Effects of Caponization on Bone Characteristics and Histological Structure in Chickens

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ABSTRACT : The aim of this study was to investigate the effects of caponization on the bone characteristics, biomechanical property and histology in Taiwan country chickens fed to market age of 26 wks. Male Taiwan country chickens D×L_2 were caponized or sham-operated at 8 wks of age, and selected healthy sham-operated and completely caponized chickens (prominent degenerated comb) were selected at 16 wks old and fed to 26 wks old for the trials. Fifteen intact male chickens (Intact), sham-operated chickens (Sham) and caponized chickens (Capon) were assigned for trial 1, and sixteen Intact and Capon were assigned for trial 2. Results in trial 1 showed that the abdominal fat and relative abdominal fat weights of Capon were significantly heavier than Intact and Sham ($p<0.05$), while the tibia weight and relative weight were the lightest ($p<0.05$). The tibia breaking strength, bending moment and stress of Capon were the poorest among groups ($p<0.05$). The trial 2 produced the similar observation that Capon were significantly lighter than Intact ($p<0.05$) in the tibia weight, relative tibia weight and their biomechanical properties. On histological determinations, Capon showed a thinner cartilage end and fewer chondrocytes (about 50%) and trabecular, and bigger marrow cavity; while decreased hemopoietic cells number with increased adipocytes than Intact observed by H&E stain and at low magnification. At high magnification, Capon showed a decrease in the chondrocyte size by 33 to 50%, with smaller nucleus located near the cell membrane, and exhibited monocellular form chondrocytes. Capon also showed a less strongly acidic sulfated mucosubstance with weaker dyeing property within cartilage zone, and smaller chondrocytes size by Alcian blue stain. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 245-251)***

Key Words : Bone Characteristic, Caponization, Histology, Male Chicken

INTRODUCTION

Capons are male chickens whose testes have been surgically removed during the immature stage. Because of the resultant androgen deficiency, the secondary male sexual features including the comb, wattle, fighting behavior and vocalization degenerate, and maturity regress to an immature stage. Lipids begin to accumulate in the body at maturity, enhancing flavor, texture, and meat juiciness compared with that of intact cockerels (Chen et al., 2000a, b; Chen et al., 2005). The capon has been popular by the Easterner since ancient time, and the annual consumptions in Taiwan has been around 4.3 millions (Deng and Wang, 2001).

Capons are normally marketed at six months of age in Taiwan and therefore required strong skeletal backbone to support the increasing body mass during long feeding period. Androgen has long been recognized as playing an important role in bone development, physiology and metabolism (Pederson et al., 1999). Depressed androgen through chemical, testectomy operation or age on the adverse effects of bone growth and development in human beings is clear (Manolagas et al., 2002). But its effects on poultry, however, are still unclear. Early studies indicated that caponized white Leghorn chickens increased bone length (Hutt, 1929). But Landauer (1937) did not find a significant effect on bone length in caponized male chickens fed to 10-months of age in his well designed trial. Ono et al. (1979) obtained similar results in broilers caponized at 9 wks of age that caponization did not influence the bone length of 31-wk-old capons. On the contrary, Lesson et al. (1976) indicated more severe sternum bending in capons than in intact male chickens, resulting in poor production efficiency and causing economic loss. Johnson and Rendano (1984) also showed that 6-wk-old caponized Leghorn male chickens were more susceptible to osteochondrodysplasia and osteodysplasia in the tibiotarsus- tarsometatarsus region than intact chickens at 35- or 47-wk-old. In fact, most studies focused on the bone length, weight and exteriority, but not on the bone biomechanical characteristics and histology.

Some contractures of capon legs were observed at commercial farms in Taiwan which affects the capons marketing value. Hence, the specific capon strain and feeding regime in the commercial production were followed in this trial to study the effects of caponization on bone biomechanical characteristics and histology in male chickens.

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Table 1. Formula and chemical composition of the basal diets

Ingredients	%
Yellow corn (grain)	69.715
Soybean meal (44%)	14.2
Wheat bran	10.0
Fish meal (65%)	2.5
Limestone, pulverized	1.4
Dicalcium phosphate	1.6
Vitamin premix[1]	0.1
Mineral premix[2]	0.1
Salts	0.3
DL-methionine	0.06
L-lysine	0.025
Total	100
Calculated analysis	
Crude protein (%)	15.9
ME (kcal/kg)	2,873
Calcium	0.08
Available phosphorus	0.035

[1] Vitamin premix supplied per kilogram of diet: retinyl acetate, 4.13 mg; cholecalciferol, 0.078 mg; dl-α-tocopherol, 34.1 mg; Vitamin K_3, 6.25 mg; Vitamin B_1, 3.75 mg; Vitamin B_2, 12.5 mg; Vitamin B_6, 10.0 mg; Ca-pantothenate, 18.8 mg; Niacin, 50 mg; Biotin, 0.06 mg; Folic acid, 1.25 mg; Vitamin B_{12}, 0.05 mg.

