



## Effect of Age and Caponization on Blood Parameters and Bone Development of Male Native Chickens in Taiwan

Cheng-Yung Lin, Jenn-Chung Hsu<sup>1</sup> and Tien-Chun Wan<sup>2,\*</sup>

Taitung Animal Propagation Station, LRI, COA. No. 30, 27 Line, Binlang Vil., Beinan Township,  
Taitung County 95445, Taiwan

**ABSTRACT:** An experiment was carried out to determine the effect of age and caponization on the development blood and bone characteristics development in male country chickens in Taiwan. A total of two hundred 8-wk-old LRI native chicken cockerels, Taishi meat No.13 from LRI-COA, were used as experimental animals. Cockerels were surgically caponized at 8 wks of age. Twelve birds in each group were bled and dressed from 8 wks to 35 wks of age at 1 to 5 wk intervals. The results indicated that the plasma testosterone concentration was significantly ( $p<0.05$ ) lower in capons after 12 wks of age (caponized treatment after 4 wks) than that of the intact males. The relative tibia weight, bone breaking strength, cortical thickness, bone ash, bone calcium, bone phosphorus and bone magnesium contents were significantly ( $p<0.05$ ) higher in intact males, while capons had higher ( $p<0.05$ ) plasma ionized calcium, inorganic phosphorus and alkaline phosphatase concentration. The plasma testosterone concentration, relative tibia weight, tibia length, breaking strength, cortical thickness, bone ash, calcium, and phosphorus contents of intact males chickens increased significantly ( $p<0.05$ ) with the advance of age. In addition, the relative tibia weight of capons peaked at 18 wks of age, and declined at 35 wks of age. The bone ash, calcium and phosphorus content increased most after 14 wks of age in male native chickens in Taiwan. Also, tibia length and cortical thickness peaked at 22 wks of age. However, the peak of bone strength was found at 26 wks of age. These findings support the assertion that androgens can directly influence bone composition fluxes in male chickens. Caponization caused a significant increase in bone loss at 4 wks post treatment, which reflected bone cell damage, and demonstrated reductions in the relative tibia weight, breaking strength, cortical thickness, bone ash, calcium, phosphorus and magnesium contents, and increases in plasma ionized calcium, inorganic phosphorus and alkaline phosphatase concentration. (**Key Words:** Age, Bone Development, Caponization, Male Chicken, Testosterone)

### INTRODUCTION

Capons are male chickens whose testes have been surgically removed. Because of the resultant androgen deficiency, secondary male sexual characteristics (the comb, wattle, fighting behavior and vocalization) degenerate, and maturity regresses to an immature stage. Androgens have demonstrated high anabolic activities in a variety of tissues that can stimulate muscle, bone, and connective tissue growth and erythropoiesis. A high androgenic activity can stimulate the reproductive system, as well as behavioral,

psychological and secondary sexual growth characteristics or changes in the males (Griggs et al., 1989; Wakley et al., 1991; Fennell and Scanes, 1992a, b; Katznelson et al., 1996; Lin, 1999; Wang, 2001). Numerous reports have been published on the influence of surgical caponization on growth performance (Lin and Hsu, 2002; Rikimaru et al., 2009; Volk et al., 2011), muscle compositions, ATP related compounds and taste panel scores (Rikimaru et al., 2009; Lin et al., 2011; Volk et al., 2011), blood traits (Chen et al., 2005; Rikimaru et al., 2009; Lin and Hsu, 2011), organ and carcass part ratios (Lin and Hsu, 2003b; Rikimaru et al., 2009; Volk et al., 2011), certain muscles' physical properties (Lin and Hsu, 2002; Lin et al., 2003a; Sirri et al., 2009), skin and muscle colors (Lin and Hsu, 2003b; Sirri et al., 2009), bone traits (Lin and Hsu, 2003a; Chen et al., 2006a, b) and behavior (Wang, 2001) in chickens. The effects of androgen treatment or castration on blood and bone traits have been reported in other studies. However, the findings

\* Corresponding Author: Tien-Chun Wan. Tel: +886-6-5911211, Fax: +886-6-5912474, E-mail: tcwan@mail.tlri.gov.tw

<sup>1</sup> Graduate Institute of Animal Science, National Chung Hsing University, Taichung, Taiwan.

<sup>2</sup> Animal Products Processing Division, Livestock Research Institute, Council of Agriculture, Tainan, Taiwan.

