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Effects of Caponization and Testosterone on Bone and Blood Parameters of SCWL Male Chickens

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ABSTRACT : This study was to investigate the caponization effects on bone characteristics in male chickens, and the optimum testosterone implantation dosage on bone characteristics improvement. Healthy Single Comb White Leghorn cockerels were caponized at 12-wk-old and selected at 16-wk-old for a 10-wk feeding experiment. Fifteen intact male and caponized male chickens (capon) respectively were assigned to trial 1. Ten sham-operated chickens and 40 capons (randomly allocated into four treatments) were implanted with cholesterol (1.62 mm i.d., 3.16 mm o.d., 9.24±0.36 mg), low (1 mm i.d., 3 mm o.d., 5.88±0.23 mg), medium (1.62 mm i.d., 3.16 mm o.d., 9.81±0.17 mg) or high dose (2 mm i.d., 4 mm o.d., 16.7±0.24 mg) of testosterone in trial 2. The results from trial 1 showed that the tibia length, relative tibia weight, breaking strength, bending moment and stress in intact males were higher than capons ($p<0.05$). The blood phosphorus concentration in capons was higher than the intact male chickens ($p<0.05$). Caponization also resulted in more antrums and osteoclasts within periosteum and cortical bone from histological observation. In trial 2, the adverse impact of caponization on the bone breaking strength, bending moment and stress could be alleviated through medium dose testosterone implantation. It appears that caponization reduced androgen secretion hence influenced the biomechanical characteristics of bone (tibia) and these adverse effects could be alleviated through appropriate dose of testosterone implantation. (**Key Words** : Bone Characteristic, Caponization, Male Chicken, Testosterone Implantation)

INTRODUCTION

Androgen has long been recognized as playing an important role in bone development, physiology and metabolism (Pederson et al., 1999). Androgen influences mammalian bone development. Depressed androgen through chemical, testectomy operation or age has adverse effects on bone growth and development in human beings (Manolagas et al., 2002). But its effects on poultry, however, are still unclear.

Hutt (1929) indicated that caponized white Leghorn chickens increased bone length. 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. 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. Ono et al. (1982) obtained similar results in broilers caponized at 9 wks of age that caponization did not influence the bone length of 31-wk-old capons. Johnson and Rendano (1984) showed that 6-wk-old caponized Leghorn male chickens were more susceptible to osteochondrodysplasia and osteodysplasia in the tibiotarsus-tarsometatarsus region than intact cockerels at 35- or 47-wk-old. However, these capons could recover to normal development as intact males through testosterone implantation. Whereas high-dose (29.2 mg) testosterone implantation in 2-wk-old caponized male Leghorn chickens decreased the tibia length (Fennell and Scanes, 1992). The shank perimeter in the capons was higher than male chicken (2006b). Chen et al. (2006a) indicated that caponized male Taiwan country chicken showed the poor tibia biomechanical characteristics and histological observations. These discordant results were due to the different age, species, caponization age and determination. Besides, there are little literatures about the

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Table 1. Composition of the basal diets

Ingredients	%
Yellow corn (grain)	68.95
Soybean meal (44%)	14.2
Wheat bran	10.0
Fish meal (65%)	2.5
Limestone, Pulverized	1.4
Dicalcium phosphate	1.6
Vitamin premix1	0.1
Mineral premix2	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	0.8
Available phosphorus	0.35

[1] Vitamin premix supplied per kilogram of diet: Vitamin A, 12,000 IU; Vitamin D_3, 3,125 ICU; Vitamin E, 37.5 IU; 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), 0.075 mg.

different doses of testosterone implantations in chickens.

This study is therefore aimed at studying the caponization effects on bone characteristics in male chickens and determining the optimum testosterone-implanted dosage for the bone characteristics improvement.