[2] Mineral premix supplied per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu), 6 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 40 mg; Zn (ZnO, 80.35% Zn), 60 mg; Se ($NaSeO_3$, 45.56% Se), 2.075 mg.

MATERIALS AND METHODS

Animal management and experimental design

Healthy male Taiwan country cockerels D×L_2 were caponized or sham operated at 8 wks of age and housed in individual 40×30 cm, 38 cm high cages for a 8-wk adaptation period. Each of sixteen intact male (Intact), sham-operated (Sham) and caponized (Capon, prominent degenerated comb) chickens in trial 1 and 15 Intact and 15 Capon in trial 2 were selected at 16 wks of age for 10 wks experimental period (feeding to 26 wks of age). Feed (Table 1) and water were available *ad libitum* during the feed period.

Testectomy

The testectomy procedure was performed according to Chen et al. (2000a). Restricted to feed and water for 12 h before the surgical operation, male chickens were restrained and the incision site was sterilized with iodine-tincture. A 1 cm lateral incision was made at the second to last rib. The testes were then removed. Iodine-tincture was applied again to the incision site.

Measurement and analysis

At the end of experiments, chickens were weighted and sacrificed. After de-plumaged, the abdominal fat and internal organs was removed, and then the plucked empty body, carcass was weighted, then removed the breast muscles and weighted. Blood samples were taken from the brachial-vein after the birds were withdrawn from feed and water for 12 h at 26 wks of age. After centrifuging, serum was stored at -40°C for further analysis. Blood serum calcium, phosphorus concentrations and alkaline phosphatase activity were analyzed by using an automatic blood chemical analyzer with Roche testing kits (Roche COBAS MIRA PLUS, Switzerland). The testosterone concentration of 26-wk chickens in trial 1 was measured according to Chen et al. (2005).

Tibias from individual chicken were dissected. After cleaning adherent tissues, the right tibia was defatted with chloroform-methanol (2:1) for 48 h referred to (Rama Rao et al., 2003), and then dried at 105°C for 24 h to measure the bone weight, length and biomechanical characteristics. The bone was held on the tension compression tester (DCS-5, Shimadzu autograph, Japan) to determine the ultimate breaking strength (kg) by three point bending test. The total distance between the two supporting ends was 6.5 cm, the test range was 0 to 100 kg and cross-head movement was 1 mm/sec. Bone bending moment (kg·cm) and stress (kg/cm^2) was calculated according to Crenshaw et al. (1981) as following:

$$\text{Bending moment (kg·cm)} = \text{breaking strength (kg)} \times 6.5 \text{ (cm)}/4$$

$$\text{Stress (kg/cm}^2\text{)} = \text{breaking strength (kg)} \times 6.5 \text{ (cm)} \times (\text{o.d.}/2) \text{ (cm)}/4 \times \text{modulus of elasticity}$$

$$\text{Modulus of elasticity (ellipse) (kg/cm}^2\text{)} = 0.0491 \times [(\text{o.d.})^3 - (\text{i.d.})^3]$$

In trial 2, the proximal epiphysis of left tibia was slit to collect 0.5 cm thickness sample. Then each sample was fixed in 10% neutral formalin, decalcified, embedded in paraffin, and 3 to 5 μm sections were made by microtome. The section was stained with hematoxylin and eosin (H&E), and histological examination was done by light microscopy.