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in these reports have not been consistent (Hutt, 1929; Vanderschueren et al., 1992; Gill et al., 1998; Mauras et al., 1999; Moghetti et al., 1999; Hsieh, 2003; Lin and Hsu, 2003a; Chen et al., 2006a, 2006b, 2007). Chen et al. (2007) showed that caponization decreased tibia length, but other studies have detected no difference (Lin and Hsu, 2003a; Chen et al., 2006a, 2006b) or even an increase in bone length (Hutt, 1929; Hsieh, 2003). In another case, Lin and Hsu (2003a) found that castration caused an increase in plasma ionized calcium, but did not influence total blood calcium concentration. Also, Chen et al. (2006b) and Moghetti et al. (1999) reported that caponization or testosterone deficiency might increase total blood calcium, but other studies have indicated no effects of caponization on total blood calcium concentration (Vanderschueren et al., 1992; Gill et al., 1998; Chen et al., 2006a, 2007). Similarly, Chen et al. (2006b, 2007) found that caponization increased the blood alkaline phosphatase concentration, but other studies have indicated no influence on blood alkaline phosphatase concentration (Mauras et al., 1999; Lin and Hsu, 2003a; Chen et al., 2006a).

Greendale et al. (1997) showed that higher bioavailable testosterone levels were associated with higher bone mineral density (BMD) in men and women. Mauras et al. (1996) also indicated that insulin-like growth factor-I (IGF-I) and sex steroid hormones impact bone formation independently. The actions of IGF-I, growth hormones, and sex steroid hormones may synergize to maximally stimulate the attainment of peak bone mass in humans. For many years it has been recognized that sex steroid hormones (androgen and estrogen) have profound effects on bone metabolism, but the paracrine mediators of androgen action on bone are at present unclear. However, little information is available concerning the influence and role of age and androgens on the fluctuation in blood and bone traits. This study was therefore conducted to investigate the effects of age and caponization on the development of blood and bone characteristics development in male country chickens in Taiwan.

## MATERIALS AND METHODS

### Animal management and experiment design

A total of two hundred country chicken cockerels (TLRI native chicken cockerels, Taishi meat No.13) bred by the Taiwan Livestock Research Institute were reared *ad libitum* in an open-sided broiler house with a conventional country chicken diet. At 8 wks of age, the cockerels were individually weighed and randomly assigned to either caponized or sham (intact) groups. Birds from each group were allocated into tetra replicates with 25 birds in each pen (200×450 cm). From 9 to 18 wks of age, birds were fed a

grower ration with 18% protein and 3,000 kcal/kg of metabolizable energy. From 19 to 35 wks, the birds were fed a finisher ration with 15% protein and 2,800 kcal/kg of metabolizable energy. Chickens received natural light. Feed and water were provided *ad libitum*.

### Testectomy

All birds in the four pens for the designated caponized group were deprived of feed for 12 h followed by caponization. The birds down feathers were removed from the lateral region just anterior to the thigh. The region was swabbed with a dilute disinfectant and the skin was incised. An incision was then made between the last two ribs and widened by a small spreader. The testis was exposed by blunt dissection and simultaneously removed by teasing its connective tissue supports free and applying gentle suction. The incision was closed using surgical silk and the operation was then repeated on the opposite side.

### Measurement and analysis

Birds were bled and dressed from 8 weeks to 35 wks of age at 1 to 5 wk intervals. After 12 h of feed deprivation, blood of 12 birds from each group (3 birds from each replicate) was collected from the brachial vein using a syringe prerinsed in a 0.15 M NaCl solution containing 1,000 IU/ml of heparin-Li. The blood samples were then placed into a tube containing 50 µl of a 1,000 IU/ml heparin-Li solution per milliliter of blood. The blood samples were kept on ice centrifuged (1,500 g for 30 min.) at 5°C. The recovered plasma was placed into three vessels. One of these vessels was held at 0 to 4°C for determination of plasma ionized calcium. The remaining vessels were frozen at -20°C until analysis of plasma total calcium, phosphorus, magnesium, alkaline phosphatase and testosterone. The plasma ionized calcium concentration was measured using a kit (Bayer, UK) and automatic analyzer (634 ISE Ca<sup>2+</sup>/pH Analyzer, Ciba Corning, England) within 72 h after blood sampling. The total calcium, inorganic phosphorus, magnesium and alkaline phosphatase concentrations of plasma were analyzed with different kits (Wako, Japan) and an automatic analyzer (Hitachi 7050, Japan). Assays for the concentrations of plasma testosterone were carried out with an ELISA microtiter reader (MRX Dynex Technologies, USA), using ELISA kits (NEOGEN Testosterone ELISA kit, USA). After 24 h of feed deprivation, 12 birds from each group (3 birds from each replicate) were weighed and sacrificed using standard procedures as reported by Koch and Rossa (1973). The left and right tibiae were removed. Bone fat was extracted with petroleum ether in a Soxhlet extractor for contiguous 48 h. Bone (free of moisture and fat, from the right and left tibia) as a percentage of body mass was taken based on the fasting

weight of the live birds. Bone ash and mineral contents as a percentage of bone mass were taken based on the free moisture and fat weight. Contents of ash mineral as a percentage of bone ash mass were also taken. Bone stress was measured with a Tension Compression Tester (HT-8116) as described by Crenshaw et al. (1981). Bone ash content was determined according to the approach of Johnson et al. (1992). Bone calcium, manganese, magnesium, and phosphorus contents were analyzed with a Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Model Z 8100, Japan) or a Spectrophotometer (Hitachi Model U-2001, Japan) by the Association of Official Analytical Chemists (1984).

