鵝生長性狀及血液生理值，可供特色化鵝隻生產用。有關飼糧中添加香椿粉對肉鵝生長、屠體性狀與肉質等影響之文獻甚少，本試驗之研究結果期能提供養鵝業者發展特色化鵝肉之參考。

材料與方法

本試驗於行政院農業委員會畜產試驗所彰化種畜繁殖場試驗鵝舍進行，試驗動物之使用、飼養管理及試驗內容皆經實驗動物照護與使用小組以畜試彰動字第 10103 號申請核准在案。

I. 試驗動物與飼養管理

使用 8 週齡肉鵝 (白羅曼鵝與白色華鵝雜交而成) 24 隻逢機分配至對照組及額外添加 1% 香椿粉組，以欄為單位，每欄 4 隻，公母各半，3 重複。鵝隻給予生長鵝料 (表 1)，飼料及水任食，香椿粉係購自許教授香椿園 (雲林縣土庫鎮)。

(1) 行政院農業委員會畜產試驗所研究報告第 2667 號。

(2) 行政院農業委員會畜產試驗所彰化種畜繁殖場。

(3) 通訊作者，E-mail: ccchang@mail.tlri.gov.tw。

表 1. 試驗飼糧組成

Table 1. The composition of experimental diet

Ingredients	Control	<i>Toona sinensis</i>
Yellow Corn, ground	62.00	62.00
Soybean meal, 44%	24.00	24.00
Wheat bran	7.00	7.00
Alfalfa meal	2.00	2.00
Molasses	2.00	2.00
<i>Toona sinensis</i> powder	—	1.00
Limestone, pulverized	0.70	0.70
Dicalcium phosphate	1.40	1.40
Salt	0.30	0.30
Choline chloride, 50%	0.10	0.10
DL-Methionine	0.15	0.15
Vitamin premix ^a	0.20	0.20
Mineral premix ^b	0.15	0.15
Total	100.00	101.00
Calculated value		
Crude protein, %	16.57	16.90
ME, kcal/kg	3,081.00	3,087.00

^a Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; vitamin E, 20 IU; vitamin B₁, 1 mg; vitamin B₂, 4.8 mg; vitamin B₆, 3 mg; vitamin B₁₂, 0.01 mg; Biotin, 0.2 mg; vitamin K₃, 1.5 mg; pantothenic acid, 20 mg; Folic acid, 0.5 mg; Nicotinic acid, 25 mg.

^b Supplied per kilogram of diet: Mn (Mn₃O₄), 80 mg; Zn (ZnSO₄ · H₂O), 50 mg; Cu (CuSO₄ · 5H₂O) 15.0 mg; Fe (FeSO₄), 80 mg; I (KI), 0.85 mg; Co (CoCO₃), 0.25 mg.

II. 調查項目與分析方法

(i) 生長性狀

肉鵝每 2 週秤重 1 次，分別於 8、10、12、14 及 16 週齡進行，試驗期間記錄各欄之剩餘飼料重，以計算飼料轉換率 (Feed conversion ratio, feed/gain)。

(ii) 血樣採集及分析

試驗鵝隻分別於 8、12 及 16 週齡進行採血，採血前禁食 15 小時，採血時以不含抗凝血劑針筒及 21 號針頭於鵝隻腳脛靜脈採集 2 mL 血液。血液樣品委由民間檢測中心以 LANNER T-900 (朕江生物科技股份有限公司) 血液分析儀配合試驗套組進行測定，項目包含肌酸酐 (Creatinine, CREA)、麩胺草醋酸轉胺酶 (Glutamic oxaloacetic transaminase, GOT)、麩胺丙酮酸轉胺酶 (Glutamic pyruvic transaminase, GPT)、三酸甘油酯 (Triglycerides, TG)、膽固醇 (Cholesterol, CHOL)、總蛋白 (Total protein, TP)、白蛋白 (Albumin, ALB)、高密度脂蛋白膽固醇 (High density lipoprotein cholesterol, HDL-C)、低密度脂蛋白膽固醇 (Low density lipoprotein cholesterol, LDL-C)，並進行 G 型免疫球蛋白 (Immunoglobulin G, IgG) 濃度測定。

(iii) 屠體性狀及肉質分析

試驗鵝隻全數於 16 週齡屠宰，依陳等 (1984) 方法進行屠體分切，剝離胸肉並冷凍保存於 -18℃，以供肉質性狀測定及一般化學成分分析。一般化學成分分析依照 A.O.A.C. (2005) 方法測定其水分、粗蛋白質、粗脂肪及粗灰分含量。色澤係利用色差儀 (Color and Color Difference Meter TC-1, Tokyo Denshoku Co., Japan) 進行測定，以 Hunter L, a, b 值分別代表胸肉之亮度、紅色度及黃色度，每塊胸肉測定 3 次後平均值代表該項數據。蒸煮失重則依 Wal *et al.* (1993) 方法進行測定，將完整胸肉樣品裝入真空袋中以 80℃ 水浴加熱 40 分鐘，以測定樣品滲出液重與蒸煮前樣品原始重量之百分比。

脂肪酸分析則委託行政院農業委員會畜產試驗所營養組測定，係參考 Griswold *et al.* (2003) 長鏈脂肪酸 (Long-chain fatty acids, LCFA) 分析方法進行。脂肪萃取參考 Hara and Radin (1978) 方法進行，脂肪甲酯化處理則參考 Christie (1989) 方法進行，經處理後所得之檢液注入氣相層析儀 (Varian CP-3800, USA) 中進行氣相層析。

(iv) 官能品評

凍存之胸肉於 4℃ 冰箱解凍 24 小時後，以鋁箔紙包覆置於 85℃ 水浴槽中水煮約 30 – 35 分鐘，當肌肉中心溫度達 80℃ 後取出。品評樣品切成 2 公分見方，由 20 位測試人員進行官能品評。品評方式採 7 分法 (1 分為非常不喜歡，7 分為非常喜歡)。品評項目包括風味 (Flavor)、嫩度 (Tenderness) 及總可接受性 (Total acceptance)。

III. 統計分析

試驗數據利用 SAS 套裝軟體 (SAS, 2004) 進行統計分析。以一般線性模式程序 (General linear model procedure) 進行變方分析，並以 Tukey's studentized range test 比較兩處理組間之差異顯著性。

結果與討論

I. 生長性狀

表 2 資料顯示，試驗期間對照組與香椿粉處理組肉鵝之生長性能無顯著差異，對照組及香椿粉處理組之平均飼料採食量與飼料轉換率分別為 228、266 g/d/bird 及 8.99、9.55。此結果與林等 (2014) 結果相似。本試驗中肉鵝於 12 週齡後增重速率下降，此與張等 (2010) 指出 11 週齡肉鵝生長速度緩慢結果相似，推測其原因可能是肉鵝於 9 – 10 週齡後之飼料消耗量減少且其飼料轉換率不佳，以致增重速率下降所致。

表 2. 飼糧中添加 1% 香椿粉對肉鵝 8 – 16 週齡生長性能之影響

Table 2. Effects of 1% *Toona sinensis* powder supplemented to diets on the th performance at 8-16 weeks of age in geese

Item	Control	<i>Toona sinensis</i>
wk of age	Body weight, kg/bird	
8	4.11 ± 0.43 ⁺	4.13 ± 0.35
10	4.66 ± 0.51	4.79 ± 0.45
12	5.01 ± 0.56	5.19 ± 0.48
14	5.28 ± 0.58	5.29 ± 0.46
16	5.55 ± 0.63	5.72 ± 0.61
	Feed consumption, g/bird/day	
8-16	228.00 ± 45.00	266.00 ± 50.00
	Feed conversion ratio, feed/gain	
8-16	8.99 ± 2.47	9.55 ± 2.95

⁺ Mean ± SD.

II. 血液生化值及免疫力

飼糧中額外添加 1% 香椿粉對 8、12 及 16 週齡肉鵝血液生化值及 IgG 濃度之影響列於表 3，對照組與香椿粉處理組肉鵝各週齡之血液生化值皆無顯著差異，惟香椿粉組之 IgG 濃度於 16 週齡顯著高於對照組者 ($P < 0.05$)。Hung *et al.* (2011) 研究指出，雞隻施打傳染性華氏囊炎病毒 (Infectious bursal disease virus, IBDV) 疫苗後，飼糧添加 0.5 – 1% 銀翹散複方有較高之 IBDV 抗體力價，由於大部分病原多透過黏膜表面入侵宿主身體，特別是腸胃、呼吸道及泌尿生殖道等，哺乳類或鳥類體內均會聚合 IgA 及 IgG 產生初級免疫反應 (Mostov and Kaetzel, 1999)，故 IgA 與 IgG 之濃度可作為評估免疫能力指標之一。本試驗以血液 IgG 濃度為免疫力指標結果顯示，飼糧額外添加 1% 香椿粉之處理組，其 16 週齡肉鵝血中 IgG 濃度顯著較高，推測具有增進鵝隻之免疫防禦機制。另 12 週齡對照組及香椿粉組之 IgG 濃度皆較 8 及 16 週齡高，推測可能與試驗期間 (11 週齡) 颱風來襲致戶外飼養鵝隻緊迫所致，因此無法預期之外力干擾試驗甚鉅，故該週齡之資料推測較無法代表實際現況。

表 3. 飼糧中添加 1% 香椿粉對肉鵝 8、12 及 16 週齡鵝隻血液生化值及 IgG 濃度之影響

Table 3. Effects of 1% *Toona sinensis* powder supplemented to diets on blood biochemical parameters and concentration of IgG at 8, 12 and 16 weeks of age in geese

	Weeks of age					
	8		12		16	
	Control	<i>Toona sinensis</i>	Control	<i>Toona sinensis</i>	Control	<i>Toona sinensis</i>
CREA ¹ , mg/dL	0.24 ± 0.05 ⁺	0.25 ± 0.03	0.30 ± 0.02	0.29 ± 0.04	0.27 ± 0.07	0.27 ± 0.07
GOT ¹ , U/L	25.00 ± 8.41	19.08 ± 8.50	22.58 ± 7.74	19.92 ± 9.86	30.58 ± 8.10	29.33 ± 4.92
GPT ¹ , U/L	7.92 ± 2.78	9.58 ± 2.39	9.58 ± 1.62	9.25 ± 2.30	11.17 ± 2.66	9.17 ± 2.72
CHOL ¹ , mg/dL	147.08 ± 59.38	167.75 ± 56.58	144.08 ± 51.43	140.92 ± 39.96	146.33 ± 36.65	150.50 ± 19.97
TG ¹ , mg/dL	144.75 ± 18.46	145.58 ± 19.12	154.00 ± 16.12	141.73 ± 46.16	138.83 ± 29.80	148.67 ± 23.89
TP ¹ , g/dL	4.19 ± 0.80	4.22 ± 0.40	4.54 ± 0.37	4.37 ± 0.67	4.66 ± 0.33	4.58 ± 0.70
ALB ¹ , g/dL	1.60 ± 0.28	1.63 ± 0.15	1.88 ± 0.14	1.89 ± 0.30	1.81 ± 0.16	1.68 ± 0.67
HDL-C ¹ , mg/dL	76.42 ± 18.96	77.25 ± 14.75	81.58 ± 10.88	85.75 ± 13.18	64.08 ± 18.81	77.58 ± 16.65
LDL-C ¹ , mg/dL	50.33 ± 16.36	49.33 ± 7.00	60.75 ± 8.95	60.58 ± 10.56	48.92 ± 6.97	47.67 ± 11.89
IgG ¹ , µg/mL	5.00 ± 0.74	4.83 ± 1.64	16.33 ± 1.23 ^a	12.58 ± 2.23 ^b	6.08 ± 1.16 ^b	7.75 ± 1.66 ^a

⁺ Mean ± SD.^{a, b} Means in the same row and at same weeks of age with different superscripts differ ($P < 0.05$).¹ CREA: creatinine, GOT: glutamic oxaloacetic transaminase, GPT: glutamic-pyruvic transaminase, CHOL: cholesterol, TG: triglycerides, TP: total protein, ALB: albumin, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, IgG: immunoglobulin G.