Statistical analysis

Analyses of variance among treatment groups (trial 1: Intact, Sham and Capon; trial 2: Intact and Capon) were calculated using the GLM procedure of the SAS (1989). Duncan's new multiple-range test was used to compare the means according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Trial 1

Carcass and bone characteristics : Table 2 shows the effects of caponization on carcass and bone characteristics in male chickens. Caponization increased ($p<0.05$) the

Table 2. The effects of caponization on carcass and bone characteristics in male chickens[1] (trial 1)

Item	Intact	Sham	Capon
Body weight (g)	2,776±307	2,813±347	2,902±314
Carcass weight (g)	2,411±240	2,339±314	2,311±300
Abdominal fat weight (g)	27±37[b]	34±25[b]	134±62[a]
Breast weight (g)	404±50	428±49	430±40
Dressing percent (g/100g BW)	86.9±3.1[a]	83.0±3.4[b]	79.6±6.0[c]
Relative abdominal fat weight (g/100 g BW)	0.947±1.30[b]	1.24±0.94[b]	4.59±1.93[a]
Relative breast weight (g/100 g BW)	14.6±1.3	15.3±1.8	14.8±1.1
Tibia length (cm)	13.5±0.4	13.1±0.8	13.1±0.9
Tibia weight (g)	17.5±2.1[a]	16.6±2.0[a]	15.0±2.1[b]
Relative tibia weight (g/100 g BW)	0.476±0.04[a]	0.459±0.05[a]	0.369±0.03[b]
Breaking strength (kg)	43±14[a]	42±14[a]	30±6[b]
Bending moment (kg·cm)	70±22[a]	71±22[a]	50±10[b]
Stress (kg/cm^2)	129±43[ab]	141±33[a]	108±35[b]

[1] Means±SD.
[a, b] Means in the same row with different superscript are significantly different ($p<0.05$).

Table 3. The effects of caponization on blood characteristics in male chickens[1] (trial 1)

Item	Intact	Sham	Capon
Testosterone (pg/ml)	1,028±301[a]	996±284[a]	85±41[b]
Calcium (mg/dl)	14.2±1.83	15.5±1.38	16.1±3.84
Phosphorus (mg/dl)	5.21±0.61	5.39±0.63	5.48±1.69
Alkaline phosphatase (U/L)	612±253[ab]	447±198[b]	742±258[a]

[1] Means±SD.
[a, b] Means in the same row with different superscript are significantly different ($p<0.05$).

abdominal fat weight and relative abdominal fat weight but decreased ($p<0.05$) the dressing percent, tibia weight, relative tibia weight, breaking strength, bending moment and stress.

The effects of caponization on abdominal fat increase was expected because of the androgen deficiency (Table 3), is consistent with previous studies (Cason et al., 1988; Chen et al., 2000a; Chen et al., 2005) and resulted in the lowest dressing percent of Capon ($p<0.05$). In the trial, Capon obtained the lightest ($p<0.05$) tibia weight but the heavier average body weight (BW) resulted in the lowest relative tibia weight ($p<0.05$). Hence, the Capon tibias bear greater BW load. Androgen promotes chondrocyte maturity and mineral sedimentation in bone of males as animal approaching mature. Since the androgen receptor mainly present in the osteoblast, androgen therefore enhences osteoblast ossification and, inhibits osteoclast corrosion (Pederson et al., 1999; Notelovitz, 2002; Kung, 2003). Hence, with androgen effects, Intact exhibited better tibia breaking strength, bending moment and stress than Capon ($p<0.05$) in this trial. Bone biomechanical characteristics including breaking strength, bending moment and stress, represent the maximum loading strength, bending degree and strength per unit of bone area, respectively. These characteristics were also influenced by several factors such as bone density, mineralization and size (Compston, 2001). Tsay et al. (2004) indicated the similar results that 26 wk SCWL chickens shown better tibia weight and biomechanical characteristics than which caponizated at 12 wk. The carcass and bone characteristics show no differences between Intact and Sham ($p>0.05$), and reflected that the operation didn't effect the carcass and bone characteristics. This was consistent with our previous study (Chen et al., 2000a) that the physiological function of male chickens would recover within 4 wk after operation.

Blood constituent : Table 3 presents the effects of caponization on blood characteristics in male chickens. Caponization increased the blood alkaline phosphatase activity ($p<0.05$), and decreased the testosterone concentration ($p<0.05$).