### Statistical analysis

Collected data were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS (SAS Institute Inc., 1988). When significant ( $p < 0.05$ ) differences were detected, means were separated using least squares means (LSMeans).

## RESULTS AND DISCUSSION

### Blood parameters

Table 1 summarizes the blood parameters data. The plasma testosterone concentration of intact male chickens increased significantly ( $p < 0.05$ ) with the advance of age, and increased most at 14 wks of age, while the plasma testosterone concentration of capons decreased significantly ( $p < 0.05$ ) with the increase of age. The plasma testosterone concentration in intact birds was higher ( $p < 0.05$ ) after 12 wks of age (caponized treatment after 4 wks) than that of capons as expected. This was in agreement with previous reports (Lin and Hsu, 2003a; Chen et al., 2005). Also, Mashaly (1984) reported a reduction in the serum testosterone concentration in 3-wks-old cockerels 2 wks after orchietomy treatment. Similarly, Peh and Lee (1985) also demonstrated that the plasma testosterone concentration in male country chickens in Taiwan increased with the advance of age.

Caponization of male chickens increased the plasma ionized calcium concentration ( $p < 0.05$ ) after 18 wks of age compared to that of intact male, but no increase was observed in total calcium concentration ( $p > 0.05$ ). These results agree with those of Lin and Hsu (2003a), who indicated that caponization increased blood ionized calcium concentration, but did not increase the total calcium concentration. Similarly, Chen et al. (2007) also demonstrated that caponization did not influence blood total calcium concentration. In contrast, Chen et al. (2006a) showed that caponization increased the total blood total calcium concentration.

Before 14 wks of age, intact male chickens had a higher plasma inorganic phosphorus concentration ( $p < 0.05$ ) than after 18 wks of age. Similarly, caponization of male chickens before 18 wks of age led to a higher plasma inorganic phosphorus concentration ( $p < 0.05$ ) than after 22 wks of age. However, caponization of cockerels increased the plasma inorganic phosphorus concentrations ( $p < 0.05$ ) compared to intact males at 18, 22 and 30 wks of age (caponized treatment after 10 wks). The results in this study agree with those of Lin and Hsu (2003a) and Chen et al. (2006a), who found that caponization increased the blood inorganic phosphorus concentrations. Similarly, Moghetti et al. (1999) showed that women with hypergonadism given GnRH agonist alone or a GnRH agonist together with antiandrogen drugs for 6 m had significantly higher serum calcium and phosphorus concentrations. In contrast, Vanderschueren et al. (1992), Gill et al. (1998) and Maura et al. (1999) reported that orchietomized rats or men with hypogonadism had significantly lower serum phosphorus concentration. Moreover, in birds exposed to androgens, there is an increase in phosphorus balance (Hervey et al., 1981; Sturike, 1986).

Caponization of male chickens decreased the plasma total calcium/inorganic phosphorus ratio ( $p < 0.05$ ) 18 and 30 wks of age. However, after another week, caponization did not affect the plasma total calcium/inorganic phosphorus ratio. The cockerels before 14 wks of age had a lower plasma calcium/inorganic phosphorus ratio ( $p < 0.05$ ) than after 26 wks of age.

Plasma magnesium concentration did not differ ( $p > 0.05$ ) between the capons and intact cockerels, but the cockerels and capons before 14 weeks of age had a lower plasma magnesium concentration than after 18 wks of age as expected, which is in agreement with previous reports (Lin and Hsu, 2003a).

Caponization of cockerels increased the plasma alkaline phosphatase concentration ( $p < 0.05$ ) between 18 and 35 wks of age. However, after another week, caponization of age did not affect the plasma alkaline phosphatase concentration. The results in this study agree with those of Chen et al. (2006b) and Chen et al. (2007), who found that caponization increased the blood alkaline phosphatase concentration. Similarly, Lin and Hsu (2003) and Chen et al. (2006a) showed that caponization did not affect the plasma alkaline phosphatase concentration. Motzok (1950) showed the plasma alkaline phosphatase level also reflected bone alkaline phosphatase activity level. However, the blood alkaline phosphatase concentration increased the numbers of bone cells which were damaged and increased bone remodeling (Kay, 1932; Hurwitz and Griminger, 1961).

