IMMUNOLOGY, HEALTH, AND DISEASE

Effects of caponization and different forms of exogenous androgen implantation on immunity in male chicks

K. L. Chen,* S. M. Tsay,* P. W. S. Chiou,† C. P. Sun,* and B. C. Weng‡[1]

*Department of Animal Science, National Chiayi University, Chiayi 600, Taiwan; †Department of Nursing, Chung Jen College of Nursing, Health Science and Management, Chiayi 600, Taiwan; and ‡Department of Microbiology and Immunology, National Chiayi University, Chiayi 600, Taiwan

ABSTRACT This study determined the caponization effects on the immune responses in male chicks. Different forms of exogenous androgen implantation on male chick immunity were compared. Healthy, uniform male Single Comb White Leghorn chicks were caponized at 3 wk of age. Birds were housed in individual cages (35 × 30 × 40 cm, length × width × height). Each of 27 sham-operated (sham) and caponized (capon) male chickens were used for trial 1. Trial 2 used 60 capons divided into 4 treatments with implants of either 1 mm i.d. × 3 mm o.d. 58 mg of cholesterol, testosterone (TES), 5α-dihydrotestosterone (5α-DHT), or 19-nortestosterone (19-NorT). The exogenous androgen was implanted immediately after caponization and resupplied every 4 wk for an entire 13-wk feeding trial. The results from trial 1 showed that the relative bursa weight increased compared with the sham treatment ($P < 0.05$). The 2 wk post-Newcastle disease virus titer and the delayed-type hypersensitivity (DTH) of 48 h post-phytohemagglutinin phosphate (PHA-P) injection were increased compared with the sham treatment ($P < 0.05$). In trial 2, implanted 5α-DHT and 19-NorT could decrease the relative bursa weight in capons ($P < 0.05$). The 2 wk post-Newcastle disease virus titer in the 5α-DHT group was higher than that in the cholesterol group ($P < 0.05$). The 19-NorT group had the highest ($P < 0.05$) PHA-P response. Peripheral blood lymphocyte subset population analysis revealed that the percentage of CD4 T cells in the TES group was lower ($P < 0.05$) compared with that of the 5α-DHT group. Differently, the percentage of CD8 T cells in the TES and 19-NorT groups was higher ($P < 0.05$) than that in the 5α-DHT group. Male chicks that were caponized had increased bursa weight and PHA-P response, whereas different forms of exogenous androgen implantation reverted the phenomena in an order of potency of 5α-DHT and 19-NorT > TES, and the PHA-P response was TES > 5α-DHT >19-NorT.

Key words: male chick, caponization, androgen, immune response

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INTRODUCTION

As animals reach sexual maturity, the sex hormone levels elevate, leading to the fundamental immune variations between sexes (Ansar Ahmed et al., 1985; Schuurs and Verheul, 1990). It is known that androgen suppresses mammalian thymus growth (Bellamy et al., 1976). In poultry, the thymus and the bursa of the Fabricius increase along with weight gain before sexual maturation and gradually regress progressively with increasing androgen levels after sexual maturation (Firth, 1977; Cecil and Bakst, 1991). Caponization or exogenous androgen implantation in capons before or after sexual maturation showed different effects on immunity. Caponization at 12 wk of age and feeding up to 26 wk of age showed hampered delayed-type hypersensitivity (**DTH**) but increased titration to SRBC. Implanting 9.81 mg of testosterone (**TES**) could produce a skewed immunity (Chen et al., 2009b). Male Leghorn chickens caponized at 3 wk of age showed a downregulated DTH and graft versus host response at 5, 10, and 15 wk of age in cell-mediated immunity (Mashaly, 1984). Nevertheless, dietary TES supplementation (0.1 mg/kg of BW) to 1-d-old male broilers produced decreased white blood cell populations and lymphocyte counts and reduced macrophage phagocytosis ability and lysosomal enzyme activity at 50 d of age (Al-Afaleq and Homeida, 1998).