Shafty (1990) reported that the calcium retention increase accompanied by the blood calcium increase, but this calcium concentration increase did not observe ($p>0.05$) in this trial. Conversely, our result agreed with the observation of Johnson and Rendano (1984) that caponization at 6-wk-old did not influence the plasma calcium content of intact male chickens at 35- and 47-wk-old. Lin and Hsu (2002) who found that caponization would increase the calcium and phosphorus concentrations, and concluded that osteocyte calcium and phosphorus could be released from bone to blood stream, while those results were not found in our trials. Since osteocyte contains large amounts of alkaline phosphatase which releases to blood during bone growth or degeneration, and alkaline phosphatase level also relates to osteogenic activity, reflecting bone characteristics and could be an indicator

Table 4. The effects of caponization on carcass and bone characteristics in male chickens[1] (trial 2)

Item	Intact	Capon
Body weight (g)	2,760±233	2,917±202
Carcass weight (g)	2,279±227	2,344±95
Abdominal fat weight (g)	36±25[b]	138±64[a]
Breast weight (g)	433±46	435±44
Relative carcass weight (g/100 g BW)	82.4±1.9	80.2±6.3
Relative abdominal fat weight (g/100 g BW)	1.34±0.94[b]	4.67±2.00[a]
Relative breast weight (g/100 g BW)	15.7±1.5	14.9±1.2
Tibia length (cm)	13.3±0.8	12.7±0.8
Tibia weight (g)	16.9±2.1[a]	14.4±2.6[b]
Relative tibia weight (g/100 g BW)	0.464±0.02[a]	0.357±0.03[b]
Breaking strength (kg)	44±12[a]	29±5[b]
Bending moment (kg·cm)	72±19[a]	47±8[b]
Stress (kg/cm^2)	144±28[a]	103±32[b]

[1] Means±SD.
[a, b] Means in the same row with different superscript are significantly different ($p<0.05$).

Table 5. The effects of caponization on blood characteristics in male chickens[1] (trial 2)

Item	Intact	Capon
Calcium (mg/dl)	15.5±1.60	16.7±4.58
Phosphorus (mg/dl)	5.40±0.65	5.94±1.90
Alkaline phosphatase (U/L)	444±157[b]	735±250[a]

[1] Means±SD.
[a, b] Means in the same row with different superscript are significantly different ($p<0.05$).

(Bell and Freeman, 1971; Galvanovskii et al., 1985). In this study, the alkaline phosphatase activity was highest in Capon. These results may be attributed to the active bone remodeling and leading to the inferior bone biomechanical characteristics.

Trial 2

Carcass and bone characteristics : Table 4 presents the effects of caponization on carcass and bone characteristics in male chickens. Capon showed heavier abdominal fat weight and relative abdominal fat weight ($p<0.05$), but the lighter tibia weight and relative tibia weight, poor breaking strength, bending moment and stress than Intact ($p<0.05$).

The results were consistent with previous result (trial 1), and again demonstrated that caponization would decrease the tibia weight, and affect the bone biomechanical characteristics.

Blood constituent : Table 5 presents the effects of caponization on blood characteristics in male chickens. Caponization increased alkaline phosphatase activity ($p<0.05$).

The blood calcium and phosphorous concentration results were also consistent with the previous trial, which did not reflect the biomechanical characteristics changes, and therefore still to be pending on further research in using it as skeletal characteristics indicator. Capon show the highest alkaline phosphatase activity ($p<0.05$), which reflected the bone cells were damaged and released alkaline phosphatase. This hypothesis could be proven through the histological observation and biomechanical characteristics assays.

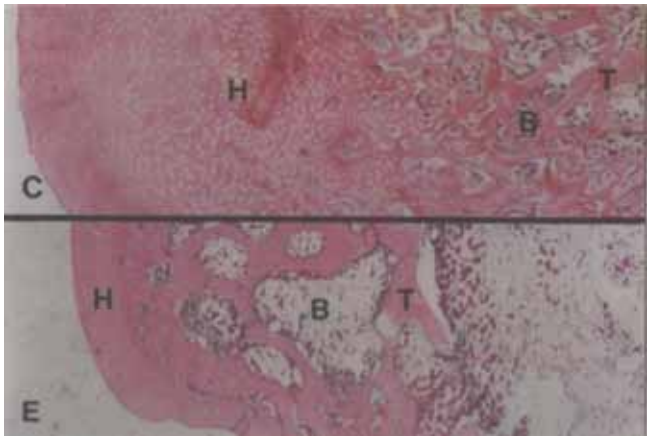

Figure 1. C: Intact, E: Capon. The effects of caponization on male Taiwan country chickens. A thinner cartilage zone and less number of chondrocyte (about 50%) and trabeculae, and bigger marrow cavity; the bony trabeculae were twice thicker decreased 33 to 50%, while decreased hemopoietic cells number with increased adipocyte in capons than the intact male chickens H&E. 13.2×. Hyaline cartilage zone (H), Bony trabeculae (T), Bone marrow cavity (B).