### Bone traits

The obtained bone trait results in this study are

**Table 1.** Effect of age and caponization on plasma traits of male country chickens in Taiwan

Weeks of age	Sham-operated	Caponized	SE	Weeks of age	Sham-operated	Caponized	SE
Testosterone (pg/ml)				Ca/P			
8 (baseline)	497.0 <sup>b</sup>	497.0 <sup>a</sup>	35.2	8 (baseline)	1.97 <sup>d</sup>	1.97 <sup>bc</sup>	0.07
9	469.3 <sup>b</sup>	419.7 <sup>a</sup>	142.1	9	2.00 <sup>d</sup>	1.95 <sup>bc</sup>	0.08
10	625.4 <sup>b</sup>	293.6 <sup>ab</sup>	176.7	10	1.97 <sup>d</sup>	1.83 <sup>c</sup>	0.08
12	753.1 <sup>bx</sup>	252.3 <sup>aby</sup>	169.2	12	1.98 <sup>d</sup>	1.93 <sup>bc</sup>	0.08
14	1,326.1 <sup>ax</sup>	251.4 <sup>aby</sup>	105.5	14	1.95 <sup>d</sup>	1.97 <sup>bc</sup>	0.07
18	1,435.7 <sup>ax</sup>	194.5 <sup>aby</sup>	85.1	18	2.22 <sup>cdx</sup>	1.82 <sup>cy</sup>	0.15
22	1,485.3 <sup>ax</sup>	150.9 <sup>aby</sup>	62.3	22	2.10 <sup>cd</sup>	2.00 <sup>bc</sup>	0.07
26	1,565.3 <sup>ax</sup>	61.5 <sup>by</sup>	39.1	26	2.40 <sup>c</sup>	2.23 <sup>b</sup>	0.10
30	1,404.5 <sup>ax</sup>	35.6 <sup>by</sup>	66.3	30	2.66 <sup>bx</sup>	2.23 <sup>by</sup>	0.08
35	1,609.8 <sup>ax</sup>	30.4 <sup>by</sup>	15.7	35	3.05 <sup>a</sup>	2.78 <sup>a</sup>	0.16
SE	117.4	90.0		SE	0.12	0.09	
Ionized calcium (mmol/L)				Magnesium (mg/dl)			
8 (baseline)	1.45 <sup>b</sup>	1.45 <sup>bc</sup>	0.01	8 (baseline)	2.15 <sup>ab</sup>	2.15 <sup>bcd</sup>	0.02
9	1.42 <sup>bc</sup>	1.40 <sup>d</sup>	0.01	9	2.02 <sup>b</sup>	2.06 <sup>cd</sup>	0.03
10	1.26 <sup>d</sup>	1.28 <sup>e</sup>	0.01	10	1.97 <sup>b</sup>	2.00 <sup>d</sup>	0.03
12	1.43 <sup>by</sup>	1.53 <sup>cx</sup>	0.03	12	2.03 <sup>b</sup>	2.00 <sup>d</sup>	0.05
14	1.41 <sup>bc</sup>	1.45 <sup>c</sup>	0.19	14	2.02 <sup>b</sup>	2.02 <sup>d</sup>	0.06
18	1.63 <sup>ay</sup>	1.80 <sup>ax</sup>	0.02	18	2.34 <sup>a</sup>	2.15 <sup>bcd</sup>	0.23
22	1.32 <sup>cdy</sup>	1.52 <sup>cx</sup>	0.02	22	2.18 <sup>ab</sup>	2.33 <sup>ab</sup>	0.08