MATERIALS AND METHODS

Animal management and experimental design

Healthy male Single Comb White Leghorn (SCWL) chickens (790±29 g) were caponized at 12 wks of age and housed in individual 40×30 cm, 38 cm high cages for a 4-wk adaptation period. Fifteen intact male and 15 caponized (capon, prominent degenerated comb) chickens were selected at 16 wks of age for a 10-wk feeding experiment in Trial 1. In Trial 2, 10 sham operated chickens (Sham) and 40 capons were randomly divided into four treatments: cholesterol implantation (1.62 mm i.d., 3.16 mm o.d., 9.24±0.36 mg), or low (1 mm i.d., 3 mm o.d., 5.88±0.23 mg), medium (1.62 mm i.d., 3.16 mm o.d., 9.81±0.17 mg) or high (2 mm i.d., 4 mm o.d., 16.7±0.24 mg) testosterone dose for the 10-wk experimental period (feeding to 26 wks of age). Feed (Table 1) and water were provided *ad libitum* during the feed period.

Testectomy

The testectomy procedure was performed according to Chen et al. (2000, 2007). 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.

Testosterone implantation

The testosterone implantation procedure was performed according to the modified method of Fennell et al. (1990). An 1 cm implantation tube (Tygon Clear Tubing R-3603, USA) was used in this trial with different inner diameter sizes to control the testosterone dose. According to the earlier study (Chen et al., 2005), testosterone was implanted subcutaneously at the back of the chickens' neck at 16, 20 and 24 wks of age to maintain the blood testosterone concentration homeostasis.

Measurement and analysis

Body weights were measured at 16 and 26 wks of age. Chickens were slaughtered at the end of trials.

Bone characteristic : Tibias from individual chicken were dissected. After cleaning adherent tissues, the right tibia was defatted with chloroform-methanol (2:1), 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).

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 μm sections were processed. The section was stained with hematoxylin & eosin and under microscopy examination (×40 and ×400).

Blood samples were taken from the brachial-vein of chickens which 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. Testosterone concentration was determined according to the method of Li et al. (1987). 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).

Statistical analysis

Analyses of variance among treatment groups (trial 1: intact male chicken and capon; trial 2: Sham, capon implanted with cholesterol and low, medium and high dose testosterone) were calculated using the general linear model

Table 2. Caponization effects on bone characteristics in male chickens (trial 1)

	Male	Capon	SEM
Tibia length (mm)	130[a]	126[b]	0.522
Tibia weight (g)	6.87	6.43	0.208
Relative tibia weight (g/100 g BW)	0.401[a]	0.364[b]	0.058
Breaking strength (kg)	23.7[a]	18.4[b]	0.675
Bending moment (kg-cm)	38.5[a]	29.9[b]	0.860
Stress (kg/cm^2)	235[a]	159[b]	2.27

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

procedure of the SAS (1985). Duncan's new multiple-range test was used to compare the means according to Steel and Torrie (1960).

RESULTS AND DISCUSSION

Trial 1

Bone characteristic : Table 2 shows the caponization effects on bone characteristics in male chickens. Caponization decreased ($p<0.05$) the tibia length, relative tibia weight, breaking strength, bending moment and stress at 26-wk-old.

Caponization resulted in the decreased tibia length, but this was not consistent with other studies that showed no influences (Ono et al., 1982; Fennell and Scanes, 1992) or increased the bone length (Hsieh, 2003). This may be attributed to the caponization time in this trial, caponization was performed before maturity from 12-wk-old to the required four wks recovery from caponization (Chen et al., 2000) Hence, caponization inhibited the tibia development in capons and therefore resulted in shorter tibia length than intact male chickens ($p<0.05$). This was consistent with Chen et al. (2006a). Besides, the androgen deficiency in capon (testosterone concentration: male, 885±571 vs. capon, 106±43 pg/ml) speeded up the abdominal fat increase (male, 5±2.6 vs. capon, 46±22 g), the body weight gain increased with 20% heavier than the intact male chickens (male, 7.8± 1.4 g/day/bird vs. capon 9.4±2.7 g/day/bird) (Chen et al., 2005), but the relative tibia weight was lower ($p<0.05$). Hence, the capon tibias bear greater body weight load.

Androgen promotes the chondrocyte maturity and mineral sedimentation in bone of males as animal approaching mature. Since the androgen receptor mainly present in the osteoblast in various bone cells, androgen therefore enhances osteoblast ossification and, inhibits osteoclast corrosion (Pederson et al., 1999; Notelovitz, 2002; Kung, 2003). Hence, with androgen effects, intact male chickens exhibited better tibia breaking strength, bending moment and stress than capons ($p<0.05$) 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).