Different forms of TES possess different anabolic and androgenic activities (Astiningsih and Rogers, 1996). Different effects were shown in lipogenesis and bone formation (Fennell and Scanes, 1992a; Chen et al., 2006b, 2009a). Moreover, TES also affected growth and

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[1]Corresponding author: brian@mail.ncyu.edu.tw

bursa weight. Two-week-old male Leghorn chickens caponized and implanted with TES or its analogs such as 5α-dihydrotestosterone (**5α-DHT**) and 19-nortestosterone (**19-NorT**) up to 12 wk of age exhibited growth inhibition in the order of 19-NorT, 5α-DHT, and TES. The bursa weights of capons decreased compared with TES when given a low dose (2.6 mg) of 5α-DHT or 19-NorT (Fennell and Scanes, 1992a; Fennell et al., 1996). The androgen effects on immunity have been shown to vary by the strains, ages of the chicken, indigenous androgen levels, forms of implanted TES, respective dosages, and ages. Overall, caponization and exogenous androgen implantation before sex maturation had greater influence on animal growth (Ansar Ahmed et al., 1985; Fennell and Scanes, 1992a).

This study investigates the caponization effect on immune organ and immune responses in male chicks and compares the effects of different forms of exogenous androgen implantation on immunity in capons.

MATERIALS AND METHODS

Bird Management and Experimental Design

Healthy male Single Comb White Leghorn chicks (85 ± 3.3 g) were caponized at 3 wk of age and selected as experimental units. Each of 27 sham-operated male chickens and capons were assigned to trial 1. Sixty capons were randomly selected for trial 2 and equally divided into 4 treatment groups of those either implanted (1 mm i.d., 3 mm o.d., 5.88 mg) with cholesterol (**CHOL**, JAH05003, Hanawa, Osaka, Japan), TES (86500, Fluka, Buchs, Switzerland), 5α-DHT (10300, Fluka), or 19-NorT (74640, Fluka). Fifteen capons in each group were housed individually in 35 × 30 × 40 cm cages for an entire 13-wk feeding trial (feeding to 16 wk of age). The exogenous androgen was implanted immediately after the caponization and resupplied every 4 wk. The testectomy procedure was performed according to Chen et al. (2000, 2005). The androgen implantation procedure was based on the modified method of Fennell et al. (1990) and performed according to Chen et al. (2009a,b). Feed (ME, 2,900 kcal/kg; CP, 18.1%) and water were provided ad libitum. All experimental protocols complied with the regulations of the Institutional Animal Care and Use Committee of National Chiayi University.

Measurement and Analysis

BW and Immune Organs. For both trial 1 and trial 2, BW and comb height and length were measured at 16 wk of age. Subsequently, 10 capons of each group were killed to collect the thymus, bursa of Fabricius, and spleen and relative weights were obtained.

Humoral Immune Response. In trials 1 and 2, chicks were injected with 1 mL of Newcastle disease virus (**NDV**; killed vaccine, Formosa Biomedical Inc., Taipei, Taiwan) into the leg muscle at 5 wk of age. Peripheral blood was collected weekly from the wing vein in the following 3 wk postchallenge. The serum was used for detection of hemagglutination inhibition (**HI**) response (Wu et al., 2004). Briefly, serial diluted serum samples containing anti-hemagglutinin antibody were added into erythrocyte solution with hemagglutinin. The serum titration of the last dilution resulting in HI was determined. The change in titer for HI was calculated using the following formula (Chen et al., 2009b): titers of postinjection − titer of initial.

DTH Test. Chicks were subjected to measure DTH response at 5 wk of age by intracutaneous injection of 0.1 mL of 100 μg of phytohemagglutinin phosphate (**PHA-P**; Sigma, St. Louis, MO) into the right side wattle. Wattle thickness was measured before injection and both at 24 and 48 h postinjection as an in vivo evaluation of cell-mediated immunity. The change in thickness for each wattle was calculated using the following formula (McCorkle and Taylor, 1994): wattle thickness of postinjection − wattle thickness of initial.

Levels of Immunoglobulin and Hematology Determination Test. Venous blood samples from 10 birds of each group were collected at 16 wk of age. Subsequently, blood was centrifuged to obtain plasma. The plasma levels of IgA, IgM, and IgG were determined according to our previous study (Chen et al., 2008). Briefly, the level of IgA, IgM, and IgG of immunoglobulin was determined by ELISA Quantization Kit (E30-103, E30-102, E30-104, Bethyl Laboratories Inc., Montgomery, TX; Kaida et al., 2003) and Starter Accessory Package I (E101) and using the ELISA reader at an optical density of 450 nm (Multiskan Ascent, Helsinki, Finland).

