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溫濕度指數對紐西蘭公兔產精性能之影響 (1)

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摘　　要

本研究旨在比較不同環境溫濕度指數 (temperature-humidity index, THI) 對紐西蘭公兔產精性能之影響，以做為兔隻熱緊迫的指標。農業部畜產試驗所紐西蘭兔舍 THI 平均值在 11 月至 4 月的涼季為 20.3 ± 2.5，5 月至 10 月熱季 THI 平均值為 27.6 ± 3.6，分析後顯示當 THI 大於 27.8 時，兔隻有 37.6% 時段處於熱緊迫狀態；公兔涼季離乳體重為 1,001.3 ± 80.5 g，顯著高於熱季離乳體重之 777.4 ± 109.0 g ($P < 0.05$)，且公兔於涼季時之騎乘行為頻度為 100.0 ± 0.0%，顯著高於熱季之 85.7 ± 7.6% ($P < 0.05$)；檢視涼季及熱季時紐西蘭公兔精液性狀，結果顯示其精液體積與精子濃度、死活比率及前進參數值等無顯著差異，僅精子活動力在涼季時為 69.3 ± 10.5%，顯著優於熱季之 46.5 ± 13.6%($P < 0.05$)；比較睪丸組織切片結果顯示，環境持續高溫影響熱季公兔生精作用。綜上所述，環境持續高溫會降低公兔之性慾、睪丸生精作用與精子活動力，可能也是造成夏季公兔生殖力降低之原因。

關鍵詞：紐西蘭白兔、產精性能、溫濕度指數。

緒　　言

熱緊迫已成為全球普遍關注的問題，其為主要的環境緊迫之一，對於農場養兔業造成巨大的經濟損失。熱緊迫對家兔的健康造成多重損害，如器官損傷、氧化緊迫、內分泌調節紊亂、免疫功能抑制和生殖障礙等，最終導致生產性能下降和死亡率增加 (Liang *et al.*, 2022)。兔子非常容易受熱緊迫影響，因為牠們幾乎沒有功能性的汗腺，導致環境溫度太高時無法排出多餘體熱 (body heat) (Marai *et al.*, 2002)。在亞熱帶維持熱季公兔 (rabbit bucks) 精液品質特性至關重要，特別是熱緊迫會延長至超過 6 個月的情況下，不利於該期間兔之繁殖生產。公兔暴露於夏季熱緊迫下，即 THI 超過 27.8，將會減低其體內平衡及精液品質，導致生育力暫時低下 (Marai *et al.*, 2001; EI-Desoky *et al.*, 2017)，因為良好的精液品質對於哺乳動物達到足夠的生育力至關重要，持續高溫環境可能導致農場動物精液品質及受精率降低。Marai *et al.* (2008) 比較冬季與夏季經過熱緊迫公兔配種後之仔兔出生情況，指出熱緊迫為環境中對公兔繁殖性能，如降低受胎率 (conception rate) 及出生窩仔數 (litter size) 之最大因素影響。且環境高溫刺激下視丘－腦下垂體－腎上腺軸活性 (hypothalamic-pituitary-adrenal axis activity) 而喚醒交感神經系統 (sympathetic system) 功能、增加抗氧化防禦系統 (antioxidant-defense system) 中自由基 (free radial) 的量且導致系統失衡 (Agarwal *et al.*, 2008; Ahmad *et al.*, 2012)。自由基之累積與精子活動力 (motility) 及精子細胞完整性 (integrity) 高度相關，且嚴重影響精子正常比例及 DNA 損壞程度而造成不孕 (Potts *et al.*, 2000)。本研究旨在調查公兔涼季與熱季精液品質之差異，以解釋公兔在熱緊迫環境可能短暫不孕及公兔性慾減少導致繁殖力下降的現象。

材料與方法

I.　試驗動物之飼養管理

試驗用紐西蘭大白兔來自農業部畜產試驗所，飼養於半開放式兔舍，籠架長 90 cm，寬 45 cm，高 50 cm；採自然光照，無溫濕度調控，飲水由自動給水裝置供應，飼料由畜產試驗所飼料廠提供，成分如表 1 所示，飼