III. 胸肉品質

飼糧中額外添加 1% 香椿粉對 16 週齡肉鵝胸肉一般化學成分、pH、L、a、b 值皆無顯著影響 (表 4)，惟感官品評有較佳之風味、嫩度及總可接受性 ($P < 0.05$)。蕭等 (2011) 指出，16 週齡雜交鵝之胸肉水分介於 71.65 – 73.67%、粗蛋白質介於 22.77 – 23.76%、粗脂肪介於 1.32 – 3.38% 及粗灰分介於 1.32 – 3.38%，其結果與本研究相近。Baeza *et al.* (1998) 進行研究指出，8 至 13 週齡於土番鴨之胸肉一般化學成分於性別間無顯著差異，惟隨飼養週齡越長，鴨胸肉水分含量下降、粗脂肪和粗蛋白質含量則上升。一般而言，粗脂肪含量愈高，類胡蘿蔔素等色素含量亦高，惟肌肉中之肌紅蛋白 (Myoglobin) 含量則愈低，故肌肉色澤之 L 值較大，a 值較小 (Lyon and Cason, 1995)。肉色直接影響消費者購買意願，亦為肉品品質判斷之標準。本試驗香椿粉組之粗脂肪含量與對照組相似，色澤方面結果亦相似。Sari *et al.* (2015) 指出，鵝隻屠宰後其胸肉 pH 值介於 5.76 – 5.93，與本試驗結果 (5.61 – 5.62) 相似。陳等 (2003) 研究指出，肉嫩度變差之原因與肌纖維面積有關，因肌纖維大小與嫩度呈負相關 (Crouse *et al.*, 1991)。另嫩度與蒸煮失重亦有密切關係，蒸煮失重高者，其官能品評之嫩度 (Van Oeckel *et al.*, 1999) 亦較差，惟本試驗結果在蒸煮失重無明顯差異，嫩度則以香椿粉組較高且具有顯著差異存在，其結果並不一致。風味可能與脂肪酸組成改變有關 (Fisher *et al.*, 2000)，推測可能與香椿具有抑制脂質過氧化功能有關 (楊, 2011)，惟待進一步證實。另肌肉之風味、嫩度及總可接受性受許多因素的影響，但主要與肌肉脂肪含量、脂肪酸比例及肌纖維大小有關。許多研究發現，肌肉脂肪含量高者較低者有較佳之適口性，特別在嫩度、風味及芳香味 (Aroma) 上 (Wood *et al.*, 1986; Cameron *et al.*, 1990; Sales, 1995)。

飼糧中額外添加 1% 香椿粉鵝隻胸肉多元不飽和脂肪酸 (Polyunsaturated fatty acid, PUFA) 比例較高 ($P < 0.05$) (表 5)。楊 (2003) 指出，鵝隻餵給狼尾草其胸肉之多元不飽和脂肪酸含量會提高，與本試驗結果相似。李等 (2019) 針對土番鴨、紅面番鴨及北京鴨比較發現，土番鴨胸肉具有最低比例 (34.92%) 之飽和脂肪酸 (Saturated fatty acid, SFA) 和最高比例 (41.16%) 之多元不飽和脂肪酸，單元不飽和脂肪酸 (Monounsaturated fatty acid, MUFA) 於該三種鴨肉間無顯著差異，顯示品種影響鴨胸肉之脂肪酸組成。潘等 (2005) 指出，不同鵝種之胸肉脂肪酸比例不同，其中以油酸 (Oleic acid, C18:1) 比例較高，本試驗測得之油酸比例介於 15.44 至 16.78(mg/100g) 亦有相同結果。對照組與香椿組就單元不飽和脂肪酸比較發現，香椿組以芥酸 (Erucic acid, C22:1) 比例較高，具有顯著差異存在。另飼糧額外添加 1% 香椿粉之處理組，其胸肉之多元不飽和脂肪酸比例增加，特別是亞麻油酸 (Linoleic acid, C18:2) 及次亞麻油酸 (Linolenic acid, C18:3)，顯示添加香椿粉具改變胸肉脂肪酸組成之效果。

表 4. 飼糧中添加 1% 香椿粉對 16 週齡鵝隻胸肉品質之影響

Table 4. Effects of 1% *Toona sinensis* powder supplemented to diets on meat quality of breast meat at 16 weeks of age in geese

Item	Control	<i>Toona sinensis</i>
Chemical composition		
Moisture, %	72.30 ± 0.63 ⁺	72.33 ± 0.48
Crude protein, %	22.90 ± 0.37	22.73 ± 0.60
Crude fat, %	2.83 ± 0.85	2.75 ± 0.99
Ash, %	1.41 ± 0.10	1.51 ± 0.11
Physical properties		
pH	5.61 ± 0.13	5.62 ± 0.11
Cooking loss, %	30.97 ± 2.24	31.69 ± 1.92
L-value	33.83 ± 1.44	33.88 ± 1.33
a-value	19.26 ± 0.87	19.16 ± 0.63
b-value	5.91 ± 0.66	5.47 ± 0.64
Sensory evaluation		
Flavor	3.38 ± 1.05 ^b	3.98 ± 1.25 ^a
Color	4.43 ± 1.20 ^a	3.98 ± 0.77 ^b
Tenderness	3.08 ± 0.94 ^b	3.85 ± 1.05 ^a
Overall acceptance	4.23 ± 1.05 ^b	4.83 ± 1.11 ^a

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

表 5. 飼糧中添加 1% 香椿粉對 16 週齡鵝隻胸肉脂肪酸組成之影響

Table 5. Effects of 1% *Toona sinensis* powder supplemented to diets of fatty acid composition of breast meat at 16 weeks of age in geese

Item	Control		<i>Toona sinensis</i>		Significance	
	Male	Female	Male	Female	Group	Sex
Unit of fatty acid: mg/100 g meat						
Myristic acid (C14:0)	0.12 ± 0.01 ⁺	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.03	NS	NS
Palmitic acid (C16:0)	9.66 ± 0.28	10.17 ± 0.37	9.78 ± 0.46	9.75 ± 0.25	NS	NS
c9-Palmoleic acid (C16:1)	1.01 ± 0.19	1.21 ± 0.22	1.06 ± 0.18	1.12 ± 0.18	NS	NS
Stearic acid (C18:0)	3.37 ± 0.32	3.50 ± 0.38	3.25 ± 0.37	3.44 ± 0.35	NS	NS
Oleic acid (C18:1)	16.78 ± 0.83	16.31 ± 0.89	16.15 ± 0.85	15.44 ± 0.85	NS	NS
Linoleic acid (C18:2)	5.22 ± 0.26 ^{ab}	4.92 ± 0.28 ^b	5.51 ± 0.47 ^{ab}	5.85 ± 0.70 ^a	**	NS
Linolenic acid (C18:3)	0.29 ± 0.02 ^{ab}	0.26 ± 0.03 ^b	0.30 ± 0.02 ^a	0.29 ± 0.02 ^{ab}	*	*
Arachidic acid (C20:0)	0	0	0	0.01 ± 0.01	NS	NS
Erucic acid (C22:1)	1.08 ± 0.34	1.24 ± 0.46	1.38 ± 0.36	1.58 ± 0.27	*	NS
Lignoceric acid (C24:0)	0	0	0	0	NS	NS
¹ SFA	13.16 ± 0.45	13.80 ± 0.47	13.16 ± 0.77	13.34 ± 0.51	NS	NS
² USFA	24.38 ± 0.37	23.94 ± 0.33	24.40 ± 0.55	24.28 ± 0.34	NS	NS
³ MUFA	18.87 ± 0.50	18.76 ± 0.48	18.59 ± 0.78	18.14 ± 0.72	NS	NS
⁴ PUFA	5.50 ± 0.25 ^{ab}	5.18 ± 0.28 ^b	5.81 ± 0.46 ^{ab}	6.14 ± 0.71 ^a	**	NS
USFA/SFA	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	NS	NS
MUFA/SFA	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	NS	NS
PUFA/SFA	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	NS	NS
C18-2/C18-3	0.44 ± 0.19	0.46 ± 0.18	0.40 ± 0.13	0.39 ± 0.10	NS	NS

¹ SFA = saturated fatty acid (C14:0 + C16:0 + C18:0 + C20:0 + C24:0)² USFA = unsaturated fatty acid (C16:1 + C18:1 + C18:2 + C18:3 + C22:1)³ MUFA = monounsaturated fatty acid (C16:1 + C18:1 + C22:1)⁴ PUFA = polyunsaturated fatty acid. (C18:2 + C18:3)⁺ Mean ± SD.

NS: not significant; *: P < 0.05; **: P < 0.01.

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

結 論

綜合本試驗結果，飼糧中額外添加 1% 香椿粉可提高肉鵝免疫力與其胸肉多元不飽和脂肪酸含量，在官能品評上有較佳之嫩度及風味，可供特色化鵝隻生產使用。

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Effects of *Toona sinensis* powder supplemented to diets on growth and immunity and carcass quality of breast meat in geese ⁽¹⁾

Chih-Chang Hsiao ⁽²⁾⁽³⁾ and Ching-Yi Lien ⁽²⁾

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Abstract

The aim of the current study was to investigate the effects of *Toona sinensis* powder supplemented to diets on growth and immunity and carcass quality of breast meat in geese. A total of twenty-four geese were randomly divided into the control and the treatment groups comprising 2 males and 2 females at 8 weeks of age per pen (3 replicates). Body weight and blood samples were collected at 2 and 4 weeks of age. Then, the carcass characteristics and breast meat quality of hybrid geese were collected at sixteen weeks of age. The results showed that there was no significant differences between the control and treatment groups in terms of growth performances, the values of blood biochemical parameters and pH, L, a, and b of breast meat quality. Notwithstanding that there were significantly higher concentration of IgG in serum, the content of polyunsaturated fatty acid in breast meat and the better flavor, tenderness and acceptability of sensory evaluation in the treatment supplemented with *Toona sinensis* powder ($P < 0.05$). In summary, the *Toona sinensis* powder supplemented to diets did not ameliorate the growth performances in geese. However, for the production of specialized goose industry, *Toona sinensis* powder could improve immunity and enhance the flavor of geese.

Key words: *Toona sinensis*, Growth, IgG, Fatty acid.

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(2) Changhua Animal Propagation Station, COA-LRI, Changhua 52149, Taiwan, R. O. C.

(3) Corresponding author, E-mail: ccchang@mail.tlri.gov.tw.

褐色菜鴨育成期黑木耳廢棄栽培介質飼糧 對其產蛋性能之影響⁽¹⁾

鄭智翔⁽²⁾ 蘇晉暉⁽²⁾ 林榮新⁽²⁾ 林芯仔⁽³⁾ 許馨云⁽³⁾⁽⁴⁾

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摘 要

本試驗旨在評估褐色菜鴨 (*Anas platyrhynchos domesticus*) 育成期餵飼黑木耳 (*Auricularia auricula-judae*) 廢棄栽培介質對其產蛋期性能之影響，以期能節省蛋鴨育成期飼料之成本。鴨隻於 1 – 4 週齡飼養於育雛室，餵飼育雛期飼糧；第 5 週齡起飼養於非開放式平飼鴨舍，並逢機分為 4 組，每組 3 重複欄，每重複欄 13 隻，共飼養 156 隻。第 5 – 8 週齡時，各處理組餵飼相同之生長期飼糧。第 9 至 21 週齡餵飼不同之育成期飼糧，分別為餵飼純碎玉米粒組 (對照組)、以烘乾粉碎之黑木耳廢棄栽培介質 (Waste cultivation medium, WCM) 取代 10% 玉米組 (WCM-10 組)、取代 20% 玉米組 (WCM-20) 及取代 30% 玉米組 (WCM-30 組)。第 22 週齡起四組皆改餵飼相同含 CP19% 及 ME 2,750 kcal/kg 之產蛋期飼糧。試驗期間 (9 – 42 週齡) 每週測定鴨隻體重、採食量及產蛋率；於 24 – 36 週齡每 3 週測定蛋重及蛋型係數；並於 33 及 36 週齡測定鴨蛋組成比例、蛋殼強度及蛋殼厚度。結果顯示，各組之採食量於育成期及產蛋期間並無明顯差異，21 週齡時四組鴨隻體重在 821 – 865 g 之間。在產蛋率方面，四組 22 – 42 週齡之平均產蛋率依序分別為 82、82、87 及 82%。在鴨蛋品質方面，各組間蛋黃、蛋白與蛋殼重量比、蛋殼厚度及蛋殼強度亦無顯著差異。綜合以上結果顯示，以黑木耳廢棄栽培介質作為蛋鴨育成期之飼糧原料，可取代玉米達 20%，不影響產蛋率及鴨蛋品質，且可節省育成期飼糧成本 17.55%，並有助於農業廢棄物循環利用。

關鍵詞：黑木耳、廢棄栽培介質、育成期、褐色菜鴨、產蛋性能。

緒 言

近年隨著養生風氣盛行，國人對於菇類產品需求大增，菇類產業之發達，廢棄資材也成為衍生之重要環境問題。目前我國每年生產之太空包數目約為 29,600 萬包，菇類栽培瓶約 7,500 萬瓶，若以每包 / 瓶之基質重量 1 公斤，食用菌平均生物效率 40% 計算，則每年產生之廢棄資材約為 22 萬噸，若無法妥善處置這些廢棄物，將對環境造成嚴重危害 (呂等, 2011)。褐色菜鴨 (*Anas platyrhynchos domesticus*) 為我國唯一的蛋鴨品種，為確保鴨隻得以在 120 日齡之後開始產蛋，並獲得較佳的蛋重及產蛋表現，鴨農會採取限飼管理。限飼的方法分為限量法，即給與正常採食量之 70 – 80% 飼糧，或限質法，即給予低營養濃度之飼糧 (陳, 2019)。目前國內蛋鴨場普遍實施之限飼方法為以玉米粒任飼一天、停餵一天。在北美，飼養過程中的非每日餵飼計畫亦是農場的常見作法，可改善肉種母雞的體重整齊度 (Zuidhof *et al.*, 2015; Carneiro *et al.*, 2019)。有研究使用可限制生長同時減少飢餓的替代餵飼方法，例如替代飼糧 (Nielsen *et al.*, 2011; Morrissey *et al.*, 2014)。替代飼糧的開發主要集中在稀釋能量含量以換得每日更大的飼糧提供量 (van Krimpen and de Jong, 2014; van Emous *et al.*, 2015)。研究發現，替代飼糧比標準的飼糧含有更高的纖維，可以減少飼養期間長期限飼造成的影響，同時可使種雞達到成熟的體重和性成熟 (Morrissey *et al.*, 2014; de Los Mozos *et al.*, 2017)。為透過調製鴨隻育成期之飼糧配方，作為維持鴨隻健康及產蛋品質之飼糧原料，並評估黑木耳 (*Auricularia auricula-judae*) 栽培廢棄物活用之商業化方式，以協助解決農業資材廢棄物處理問題。本試驗旨在評估育成期餵飼黑木耳廢棄栽培介質對褐色菜鴨產蛋性能之影響，以期能節省蛋鴨育成期飼糧之成本。

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(2) 行政院農業委員會畜產試驗所宜蘭分所。

(3) 東海大學畜產與生物科技學系。

(4) 通訊作者，E-mail: hyh@thu.edu.tw。

材料與方法

本試驗於行政院農業委員會畜產試驗所宜蘭分所試驗鴨舍進行，試驗動物之使用、飼養管理及試驗內容，均經畜產試驗所宜蘭分所實驗動物管理小組以畜試宜動字第 107012 號核准在案。