Histological change : To understand the effects of caponization on bone histological structure, tibia sections were determined. Results showed that a thinner cartilage zone and less number of chondrocyte (about 50%) and trabeculae, and bigger marrow cavity; while decreased hemopoietic cells number with increased adipocyte in Capon than the Intact by H&E stain and at low magnification (Figure 1), while the bony trabeculae were twice thicker decreased 33 to 50% in the chondrocytes size with smaller nucleus located near the cell membrane (Figure 2), and exhibited in nest by monocellular form and

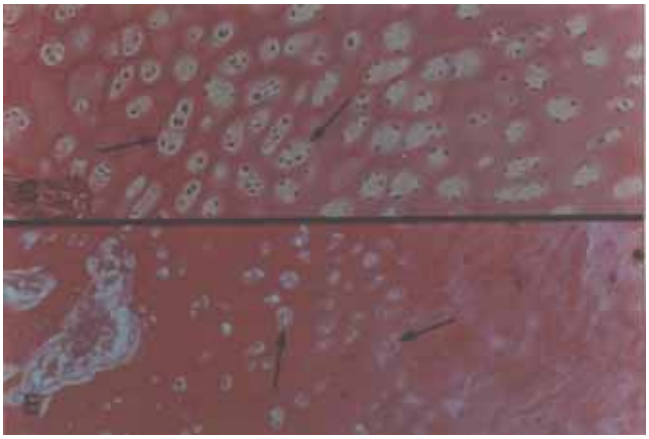

Figure 2. C: Intact, E: Capon. The effects of caponization on male Taiwan country chickens. Chondrocytes size with smaller nucleus located near the cell membrane, and exhibited in nest by monocellular form and eosinophilic stain at high magnification. But 2 to 6 chondrocytes exhibted in nest form osogenic chondrocyte in intact male chickens, also can be found 8 chondrocytes in nest form isogenic group. H&E. 66×. Isogenic chondrocyte (arrow).

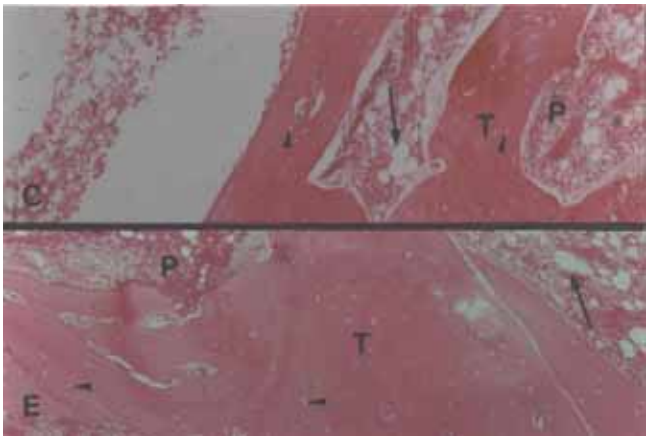

Figure 4. C: Intact, E: Capon. The effects of caponization on male Taiwan country chickens. The bony trabeculae were twice thicker decreased 33 to 50%. It exhibited in nest by monocellular form and eosinophilic stain at high magnification. But 2 to 6 chondrocytes exhibted in nest form isogenic chondrocyte in intact male chickens, also can be found 8 chondrocytes in nest form isogenic group. H&E. 66×. Bony trabeculae (T), Hemopoietic cell (P), Adipocyte (arrow).

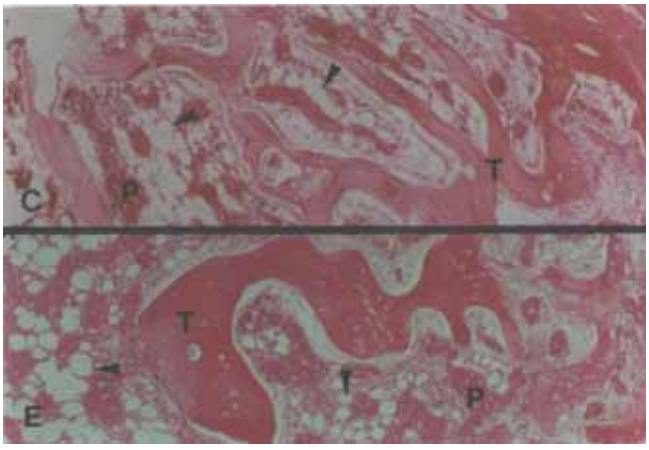

Figure 3. C: Intact, E: Capon. The effects of caponization on male Taiwan country chickens. The bony trabeculae were twice thicker decreased 33 to 50%. It exhibited in nest by monocellular form and eosinophilic stain at high magnification. But 2 to 6 chondrocytes exhibted in nest form isogenic chondrocyte in intact male chickens also can be found 8 chondrocytes in nest form isogenic group. H&E. 66×. Bony trabeculae (T), Hemopoietic cell (P), Adipocyte (arrowhead).