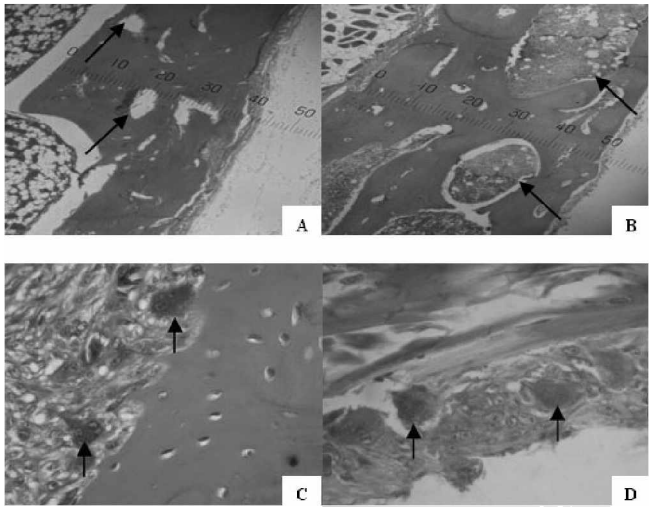

Figure 1. Micrographs of the tibia section. Caponization resulted in more antrums (B) and osteoclasts (D) within periosteum and cortical bone as compared to intact male chickens (A and C). The section was stained with hematoxylin-eosin. Magnification: ×40 (A and B) and ××00 (C and D).

Figure 1 presents the histological observation. Caponization did not show an influence on the growth plate, secondary ossification center and bone thickness, but showed more antrums and osteoclasts (male, 1 to 2 vs. capon, 2 to 3) within periosteum and cortical bone, reflected that the bone density was lower and resulted in poor biomechanical characteristics in the capons. Caponization effects on the biomechanical characteristics reflect the androgen effect on the bone structure.

Blood constituent : Table 3 presents the caponization effects on blood characteristics in male chickens. Caponization did not influence blood calcium concentration and alkaline phosphatase activity ($p>0.05$), but increase the blood phosphorus concentration ($p<0.05$).

Since bone cell contains large amounts of alkaline phosphatase which released to blood during bone growth or degeneration, the alkaline phosphatase was related to osteogenic activity and phosphorous concentration and could be an indicator for bone characteristics (Bell and Freeman, 1971; Galvanovskii et al., 1985). However, the alkaline phosphatase activity was not affected ($p>0.05$) in this trial. The observation of the increase ($p<0.05$) blood phosphorus concentration at 26-wk-old capons agreed with Lin and Hsu (2002) who concluded that phosphorus can be released from bone and flow to blood stream after caponization. Shafty (1990) reported that the calcium retention would be increased with the increase of blood calcium, but the calcium concentration was not affected ($p>0.05$) in this trial. Conversely, Johnson and Rendano

Table 3. Caponization effects on blood characteristics in male chickens (trial 1)

	Male	Capon	SEM
Calcium (mg/dl)	10.9	10.8	0.458
Phosphorus (mg/dl)	5.08 [b]	5.78 [a]	0.238
Alkaline phosphatase (U/L)	726	872	3.93

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

(1984) showed that caponization at 6-wk-old did not influence the plasma calcium content of male chickens at 35- and 47-wk-old, and is agreed with the trial 1.

Trial 2

Bone characteristic : Table 4 presents the testosterone implantation effects on bone characteristics in capons. The medium testosterone implanted capons showed higher tibia length, breaking strength, bending moment and stress ($p<0.05$) than the cholesterol implanted capons, and reached a similar level with the Sham ($p>0.05$).