I. 試驗方法

- (i) 試驗動物與試驗設計：使用宜蘭分所自行育成之褐色菜鴨供試。鴨隻於 0－4 週齡時飼養於育雛室，餵飼 CP 及 ME 分別為 19.5% 及 2,900 kcal/kg 之育雛期 (Starter) 飼糧；第 5 週齡起飼養於非開放式平飼鴨舍，並將鴨群逢機分為 4 組，每組 3 重複欄，每重複欄 13 隻，共飼養 156 隻。第 5－8 週齡時，各處理組均餵飼 CP 及 ME 分別為 13.5% 及 2,650 kcal/kg 之生長期 (Grower) 飼糧；第 9 至 21 週齡為試驗期，4 組各餵飼不同處理之育成期 (Developer) 飼糧，分別為：餵飼純碎玉米粒 (對照組)、以黑木耳廢棄栽培介質取代 10% 玉米組 (WCM-10 組)、以黑木耳廢棄栽培介質取代 20% 玉米組 (WCM-20) 及以黑木耳廢棄栽培介質取代 30% 玉米組 (WCM-30 組)。第 22 週齡改餵食玉米－大豆粕為主之粒狀產蛋期飼糧 (蛋白質含量 19%、代謝能 2,750 kcal/kg、鈣 3.0%、有效磷 0.46%)，試驗期間採平飼、自然光照及自由飲水，且各階段之飼糧均為任食。
- (ii) 黑木耳廢棄栽培介質製備：黑木耳廢棄栽培介質取自宜蘭縣三星鄉之有機黑木耳生產農場，取回廢棄栽培介質，進行粉碎及乾燥備用。經進行乾燥條件測試並製成乾燥曲線，之後依乾燥條件 90℃ 乾燥 5 小時，使水分含量低於 20% 以下後備用，乾燥後之廢棄栽培介質檢測水分、粗脂肪、粗蛋白、粗纖維及粗灰分含量，並為確保試驗動物之安全無虞，將樣品送財團法人中央畜產會進行黴菌毒素之檢測，檢測項目包含黃麴毒素 B1、B2、G1、G2 (Aflatoxin B1, B2, G1, G2)、赭麴毒素 A (Ochratoxin A)、伏馬毒素 B1 (Fumonisin B1)、伏馬毒素 B2 (Fumonisin B2)、玉米赤黴毒素 (Zearalenone)、脫氧雪腐鐮刀菌烯醇 (Deoxynivalenol)、T-2 毒素 (T2-toxin) 及 HT-2 毒素 (HT2-toxin)。

II. 測定項目

- (i) 飼糧成分分析：蛋鴨育成期之飼糧參照 A.O.A.C. (2005) 方法進行粗蛋白質、粗脂肪、粗灰分、粗纖維及總碳水化合物含量等分析。
- (ii) 鴨隻體重：於鴨群 5 週齡進行逢機分組起至 21 週齡止，每週秤量各重複欄鴨隻體重，以計算當週齡之平均體重。
- (iii) 採食量：於鴨群 5 週齡更換生長期飼糧起至試驗結束，每週秤量各重複欄之飼糧給予量與剩餘量重，以計算當週隻日採食量。
- (iv) 存活率：自 5 週齡進行逢機分組起至試驗結束，每週記錄每重複欄每日之死亡數，並計算當週齡之累計存活率 (Cumulative survival rate)。
- (v) 產蛋率：自產蛋期開始至試驗結束，每週記錄每重複欄每日之產蛋數，並計算當週之平均隻日產蛋率 (Hen-day egg production) 及隻舍產蛋率 (Hen-housed egg production)。
- (vi) 鴨蛋品質測定：於鴨隻第 24、27、30、33 及 36 週齡時，每重複欄連續收集 2 日的鴨蛋，秤量鴨蛋重量；以游標尺測量鴨蛋之短徑及長徑，並計算蛋型係數 (蛋的短徑 / 長徑 × 100)。於第 33 及 36 週齡時，將鴨蛋以蛋殼強度器 (HT-8116, Hung-Ta Instrument Co. Ltd., Taichung, Taiwan) 測定蛋殼強度後，打開蛋殼取出內容物，分離蛋白後測量蛋黃重量。打開之蛋殼擦乾後，秤量蛋殼重量，再去除蛋殼膜，分別在鈍端、尖端與赤道區域 (Equatorial area) 各取一塊蛋殼，以蛋殼厚度計 (FN595, FHK Fujihira Industry Co., Ltd, Tokyo, Japan) 測量得平均蛋殼厚度 (Nordskog and Farnsworth, 1953)。由以上測量之數據，分別計算得蛋殼重、蛋白重與蛋黃重，再分別計算其占鴨蛋之比重。

III. 統計分析：試驗設計為完全逢機設計試驗，試驗測定之結果使用 SAS 統計套裝軟體 (SAS, 2016) 分析，以一般線性模式 (GLM procedure) 進行主效應的變方分析，並以特奇公正顯著差異法 (Tukey's honest significant difference)，比較各組平均值間之差異顯著性，本試驗以 $P < 0.05$ 為顯著差異標準。

結果與討論

I. 飼糧分析結果

各組飼糧之成分分析結果如表 1 所示。在粗蛋白及粗脂肪部分，以對照組含量最高，WCM-10 組、WCM-20 組及 WCM-30 組之間無顯著差異；粗灰分及粗纖維則以對照組最低而 WCM-30 組最高；在總碳水化合物部分，

以對照組含量最低，WCM-10 組、WCM-20 組及 WCM-30 組之間無差異；總能部分各組間無差異。飼糧分析的結果顯示，添加黑木耳廢棄栽培介質會降低粗蛋白及粗脂肪含量，並增加粗灰分及粗纖維的含量。由於黑木耳栽培介質主要材料為闊葉樹為主之木屑，棉籽殼、玉米軸、稻草、野草等次之，配方主料占 84 – 74%、輔料占 15 – 25%，輔料有米糠、麥麩、粉頭 (Wheat middlings)、玉米粉、大豆粉等 (吳，2019)，栽培介質之原料，為造成粗纖維及粗灰分含量增加之因素。

II. 對鴨隻各階段日採食量及產蛋前體重之影響

四組鴨隻於各週齡日採食量的影響方面列如圖 1 所示。四組鴨隻於生長期 (5 – 8 週齡) 之平均日採食量分別為 91.2、96.4、99.0 及 95.7 g；於試驗期 (育成期 9 – 21 週齡) 之平均採食量分別為 93.2、89.9、88.3 及 99.5 g；於產蛋期 (22 – 42 週齡) 之平均採食量分別為 155.5、151.0、155.4 及 152.1 g。第 5 至 8 週齡期間，各組之採食量隨鴨隻週齡增加而增加，同週齡之四組鴨隻之採食量無差異。對照組之採食量於 9 週齡時明顯低於 8 週齡，而其他組之採食量則無降低之情形，其原因不明。第 22 週齡開始，各組之採食量隨週齡增加而增加，除第 23 週齡及第 33 週齡之外，不同週齡之各組鴨隻採食量均無差異。結果顯示，育成期飼餵不同比例之黑木耳廢棄栽培介質，不影響鴨隻育成期與產蛋期之採食量。對產蛋前的體重影響方面 (圖 2)，第 5 – 8 週齡飼餵生長期飼糧期間，各組之體重隨週齡增加而增加，各週齡之組間體重無差異，第 8 週齡時，對照組、WCM-10 組、WCM-20 組及 WCM-30 組之體重分別為 936、984、989 及 961 g。第 9 – 21 週齡飼餵不同處理之育成期飼糧期間，各週齡不同組間之體重無差異，第 21 週齡時，四組體重分別為 852、821、865 及 821 g，且以 WCM-20 組有較高之趨勢。

表 1. 黑木耳廢棄栽培介質添加比例之成分分析結果 (所有組成皆以乾物基表示)

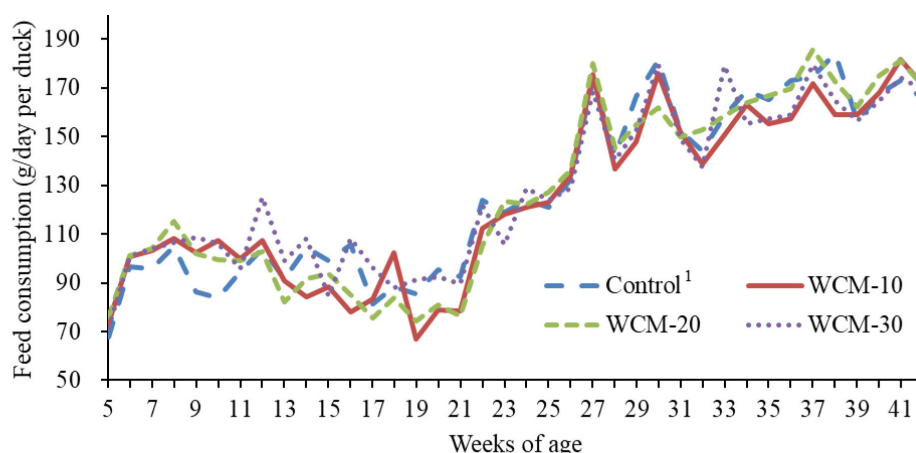
Table 1. Approximate analysis results of waste black fungus cultivation medium addition ratio (All compositions are shown as dry matter basis)

Items	Control ¹	WCM-10	WCM-20	WCM-30
Cru ^d e protein (%)	8.26 ± 0.03 ^a	7.63 ± 0.02 ^b	7.51 ± 0.07 ^b	7.50 ± 0.02 ^b
Cru ^d e fat (%)	5.06 ± 0.03 ^a	2.49 ± 0.37 ^b	1.73 ± 0.02 ^b	2.00 ± 0.02 ^b
Ash (%)	1.22 ± 0.00 ^c	1.36 ± 0.05 ^{bc}	1.49 ± 0.03 ^b	2.07 ± 0.03 ^a
Cru ^d e fiber (%)	2.14 ± 0.44 ^d	3.86 ± 0.10 ^c	6.60 ± 0.08 ^b	10.68 ± 0.26 ^a
Total carbohy ^d rates (%)	85.46 ± 0.31 ^b	88.52 ± 0.40 ^a	89.27 ± 0.12 ^a	88.44 ± 0.01 ^a
Total energy (kcal/kg)	4,087 ± 56	4,050 ± 9	4,073 ± 29	4,096 ± 19

¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

^{a, b, c, d} Means in the same row without the same superscript differ significantly ($P < 0.05$).

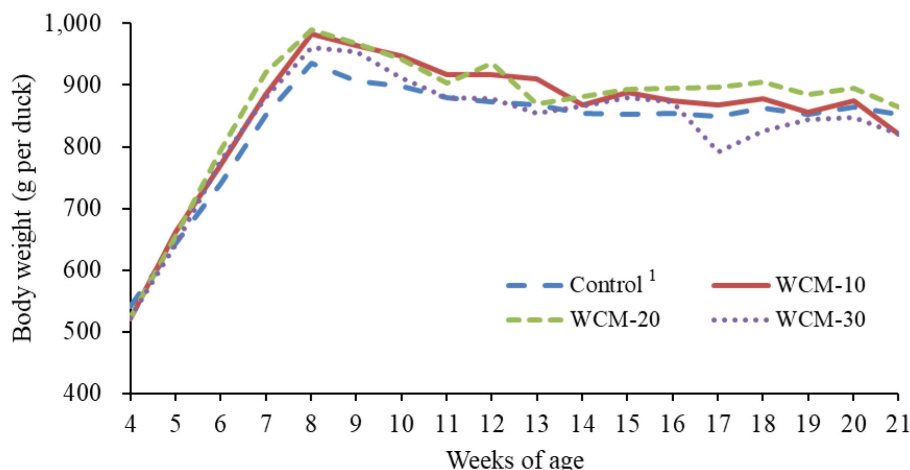
Means ± SE. (n = 3).



¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

圖 1. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其採食量之影響。

Fig. 1. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on feed consumption of Brown Tsaiya ducks (n = 3).



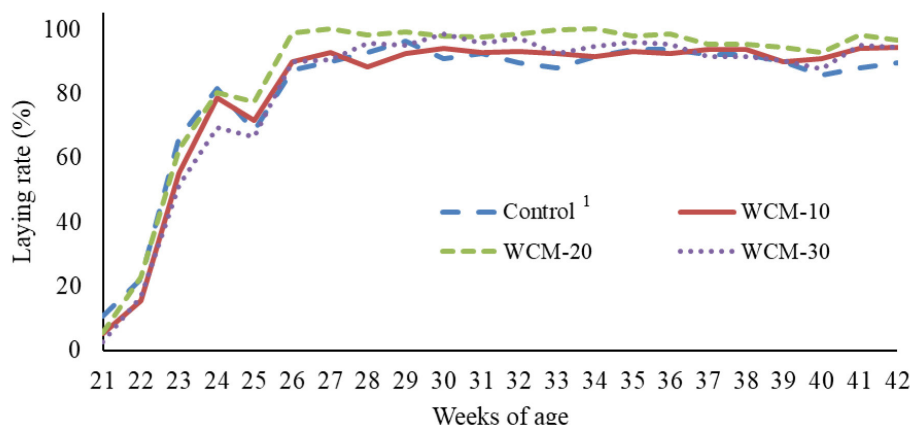
¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

圖 2. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其 9 至 21 週齡體重之影響。

Fig. 2. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on body weights of Brown Tsaiya ducks from 9 to 21 weeks of age (n = 3).