An aliquot of whole blood sample was used to measure hematocrit (**HCT**), numbers of erythrocyte and leukocyte populations, and leukocyte differential counts. The methods were conducted as described in Chen et al. (2009b).

Analysis of Phagocytic and Bactericidal Capability. In trial 1, at 7 wk of age, 2 chicks from each group were killed every day for 6 d to collect phagocytic cells for determination of the bactericidal capability. The method used in Chen et al. (2008) was adopted. Briefly, the *Salmonella enterica* serovar Enteritidis was inoculated to macrophage cultures. The live bacterial count at 0 h (**T0**) represented the phagocytosis ability. The live bacteria counts at 1 and 4 h (**T1** and **T4**, respectively) represented the bactericidal activity.

In trial 2, venous blood collected in EDTA vacuum tubes from 10 birds of each group were subjected to lymphocyte purification according to the methods of Merendino et al. (1998) and Gehad et al. (2002). Harvested lymphoyctes were first adjusted to a cell suspension of 1×10^7/mL of cell stocks with RPMI medium (Sigma). A 100-μL cell suspension was pipetted into each well of a 96-well plate. Based on the recommendations of the manufacturers, the primary antibodies (Southern Biotech, Birmingham, AL) of mouse anti-chicken CD5 (purified unlabeled $IgG_{1\kappa}$, 8360-01), CD3

(purified unlabeled $IgG_{1\kappa}$, 8200-01), CD4 (purified unlabeled $IgG_{1\kappa}$, 8210-01), and CD8 (R-phycoerythrin-conjugated IgG_{2b}, 8390-09) were added into respective wells assigned for the experiment. After 40 min of incubation at 4°C, the plate was then centrifuged at 300 × *g* for 15 min. The supernatant was discarded. Another 200 μL of PBS containing 0.02% sodium azide was added to wash the cell pellet one more time. Subsequently, the fluorescence goat anti-mouse $IgG_{1\kappa}$ secondary antibodies (1070, Southern Biotech) were added and incubated for additional 60 min at 4°C. Labeled cells were washed 2 times with PBS and fixed with 4% formalin by adding 100 μL/well. Finally, samples from each well were analyzed using flow cytometry (FACScan, Becton Dickinson, San Jose, CA) and Cell Quest software (Becton Dickinson).

Statistical Analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1985). Treatment differences (trial 1: sham and capons; trial 2: capon implanted with CHOL, TES, 5α-DHT, or 19-NorT) were compared using Duncan's new multiple-range test according to Steel and Torrie (1997).

RESULTS AND DISCUSSION

Trial 1

BW, Comb, and Immune Organs. The caponization effects on BW, comb, and immune organs are shown in Table 1. Significant decreases in comb height and length and increases in relative bursa weight were observed after caponization compared with intact male chicks ($P < 0.05$).

Similar to the previous result (Chen et al., 2006a, 2009b), the capons deficient in TES had decreased ($P < 0.05$) comb height and length as compared with sham. In poultry, the thymus and bursa are the primary lymphoid organs, which are gradually undergoing atrophy in male chickens along with sexual maturation (Cecil and Bakst, 1991; Glick, 2000). When birds are caponized before sexual maturation, the immune organ weights keep developing without influence by TES. It has been reported that the increase in TES concentration inhibits the growth of immune organs (Mase and Oishi, 1991). In this trial, the male chicks caponized at 3 wk of age had a higher ($P < 0.05$) relative bursa weight at 16 wk of age than the sham group, which was similar to that in Fennell and Scanes (1992a) and Fennell et al. (1996). Moreover, it is also possible that the TES of intact male chickens at 16 wk of age might not be old enough to reach the TES inhibition concentration threshold on thymus development. In this trial, the spleen weight and relative spleen weight of capons were not different ($P > 0.05$) from those of intact males in the sham group. Similar results were also demonstrated by Chen et al. (2009b) using capons from 12 wk of age and fed up to 26 wk of age.