The capons implanted with the increase amount of testosterone up to the high dose had increased ($p<0.05$) serum testosterone concentration compared with capons implanted with CHOL. This value was still lower ($p<0.05$) than that of the Sham (Sham, 817±137; CHOL, 139±16.5; Low, 266±28.8; Medium, 342±45.4; High, 405±53 pg/ml) (Chen et al., 2005). The medium testosterone implantation did improve the poor bone characteristics resulted from androgen deficiency due to caponization. Consequently, the tibia length, weight, relative tibia weight and biomechanical characteristics achieved close to the Sham level ($p>0.05$). Rath et al. (1996) indicated that testosterone implantation (10 mg/kg BW/wk) rather than the other sex steroids could improve tibia physical properties of 6-wk-old broilers for three wks, and concluded that androgen stimulated bone development. Although the high dose implantation could increase the tibia weight, relative tibia weight and stress to the level close to Sham ($p>0.05$), the length, breaking strength and bending moment were lower than medium dose implantation ($p<0.05$). Fennell and Scanes (1992) reported that only the high dose testosterone (29.2 mg, 3 cm length and 1.57 mm i.d.) inhibited the tibia growth. In their study, 2-wk-old caponized male chicks were implanted different doses of testosterone (2.6, 8.9 or 29.2 mg) until 12 wks of ages. These results agreed to this current study, and reflected an androgen threshold on tibia growth. Excess threshold dose androgen would adverse affect on tibia performance. Comparison of the bone characteristics results between the Sham and cholesterol implanted showed similarity in both trials. This again proved that androgen affect on bone development.

The histological observation was agreed to both trials that more osteoclast was observed in cholesterol-implanted capons than in the Sham, while no improvement after testosterone implantations was found. Whether this could be attributed to the effect of caponization age on the trial, chickens were caponized at 12-wk-old and implanted at 16-wk-old, needed to be proven. Although testosterone implantations did not depress the number of infiltrating osteoclasts, testosterone (by medium dose implantation) might improve the biomechanical characteristics via stimulate the osteoblast activity.

Blood constituent : presents the testosterone implantation effects on blood characteristics in capons. The cholesterol implanted capons showed the highest ($p<0.05$) blood phosphorus concentration and the high dose testosterone implanted capons showed the highest alkaline phosphatase activity ($p<0.05$).

In this trial, blood calcium concentration of the Sham showed no significant difference as compared to the cholesterol-implanted capons, and agreed with the comparison between intact male chickens and capons (trial 1). On the other hand, with dose increase, testosterone did

Table 4. Testosterone implantation effects on bone characteristics in capons (trial 2)

	Sham	Implantation				SEM
		CHOL	Low	Medium	High	
Tibia length (mm)	126 [ab]	121 [b]	125 [ab]	129 [a]	123 [b]	0.739
Tibia weight (g)	6.52 [a]	5.74 [b]	6.31 [ab]	6.41 [ab]	5.91 [ab]	0.277
Relative tibia weight (g/100 g BW)	0.381 [ab]	0.345 [b]	0.374 [ab]	0.375 [ab]	0.398 [a]	0.033
Breaking strength (kg)	24.5 [a]	15.4 [b]	14.0 [b]	21.7 [a]	13.6 [b]	0.759
Bending moment (kg·cm)	39.8 [a]	25.0 [b]	22.7 [b]	35.3 [a]	22.1 [b]	0.968
Stress (kg/cm^2)	235 [a]	154 [b]	184 [ab]	236 [a]	175 [ab]	2.77

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

Table 5. Testosterone implantation effects on blood characteristics in capons (trial 2)

	Sham	Implantation				SEM
		CHOL	Low	Medium	High	
Calcium (mg/dl)	9.09	12.2	10.4	11.4	9.31	0.55
Phosphorus (mg/dl)	5.60 [ab]	6.27 [a]	5.78 [ab]	5.86 [ab]	5.00 [b]	0.33
Alkaline phosphatase (U/L)	546 [b]	538 [b]	576 [b]	595 [b]	833 [a]	3.44

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

not decrease the blood calcium concentration. Whether the blood calcium and phosphorous concentrations can be used as an indicator of chicken skeletal characteristics is still pending on further research. The phosphorus concentration of cholesterol-implanted capons showed a trend higher than the Sham, similar to the comparison between the intact males and capons in trial 1. As the testosterone implantation increases, the blood phosphorus concentration was increased to the identical level as Sham ($p>0.05$). This implied that testosterone could slow down the release of bone phosphorus. Furthermore, high dose testosterone implantation increased the alkaline phosphatase activity. It implicates that, high dose testosterone implantation adversely affects on bone and reflected on the poor breaking strength and bending moment than intact male chickens.

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