III. 對產蛋性能及累計存活率之影響

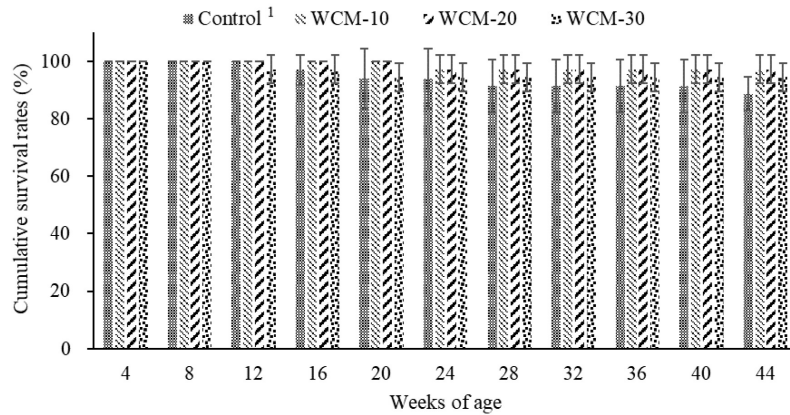
產蛋率的結果整理如圖 3 所示。四組鴨隻第 21 週齡時之產蛋率分別為 11、5、6 及 3%；第 22 週齡起，各組產蛋率隨週齡增加而顯著上升，第 25 週齡時因試驗鴨舍旁進行新鴨舍地基工程，造成試驗鴨隻因緊迫致產蛋率短暫下降；第 26 週齡時，四組產蛋率分別為 87、90、96 及 90%，各組間產蛋率無顯著差異。WCM-20 組於第 26 – 42 週齡期間，產蛋率有高於其他組之趨勢。以產蛋期間 (21 – 42 週齡) 之平均產蛋率來看，各組分別為 82、82、87 及 82%，各組間無顯著差異，但仍以 WCM-20 組有較高之產蛋率數值 (P = 0.38)。許多研究指出，延遲雞隻性成熟，產蛋高峰後有較高的產蛋率 (Walter and Aitken, 1961; McDaniel, 1983; Kling *et al.*, 1985)。在累計存活率方面 (圖 4)，第 9 週齡育成期試驗開始時，各組之累計存活率均為 100%；第 21 週齡更換產蛋期飼糧時，各組之累計存活率分別為 94、97、97 及 94%，且各組間無差異，顯示育成期飼糧不同比例之黑木耳廢棄栽培介質飼糧，並不影響蛋鴨限飼期間之存活率，此與林及徐 (1995) 指出育成期採用低蛋白質飼糧，可減輕生長期體重及延遲生殖器官發育，而對雞群整齊性及死亡率無顯著影響的結果一致。第 44 週齡時，四組累計存活率相近，分別為 89、97、97 及 94%，而各組之平均隻舍產蛋率分別為 82、81、87 及 83%。由鴨隻存活率的結果顯示，進行限飼處理期間，不同比例之廢棄栽培介質飼糧並不影響蛋鴨的累計存活率，且產蛋期間之累計存活率亦不受影響。



¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

圖 3. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其 21 至 42 週齡產蛋率之影響。

Fig. 3. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on laying rates of Brown Tsaiya ducks from 21 to 42 weeks of age (n = 3).



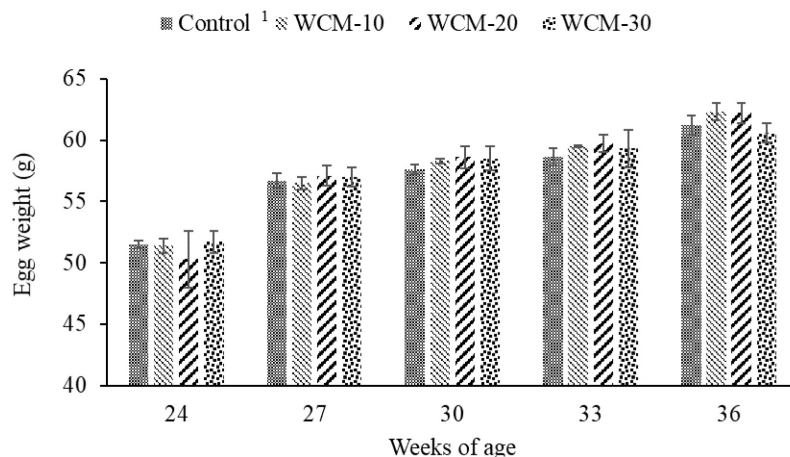
¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

圖 4. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其 4 至 44 週齡累計存活率之影響。

Fig. 4. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on cumulative survival rates of Brown Tsaiya ducks between 4 to 44 weeks of age (n = 3).

IV. 對鴨蛋品質之影響

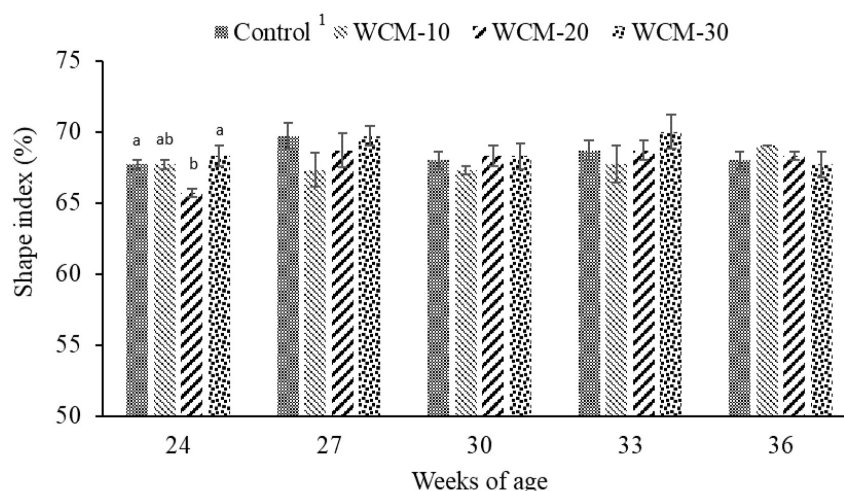
在蛋重方面 (圖 5)，第 24 週齡四組蛋重分別為 51.5 ± 0.3 、 51.4 ± 0.6 、 50.3 ± 2.3 及 51.7 ± 0.9 g，各組間無差異。各組之蛋重隨產蛋週齡增加而上升，至第 36 週齡時，四組之蛋重分別為 61.2 ± 0.8 、 62.3 ± 0.7 、 62.2 ± 0.8 及 60.6 ± 0.8 g，且以 WCM-10 組及 WCM-20 組之蛋重有較高之趨勢 ($P > 0.05$)。在蛋型係數方面 (圖 6)，第 24 週齡時，以對照組及 WCM-30 組較高而 WCM-20 組較低，各組之蛋型係數介於 65.7 – 68.3% 之間，由於第 24 週齡時鴨隻為產蛋前期，所產之鴨蛋大小及形狀差異較大，推測為造成產蛋前期蛋型係數差異之原因；第 27、30、33 及 36 週齡時，各組之蛋型係數皆無差異。第 33 及 36 週齡部分的鴨蛋品質測定結果顯示 (表 2)，在蛋黃、蛋白及蛋殼重量比例部分，蛋殼重量比例有隨鴨隻週齡增加而增加之現象，四組的蛋殼重量比於 33 週齡之 14.6 – 17.0%，上升至 36 週齡之 17.3 – 18.7% 之間。在蛋殼厚度的結果發現，36 週齡之對照組、WCM-10 組及 WCM-20 組其蛋殼厚度在數值上有高於 33 週齡之現象，WCM-30 組則有較低與穩定之趨勢。在蛋殼強度方面，各組間無差異。研究顯示肉種母雞於產蛋期限飼，可改善蛋殼品質 (McDaniel *et al.*, 1981; Wilson *et al.*, 1983; Robbins *et al.*, 1986)，此與體重增加受到抑制，致體脂肪較低有關。Bish *et al.* (1985) 之研究顯示，性成熟體重輕者蛋殼品質顯著較體重重者佳，又 Spratt and Lesson (1987) 指出，蛋黃比例會隨每日能量攝取量增加而增加。本試驗中鴨隻產蛋前體重及產蛋期採食量無差異，應為各組鴨蛋品質無差異之原因。綜合以上結果顯示，育成期餵飼不同比例之黑木耳廢棄栽培介質，不影響褐色菜鴨產蛋期之鴨蛋品質。



¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

圖 5. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其蛋重之影響。

Fig. 5. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on egg weights of Brown Tsaiya ducks (n = 3).



¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

圖 6. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其蛋型係數之影響。

Fig. 6. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on egg shape index of Brown Tsaiya ducks (n = 3).

表 2. 褐色菜鴨育成期飼糧以黑木耳廢棄栽培介質取代不同比例玉米對其 33 與 36 週齡鴨蛋品質之影響

Table 2. Effects of replacing different ratios of corn with black fungus waste cultivation medium during the developer period on egg quality of Brown Tsaiya ducks at 33 and 36 weeks of age

Weeks of age	Treatment			
	Control ¹	WCM-10	WCM-20	WCM-30
----- Egg yolk weight ratio (%) -----				
33	32.0 ± 0.6	31.3 ± 0.3	32.0 ± 0.0	32.7 ± 0.7
36	32.7 ± 0.3	32.3 ± 0.3	32.3 ± 0.3	32.0 ± 0.6
----- Egg white weight ratio (%) -----				
33	51.0 ± 0.6	52.0 ± 1.5	51.7 ± 0.3	52.7 ± 0.3
36	49.7 ± 0.3	50.7 ± 0.3	49.3 ± 0.7	49.3 ± 0.3
----- Egg shell weight ratio (%) -----				
33	17.0 ± 0.6	16.7 ± 1.2	16.3 ± 0.3	14.6 ± 0.7
36	17.7 ± 0.3	17.3 ± 0.3	18.0 ± 0.6	18.7 ± 0.7
----- Egg shell thickness (μm) -----				
33	390.0 ± 0.0	396.7 ± 6.7	386.7 ± 8.8	380.0 ± 0.0
36	400.0 ± 5.8	403.3 ± 8.8	396.7 ± 3.3	380.0 ± 0.0
----- Egg shell strength (kg/cm ²) -----				
33	4.46 ± 0.13	4.64 ± 0.31	4.53 ± 0.24	4.29 ± 0.06
36	4.41 ± 0.13	4.42 ± 0.15	4.44 ± 0.10	4.30 ± 0.10

¹ Control = 100% corn; WCM-10, WCM-20 and WCM-30 = replace 10, 20 or 30% corn with black fungus waste cultivation medium, respectively.

Means ± SE. (n = 3).

There is no significant difference between the above groups (P > 0.05).

IV. 育成期之飼糧成本

本試驗之碎玉米於 109 年度之平均價格為每公斤 8.545 元，四組試驗鴨隻於育成期之飼養天數為 91 天 (9 – 21 週齡)、平均隻日採食量分別為 93.2、89.9、88.3 及 99.5g，及國內蛋鴨場平均飼養隻數 6,000 隻鴨來計算，則四組所需蛋鴨育成期飼糧分別需 50,887、49,054、48,212 及 54,347 公斤。若以黑木耳廢棄栽培介質之運輸及乾燥成本每公斤 3 元來計算，四組平均飼糧價格每公斤分別為 8.545、7.991、7.436 及 6.882 元，經計算育成期

飼糧分別需 434,829、391,991、358,504 及 373,878 元。結果顯示，育成期飼糧以黑木耳廢棄栽培介質取代玉米 10、20 及 30%，分別可節省飼糧成本 9.85%、17.55% 及 14.02%。

結 論

試驗結果顯示，褐色菜鴨 9—21 週齡育成期餵飼不同比例之黑木耳廢棄栽培介質飼糧，不影響鴨隻育成期與產蛋期之採食量、產蛋前之體重、產蛋率及累計存活率。試驗四組於 26—42 週齡平均產蛋率分別為 82、82、87 及 82%，以 WCM-20 組有較高數值之趨勢，且四組至產蛋期之累計存活率分別達 89、97、97 及 94%。在鴨蛋品質部分，各組之蛋重、蛋型係數、組成比例及蛋殼強度等，皆無顯著差異。綜上所述，以產蛋率趨勢及飼糧成本來看，推薦以黑木耳廢棄栽培介質取代 20% 玉米作為褐色菜鴨育成期飼糧，可維持褐色菜鴨產蛋期之產蛋率及鴨蛋品質，且可降低蛋鴨飼養成本達 17.55%。黑木耳廢棄栽培介質可應用於產蛋鴨育成期飼糧，對於降低黑木耳產業之農業廢棄物問題，應有所助益。

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Effect of waste cultivation medium of black fungus during developer period on laying performance of Brown Tsaiya ducks ⁽¹⁾

Chih-Hsiang Cheng ⁽²⁾ Chin-Hui Su ⁽²⁾ Jung-Hsin Lin ⁽²⁾ Hsin-Yu Lin ⁽³⁾ and Hsin-Yun Hsu ^{(3) (4)}

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Abstract

The purpose of this experiment was to evaluate the effect of feeding waste cultivation medium of black fungus (*Auricularia auricula-judae*) on the laying performance in Brown Tsaiya ducks (*Anas platyrhynchos domesticus*) during development period, thereby to establish the reference on saving feed costs of laying ducks. Ducks are raised in brooding rooms at 1 - 4 weeks of age and fed starter feed; from the 5 weeks of age, the ducks are raised in the experimental duck house. The duck flock is divided into 4 testing groups, namely corn group (control), while the corn group is replaced by 10, 20 and 30% waste cultivation medium, respectively (WCM-10, WCM-20 and WCM-30 group). Each group consists of 3 repeating pens, with 13 ducks per repeating pen. A total of 156 ducks are raised. At 5 to 8 weeks of age, each group was fed CP and ME of 13.5% and 2,650 kcal/kg grower feed, respectively. From the 9 to 21 weeks of age, they were fed different treatments for development period. At 22 weeks of age, ducks were fed 19% of crude protein and 2,750 kcal/kg layer feed. During the test period (9 - 42 weeks of age), data on body weight, feed consumption and laying rate were collected every week, and egg weight and shape index were measured every 3 weeks from 24 to 36 weeks of age. Data on the composition ratio of duck eggs, eggshell strength and eggshell thickness were collected at 33 and 36 weeks of age. The results showed the follows: there was no significant difference in the feed consumption of groups between the limited feeding period and the laying period. The ducks weighed between 821 to 864 g at 21 weeks of age. In terms of laying rate, the average egg production rates for each group at 22 - 42 weeks of age were 82, 82, 87 and 82%, respectively. In terms of duck egg quality, there was no significant difference between the groups. The results showed that the waste cultivation medium of black fungus can be used as a feed ingredient in the laying duck's limited feeding period, which can be replaced by corn up 20% without affecting laying performance and egg quality and can save 17.55% of the feed cost during the development period.

Key words: Black fungus, Waste cultivation medium, Developer period, Brown Tsaiya duck, Laying performance.

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(2) Ilan Branch, COA-LRI, Ilan 26846, Taiwan, R. O. C.

(3) Department of Animal Science and Biotechnology, Tunghai University, Taichung 40704, Taiwan, R. O. C.

(4) Corresponding author, E-mail: hyh@thu.edu.tw.