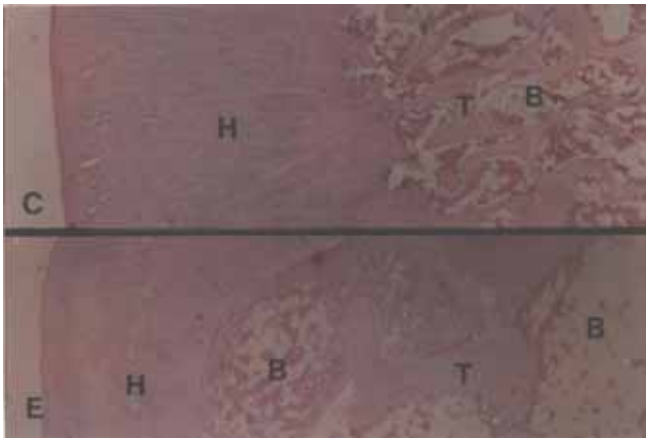

Figure 5. C: Intact, E: Capon. The effects of caponization on male Taiwan country chickens. Capon also showed less strong acidic sulfated mucosubstance with weaker dyeing property within cartilage layer. H&E. 26.4×. Hyaline cartilage zone (H), Bony trabeculae (T), Bone marrow cavity (B).

eosinophilic stain at high magnification. But 2 to 6 chondrocytes exhibted in nest form osogenic chondrocyte in Intact, also can be found 8 chondrocytes in nest form isogenic group (Figures 2, 3 and 4). Capon also showed less strong acidic sulfated mucosubstance with weaker dyeing property within cartilage layer (Figure 5), and smaller size chondrocytes and basophilic stain by alcian blue stain, but larger and eosinophilic stain chondrocytes in the Intact (Figure 6).

In general, the ground substance contains large amount of sulfated polysaccharide (Wheater et al., 1987). In the trial, Capon showed less strong acidic sulfated mucosubstance in the ground substance, the thinner cartilage zone, less number and smaller size of chondrocyte, and decreased isogenic chondrocyte numbers, but show no different in the osteoclasts number. The androgen receptor mainly presents on the nucleus membrane in the osteoblast or osteoclast (Pederson et al., 1999; Notelovitz, 2002; Kung, 2003). Hence, these results showed that because of androgen deficiency, there was insufficient androgen to bind with the osteocyte receptor, and which resulted in the degeneration of tibia proliferation and the acceleration of ossification. These histological phenomenons again agreed with the observation of the lighter tibia weight and poor

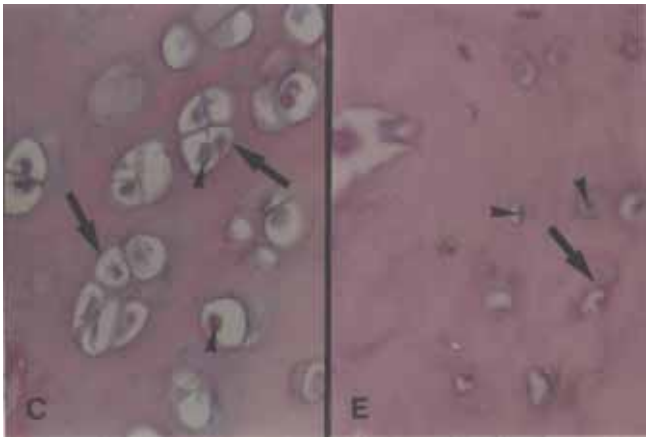

Figure 6. C: Intact, E: Capon. The effects of caponization on male Taiwan country chickens. Smaller size chondrocytes and basophilic stain by H&E stain, but larger and eosinophilic stain chondrocytes in the intact male chickens. H&E. 26.4×. Nucleu of chondrocyte on hyaline cartilage (arrowhead), Isogenic chondrocyte (arrow).

biomechanical characteristics of Capon as compared to the Intact in both trials. Hence, caponization would have inverse impacts on bone structure, and furthermore, to influence the bone characteristics in 26-wk-old male chickens.

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