(1) 農業部畜產試驗所研究報告第 2784 號。
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料餵飼採任飼，公及母兔於 5 週齡離乳，為避免打鬥受傷，公兔於 3 月齡以上開始單獨飼養。本研究之動物試驗於農業部畜產試驗所進行，動物之使用、飼養及實驗內容皆符合農業部畜產試驗所實驗動物照護之規定，並經使用小組審查同意進行 (動物實驗申請表暨同意書編號：107-1)。

表 1. 飼糧組成

Table 1. The composition of experimental diets

Ingredients	Percentage
Yellow Corn	18.05
Soybean meal, cp 44%	17.0
Wheat bran	12.0
Alfalfa meal	46.0
Soybean oil	2.0
Molasses	3.0
Dicalcium phosphate	1.0
Salt	0.5
DL- Methionine	0.15
Vitamin premix[1]	0.2
Mineral premix[2]	0.1
Total	100.00
Analyzed value	
Moisture,%	11.7
GE, kcal/kg	3,965.00
ADF,%	14.8
NDF,%	23.8
Crude protein,%	18.9
Crude fiber,%	11.6
Ether extract,%	4.2
Calcium,%	1.2

[1] Mineral premix composition (g/kg): Fe, 80; Cu, 15; Mn, 80; Zn, 50; I, 0.85; Co, 0.25.

[2] Vitamin premix provided per kilogram of diet as follows: Vitamin A, 12,000 IU; Vitamin D_3, 3,125 IU; Vitamin E, 37.5 IU; Vitamin K_3, 1.5 g; Vitamin B_1, 1 g; Vitamin B_2, 4.8 g; Vitamin B_6, 3 g; Vitamin B_{12}, 0.01 g; Niacin, 25 g; Pantothenic acid, 10 g; Folic acid, 0.5 g; Biotin, 0.2 g.

II. 環境溫濕度指數紀錄

畜舍內吊掛自動溫濕度紀錄器 (Microlog EC650, Fourtec, USA) 以每兩小時一次的頻率測定畜舍內溫濕度，測定期間涼季為 11 至 4 月、熱季為 5 至 10 月，測得之數據再依 Marai *et al.* (2001) 公式換算成 THI，用於評估環境溫度與濕度對兔隻造成之熱緊迫程度。THI 計算公式為：THI = dbºC – [(0.31 – 0.31 RH) (dbºC – 14.4)]。

公式中：db 表示乾球攝氏溫度，RH 表相對濕度百分率。Marai *et al.* (2001) 指出 THI 小於 27.8 表示無緊迫狀態，27.8 － 28.9 表示輕微熱緊迫，28.9 － 30.0 表示嚴重熱緊迫狀態，大於 30.0 表示非常嚴重熱緊迫狀態。

III. 精液採集

在臺灣熱帶地區半開放式的養兔環境下，本試驗分別在 107 － 108 年不同月份採精，再將精液測定資料分為涼季 (11 月至 4 月) 及熱季 (5 月至 10 月) 兩組，每週採樣 1 － 2 次。採精時，選定 9 隻 7 個月齡以上性成熟之紐西蘭白公兔，及 6 月齡以上外陰部紅腫的紐西蘭白母兔，利用圓筒形矽膠做成之假陰道，並套上 1.7 mL 離心管以收集精液 (圖 1)，使用前先預熱至 50℃，待公兔駕乘母兔時套在公兔陰莖進行精液收集。採集的兔精液顏色，正常者為白色至米白色，若呈黃色為尿液污染，呈紅棕色則為血液污染，污染者不進行後續分析。

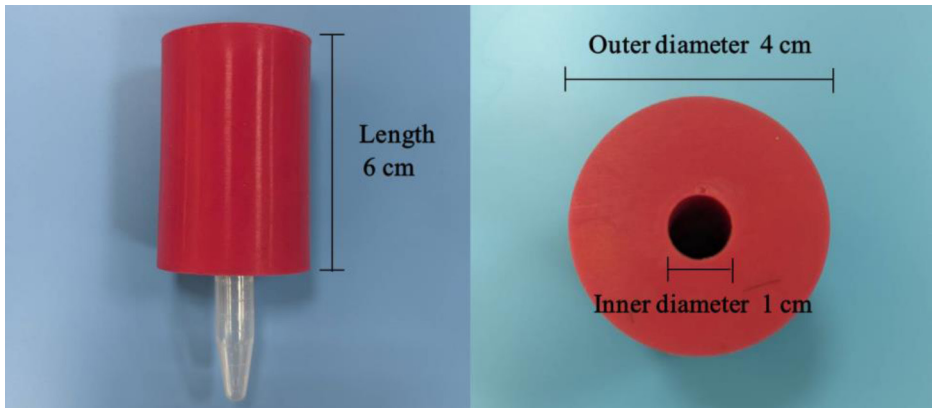

圖 1.　採集兔精液之假陰道，一端接 1.7 mL 微量離心管。

Fig. 1. Artificial vagina for rabbit semen collection. One end connects 1.7 mL microcentrifuge tube.