Table 1. Effects of caponization on BW, comb, and immune organs in male chicks[1] (16 wk) (trial 1)

Item	Sham	Capon
BW, g	1,144 ± 28.4	1,192 ± 23.6
Comb height, mm	59.0 ± 2.56[a]	24.1 ± 2.36[b]
Comb length, mm	103 ± 4.65[a]	43.8 ± 3.14[b]
Spleen, g/100 g of BW	0.550 ± 0.055	0.454 ± 0.085
Thymus, g/100 g of BW	0.505 ± 0.058	0.478 ± 0.075
Bursa, g/100 g of BW	0.008 ± 0.005[b]	0.082 ± 0.018[a]

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

[1]Means ± SE.

Immune Traits and Hematology. Effects of caponization on immune traits and hematology in male chicks are shown in Table 2. Capons of 5 wk of age had increased HI antibody titer by 2 wk after injecting with NDV. The PHA-P response of capons at 48 h postinjection was higher ($P < 0.05$) than that from the sham group. Hematological results revealed a reduction on the total number of leukocytes at 16 wk of age ($P < 0.05$).

When chicks were caponized at 3 wk of age, there was no ($P > 0.05$) influence on γ-globulin level measured at 16 wk of age. This result was similar to that of Chen et al. (2009b) with chicks caponized at 12 wk of age and measured at 26 wk of age. In addition, caponization exhibited an increase in ($P < 0.05$) HI antibody titer at 2 wk post-NDV injection. Similar results were reported in Chen et al. (2009b) using 12-wk-old capons fed to 26 wk of age. Thus, these results indicate that the timing of caponization (before or after sexual maturity) may affect antibody production to NDV.

The wattle skin swelled post-PHA-P injection and was used to evaluate the cell-mediated immune response. The results showed that caponization significantly increased PHA-P responses ($P < 0.05$), which was in disagreement with Chen et al. (2009b) that male Leghorn chickens caponized at 12 wk of age exhibited no influence on the DTH response by PHA-P injection. Thus, the timing of caponization before or after sexual maturity may affect the outcomes of the DTH response, which resembled the cell-mediated immunity.

Androgen increases turkey hemoglobin production and enhances the HCT and total erythrocyte numbers (Burton and Smith, 1972; Cecil and Bakst, 1991). Chen et al. (2009b) indicated that capons deficient in TES induced HCT decrease compared with intact male chickens at 26 wk of age, and erythrocyte numbers were also lower in concert with the above results. However, their results disagreed with this trial in that there was no significant difference between the sham and capon groups on HCT and erythrocyte numbers. This discrepancy may be due to the timing of caponization at a younger age in male chicks (16 wk of age); thus, androgen might exert less effect on them. In leukocyte differential counts, the blood of male chickens contains higher heterophils and fewer lymphocytes than that of female chickens.

Table 2. Effects of caponization on immunoglobulin, immune response, and hematology in male chicks[1] (trial 1)

Item (age)	Sham	Capon
γ-Globulin, mg/dL (16 wk)	1,928 ± 59.5	1,981 ± 59.4
Immunoglobulin, mg/dL (16 wk)		
IgM	65.2 ± 16.7	76.2 ± 7.13
IgA	208 ± 43.7	201 ± 37.1
IgG	701 ± 41.9	960 ± 120
Hemagglutination inhibition titer, $\log_2$ (5 wk)		
First week	4.46 ± 0.514	5.77 ± 0.545
Second week	6.16 ± 0.561[b]	7.85 ± 0.318[a]
Third week	7.22 ± 0.982	7.18 ± 0.344
Phytohemagglutinin phosphate response, mm (5 wk)		
24 h	0.144 ± 0.014	0.166 ± 0.014
48 h	0.076 ± 0.013[b]	0.137 ± 0.014[a]
Hematology (16 wk)		
Hematocrit, %	31.6 ± 1.96	30.2 ± 1.56
Red blood cell, $10^6/\mu L$	2.82 ± 0.213	2.70 ± 0.179
White blood cell, $10^3/\mu L$	38.8 ± 2.79[a]	27.2 ± 2.28[b]
Lymphocyte, %	93.6 ± 1.14	93.0 ± 0.577
Heterophil, %	6.33 ± 1.14	7.00 ± 0.577

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

[1]Means ± SE.