飼料添加凝結芽孢桿菌與狼尾草粉對離乳仔豬 生長性能之影響⁽¹⁾

王錦盟⁽²⁾ 劉芳爵⁽²⁾ 林秉憲⁽³⁾ 林幼君⁽⁴⁾⁽⁵⁾

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摘 要

本試驗旨在探討飼糧中添加凝結芽孢桿菌 (*Bacillus coagulans*) 及狼尾草粉對離乳仔豬生長性能的影響。以 48 頭 4 週齡藍瑞斯 × 杜洛克離乳仔豬為試驗動物，逢機分為 3 組，分別予對照組飼糧 (A)、對照組飼糧額外添加凝結芽孢桿菌 2×10^5 CFU/kg (B)，B 組飼糧再額外添加台畜草二號狼尾草粉 3.2 g/kg (C)。試驗為期 4 週，期間測定飼料採食量、體重、血液學各種血球及血紅素。結果顯示，A、B 與 C 組的仔豬隻日採食量分別為 0.47、0.57 與 0.51 kg，B 組顯著高於對照組 ($P < 0.05$)，B 組增重亦顯著高於對照組 ($P < 0.05$)，三組間飼料利用效率則無顯著差異。在血液學生化值分析方面，各檢測項目於各組間均無顯著差異。綜上所述，飼料中添加凝結芽孢桿菌，可增加仔豬採食量與增重。

關鍵詞：離乳仔豬、凝結芽孢桿菌、狼尾草粉、生長性能。

緒 言

2001 年聯合國糧食及農業組織 (The Food and Agriculture Organization of the United Nations, FAO) 將益生菌定義為「活的微生物，當給予足夠量時可對宿主產生健康益處 (Reid, 2016)」。一般常見的益生菌可區分為細菌及真菌兩大類。益生菌需能抵抗胃酸與膽鹽的破壞，無病原性，能定殖於腸壁細胞及能在腸道上進行繁殖，才能產生良好的作用 (Fuller, 1992)。

芽孢桿菌屬於中度嗜熱菌，固態發酵的耐受溫度可達 50 – 55°C，可以降低其在發酵生產過程被其他微生物污染的風險 (Abdel-Banat *et al.*, 2010; Lidan *et al.*, 2013)。一般微生物無法有效分解五碳糖，微生物利用磷酸乙酮醇酶途徑 (Phosphoketolase pathway) 發酵五碳糖時，可產生等當量之醋酸與左旋乳酸，但其產生效率大約 60%。芽孢桿菌將五碳糖或六碳糖水解產生左旋乳酸與醋酸的效率接近 100% (Patel *et al.*, 2006)。凝結芽孢桿菌為芽孢桿菌屬之菌種，凝結芽孢桿菌菌株具分解纖維與產生左旋乳酸之能力 (劉及林, 2016)。因此推測具利用狼尾草粉之能力，基於狼尾草粉取得相對較容易與未來的實用性之考量，本試驗於飼糧中添加凝結芽孢桿菌 S10，期能提升仔豬的生長性能。

飼糧中使用 5% 苜蓿粉不會影響仔豬的營養與能量消化，迴腸中的揮發性脂肪酸濃度隨著飼料中牧草使用量的增加而提升 (Chen *et al.*, 2013)。纖維在維持豬隻腸道菌群方面，具有正面的作用，牧草富含纖維，飼糧中添加紫色狼尾草會影響母豬腸道菌群的菌相，尤其是增加桿菌科 (*Coriobacteriaceae*) 的相對量 (Huang *et al.*, 2021)。一般而言，益生菌定殖在動物的消化道中，以小腸部分的數量最多，主要的作用在抑制腸道致病菌與維持消化道機能等。大腸部位之微生物菌相，對豬隻健康扮演非常重要的角色，主要由於微生物產生之短鏈脂肪酸，可降低腸道中的 pH 值，避免有害微生物的作用 (Blottiere *et al.*, 2003; Biagi *et al.*, 2006)。

由於凝結芽孢桿菌在動物消化道中能分解飼料原料的纖維素，為增加凝結芽孢桿菌的營養源及碳源比例，以提升其在動物消化道中的增殖。本試驗於離乳仔豬飼糧中使用凝結芽孢桿菌與狼尾草粉，探討其對離乳仔豬生長性能

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(2) 行政院農業委員會畜產試驗所產業組。

(3) 農業科技研究院動物科技研究所。

(4) 行政院農業委員會畜產試驗所營養組。

(5) 通訊作者，E-mail: hiujj@mail.tlri.gov.tw。

的影響。

材料與方法

本試驗於行政院農業委員會畜產試驗所產業組的試驗豬舍進行，試驗動物之使用、飼養管理及試驗內容，經畜產試驗所實驗動物管理小組以畜試動字第 109019 號申請核准在案。

I. 凝結芽孢桿菌飼料添加物及狼尾草粉

篩選自豬隻糞便的凝結芽孢桿菌 S10 菌株，具耐酸 (pH 2)、耐膽鹽 (2%)、耐高溫 (90°C) 及產孢能力等特性 (劉及林, 2016)。以此凝結芽孢桿菌之發酵產物 (含菌液) 進行冷凍乾燥，製成凝結芽孢桿菌粉，再依菌粉濃度稀釋製成凝結芽孢桿菌添加物。另以台畜草二號狼尾草粉 (CP 8.7%，ADF 45.1%，NDF 70.3%；黃等, 2021) 額外添加於飼料中。

II. 動物試驗

採用 4 週齡二品種雜交離乳仔豬 (藍瑞斯 × 杜洛克) 48 頭為試驗動物，逢機分為 3 組，分別為對照組 (A) 與凝結芽孢桿菌組 (B 組) 與凝結芽孢桿菌 + 狼尾草粉組 (C 組) 等 2 處理組，三組仔豬離乳平均體重分別為 7.20 ± 0.40 、 7.19 ± 0.23 與 7.04 ± 0.13 kg (mean \pm SD)。仔豬飼養於傳統高床保育豬舍，每組 4 重複，每欄 4 頭 (公母各半)，每欄面積 2.55 平方公尺。對照組 (A) 仔豬給飼保育豬基礎試驗飼糧 (表 1)；B 組，給飼糧加凝結

表 1. 凝結芽孢桿菌基礎飼糧配方組成

Table 1. Formulation and compositions of basal diet at *Bacillus coagulans* experiment

Ingredients	%
Yellow corn meal	67.65
Soybean meal, CP 44%	19.00
Fish meal	5.00
Skimmed milk	2.00
Whey powder	2.00
Soybean oil	1.00
Salt	0.50
Dicalcium phosphate	1.60
Limestone, pulverized	0.80
L-Lysine, 98.5%	0.10
Choline chloride, 50%	0.10
Vitamin premix ¹	0.15
Mineral premix ²	0.10
Total	100.00
Calculated values	
Crude protein, %	17.40
ME, kcal/kg	3,217
Analyzed values	
Crude protein, %	17.53
Calcium, %	1.16
Total phosphorus, %	0.75
Crude fiber, %	2.61

¹ Vitamin premix provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D₃, 600 IU; vitamin E, 60 IU; vitamin K, 3 mg; vitamin B₁, 3 mg; vitamin B₂, 9 mg; vitamin B₆, 4.5 mg; vitamin B₁₂, 0.045 mg; nicotinic acid, 45 mg; calcium pantothenate, 45 mg; folic acid, 0.9 mg and biotin, 0.3 mg.

² Mineral premix provided per kilogram of diet: Cu, 5 mg; Mn, 6 mg; Co, 0.35 mg; Zn, 40 mg; I, 0.2 mg; Se 0.1 mg and Fe, 80 mg.

芽孢桿菌添加物之飼糧，飼料中凝結芽孢桿菌濃度為 2×10^5 CFU/kg；C 組飼料如同 B 組，再額外添加狼尾草粉 3.2 g/kg，此使用量係參考 Al-Khalaifa *et al.* (2019)。試驗為期 4 週，試驗於仔豬 8 週齡結束。試驗於春季進行，期間採任食，每日補充飼料 2 次。以乳頭式或碗式飲水器給水，每欄 2 個飲水器。每週測定仔豬的飼料採食量及體重，7 週齡時，於上午 9 – 10 時，豬隻以人工保定後，由頸靜脈採集 3 mL 血液，以血液分析儀 (XT-1800i Hematology Analyzer Sysmex Corporation, Co., Japan) 進行血液學的全血細胞計數 (Complete blood count) 檢測各類血球及血紅素。

III. 統計分析

本試驗採完全隨機設計 (Completely randomized design; CRD)，所得試驗資料使用 SAS 統計套裝軟體 (SAS, 2002)，利用一般線性模式程序 (General linear model procedure) 進行變方分析，如有顯著差異存在，再以最小平方方法 (Least squares means) 比較處理組間之差異顯著性，本試驗顯著差異水準為 ($P < 0.05$)。

結果與討論

I. 添加凝結芽孢桿菌與狼尾草粉對仔豬生長性能的影響

(i) 飼料採食量

仔豬於試驗全期及各週齡隻日採食量如表 2 所示，對照組 (A 組)、凝結芽孢桿菌組 (B 組) 與凝結芽孢桿菌 + 狼尾草粉組 (C 組) 的仔豬全期隻日採食量分別為 0.47、0.57 與 0.51 kg。以 B 組的採食量顯著高於對照組 ($P < 0.05$)。各週齡的採食量趨勢亦以 B 組高於對照組及 C 組，且在 6 週齡與 8 週齡時，B 組的採食量顯著高於對照組及 C 組 ($P < 0.05$)。顯示飼料添加凝結芽孢桿菌可促進離乳仔豬的食慾，然而再額外添加台畜草二號狼尾草粉 0.32%，對採食量無顯著影響。

表 2. 飼糧中添加凝結芽孢桿菌與狼尾草粉對離乳仔豬 5 – 8 週齡生長性能之影響

Table 2. Effects of adding *Bacillus coagulans* and napier grass powder in diet on growth performances of weaned pigs during 5 - 8 wk of age

Group	A	B	C
	Control	A diet added <i>Bacillus coagulans</i> at 2×10^5 CFU/kg	B diet added 0.32% Napier- grass Taishigrass No.2 powder
	Feed intake (kg/piglet/day)		
5-wk-old	0.15 ± 0.06^a	0.20 ± 0.02	0.15 ± 0.03
6-wk-old	0.34 ± 0.07^b	0.44 ± 0.08^a	0.40 ± 0.03^b
7-wk-old	0.56 ± 0.06	0.64 ± 0.07	0.59 ± 0.10
8-wk-old	0.82 ± 0.11^b	1.01 ± 0.10^a	0.88 ± 0.11^b
Whole period	0.47 ± 0.06^b	0.57 ± 0.05^a	0.50 ± 0.06^{ab}
	Body weight gain (kg/piglet/day)		
5-wk-old	0.00 ± 0.05	0.05 ± 0.01	0.01 ± 0.01
6-wk-old	0.17 ± 0.05	0.21 ± 0.05	0.20 ± 0.04
7-wk-old	0.30 ± 0.03	0.33 ± 0.04	0.29 ± 0.06
8-wk-old	0.39 ± 0.08	0.52 ± 0.10	0.45 ± 0.06
Whole period	0.22 ± 0.04^b	0.28 ± 0.03^a	0.24 ± 0.03^{ab}
	Feed efficiency (Gain/Feed)		
5-wk-old	0.00 ± 0.43	0.25 ± 0.05	0.07 ± 0.08
6-wk-old	0.50 ± 0.22	0.48 ± 0.14	0.50 ± 0.08
7-wk-old	0.54 ± 0.06	0.52 ± 0.10	0.49 ± 0.11
8-wk-old	0.49 ± 0.13	0.51 ± 0.12	0.51 ± 0.08
Whole period	0.47 ± 0.12	0.48 ± 0.08	0.47 ± 0.08

* Mean \pm SD.

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

Pu *et al.* (2018) 指出於飼料中添加凝結芽孢桿菌，可增加仔豬的隻日飼料採食量 (0.406 vs. 0.486 kg)。吳等 (2019) 在 5 – 7 週齡離乳仔豬飼糧中添加凝結芽孢桿菌，可顯著提高全期隻日飼料採食量 (0.40 vs. 0.50 kg)。本試驗獲得類似的結果，推測由於飼料添加凝結芽孢桿菌能影響腸道微生物菌相並改善腸道吸收，進而提升仔豬採食量。同時凝結芽孢桿菌可產生有機酸—左旋乳酸 (劉及林, 2016)，有機酸於解離前以親脂性型態存在，具穿透革蘭氏陰性菌細胞膜的能力，於細菌細胞內有機酸解離成氫離子和羧基陰離子，氫離子可降低細菌細胞內 pH 值，羧基陰離子則具有抑制細菌 DNA 和蛋白質合成的作用 (Stratford and Anslow, 1996; Russel and Diez-Gonzales, 1998)，推測凝結芽孢桿菌產生之有機酸能抑制革蘭氏陰性菌的繁殖，有助於改善腸菌相與提升仔豬採食量。

(ii) 仔豬增重

A、B 與 C 組仔豬的全期隻日增重分別為 0.22、0.28 與 0.24 kg，以 B 組最高，C 組次之，且 B 組仔豬隻日增重顯著高於對照組 ($P < 0.05$)。三組於各週齡的仔豬增重，具有類似趨勢，均以 B 組為最高，A 或 C 組次之。

飼糧中添加苜蓿有助於仔豬隻腸道中有益菌的增殖，抑制有害菌的增殖 (Wang, *et al.*, 2018)，飼糧中添加 5% 紫色狼尾草降低母豬腸道中大腸桿菌 (*Escherichia*) 與志賀氏菌 (*Shigella*) 的增殖 (Huang *et al.*, 2021)。Guerra *et al.* (2006) 指出，仔豬飼料中添加乳酸桿菌，具有提升仔豬日增重的效果。飼料中添加乳酸桿菌的代謝產物，亦具有提升仔豬日增重的結果 (Thu *et al.*, 2011)。由於纖維素在大腸道中可作為腸道微生物之營養來源，讓腸道內多種的細菌數量快速增加，包括好氧性與厭氧性之菌種 (Schnabel *et al.*, 1983)，因此分纖維素之凝結芽孢桿菌，可使用做為提升豬隻生長性能的益生菌來源。Pu *et al.* (2018) 指出，凝結芽孢桿菌可以改善腸粘膜屏障的完整性，以減緩大腸桿菌的攻擊，促進仔豬的生長。吳等 (2019) 在飼料中添加凝結芽孢桿菌，有提高 5 至 7 週齡離乳仔豬日增重的趨勢，綜上所述，使用凝結芽孢桿菌等益生菌可提升仔豬的增重。本試驗結果亦顯示，於飼料中添加凝結芽孢桿菌添加物可提升仔豬的日增重，但額外再添加狼尾草粉組 (C 組) 之仔豬增重與 B 組間無顯著差異，顯示飼料中添加凝結芽孢桿菌再額外添加狼尾草粉 0.32%，未有進一步提升仔豬增重的效果。