IV. 離乳體重

兔隻於出生經識別公母後於耳朵刺青標示識別號碼，並且個別秤重，於五週齡離乳比較涼熱季公兔離乳體重差異。

V. 駕乘行為

公兔交配行為又叫做駕乘 (mounting behavior)，為兩隻兔子間的行為，一隻兔子從側邊或後面接近另一隻兔子，然後環抱對方的背部或頭頸部，可能做出骨盆推力動作 (pelvic thrusting motion)，在 10 分鐘內公兔無任何爬跨母兔的行為，即視為無駕乘。每次採精時公兔之駕乘行為頻度將其數據化，有駕乘者計為 1 (100%)，沒駕乘者計為 0 (0%)。

VI. 精子性狀分析

兔精子性狀分析係參考 Rosato and Iaffaldano (2013) 之步驟進行，將新鮮或解凍後的兔精液使用電腦輔助精子分析儀 (Computer Assisted Sperm Analyzer, CASA, Microptic S. L., Spain) 之精子分類分析器 (Sperm Class Analyzer®, SCA evolution, Microptic S. L., Spain) 檢測分析精子活動力及前進值，亦即將精液原液以 TCG 基礎液 (250 mM TRIS-hydroxymethylaminomethane, 88 mM citric acid, 47 mM glucose, pH 6.9) 稀釋成 106 sperm/mL，再於 37℃加熱活化 7 min 後取 3 μL 注入分析玻片 (standard count 4 chamber slide, Leja, 301115, Netherland)，於顯微鏡 (ECLIPSE Ci, Nikon, Japan) 下對焦後，在 CASA 系統中選取 mot (即活動力) 測定，讓 CASA 系統同時換算出精子濃度並分析活動力及前進值。而精子存活率測定則是將精子以 1 μg/mL 之 Hoechst 33342 (Sigma-Aldrich, B2261, USA) (藍色螢光 EX/EM = 355/465 nm) 及 0.2 mM 之 propidium iodide (Life, L7011, USA) (紅色螢光 EX/EM = 488/615 nm) 在 37℃下染色 5 min；精子呈現藍色為存活，橘紅色則為死亡，染色後精子則在螢光顯微鏡下利用濾鏡 Green filter/ Bandpass filter (UV-2A, EX 330-338, DM:400, BA:420) 以 CASA 進行分析，計算出存活率百分比。

VI. 睪丸組織切片分析

在涼季 (2 月) 及熱季 (8 月) 時，取出生滿一年外觀健康之種公兔各 4 隻將其犧牲，取下睪丸及附睪，於磷酸鹽緩衝生理食鹽水 (phosphate buffered saline) 中浸潤多次，以去除所有血液，再於 4% paraformaldehyde 中固定 1 個星期，送至拓生科技公司進行石蠟包埋、切片、蘇木精及伊紅染色，在於高倍率顯微鏡下拍照記錄睪丸及附睪體內精子生成狀況。

VII. 統計分析

試驗結果以 SAS (2009) 套裝軟體 (SAS 9.3) 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，再以最小平方平均法 (least square means, LSM) 比較差異性，並以 $P < 0.05$ 為具顯著差異。

結果與討論

I. 涼熱季畜舍溫度、相對濕度、溫濕度指數 (THI) 變化與離乳體重與駕乘率比較

試驗兔舍涼季溫度、相對溼度及 THI 之平均值如表 2，涼季之平均溫度為 21.2 ± 6.7 ℃ (13.3 － 29.0℃)，濕

度為 74.3 ± 4.9% (46.2 － 94.0%)，平均 THI 為 20.3 ± 2.5 (13.6 － 28.2)；熱季之平均溫度為 29.5 ± 5.3℃ (20.5 － 36.2℃)，濕度為 81.9 ± 7.2% (42.2 － 100.0%)，平均 THI 為 27.6 ± 3.6 (20.5 － 33.6)。