26	1.50 <sup>by</sup>	1.63 <sup>bx</sup>	0.04	26	2.20 <sup>ab</sup>	2.23 <sup>bc</sup>	0.07
30	1.31 <sup>dy</sup>	1.44 <sup>cx</sup>	0.05	30	2.16 <sup>ab</sup>	2.43 <sup>a</sup>	0.11
35	1.26 <sup>dy</sup>	1.46 <sup>cdx</sup>	0.04	35	2.38 <sup>a</sup>	2.44 <sup>a</sup>	0.05
SE	0.04	0.03		SE	0.07	0.06	
Total calcium (mg/dl)				Alkaline phosphatase (U/L)			
8 (baseline)	11.3 <sup>ab</sup>	11.3 <sup>bc</sup>	0.12	8 (baseline)	2,374.2 <sup>a</sup>	2,374.2 <sup>ab</sup>	35.2
9	11.7 <sup>ab</sup>	11.8 <sup>ab</sup>	0.32	9	1,567.8 <sup>abc</sup>	1,958.2 <sup>abc</sup>	608.7
10	12.1 <sup>a</sup>	11.6 <sup>ab</sup>	0.33	10	2,050.2 <sup>ab</sup>	2,455.5 <sup>ab</sup>	615.8
12	11.8 <sup>a</sup>	11.6 <sup>ab</sup>	0.22	12	1,555.8 <sup>abc</sup>	1,919.5 <sup>abc</sup>	544.4
14	11.7 <sup>ab</sup>	12.0 <sup>ab</sup>	0.31	14	1,344.5 <sup>abcd</sup>	2,111.0 <sup>abc</sup>	533.9
18	10.3 <sup>ab</sup>	12.1 <sup>a</sup>	1.45	18	1,428.2 <sup>abcy</sup>	2,595.7 <sup>ax</sup>	583.2
22	9.45 <sup>by</sup>	10.7 <sup>cdx</sup>	0.26	22	1,126.2 <sup>bcd</sup>	1,196.8 <sup>abc</sup>	180.5
26	10.1 <sup>ab</sup>	9.90 <sup>e</sup>	0.18	26	780.2 <sup>cd</sup>	908.0 <sup>bc</sup>	197.2
30	10.6 <sup>ab</sup>	10.5 <sup>de</sup>	0.31	30	585.8 <sup>cd</sup>	576.7 <sup>c</sup>	90.0
35	10.6 <sup>ab</sup>	10.5 <sup>de</sup>	0.14	35	363.8 <sup>dy</sup>	529.3 <sup>cx</sup>	50.3
SE	0.23	0.23		SE	322.8	517.6	
Inorganic phosphorus (mg/dl)							
8 (baseline)	5.88 <sup>a</sup>	5.88 <sup>ab</sup>	0.17				
9	5.88 <sup>a</sup>	6.03 <sup>ab</sup>	0.22				
10	6.20 <sup>a</sup>	6.35 <sup>a</sup>	0.25				
12	6.02 <sup>a</sup>	6.02 <sup>ab</sup>	0.21				
14	6.02 <sup>a</sup>	6.20 <sup>a</sup>	0.27				
18	4.52 <sup>by</sup>	6.62 <sup>ax</sup>	0.75				
22	4.50 <sup>by</sup>	5.33 <sup>bcx</sup>	0.17				
26	4.25 <sup>b</sup>	4.58 <sup>d</sup>	0.17				
30	3.96 <sup>by</sup>	4.72 <sup>cdx</sup>	0.17				
35	3.53 <sup>b</sup>	3.83 <sup>d</sup>	0.21				
SE	0.37	0.24					