由於離乳、運輸及飼養環境設施改變等因素，常造成仔豬的緊迫，緊迫通常持續 4 到 24 小時 (Lewis and Berry, 2006)。在離乳過程中，仔豬與母豬分離，混合仔豬群和重排社會序位，經常造成仔豬離乳後 48 小時內採食量下降甚至未採食的情形 (Brooks *et al.*, 2001)。王等 (2020) 指出，飼料中添加凝結芽孢桿菌對仔豬離乳後第一週的增重無顯著助益，且仔豬離乳後第一週的增重為負值。本試驗有類似的結果，添加凝結芽孢桿菌對仔豬離乳後第一週的增重無顯著助益，但可降低仔豬負增重的頭數比率。

(iii) 飼料效率

A、B 與 C 組仔豬於試驗全期飼料效率分別為 0.46、0.48 與 0.47，各組間無顯著差異，各組仔豬於各週齡的飼料效率亦無顯著差異。有研究指出仔豬飼糧中添加液化澱粉芽孢桿菌 (*Bacillus amyloliquefaciens*) 或凝結芽孢桿菌對離乳仔豬飼料效率無顯著影響 (曾等, 2017; 吳等, 2019)，本試驗獲得類似結果。

II. 添加凝結芽孢桿菌與狼尾草粉對仔豬血液學檢測值的影響

仔豬於 7 週齡時，各組的血液學數值測定結果如表 3 所示，各組間之各檢測項目數值均無顯著差異，其中各組平均紅血球血紅素濃度 (Mean corpuscular cell hemoglobin concentration, MCHC) 介於 27.0 – 27.4% 之間，除此項略低於 Ježek *et al.* (2018) 參考值的最低值 28.8%。其餘各檢測項目之數值均落在 Ježek *et al.* (2018) 的推薦參考值內，顯示各項檢測值均在一般合理範圍內。

在不同的降溫設施下，母豬血液中的嗜中性白血球與淋巴球比值 (NET/LYM) 是良好的中長期壓力指標，緊迫提高嗜中性球與淋巴球的比值 (Quiñonero *et al.*, 2009)。王等 (2020) 飼養之對照組與添加凝結芽孢桿菌組仔豬，其 NET/LYM 比值分別為 0.79 與 0.71。本試驗 A、B 與 C 組的 NET/LYM 比值分別為 0.73、0.72 與 0.70，各組間無顯著差異，與王等 (2020) 的比值相近，推測兩試驗的仔豬所受到的中長期壓力相似。

表 3. 飼糧中添加凝結芽孢桿菌與狼尾草粉對 7 週齡仔豬血液學分析值之影響

Table 3. Effects of adding *Bacillus coagulans* napier grass powder in diet on hematological profile of piglets at 7 wk of age

Group	A Control	B A diet added <i>Bacillus coagulans</i> at 2×10^5 CFU/kg	C B diet added 0.32% Napiergrass Taishigrass No.2 powder	Ježek <i>et al.</i> (2018) Reference ranges (7 – 14 weeks of age)
No.	16	16	16	
RBC, 10^{12} cell/uL	$6.3 \pm 0.3^*$	6.2 ± 0.3	6.3 ± 0.3	5.40 – 7.28
WBC, 10^9 cell/uL	21.4 ± 1.6	22.1 ± 0.9	22.7 ± 4.5	13.70 – 34.12
PLT, 10^3 /uL	469.6 ± 60.4	558.8 ± 75.4	533.8 ± 72.7	273 – 730
NET, %	39.6 ± 1.4	38.7 ± 3.6	37.6 ± 4.0	30 – 71
LYM, %	55.3 ± 2.2	56.3 ± 3.2	56.9 ± 2.9	22 – 69
MON, %	4.2 ± 1.0	3.8 ± 0.8	4.3 ± 1.6	0 – 7
EOS, %	0.61 ± 0.18	0.73 ± 0.25	0.73 ± 0.27	0 – 9
BASO, %	0.39 ± 0.04	0.44 ± 0.05	0.46 ± 0.05	0 – 2
Hgb, gm%	10.3 ± 0.5	10.2 ± 0.4	10.3 ± 0.2	9.2 – 12.5
Hct, %	38.2 ± 1.3	37.8 ± 1.8	37.4 ± 1.9	28.0 – 41.7
MCV, fL	60.8 ± 1.9	60.9 ± 0.6	59.3 ± 1.3	47.7 – 63.0
MCH, pg	16.4 ± 0.8	16.4 ± 0.4	16.2 ± 0.2	14.0 – 18.5
MCHC, g/dL	27.0 ± 0.5	27.0 ± 0.8	27.4 ± 0.7	28.8 – 33.5
NET/LYM	0.73 ± 0.06	0.72 ± 0.10	0.70 ± 0.13	

* Mean \pm SD.

RBC, red blood cells; WBC, white blood cells; PLT, platelets; NET, neutrophils; LYM, lymphocytes; MON, mononuclear balls; EOS, eosinophilic white blood cells; BASO, basophilic white blood cells; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular cell hemoglobin concentration.

結 論

離乳仔豬飼糧中添加凝結芽孢桿菌 2×10^5 CFU/kg，可提升仔豬飼料採食量與增重，但對飼料效率則無顯著影響。

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Effects of applying *Bacillus coagulans* and Napier grass powder on the growth performances of weaned piglets ⁽¹⁾

Chin-Meng Wang ⁽²⁾ Fang-Chieh Liu ⁽²⁾ Bing-Sian Lin ⁽³⁾ and You-Jun Lin ⁽⁴⁾⁽⁵⁾

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Abstract

The purpose of this study was to evaluate the effects of *Bacillus coagulans* products and Napier grass meal on growth performance of weaned piglets. A total of 48 four-week-old (Landrace × Duroc) weaned piglets were used. Pigs were randomly divided into 3 groups and provided with control diet (A), control diet added 2×10^5 CFU/kg *Bacillus coagulans* products (B), and B diet added with Napier grass Taishigrass No.2 meal 3.2 g/kg (C). During the 4 weeks experiment, feed intake, body weight and hematology of the piglets were measured. The result showed that the daily feed intake of pigs at A, B, and C groups were 0.47, 0.57 and 0.51 kg, respectively. Pigs at B group had significantly ($P < 0.05$) larger feed intake than the control group. The body weight gain of pigs in B group was also significantly ($P < 0.05$) larger than those in the control group. There were no differences on the feed efficiency among groups. In hematological profile, no significant differences were observed among groups. In conclusion, the dietary application of *Bacillus coagulans* products significantly increased feed intake and body weight gain of the weaned piglets.

Key words: Weaned piglet, *Bacillus coagulans*, Napier grass powder, Growth performance.

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(2) Animal Industry Division, COA-LRI, Tainan 71246, Taiwan, R. O. C.

(3) Animal technology research center, Agricultural Technology Research Institute. Hsinchu 300100, Taiwan, R. O. C.

(4) Nutrition Division, COA-LRI, Tainan 71246, Taiwan, R. O. C.

(5) Corresponding author, E-mail: hiujj@mail.tlri.gov.tw.

應用綠球藻去除養豬廢水中氮磷與沼氣中二氧化碳⁽¹⁾

蘇天明⁽²⁾⁽⁴⁾ 翁義翔⁽²⁾ 廖仁寶⁽²⁾ 蕭庭訓⁽²⁾ 程梅萍⁽³⁾

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摘 要

養豬廢水經過三段式處理後水質的氮和磷濃度仍高，厭氧處理階段產生的沼氣含有高比例的二氧化碳，皆可提供藻類作為營養源。因此，本試驗旨在探討應用三段式處理後的養豬廢水，及厭氧階段產生的沼氣培養綠球藻 (*Chlorella* sp.)，對廢水中氮磷去除與沼氣中二氧化碳減量的效果，期予以資源再利用，並減輕對承受水體和溫室效應之負面影響。使用經三段式處理後氮和磷濃度分別為 324 mg/L 和 84 mg/L 的養豬廢水作為綠球藻培養液，採取靜置 (A 組)、空氣曝氣 (B 組)，及沼氣曝氣 (C 組) 等三種不同二氧化碳供應方式進行綠球藻培養。培養 2 週後，C 組培養液中所含氮 (200 mg/L) 和磷 (49 mg/L) 濃度顯著較 A 組及 B 組為低，氮和磷的去除率分別為 38.4% 和 41.4%，綠球藻產量較 A 組及 B 組為高 ($P < 0.05$)，每生產 1 g 綠球藻約消耗 4.84 L 的二氧化碳。各組生產的綠球藻乾基粗蛋白和磷含量分別介於 41 – 64% 及 2.18 – 2.68% 之間。綜上所述，使用三段式處理後的養豬廢水及沼氣培養綠球藻，可有效促進其生長並具有降低廢水中氮和磷含量及沼氣中的二氧化碳效果，且藻體之粗蛋白質及磷含量與大豆粕和魚粉相近，具供作飼糧原料之可能性。但需精進藻體收穫技術及細胞壁破壁技術方具實用性，而藻液以離心方法收穫藻體，培養液上層仍有藻體懸浮，必須再經適當處理。

關鍵詞：綠球藻、養豬廢水、氮磷去除。

緒 言

全球人口正快速增加，美國人口普查局預估 2050 年全球人口將達到驚人的 99 億，糧食需求將增加 70 – 100% (Prosekov and Ivanova, 2018)。豬肉是世界上消耗量最多的畜產品 (Dennehy *et al.*, 2017)，為了滿足人類對蛋白質需求的不斷增加，世界各地的養豬頭數都呈指數增加，因此也產生大量的養豬廢水 (Nagarajan *et al.*, 2019a)，致衍生廢水的處理問題 (Dennehy *et al.*, 2017)。養豬廢水所含的氮和磷濃度分別為 200 – 2,055 mg/L 和 100 – 620 mg/L (Cheng *et al.*, 2019)，經三段式廢水處理後氮和磷的濃度則大約介於 200 – 400 mg/L 和 20 – 100 mg/L 之間 (曾等, 2003；蔡及周, 2005；蘇等, 2020)，顯示經三段式處理後養豬廢水中仍富含高濃度的氮和磷等營養鹽。依照現行的放流水標準 (行政院環境保護署, 2019)，氨氮與正磷酸鹽之最大限值分別為 10.0 與 4.0 mg/L，雖然養豬業放流水目前尚不適用該項標準，但放流水氮、磷過高將衍生後續之環境污染與河川水質優養化等環保問題 (Giannuzzi *et al.*, 2011)，仍須加強放流水氮磷減量技術之研發。

養豬廢水若未妥善處理，將造成水源與土壤污染、溫室氣體排放，以及對人類健康產生負面影響 (Dennehy *et al.*, 2017; Wang *et al.*, 2020)。Hu *et al.* (2021) 指出，厭氧處理是最有效的豬糞尿處理方法之一，包括沼氣的產生、溫室氣體的減量排放及病原體的減少等。De la Noüe and De Pauw (1988) 研究，以微藻進行生物處理可藉太陽能將二氧化碳及氮磷轉化為有用的生物質。由於藻類具有利用無機氮和磷以促進其生長的能力，故可利用藻類培養進行廢水的三級處理 (Larsdotter, 2006; Abdel-Raouf *et al.*, 2012)，Nagarajan *et al.* (2019a) 研究發現，微藻可耐受養豬廢水中的高氮含量，同時還可以去除磷。養豬廢水三段式處理在厭氧階段會有沼氣產生，Harasimowicz *et al.* (2007) 指出，沼氣含有 55 – 65% 的甲烷、30 – 45% 的二氧化碳 (carbon dioxide, CO₂) 和微量的硫化氫及水氣。張等 (2015) 指出，自營性藻類具有光合作用色素可以行光合作用來捕獲與固定 CO₂，且利用效率高，具有固碳及減少碳排的效果。利用 CO₂ 來培養微藻具有極大的發展潛力 (Abinandan and Shanthakuma, 2015)。廢水中有機物經過微生物分解消

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(2) 行政院農業委員會畜產試驗所經營組。

(3) 行政院農業委員會畜產試驗所主任秘書室。

(4) 通訊作者，E-mail: tmsu@mail.tlri.gov.tw。

化後，產生小分子的有機碳和無機鹽，為藻類極佳之營養物質 (Travieso *et al.*, 2006)。Nagarajan *et al.* (2019b) 指出，沼氣是在厭氧消化過程中獲得的氣態副產物，富含甲烷以及大量其他氣體，而 CO₂ 去除對於沼氣純化至關重要，利用微藻耐高 CO₂ 濃度的特性，藻液可供為 CO₂ 洗滌液。此外，Colla *et al.* (2007) 也指出，藻類富含蛋白質、脂質、維生素、礦物質、微量元素等營養素，可作為動物飼料原料，但微藻具有非常堅韌的細胞壁，單胃動物很難消化微藻細胞壁，必須開發適當的技術來提高動物體內微藻營養物的生物利用率 (Austic *et al.*, 2013; Lum *et al.*, 2013)。

將廢棄物資源化的循環經濟是時代所趨，為了利用養豬廢水的氮、磷，以及降低沼氣中 CO₂ 含量，本研究應用三段式處理後的養豬廢水和沼氣培養綠球藻，探討本程序對降低放流水氮磷及沼氣中 CO₂ 濃度的效果。

材料與方法

I. 綠球藻來源

本試驗使用蘇等 (2016) 培養的綠球藻 (*Chlorella* sp.)。蘇等 (2016) 利用養豬廢水以專一性培養基 (BCRC Number: AL20008, 生物資源及研究中心; 表 1) 培養，再參考蔡等 (2010) 方法，取藻液以 DNA 套組萃取藻體 DNA，以聚合酶連鎖反應 (polymerase chain reaction, PCR) 進行 DNA 複製，而後以 18S rDNA 分子選殖 (clone)，選擇適當的勝任細胞，插入藻體的 DNA 片段，最後利用核酸自動定序儀定序，再將核酸序列與美國國家生物技術資訊中心 (National Center for Biotechnology Information, NCBI; <http://www.ncbi.nlm.nih.gov/>) 資料庫比對得到與綠球藻核酸序列的相似度達 99%，確定培養的藻體為綠球藻。

表 1. 綠球藻培養基組成分

Table 1. The component of culture medium for *Chlorella* sp

Items	Usage amount/100 mL ¹
Medium	
Ca (NO ₃) ₂ · 4H ₂ O	15 mg
KNO ₃	10 mg
β-Na ₂ glycerophosphate · 5H ₂ O	5 mg
MgSO ₄ · 7H ₂ O	4 mg
Tris (hydroxymethyl) aminomethane	50 mg
Vitamin B ₁₂	0.01 µg
Biotin	0.01 µg
Thiamine HCl	1 µg
P IV metals (components as follow)	0.3 mL
Distilled water	99.7 mL

P IV metals	
Na ₂ EDTA · 2H ₂ O	100 mg
FeCl ₃ · 6H ₂ O	100 mg
MnCl ₂ · 4H ₂ O	3.6 mg
ZnCl ₂	1.04 mg
CoCl ₂ · 6H ₂ O	0.4 mg
Na ₂ MoO ₄ · 2H ₂ O	0.25 mg
Distilled water	100 mL

¹ Refer to the medium formula of BCRC Biological Resources and Research Center (BCRC Number: AL20008).