Therefore, a higher heterophil:lymphocyte ratio occurs in male than in female chickens (Maxwell et al., 1992; Campo and Davila, 2002; Khajavi et al., 2003). Dietary TES supplementation (0.1 mg/kg of BW) in 1-d-old male broilers has been shown to decrease total white blood cell populations and reduce lymphocyte count, macrophage phagocytic activity, and lysosomal enzyme activity at 50 d of age (Al-Afaleq and Homeida, 1998). Chen et al. (2009b) has also indicated that caponization at 12 wk of age and feeding to 26 wk of age had no effect on total leukocyte count, which was not in agreement with this study. It is commonly known that when animals suffer from serious inflammations or infections, there is an increase in total leukocyte count (McFarlane and Curtis, 1989; Maxwell et al., 1992; Maxwell, 1993). In this study, chicks were not found with any infectious diseases or signs of stress during the 13-wk feeding; the specific reason for the decreased total white blood cell count of the 3-wk-old chicks (before sexual maturity) caponized and fed to 16 wk of age may require further investigation.

Analysis of Phagocytic and Bactericidal Capability. Caponization effects on phagocytic and bactericidal capabilities in male chicks are shown in Table 3. Caponization did not affect phagocytosis and bactericidal capability ($P > 0.05$).

Table 3. Effects of testosterone implantation on phagocytosis and bactericidal activity of macrophage in capons[1] (trial 1)

Item	Sham	Capon
T0, 10^6 cfu/well[2]	6.00 ± 1.14	4.65 ± 0.525
T1, 10^6 cfu/well[2]	6.01 ± 1.04	5.37 ± 0.514
T4, 10^8 cfu/well[2]	4.62 ± 1.48	3.23 ± 1.32

[1]Means ± SE.

[2]The colony-forming units measured at 0 h (T0) represented the phagocytosis and the colony-forming units measured at 1 and 4 h (T1 and T4, respectively) represented the bactericidal activity.

Macrophages are the most important phagocytes in the immune system. They are capable of producing large amounts of reactive oxygen species and a variety of lysosomal enzymes to eliminate engulfed bacteria. They are also able to secrete cytokines to initiate the adaptive cell-mediated immunity (Qureshi et al., 1994; Tizard, 2000). Poultry lack resident macrophages in the peritoneal cavity, which is one of the primary differences in innate immunity between mammalian and avian species. Therefore, harvesting peritoneal macrophages requires bacteria or Sephadex (Sigma) induction (Nicolas-Bolnet et al., 1995; Qureshi, 2003). *Salmonella enterica* serovar Enteritidis is the most notorious *Salmonella* causing gastroenteritis. A lack of obvious signs when poultry contract the enteritidis means that *Salmonella* Enteritidis often contaminates poultry products such as meats and eggs, leading to food poisoning and salmonellosis (Brock and Madigan, 1988; Guiney et al., 1995). The current study used Sephadex to enumerate macrophages and subsequently harvest for ex vivo study of peritoneal macrophages' defense to *Salmonella*. *Salmonella* were inoculated into peritoneal macrophage cultures to evaluate phagocytosis (T0) and bactericidal activity (T1 and T4). The results showed that T4 had a higher bacteria count than T1 and T0, which indicated an insufficient bactericidal capacity. Yang et al. (2001) reported that the optimal time for harvesting macrophages was at around 3 to 4 wk of age. The collection of sufficient peritoneal macrophages could be difficult from chicks 7 to 8 wk of age; thus, the optimal bacteria:macrophage ratio might have been compromised.

Trial 2

BW, Comb, and Immune Organs. The effects of different androgen implantations on BW, comb, and

immune organs of capons are shown in Table 4. The comb length in the 19-NorT group was significantly ($P < 0.05$) increased when compared with the CHOL and TES groups. The 19-NorT group had higher relative spleen weight than those of the other groups ($P < 0.05$). Moreover, the 5α-DHT and 19-NorT groups had lower relative bursa weight than the TES and CHOL groups ($P < 0.05$).