表 2.　涼季及熱季兔舍內溫度、相對濕度及 THI 之平均值 (平均值 ± 標準偏差)

Table 2.　Average temperature, relative humidity and THI in rabbit house during cool season and hot season (mean ± SD)

Items	Cool season (Nov.-Apr.)	Hot season (May-Oct.)
Average temperature (ºC)	21.2 ± 6.7	29.5 ± 5.3
Average relative humidity (%)	74.3 ± 4.9	81.9 ± 7.2
Average THI*	20.3 ± 2.5[a]	27.6 ± 3.6[b]

* THI = dbºC – [(0.31 – 0.31 RH) (dbºC – 14.4)], dbºC = dry bulb temperature in Celsius and RH = relative humidity percentage/100.

[a, b] Means within the same row without the same superscripts differ significantly (P < 0.05).

試驗期間畜舍涼季及熱季之平均溫度、相對濕度及 THI 之變化如圖 2 所示，不論涼季或熱季畜舍內溫度均於上午 8 點逐漸升高，且都在 14 點左右達最高溫，而相對濕度走勢與溫度相反，大約於 12 － 14 點達最低點，清晨 6 點時最高，可知 THI 數值主要隨溫度上升而增加，而在相同溫度時，較低的相對濕度具較低的 THI，即降低濕度可降低熱緊迫。

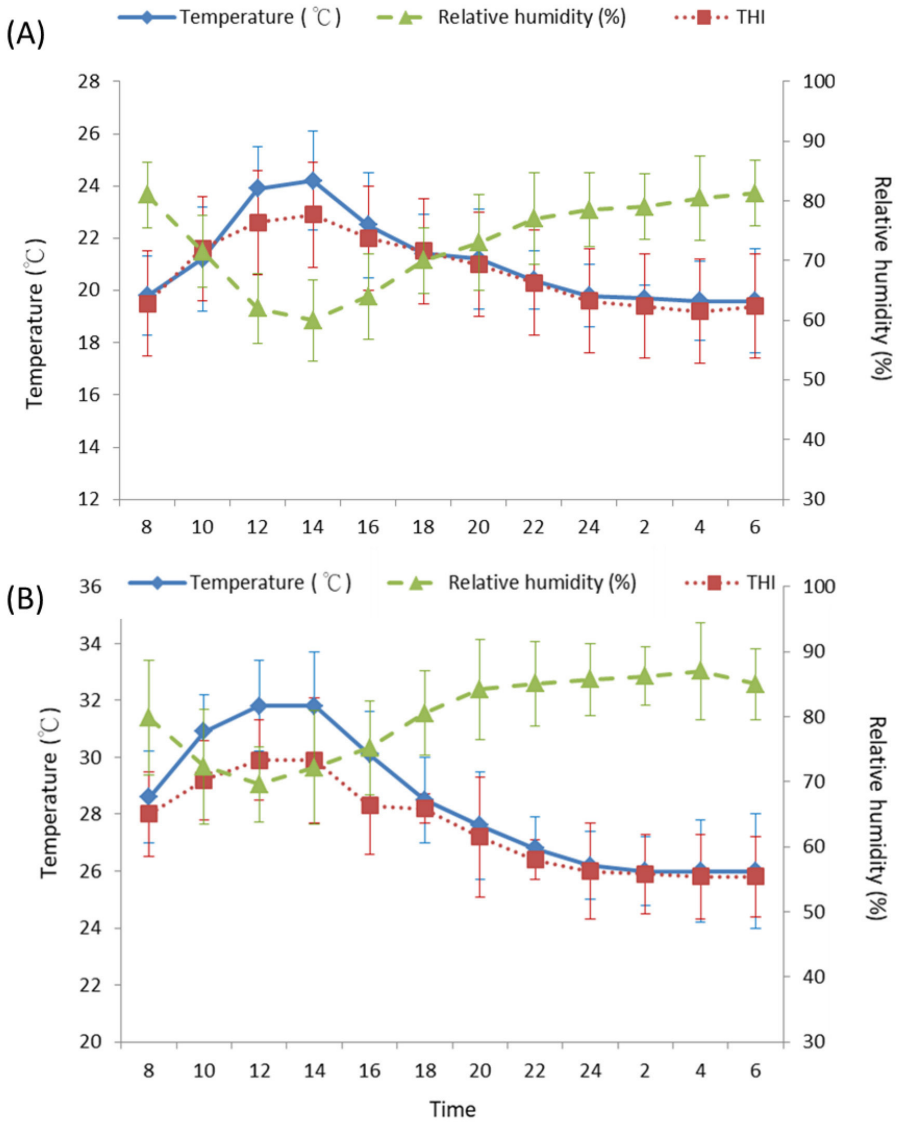

圖 2.　涼季 (A) 與熱季 (B) 兔舍的溫度、相對濕度及 THI 變化。

Fig. 2.　The changes of temperature, relative humidity and THI in the rabbit house during cool season (A) and hot season (B).