<sup>a,b,c,d</sup> Means within the same column without the same superscript are significantly different ( $p < 0.05$ ).

<sup>x,y</sup> Means within the same row without the same superscript are significantly different ( $p < 0.05$ ).

displayed in Table 2. Caponization of cockerels led to a decrease in the relative tibia weight, cortical thickness ( $p < 0.05$ ) after 18 wks of age (orchietomy treatment after 6 wks) and breaking strength ( $p < 0.05$ ) after 22 wks of age (orchietomy treatment after 14 wks). Also, caponization of

male chickens led to an increase in the tibia length ( $p < 0.05$ ) before 18 wks of age (orchietomy treatment within 10 wks), but caponization had no effect on tibia length after 22 wks of age (orchietomy treatment after 14 wks). Moreover, the relative tibia weight reached peak at 18 wks of age and

**Table 2.** Effect of age and caponization on bone characteristics of male native chickens in Taiwan

Weeks of age	Sham-operated	Caponized	SE	Weeks of age	Sham-operated	Caponized	SE
Relative tibia weight (g/100 g BW)				Bone breaking strength (kg)			
8 (baseline)	0.79 <sup>c</sup>	0.79 <sup>ab</sup>	0.02	8 (baseline)	6.71 <sup>d</sup>	6.71 <sup>c</sup>	2.15
14	0.83 <sup>bc</sup>	0.83 <sup>a</sup>	0.04	14	9.75 <sup>cd</sup>	9.24 <sup>abc</sup>	1.06
18	0.85 <sup>abx</sup>	0.73 <sup>by</sup>	0.01	18	10.4 <sup>cd</sup>	8.31 <sup>bc</sup>	0.90
22	0.87 <sup>ax</sup>	0.74 <sup>by</sup>	0.02	22	12.9 <sup>bcx</sup>	7.98 <sup>bcy</sup>	1.25
26	0.85 <sup>abx</sup>	0.77 <sup>by</sup>	0.01	26	16.8 <sup>abx</sup>	12.4 <sup>ay</sup>	1.13
30	0.85 <sup>abx</sup>	0.74 <sup>by</sup>	0.02	30	19.2 <sup>ax</sup>	10.5 <sup>aby</sup>	2.21
35	0.79 <sup>cx</sup>	0.67 <sup>cy</sup>	0.03	35	19.6 <sup>ax</sup>	11.9 <sup>aby</sup>	1.83
SE	0.02	0.02		SE	1.75	1.13	
Tibia length (mm)				Cortical thickness (µm)			
8 (baseline)	90.8 <sup>d</sup>	90.8 <sup>d</sup>	2.34	8 (baseline)	701.3 <sup>d</sup>	701.3 <sup>d</sup>	42.2
14	114.7 <sup>cy</sup>	122.2 <sup>cx</sup>	1.75	14	992.8 <sup>c</sup>	947.9 <sup>a</sup>	39.6
18	127.7 <sup>by</sup>	132.8 <sup>bx</sup>	1.45	18	996.1 <sup>bcx</sup>	931.7 <sup>ay</sup>	32.5
22	134.9 <sup>a</sup>	135.2 <sup>ab</sup>	2.33	22	1,048.8 <sup>abcx</sup>	910.2 <sup>aby</sup>	24.5
26	133.0 <sup>ab</sup>	137.2 <sup>a</sup>	1.53	26	1,126.1 <sup>ax</sup>	843.8 <sup>bcy</sup>	38.8
30	130.0 <sup>ab</sup>	132.9 <sup>ab</sup>	2.02	30	1,093.8 <sup>abx</sup>	839.8 <sup>bcy</sup>	22.2
35	129.9 <sup>ab</sup>	133.0 <sup>ab</sup>	1.82	35	1,129.7 <sup>ax</sup>	777.8 <sup>cdy</sup>	28.8
SE	1.91	1.86		SE	34.5	26.0	

<sup>a,b,c,d</sup> Means within the same column without the same superscript are significantly different ( $p < 0.05$ ).

<sup>x,y</sup> Means within the same row without the same superscript are significantly different ( $p < 0.05$ ).

declined at 35 wks of age. The peak of tibia length was reached at 22 wks of age in male native chickens. However, the bone strength of male native chickens increased most at 14 wks of age.

The results in this study agree with those of Lin and Hsu (2003a), and Chen et al. (2007), who found that caponizing cockerels lowered the relative tibia weight, bone breaking strength and cortical thickness. However, Turner et al. (1989), Wakley et al. (1991) and Vandeschueren et al. (1992) found that orchiectomy caused an incremental increase in the skeletal remodeling and a loss of cancellous bone in the tibia in young and old rats. This loss could be prevented by testosterone replacement or dihydrotestosterone. Similarly, Greendale et al. (1997) showed that higher bioavailable testosterone levels were associated with higher bone mineral density in men and women. Furthermore, Puche and Romano (1968; 1969) also demonstrated that the addition of testosterone to *in vitro* cultures of embryonic fowl bone enhanced calcification and the synthesis of osteoid tissue. Also, Hutt (1929) and Hsieh (2003) reported that caponization of cockerels increased the bone length. However, Landauer (1937), Burke and Edwards (1994), Lin and Hsu (2003a) and Chen et al. (2006a) observed no difference in bone length between caponized and intact fowls. Also, the bone mass significantly increased post puberty in humans (Gilbanz et al., 1988; Bonjour et al., 1991). Other studies have shown that the bone mass reaches a peak in postpubertal humans (Rico et al., 1993; Matkovic et al., 1994). Similarly, Lin and

Hsu (2008) indicated that the plasma testosterone concentration, testicles weight, testicles percentage and tibiae traits of male native chickens in Taiwan, increased significantly with the advance of age, the peak of plasma testosterone concentration and a percentage of testicle to body weight occurred at 22 wks of age and 18 wks of age, respectively. Pederson et al. (1999) showed that androgen receptors are present in the avian skeleton, as well as in the mammalian skeleton. Since androgen enhances osteoblast ossification and inhibits osteoclast corrosion, it is therefore reasonable to expect intact birds to have relatively higher tibia weight, breaking strength and cortical thickness associated with higher plasma testosterone concentration (Wakley et al., 1991; Vandeschueren et al., 1992; Mauras et al., 1999). On the other hand, the reduction in relative tibia weight of breaking strength and cortical thickness in capons is probably due to a decrease in bone ash and calcium contents (Crenshaw, 1986).