II. 綠球藻先期培養過程：如圖 1 所示。

- 將綠球藻液 40 mL 放入 1 L 螺旋蓋血清瓶，加入經過厭氧及曝氣處理後的養豬廢水 (培養液) 460 mL，以小型曝氣機輔以曝氣石，通入空氣進行培養。
- 培養 2 週後，將 (i) 綠球藻液倒入 8 L 裝的透明桶，加入 3.5 L 綠球藻培養液，進行培養。經培養 2 週後，約

獲得 4 L 藻液，平均分裝於 4 個 8 L 裝的透明桶，再加入 3 L 培養液。再經培養 2 週後，約獲得 16 L 藻液，再平均分裝於 16 個 8 L 裝的透明桶，加 3 L 培養液。再經培養 2 週後，於每一透明桶再加 3 L 培養液，期間皆以小型曝氣機輔以曝氣條提供空氣，共生產 100 L 的藻液。

- (iii) 將 (ii) 之藻液平均分裝於 4 個容量 100 L 培養桶 (每桶約 25 L) 後，加入 60 L 培養液，以曝氣條提供空氣培養 2 週供試驗用。

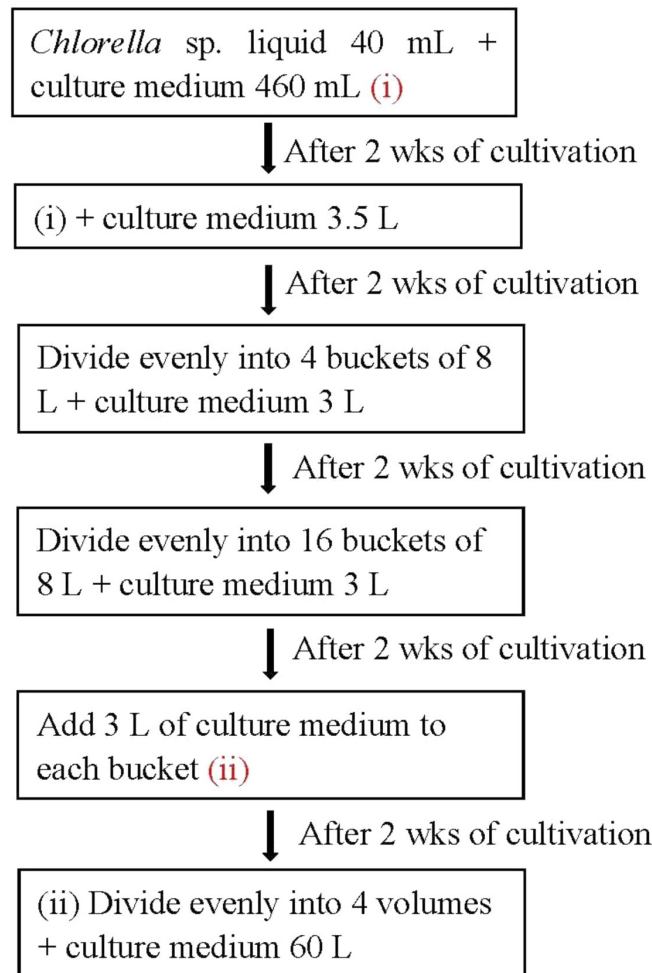


圖 1. 綠球藻先期培養過程。

Fig. 1. Culture process of *Chlorella* sp. in the pre-trial stage.

III. 二氧化碳供應方式

- (i) 試驗開始將前述藻液集中並注入空氣混合均勻後，平均分裝於 6 個加入 25 L 培養液、容量 100 L 的培養桶 (每桶約 80 L)，分別採取靜置 (A 組)、以空氣供應 CO₂ (B 組) 及以沼氣供應 CO₂ (C 組) 等 3 種不同 CO₂ 供應方式培養 2 週，每組 2 重複。
- (ii) 二氧化碳供應濃度：A 組及 B 組培養桶上方皆不加蓋，A 組採取靜置方式僅表面可接觸空氣，故二氧化碳供應量以 0 估算，B 組以曝氣機注入空氣供應，二氧化碳供應量以空氣的二氧化碳含量 0.0412% (Buis, 2019) 估算。C 組沼氣源自畜產試驗所總廢水處理場，從污泥消化池恆壓筒接管路收集至容積約 12.723 m³ 的貯氣袋 (長 × 直徑 = 5 m × 1.8 m)，試驗期間不再通入外源沼氣，培養桶底部放置曝氣條與貯氣袋連接，以塑膠軟管建置迴路，以加壓馬達抽取沼氣使沼氣循環於培養桶與貯氣袋間，通入培養桶內之曝氣條以供應二氧化碳並攪拌培養液，培養桶上方則加蓋並輔以迫緊使密合不洩氣。
- (iii) C 組 (以沼氣供應) 二氧化碳消耗量估算
 1. 以二氧化碳和甲烷的標準品，配製 5 種不同比例的混合氣體 (二氧化碳：甲烷 = 0：100、20：80、40：60、60：40、80：20、100：0)，以 500 μL 的氣密注射針將混合氣體注入氣相層析儀 (Trace 2000, Thermo Scientific, UK)，以 TCD 為偵測器、攜帶氣體 (carrier gas) 為氮氣，以積分儀計算峰面積 (peak area) 後製作檢量線 (圖 2)。

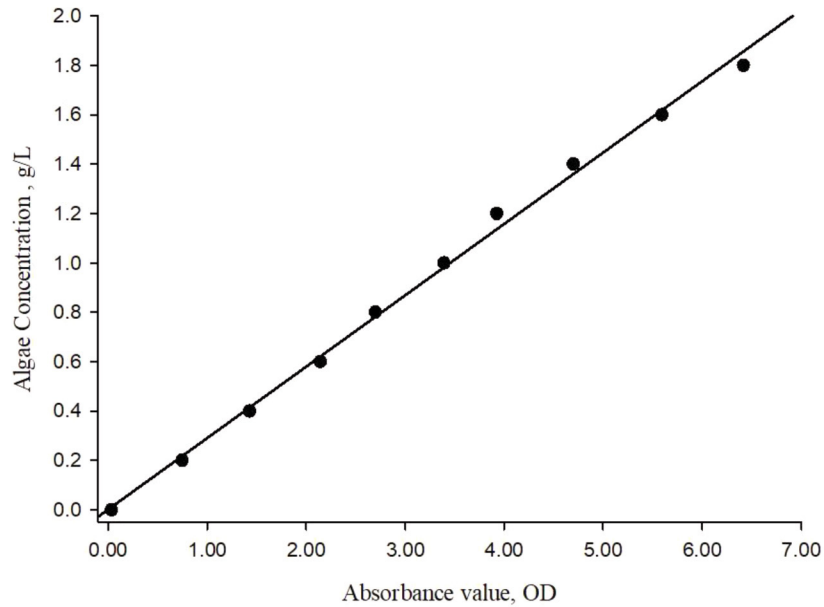


圖 2. 藻液吸光值及藻體質量之檢量線 ($y = 0.2891x + 0.0015$; $R^2 = 0.9967$)。

Fig. 2. Calibration line of algae liquid absorbance value and biomass quantity.

2. 試驗前、後分別從貯氣袋與塑膠軟管連接處，以注射針筒採集沼氣樣品收集在 1 L 的氣體採樣袋中，試驗前後樣品各採集 3 袋後，立即攜回實驗室進行分析。

3. 樣品分析：以 500 μL 的氣密注射針抽取收集的氣體注入氣相層析儀，以積分儀計算峰面積，線性迴歸公式： y (二氧化碳濃度, %) = $8\text{E-}07x$ (峰面積) + 2.2437, $R^2 = 0.9951$ ，估算排出氣體之二氧化碳濃度。

IV. 藻液綠球藻乾物量檢量線製作與乾物量估算

- (i) 從 C 組取 5 L 培養後藻液，以離心機 (Himac CR22G, Hitachi, Japan) 在 3,000 rpm 條件下離心 15 分鐘，收集下層藻體後，於 105°C 下進行烘乾。
- (ii) 精秤乾物重 0.200、0.400、0.600、0.800、1.000、1.200、1.400、1.600 及 1.800 g 之藻體，先以 10 mL 綠藻液態培養基 (表 1) 浸泡 30 分鐘，而後以電磁攪拌器 (MS-11) 輔以攪拌石混合均勻後，再以綠藻液態培養基定量至 1 L 並繼續攪拌。
- (iii) 參考陳 (2013) 方法，利用分光光譜儀 (Hitachi U-2900, Japan) 在 682 nm 波長下，量測到的吸光值對應綠球藻濃度製作檢量線 (圖 3)，線性迴歸公式： y (綠球藻濃度, g/L) = $0.2891x$ (吸光值) + 0.0015, $R^2 = 0.9967$ ，以吸光值估算藻液中綠球藻濃度。

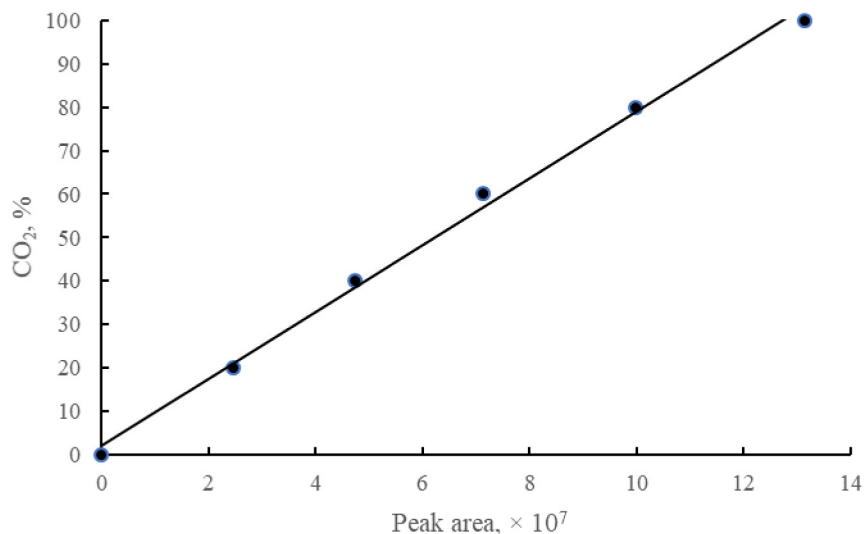


圖 3. 二氧化碳濃度檢量線 ($y = 8\text{E-}07x + 2.2437$; $R^2 = 0.9951$)。

Fig. 3. Calibration curve of carbon dioxide concentration.