表 3 將 Marai *et al.* (2001) 歸納 THI 數值對於兔之熱緊迫定義，用於本試驗中涼季及熱季畜舍 THI 進行分析，得知兔隻熱緊迫情形，顯示涼季時有 0.4% 時段兔隻處於輕微熱緊迫，而 99.6% 時段的兔隻都沒有熱緊迫的現象；反觀熱季時 15.6% 時段之兔隻處於非常嚴重熱緊迫、10.8% 時段之兔隻處於嚴重熱緊迫、11.2% 時段之兔隻處於輕微熱緊迫現象，而 62.4% 時段之兔隻沒有熱緊迫現象，與涼季差距甚大，即熱季時有 37.6% 時段之兔隻處於熱緊迫中。

表 3.　涼季及熱季兔舍 THI 分布與熱緊迫定義範圍

Table 3.　THI distribution and definition range of heat stress in rabbit house during cool and hot season

Definition range of heat stress	THI distribution	
	Cool season (Nov.-Apr.)	Hot season (May-Oct.)
Very severe heat stress (THI* > 30.0)	0%	15.6%
Severe heat stress (28.9 < THI < 30.0)	0%	10.8%
Moderate heat stress (27.8 < THI < 28.9)	0.4%	11.2%
Absence of heat stress (THI < 27.8)	99.6%	62.4%

* THI = dbºC – [(0.31 – 0.31 RH) (dbºC – 14.4)], dbºC = dry bulb temperature in Celsius and RH = relative humidity percentage/100.

比對季節對公兔個體離乳體重及駕乘率之影響，結果 (表 4) 顯示涼季離乳體重 1,001.3 ± 80.5 g 顯著高於熱季離乳體重 777.4 ± 109.0 g (P < 0.05)，此結果與 McNitt and Lukefahr (1993) 及 Roberts and Lukefahr (1992) 研究相符，皆顯示不同季節出生的小兔生長速率不同，在熱季出生及離乳者體重較低，而涼季出生及離乳者體重較高 (Shehata *et al.*, 1998)。且 Chiericato *et al.* (1996) 指出，夏季高溫時導致兔平均日增重減少 18%，兔平均日增重 (average daily body gain) 在熱季比涼季低 (Habeeb *et al.*, 1993)。

表 4.　比較涼季及熱季之公兔離乳體重及駕乘率 (平均值 ± 標準偏差)

Table 4.　Comparison of weaning weight and mounting behavior of male rabbits during cool and hot season (mean ± SD)

Items	Cool season (Nov.-Apr.)	Hot season (May-Oct.)
No. of trials	39	77
Weaning weight (g)	1,001.3 ± 80.5[a]	777.4 ± 109.0[b]
Mounting behavior (%)	100.0 ± 0.0[a]	85.6 ± 7.6[b]

[a, b] Means within the same row without the same superscripts differ significantly (P < 0.05).

另外，採精時公兔駕乘行為 (性慾評估) 統計，分別於涼季及熱季時統計公兔駕乘行為，在本研究中 10 分鐘內公兔無駕乘成功或無駕乘動作者，駕乘率紀錄為 0。統計結果，顯示涼季時駕乘率 100.0 ± 0.0% 顯著優於熱季之駕乘率 85.6 ± 7.6% (P < 0.05) 如表 4 所示。

文獻指出，公兔在高溫下可能會有暫時不孕的現象 (El-Gaafary, 1994; Daader *et al.*, 1997; Zeidan *et al.*, 1997)，因公兔繁殖力在 7 月到 9 月間因性慾降低而減少 (Nalbandov, 1970)。