#### Bone ash and mineral contents

Table 3 shows the caponization and age effects on bone ash and mineral contents of male native chickens in Taiwan. The bone ash, calcium and phosphorus content increased most after 14 wks of age in male native chickens in Taiwan. Caponization of male chickens decreased the bone ash, calcium, phosphorus and magnesium after 14, 18, 22, 26 and 30 wks of age (orchiectomy treatment after 6, 10, 14 and 18 wks), but the values had no influence on bone manganese contents as expected, which is in agreement

**Table 3.** Effect of age and caponization on bone composition of male native chickens in Taiwan

Weeks of age	Sham-operated	Caponized	SE	Weeks of age	Sham-operated	Caponized	SE
<b>Bone ash (%)</b>				<b>Bone magnesium (%)</b>			
8 (baseline)	51.7 <sup>c</sup>	51.7 <sup>d</sup>	1.29	8 (baseline)	0.52 <sup>b</sup>	0.52 <sup>b</sup>	0.02
14	56.6 <sup>bx</sup>	52.4 <sup>cdy</sup>	0.43	14	0.48 <sup>c</sup>	0.47 <sup>c</sup>	0.01
18	64.4 <sup>ax</sup>	63.2 <sup>ay</sup>	0.46	18	0.49 <sup>c</sup>	0.47 <sup>c</sup>	0.01
22	63.9 <sup>ax</sup>	62.7 <sup>ay</sup>	0.38	22	0.68 <sup>ax</sup>	0.61 <sup>ay</sup>	0.02
26	64.8 <sup>ax</sup>	56.3 <sup>bcy</sup>	1.01	26	0.50 <sup>cx</sup>	0.46 <sup>cy</sup>	0.01
30	63.2 <sup>ax</sup>	57.8 <sup>by</sup>	0.81	30	0.50 <sup>cx</sup>	0.44 <sup>cy</sup>	0.02
35	66.3 <sup>a</sup>	65.8 <sup>a</sup>	0.48	35	0.47 <sup>c</sup>	0.45 <sup>c</sup>	0.01
SE	1.04	1.06		SE	0.02	0.01	
<b>Bone calcium (%)</b>				<b>Bone manganese (ppm)</b>			
8 (baseline)	22.0 <sup>c</sup>	22.0 <sup>d</sup>	0.47	8 (baseline)	1.29	1.29	0.27
14	25.2 <sup>b</sup>	25.0 <sup>b</sup>	0.26	14	1.52	1.59	0.17
18	26.2 <sup>ab</sup>	25.7 <sup>ab</sup>	0.26	18	1.73	1.64	0.16
22	27.2 <sup>a</sup>	25.9 <sup>ab</sup>	0.66	22	1.01	1.16	0.16
26	27.2 <sup>ax</sup>	23.5 <sup>cy</sup>	0.47	26	1.59	1.51	0.14
30	25.6 <sup>bx</sup>	23.1 <sup>cdy</sup>	0.34	30	1.44	1.31	0.14
35	27.0 <sup>a</sup>	26.3 <sup>ab</sup>	0.28	35	2.10	1.68	0.21
SE	0.40	0.38		SE	0.16	0.15	
<b>Bone phosphorus (%)</b>							
8 (baseline)	9.93 <sup>d</sup>	9.93 <sup>e</sup>	0.23				
14	11.6 <sup>c</sup>	11.7 <sup>bcd</sup>	0.09				
18	12.7 <sup>ax</sup>	12.1 <sup>aby</sup>	0.23				
22	12.1 <sup>bc</sup>	11.8 <sup>bc</sup>	0.28				
26	12.8 <sup>ax</sup>	10.8 <sup>dy</sup>	0.24				
30	12.5 <sup>abx</sup>	11.4 <sup>cdy</sup>	0.24				
35	12.4 <sup>ab</sup>	12.4 <sup>a</sup>	0.12				
SE	0.18	0.19					

<sup>a,b,c,d</sup> Means within the same column without the same superscript are significantly different ( $p < 0.05$ ).

<sup>x,y</sup> Means within the same row without the same superscript are significantly different ( $p < 0.05$ ).

with previous reports (Chen et al., 2006). Similarly, Lin and Hsu (2003a) showed that caponization of cockerels decreased the bone calcium and manganese contents, but had no influence on bone phosphorus and manganese contents. Mauras et al. (1996) also indicated that insulin-like growth factor-I (IGF-I) and sex steroid hormones impact bone formation independently and that the actions of IGF-I, growth hormones, and sex steroid hormones might synergize to maximally stimulate the attainment of peak bone mass in humans. The reduced bone ash, calcium, phosphorus and magnesium contents in capons might have been due to increased bone calcium loss and decreased kinetic markers in the bone calcium deposition (Mauras et al., 1999). Since carbonate calcium, phosphate calcium and phosphate magnesium are the most abundant in cortical bone, it was expected that capons would have lower bone ash, calcium, phosphorus and magnesium contents, which are associated with lower plasma testosterone concentration in comparison to intact birds.