(iv) 試驗前、後取各組藻液，以「藻液中綠球藻濃度 × 藻液容積」公式，估算綠球藻乾物量。

V. 二氧化碳消耗量估算

以下列公式估算試驗期間 C 組每生產 1 g 綠球藻之二氧化碳消耗量， CO_2 消耗量 ($\text{L CO}_2/\text{g Chlorella sp.}$) = $[(\text{試驗前沼氣中 CO}_2 \text{ 含量}(\%) - \text{試驗後沼氣中 CO}_2 \text{ 含量}(\%)) \div 100 \times 12,723 (\text{貯氣袋容積}(\text{L}))] \div (\text{試驗後藻體乾物重}(\text{g}) - \text{試驗前藻體乾物重}(\text{g}))$ ，其中沼氣容積皆以貯氣袋容積估算之。

VI. 採集各處理組培養前、後之藻液樣品，經 105℃ 乾燥後，分析藻體之總氮、總磷、銅、鋅、鐵、錳含量，分析方法：

- (i) 總氮：參照行政院環境保護署環境檢驗所 NIEA W423.52C (2004) 方法計算之。水中總氮濃度 = 水中硝酸鹽氮濃度 + 水中亞硝酸鹽氮濃度 + 水中凱氏氮濃度。水中硝酸鹽氮、亞硝酸鹽氮及凱氏氮之測定，分別參照行政院環境保護署環境檢驗所 NIEA W419.51A (2006a)、NIEA W418.53C (2019) 及 NIEA W451.51A (2006b) 方法分析之。
- (ii) 總磷：參照行政院環境保護署公告之水中磷檢測方法—分光光度計 / 維生素丙法 (NIEA W423.52C27.53B；行政院環境保護署環境檢驗所，2010) 方法分析之。
- (iii) 礦物質濃度分析：精秤藻體乾重後，放入灰化爐 (NEYTECH-2-525)，在 550 – 650℃ 溫度下灰化約 6 小時。樣品灰化、冷卻、精秤，記錄灰分濃度後，加入 3 N 的鹽酸 10 mL，以鈹玻璃覆蓋置 350℃ 電熱板進行酸解、過濾並定量後，以原子吸收光譜儀 (Atomic absorption spectrometer Z8100, Hitachi, Japan) 測定銅、鋅、鐵及錳濃度。

VII. 統計分析

二氧化碳供應方式對綠球藻產量、二氧化碳消耗量及藻體成分等分析資料，利用 SAS 統計分析套裝軟體的一般線性模式程序 (General linear model procedure) 進行變方分析 (SAS, 2002)，若有處理效應 ($P < 0.05$)，再以鄧肯新多域顯著性測驗法 (Duncan's new multiple range test)，檢定各處理組間的差異顯著性。

結果與討論

I. 二氧化碳供應方式對綠球藻產量之影響

藻液濃度愈高，其吸光值相對愈高 (陳，2013)。試驗前各組每桶藻液量約 80 L，藻液吸光值在 0.631 – 0.664 間，以圖 2 公式 (綠球藻濃度， $\text{g/L} = 0.2891 \times \text{吸光值} + 0.0015$) 估算，各組培養前的藻液濃度在 0.202 – 0.213 g/L 間，每桶的綠藻乾物重則介於 16.2 – 17.1 g (表 2)。經 2 週培養後，A 組藻液量顯著地較 B 組及 C 組為多，係因 A 組未提供氣體靜置於培養桶，期間的藻液量僅因表面蒸發略減，C 組則為了瞭解試驗期間 CO_2 消耗量，故設計為加蓋密合，B 組藻液量較 C 組少，係因未加蓋並提供空氣，致蒸發量較大所致。試驗結束時，A 組、B 組及 C 組的藻液濃度分別為 0.409、0.794 及 1.492 g/L，估算藻體乾物重則為 32.6、61.5 及 117 g/桶，C 組藻體產量顯著地 ($P < 0.05$) 較 A 組及 B 組為多，此與藻類具有光合作用色素可以行光合作用來捕獲與固定 CO_2 (張等，2015)，而沼氣含有 30 – 45% 的 CO_2 (Harasimowicz *et al.*, 2007)，空氣的 CO_2 含量僅約 0.0412% (Buis, 2019) 應有關係。

II. 二氧化碳消耗量

張等 (2015) 指出，藻類培養因種類或生長環境的不同，人工培養可採取自營、異營或混營等方式。藻類大多僅利用溶在水中的 CO_2 ，自營培養最大的問題在於 CO_2 的溶解速率低，致藻類生長速率緩慢，異營培養是必須提供碳源與其他營養源致成本較高，而混營培養可同時進行自營生長和異營生長 (張等，2015)。本試驗 C 組利用養豬廢水及沼氣提供綠球藻培養所需之氮、磷及微量礦物質，並從培養桶底部通入沼氣希提高 CO_2 的溶解效率供作綠球藻營養源。採集 C 組試驗前、後沼氣進行二氧化碳濃度分析，結果試驗前 CO_2 濃度為 23.15%，較 Harasimowicz *et al.* (2007) 指出的 30 – 45% 為低，試驗後則為 7.94%，進行 2 週的綠藻培養約消耗 15.21% 的二氧化碳。本試驗未測定試驗後貯氣袋內的沼氣容積而以試驗前之容積再依照圖 3 的檢量線估算，獲得每生產 1 g 藻體乾物質約消耗 4.84 L 的 CO_2 ，而試驗期間 CO_2 去除率約 65.70% 的推論值。此與 Nagarajan *et al.* (2019b) 指出，微藻具有耐高 CO_2 濃度的特性，可利用沼氣所含 CO_2 供為藻類營養源之研究結果一致。

III. 綠球藻培養前、後藻液成分變化

綠球藻培養前分析培養液 (經過厭氧及曝氣處理後的養豬廢水) 成分，結果其總氮、總磷、銅、鋅、鐵和錳的含量分別為 324、84、2.1、10.2、25.8 和 4.3 mg/L。經 2 週培養後，C 組培養液的氮和磷濃度皆顯著地較 A

組及 B 組為低 (表 3)，A 組經 2 週培養後培養液成分與培養前相近，而 B 組培養後之培養液除了 N 濃度較培養前為低 ($P < 0.05$) 外，其他成分皆與培養前相近，此應與 C 組藻體產量顯著高於 A 組及 B 組有關，本結果與渠等研究指出藻類可耐受養豬廢水中的高氮含量，利用氮和磷作為營養物質之論述一致 (De la Noüe and De Pauw, 1988; Larsdotter, 2006; Abdel-Raouf *et al.*, 2012; Nagarajan *et al.*, 2019a)。C 組使用氮和磷濃度分別為 324 mg/L 和 84 mg/L 的養豬廢水作為綠球藻培養液，經培養 2 週後培養液中氮和磷濃度降為 200 mg/L 和 49 mg/L，期間去除約 124 mg/L 和 35 mg/L 的氮和磷，去除率分別為 38.4% 和 41.4%，顯示綠球藻具有利用氮和磷的能力 (Larsdotter, 2006; Abdel-Raouf *et al.*, 2012)，可利用於養豬廢水的氮磷去除。本試驗經培養綠球藻 2 週後，培養液氮和磷濃度仍高，後續將嘗試延長培養期間，以了解綠球藻去除養豬廢水氮磷的最佳效果。各組培養液在培養後的銅、鋅、鐵和錳含量差異皆相近，而去除率皆以 C 組顯著地較 A 組及 B 組顯著為高，與 C 組藻體產量較 A 組及 B 組皆高應有相關性。

表 2. 二氧化碳供應對綠球藻產量之影響

Table 2. Effect of carbon dioxide supply on *Chlorella* sp. production

Items	Group A ¹	Group B	Group C	SE
Volume, L/tub				
Before cultivation	80.1	80.2	80.1	0.1
After cultivation	79.6 ^a	77.4 ^c	78.5 ^b	0.1
Algae concentration ² , g/L				
Before cultivation	0.212 ^a	0.202 ^b	0.213 ^a	0.01
After cultivation	0.409 ^c	0.794 ^b	1.492 ^a	0.02
Algae dry weight ² , g/tub				
Before cultivation	16.98	16.20	17.06	0.05
After cultivation	32.58 ^c	61.47 ^b	117.09 ^a	0.07
Difference	15.59 ^c	45.27 ^b	99.98 ^a	0.06

¹ Group A: static state; Group B: CO₂ supplied by air bubbling; Group C: CO₂ supplied by biogas bubbling.

² Estimated value, algae concentration = $0.2891 \times \text{absorbance value} + 0.0015$; algae dry weight = algae concentration \times volume.

^{a, b, c} Means in the same row without the same superscripts are significantly different ($P < 0.05$).

表 3. 綠球藻培養前後培養液成分變化

Table 3. Compositions of culture medium before and after cultivation of *Chlorella* sp

Items	Before cultivation	After cultivation			SE
		Group A ¹	Group B	Group C	
Concentration, mg/L					
Nitrogen	324 ^a	313 ^a	280 ^b	200 ^c	12.0
Phosphorus	84 ^a	80 ^a	72 ^a	49 ^b	6.0
Copper	2.1	2.0	1.9	1.8	0.2
Zinc	10.2	9.9	10.0	9.4	0.2
Iron	25.8	24.9	25.0	23.5	0.3
Manganese	4.3	4.0	3.9	3.6	0.2
Removal rate, %					
Nitrogen		3.37 ^c	13.54 ^b	38.36 ^a	4.30
Phosphorus		4.46 ^c	14.35 ^b	41.35 ^a	3.90
Copper		6.03 ^b	7.16 ^b	16.17 ^a	0.35
Zinc		2.48 ^b	1.91 ^b	7.58 ^a	0.21
Iron		3.56 ^b	3.04 ^b	8.93 ^a	0.33
Manganese		7.67 ^b	8.24 ^b	16.05 ^a	0.68

¹ Described as in Table 2.

^{a, b, c} Means in the same row without the same superscripts are significantly different ($P < 0.05$).

IV. 綠球藻乾物質成分

C 組乾物質氮、鐵和錳含量皆顯著地較 A 組及 B 組為高，磷含量也較 A 組為高 ($P < 0.05$)，而銅和鋅含量各組間相近 (表 4)，此與 C 組 CO_2 供應量較 A 組及 B 組皆多 (張等, 2015) 應有關係。各組乾物質氮含量達 6.61 – 10.3%，估算粗蛋白質含量介於 41 – 64% 間，與大豆粕及魚粉相近，而磷含量 (2.18 – 2.86%) 也與魚粉的 2.98% (行政院農業委員會畜產試驗所, 2011) 相近。銅、鋅、鐵和錳的含量分別為 20 – 22ppm、59 – 61 ppm、140 – 158 ppm 及 28 – 37 ppm。Marta *et al.* (2017) 整理了多篇研究報告指出，綠球藻的粗蛋白與磷分別為 37.7 – 47.8% 與 0.96 – 1.76%，而鐵、錳和鋅的含量則介於 400 – 5,500、20 – 100 和 6 – 50 mg/kg 間，本試驗綠球藻的磷含量較 Marta *et al.* (2017) 整理的文獻數據為高，而鐵的含量則明顯較低。Colla *et al.* (2007) 指出，藻類具供為畜禽飼糧原料之可行性，但由於單胃動物很難消化微藻細胞壁，必須開發適當的技術以提高營養分的生物可利用率 (Austic *et al.*, 2013; Lum *et al.*, 2013)。

表 4. 培養後綠球藻乾物質成分

Table 4. The dry matter composition of *Chlorella* sp. after cultivation

Items	Group A ¹	Group B	Group C	SE
Nitrogen, %	6.61 ^c	9.50 ^b	10.27 ^a	0.24
Phosphorus, %	2.18 ^b	2.58 ^{ab}	2.86 ^a	
Copper, ppm	20	21	22	1.3
Zinc, ppm	59	61	61	2.5
Iron, ppm	140 ^c	152 ^b	158 ^a	1.3
Manganese, ppm	28 ^c	34 ^b	37 ^a	1.6
Crude protein ² , %	41.31	59.37	64.19	—

¹ Described as in Table 2.

² Estimated value, Crude protein = Nitrogen \times 6.25.

^{a, b, c} Means in the same row without the same superscripts are significantly different ($P < 0.05$).

結 論

本試驗以三種不同二氧化碳供應方式 (靜置、空氣曝氣及沼氣曝氣)，發現應用沼氣供應二氧化碳組 (C 組) 在為期 2 週的培養期間藻體產量最高，而生產的綠球藻粗蛋白含量達 64%，總氮 (38.4%) 和總磷 (41.4%) 的去除率皆較 A 組及 B 組為高，每生產 1 g 綠球藻估計約消耗 4.84 L 二氧化碳，藻體粗蛋白含量與目前國內常用的蛋白質飼料原料相近。但藻液以離心方法收穫藻體，培養液上層仍有藻體懸浮，必須再經適當處理後始得放流，以符合放流水標準。研究結果後續在廢水處理方面，可嘗試應用於養豬廢水氮磷去除、利用藻液作為沼氣去除二氧化碳之洗滌液，以及藻體收穫後培養液處理模式的研究。在藻體應用方面，將朝精進藻體收穫技術與細胞壁破壁技術，並進一步將藻體開發作為畜禽飼糧替代原料方面進行研究。

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Application of *Chlorella* sp. to the removal of nitrogen and phosphorus in pig wastewater and carbon dioxide in biogas ⁽¹⁾

Tein-Ming Su ^{(2) (4)} Yi-Hsiang Weng ⁽²⁾ Ren-Bao Liaw ⁽²⁾ Ting-Hsun Hsiao ⁽²⁾ and Mei-Ping Cheng ⁽³⁾

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Abstract

After the three-stage treatment of pig wastewater, concentrations of nitrogen (N) and phosphorus (P) of the water were still high. The biogas produced in the anaerobic treatment stage contained a high proportion of carbon dioxide (CO₂), which could provide algae as a carbon source. Therefore, the aim of this study was to explore the effects of application of the discharged pig wastewater from the three-stage treatment system and the biogas produced from the anaerobic stage to cultivate *Chlorella* sp., with regards to the removal of N and P in the wastewater and the consumption of CO₂ in the biogas. The reuse of resources is expected to reduce the negative impacts of the discharged wastewater on the water body and of the biogas on global warming. A sample of treated pig wastewater with N and P concentrations of 324 mg/L and 84 mg/L, respectively, was used as the culture medium for *Chlorella* sp. Three different carbon dioxide supply methods including static state (group A), forced air bubbling (group B) and forced biogas bubbling (group C) were used on the cultivation of *Chlorella* sp. After two weeks of cultivation, the concentration of N (200 mg/L) and P (49 mg/L) in the culture medium of group C was significantly lower ($P < 0.05$) than those of groups A and B. The removal efficacies of N and P of group C were 38.4% and 41.4%, respectively. The biomass production of algae of group C was higher ($P < 0.05$) than those of groups A and B, and the carbon dioxide consumption of *Chlorella* sp. was estimated to be 4.84 L/g. Besides. The contents of crude protein (CP) and P of algae produced from all the three groups were between 41 to 64% and 2.18 to 2.86% on a dry matter basis, respectively. In summary, the use of the three-stage treated pig wastewater and biogas to cultivate *Chlorella* sp. can effectively promote its growth and reduce the N and P contents of wastewater and the CO₂ in the biogas. The CP and P content of *Chlorella* sp. are similar to those of soybean meal and fish meal, which could likely be used as one of feedstuff ingredients. However, it is necessary to refine the algae harvesting and the cell wall breaking technology, in practice, and there were still some algae suspended in the upper layer of harvested culture liquid after the centrifugation operation, which must be properly treated.

Key words: *Chlorella* sp., Pig wastewater, Nitrogen and phosphate removal.

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(2) Livestock Management Division, COA-LRI, Tainan 71246, Taiwan, R. O. C.

(3) Chief Secretary Office, COA-LRI, Tainan 71246, Taiwan, R. O. C.

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

行政院農業委員會畜產試驗所「畜產研究」稿約

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