前人研究公兔性慾 (sexual desire) 評估方法，自母兔放到公兔籠至公兔開始駕乘及射精的時間，以秒為單位計算反應時間，研究指出性慾會依周遭環境溫度上升而降低 (El-Gaafary, 1994; Daader and Seleem, 1999)。Tharwat *et al.* (1994) 發現暴露於氣溫攝氏 40 度和相對溼度 60 － 65% 的公兔，性慾延遲了 11.9 － 18.5 秒，最多延遲 40 分鐘。

II. 涼熱季精液性狀比較

9 隻公兔在不同月份採精後分析精子性狀，再將資料依月份分成涼熱季，分別比較每次採精精液體積、濃度、精子活動力、精子存活率、精子前進值如表 5，結果顯示， 除了精子活動力於涼季 69.3 ± 10.5%，顯著高於熱季 46.5 ± 13.6% 外 (P < 0.05)，其餘體積、濃度、存活率、前進值在統計上差異並不顯著。文獻指出精子濃度及射出精液體積範圍分別為 150 － 500 × 10^6 sperm/mL 及 0.3 － 0.6 mL 的平均範圍 (Adams and Singh, 1981; Lebas *et al.*, 1997)，影響因子如飼糧、採精頻率、年齡、射精次數及氣溫。而精子活動力測定結果與 Marai *et al.* (1996) 及 Daader *et al.* (1997) 結果相符，都是涼季較佳，精子活動力對於精子在生殖道中的移動 (transportation)

及穿透性 (penetration) 至關重要 (Holt and Van Look, 2004)，這也解釋了熱季懷孕受胎率較低之原因。

表 5. 比較涼季及熱季精液品質 (平均值 ± 標準偏差)

Table 5. Compared the semen quality of male rabbits during cool and hot season (mean ± SD).

Items	Cool season (Nov.-Apr.)	Hot season (May-Oct.)
No. of trials	39	77
Semen volume (mL)	1.1 ± 0.2	0.8 ± 0.2
Semen conc. (10^6 sperm / mL)	882.3 ± 342.4	914.0 ± 144.1
Motility (%)	69.3 ± 10.5[a]	46.5 ± 13.6[b]
Vitality (%)	67.4 ± 11.1	62.6 ± 8.0
Progressive motility (%)	33.4 ± 9.4	24.4 ± 11.5

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

III. 涼熱季睪丸組織切片分析比較

涼熱季公兔睪丸組織切片分析結果比較如圖 3，結果顯示，涼季時公兔睪丸之生精細管充滿精原細胞、發育中的精母細胞與精子 (圖 3A)，顯示生精作用正常；而熱季時之公兔生精細管中所有精母細胞、精細胞與精子數驟減而呈現空洞化現象 (圖 3B)。另一方面，涼季公兔附睪中充滿精子 (圖 3C)，為繁殖力旺盛的象徵，反之熱季公兔中附睪中只有零星的精子 (圖 3D)，與涼季附睪切片觀察中充滿精子之結果形成相當大的對比。試驗結果與 Marai *et al.* (2002) 指出熱緊迫會造成兔睪丸內精子生成減少一致，也是導致熱季時公兔短暫不孕的原因。