### Mineral contents of bone ash

Table 4 presents the effects of caponization and age on mineral contents of bone ash. Caponizations of cockerels decreased the ash calcium after 14 wks and lowered the phosphorus content between 18 and 26 wks. Ash calcium and phosphorus contents at other weeks of age, and ash magnesium and manganese contents did not differ between the caponized and intact birds. The results from this study show that the calcium:phosphorus ratio was nearly 2.15:1 and the ash was made up of calcium, 41.95%; phosphorus, 19.48%; magnesium, 0.85%, and manganese, 2.36 ppm. Similarly, Hegsted (1973) reported that the calcium:phosphorus ratio was nearly constant and somewhat greater than 2:1. He reported that the ash was made up of calcium, 36%; phosphorus, 17%; and magnesium, 0.8% in mammals. Also, Lin and Hsu (2003a) found that caponization decreased bone ash calcium and manganese contents, but had no influence on bone ash phosphorus and magnesium contents.

In this study, our findings support the assertion that

**Table 4.** Effect of age and caponization on mineral composition of bone ash of male native chickens in Taiwan

Weeks of age	Sham-operated	Caponized	SE	Weeks of age	Sham-operated	Caponized	SE
<b>Calcium (%)</b>				<b>Magnesium (%)</b>			
8 (baseline)	42.6 <sup>b</sup>	42.6 <sup>b</sup>	1.32	8 (baseline)	1.01 <sup>a</sup>	1.01 <sup>a</sup>	0.04
14	44.5 <sup>ay</sup>	47.7 <sup>ax</sup>	2.06	14	0.87 <sup>b</sup>	0.90 <sup>b</sup>	0.04
18	40.7 <sup>b</sup>	40.7 <sup>b</sup>	0.25	18	0.76 <sup>c</sup>	0.74 <sup>cd</sup>	0.02
22	42.6 <sup>b</sup>	41.4 <sup>b</sup>	1.08	22	1.07 <sup>a</sup>	0.97 <sup>ab</sup>	0.05
26	42.0 <sup>b</sup>	41.7 <sup>b</sup>	0.43	26	0.77 <sup>bc</sup>	0.81 <sup>c</sup>	0.02
30	40.6 <sup>b</sup>	40.0 <sup>b</sup>	0.31	30	0.79 <sup>bc</sup>	0.77 <sup>c</sup>	0.03
35	40.7 <sup>b</sup>	40.0 <sup>b</sup>	0.39	35	0.71 <sup>c</sup>	0.68 <sup>d</sup>	0.01
SE	1.08	0.92		SE	0.04	0.02	
<b>Phosphorus (%)</b>				<b>Manganese (ppm)</b>			
8 (baseline)	19.2 <sup>b</sup>	19.2 <sup>b</sup>	0.57	8 (baseline)	2.46 <sup>ab</sup>	2.46 <sup>ab</sup>	0.49
14	20.4 <sup>ay</sup>	22.3 <sup>ax</sup>	0.88	14	2.92 <sup>a</sup>	2.99 <sup>a</sup>	0.32
18	19.7 <sup>ab</sup>	19.1 <sup>b</sup>	0.34	18	2.69 <sup>a</sup>	2.58 <sup>ab</sup>	0.25
22	18.9 <sup>b</sup>	18.8 <sup>b</sup>	0.45	22	1.60 <sup>b</sup>	1.86 <sup>b</sup>	0.25
26	19.7 <sup>ab</sup>	19.3 <sup>b</sup>	0.20	26	2.47 <sup>ab</sup>	2.52 <sup>ab</sup>	0.22
30	19.7 <sup>ab</sup>	19.7 <sup>b</sup>	0.38	30	2.29 <sup>ab</sup>	2.27 <sup>ab</sup>	0.23
35	18.7 <sup>b</sup>	18.8 <sup>b</sup>	0.12	35	2.10 <sup>ab</sup>	2.54 <sup>ab</sup>	0.21
SE	0.47	0.46		SE	0.28	0.24	

<sup>a,b,c</sup> Means within the same column without the same superscript are significantly different ( $p < 0.05$ ).

<sup>x,y</sup> Means within the same row without the same superscript are significantly different ( $p < 0.05$ ).

androgens directly influence bone composition fluxes in male chickens. Caponization caused a significant increase in bone loss at 4 wks post treatment, which reflected that the bone cells were damaged, and demonstrated that relative tibia weight, breaking strength, cortical thickness, bone ash, calcium, phosphorus and magnesium contents were reduced, and the plasma ionized calcium, inorganic phosphorus and alkaline phosphatase concentrations were increased. The androgens clearly had significant skeletal effects. The actions of the paracrine mediators of androgen that played on bones included suppressing osteoblast interleukin-6 production (Hofbauer et al., 1999), ensuring antiresorptive effect on bones (Hofbauer et al., 1999), reducing bone remodeling (Katznelson et al., 1996), and increasing  $\beta$ -transforming growth factor production and bone deposition (Gill et al., 1998; Pederson et al., 1999).

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