Field observations revealed that capons implanted with 19-NorT had a quickly increased comb and wattle size in the first week postimplantation, whereas 6 capons died during this period. The result was partially consistent with the results of Fennell and Scanes (1992b) that implantation of TES, 5α-DHT, or 19-NorT in 2-wk-old capons then feeding to 12 wk of age showed inhibition in growth and an elevation in mortality by 19-NorT. The same group also reported that the 19-NorT and 5α-DHT groups had a stronger BW gain inhibition than the TES group. In this study, chickens fed to 16 wk of age were already close to their sexual maturity, which might explain why TES implantation had no effect ($P > 0.05$) on BW gain. A similar result was also observed in Chen et al. (2005) using 16-wk-old capons implanted with different doses of TES (5.88 to 16.7 mg/4 wk) or different forms (TES, 5α-DHT, and 19-NorT; 10.4 mg/4 wk) to 26 wk of age.

The secondary sex characteristics of male chickens diminished along with decreasing TES after caponization. The implantation of exogenous androgen rejuvenates the comb growth in capons (Fennell and Scanes, 1992a; Astiningsih and Rogers, 1996). Fennell and Scanes (1992a) using 2-wk-old capons to compare 3 kinds of exogenous androgen implantations to 12 wk of age was shown to promote comb and wattle growth and the order was 19-NorT, 5α-DHT, and TES. In this trial, the 19-NorT group had a higher ($P > 0.05$) comb length than those of other groups. The 5α-DHT is more effective on accessory organs such as the comb and wattle, resulting in a masculine appearance. Therefore, it showed more effect on comb growth than TES. Moreover, the 19-NorT group increased comb and wattle size within a shorter period, appearing more reddish in color and thicker than those of other groups. These results are similar to that obtained by Fennell and Scanes (1992a).

Al-Afaleq and Homeida (1998) indicated that feeding the TES (0.1 mg/kg of BW) or DHT (0.2 mg/kg of BW) to 1-d-old male broiler up to 50 d of age does not affect relative spleen weight. However, Leitner et al. (1996) found that injecting DHT into 2-d-old broilers significantly increased relative spleen weight at 25 d of age. It was previously (Chen et al., 2009b) found that 12-wk-old capons implanted with a middle dose of TES (9.81 mg/4 wk) then fed to 26 wk of age had a higher ($P < 0.05$) relative spleen weight than the sham. This result was demonstrated again in this study that the relative spleen weight of the 19-NorT group was higher ($P < 0.05$) than other groups at 16 wk of age. Overall, androgen supplementation may increase spleen weight, whereas the level of effectiveness is based on the TES form and dose level.

Schuurs and Verheul (1990) indicated that injected TES and synthesized TES (nandrolone and mibolerone) into the embryo did not affect thymus development. Similar results were also obtained in this study. The relative spleen weights of the 5α-DHT and 19-NorT groups were lower than the TES and CHOL groups ($P < 0.05$). Implanted 5α-DHT and 19-NorT also suppressed the bursa growth. As to the secondary sex characteristics and accessory organs, TES has to be converted to 5α-DHT to exert its bioactivity (Swerdloff et al., 1992). In addition, it is known that the 5α-DHT and 19-NorT have higher binding affinity to TES receptors than that of TES (Astiningsih and Rogers, 1996). Sullivan and Wira (1979) showed the existence of sex hormone receptors on the bursa. Testosterone may inhibit bursa cells proliferation (Novotny et al., 1983) and growth of bursa (Deyhim et al., 1992; Fennell and Scanes, 1992a; Fennell et al., 1996; Leitner et al., 1996; Rath et al., 1996). Nevertheless, Fennell and Scanes (1992a) indicated that implanted different forms of TES in 2-wk-old capons feeding to 12 wk of age and implanted low doses (2.6 mg) of 5α-DHT and 19-NorT may inhibit bursa growth. Similar results were also obtained in this study.