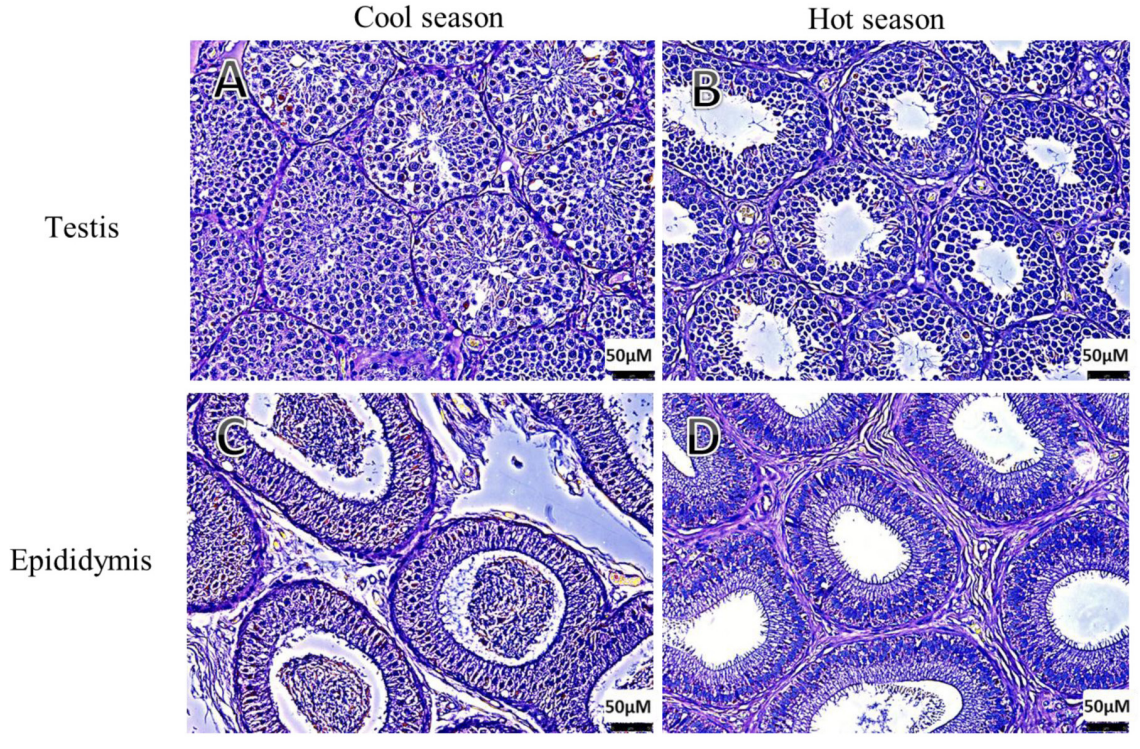

圖 3. 涼季與熱季之一歲齡之公成兔睪丸生精細管及附睪切片組織化學染色切片結果。
A：涼季的公兔生精細管中有各種不同發育階段的精母細胞、精細胞與精子；B：熱季公兔生精細管發育中的精母細胞、精細胞與精子數驟減，造成空洞化；C：涼季的公兔附睪內充滿精子；D：熱季公兔附睪稀少的精子數量形成對比。(Bar = 50 μM)

Fig. 3. Immunohistochemistry staining of 1 year-aged rabbit testis and epididymis in cool and hot season. There are different developing stage of spermatocytes, spermatids and spermatozoa within the lumina of seminiferous tubules of cool season male testis (A), but are decrease in the seminiferous tubules of hot season male testis (B). Epididymis of cool season buck showing presence of large numbers of sperm (C) which constracts with lack of sperm in epididymis of hot season buck (D). (Bar = 50 μM).

結　　論

本研究結果顯示，半開放飼養兔隻在熱季時因熱緊迫會顯著影響公兔離乳體重及駕乘頻度，在精液性狀方面嚴重降低精子活動力，且由熱季公兔睪丸及附睪組織切片與涼季比較結果得知，持續高溫環境會減少精子生成作用。

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The effect of temperature-humidity index on semen production of male New Zealand rabbits [1]

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Abstract

The purpose of this study was to compare the effects of temperature-humidity index (THI) on the semen production of New Zealand rabbits, as an indicator heat stress in rabbits. The average THI of rabbit houses in Taiwan Livestock Research Institute during the cool season from November to April was 20.3 ± 2.5, and the average THI in the hot season from May to October was 27.6 ± 3.6. Analysis shows that when THI was higher than 27.8, rabbits suffered heat stress during 37.6% of the time. The weaning weight of male rabbits in cool season was 1,001.3 ± 80.5 g, which was significantly higher than that of 777.4 ± 109.0 g in hot season ($P < 0.05$). Moreover, the frequency of mating behavior in male rabits in cool season was 100.0 ± 0.0%, which was significantly higher than the 85.7 ± 7.6% in the hot season ($P < 0.05$). The examination results of the semen traits of New Zealand male rabbits in the cool season and hot season showed that there was no significant difference in the parameters of semen volume, sperm concentration, vitality/mortality rate, and progressive motility, but the sperm motility during the cool season was 69.3 ± 10.5%, which was significantly better than that in the hot season (46.5 ± 13.6%) ($P < 0.05$). The comparison of testicular tissue sections showed that continuous high temperature in the environment affected spermatogenesis in male rabbits. In summary, continuous high temperature decreases sexual desire, testis spermatogenesis and sperm motility in male rabbits, which could also be the reason for the decrease in male rabbit fertility in the summer.

Key words: New Zealand White rabbit, Semen production, Temperature-Humidity Index (THI).

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