Immune Traits and Hematology. The effects of different TES implantation forms on immunoglobulin, immune response, and hematology in capons are shown in Table 5. The HI titers of the 5α-DHT group were significantly higher ($P < 0.05$) than that for the CHOL group after injection with NDV for 2 wk. All TES implantation forms showed a higher NDV titer than the CHOL group by 3 wk post-NDV injection. The PHA-P response at 24 h was higher ($P < 0.05$) in the 19-NorT

Table 4. Effects of different forms of androgen implantations on BW, comb, and immune organs in capons[1] (16 wk) (trial 2)

Item	CHOL	TES	5α-DHT	19-NorT
BW, g	1,047 ± 46.4	1,152 ± 35.7	992 ± 48.3	1,035 ± 23.7
Comb height, mm	31.2 ± 2.45	30.1 ± 1.96	39.8 ± 2.64	41.3 ± 4.09
Comb length, mm	59.0 ± 4.84^{bc}	57.6 ± 4.29^{c}	72.4 ± 5.33^{ab}	75.8 ± 5.22^{a}
Spleen, g/100 g of BW	0.463 ± 0.040^{bc}	0.346 ± 0.055^{c}	0.560 ± 0.062^{b}	0.840 ± 0.053^{a}
Thymus, g/100 g of BW	0.497 ± 0.049	0.526 ± 0.086	0.490 ± 0.047	0.522 ± 0.081
Bursa, g/100 g of BW	0.128 ± 0.019^{a}	0.048 ± 0.008^{a}	0^{b}	0^{b}

$^{a–c}$Means in the same row with different superscripts are significantly different ($P < 0.05$).

[1]Means ± SE. CHOL = cholesterol; TES = testosterone; 5α-DHT = 5α-dihydrotestosterone; 19-NorT = 19-nortestosterone.

Table 5. Effects of different forms of androgen implantations on immunoglobulin, immune response, and hematology in capons[1] (trial 2)

Item (age)	CHOL	TES	5α-DHT	19-NorT
γ-Globulin, mg/dL (16 wk)	1,756 ± 85.0	1,902 ± 60.1	1,970 ± 37.1	1,933 ± 124
Immunoglobulin, mg/dL (16 wk)				
IgM	111 ± 25.2	60.0 ± 7.14	91.0 ± 11.2	94.4 ± 34.9
IgA	228 ± 39.8	217 ± 72.7	171 ± 35.7	143 ± 55.0
IgG	734 ± 69.2	881 ± 71.6	761 ± 102	1,069 ± 81.5
Hemagglutination inhibition titer, $\log_2$ (5 wk)				
First week	5.77 ± 0.508	5.92 ± 0.499	6.09 ± 0.457	4.83 ± 0.534
Second week	7.59 ± 0.268^{b}	8.38 ± 0.615^{ab}	8.90 ± 0.315^{a}	8.50 ± 0.453^{ab}
Third week	6.95 ± 0.418^{b}	9.30 ± 0.746^{a}	9.00 ± 0.471^{a}	8.66 ± 0.645^{a}
Phytohemagglutinin phosphate response, mm (5 wk)				
24 h	0.140 ± 0.012^{bc}	0.126 ± 0.012^{c}	0.193 ± 0.014^{ab}	0.238 ± 0.043^{a}
48 h	0.100 ± 0.013^{ab}	0.054 ± 0.015^{b}	0.090 ± 0.012^{ab}	0.126 ± 0.024^{a}
Hematology (16 wk)				
Hematocrit, %	31.5 ± 1.04	31.4 ± 1.28	31.2 ± 1.73	28.0 ± 2.16
Red blood cell, $10^6/\mu L$	2.49 ± 0.153	2.98 ± 0.224	2.27 ± 0.255	2.44 ± 0.443
White blood cell, $10^3/\mu L$	25.3 ± 2.16	31.0 ± 4.86	26.1 ± 3.34	26.9 ± 3.64
Lymphocyte, %	90.2 ± 1.60	95.0 ± 0.816	92.0 ± 2.42	93.0 ± 2.00
Heterophil, %	9.80 ± 1.60	5.00 ± 0.816	8.00 ± 2.42	7.00 ± 2.00

[a–c]Means in the same row with different superscripts are significantly different ($P < 0.05$).

[1]Means ± SE. CHOL = cholesterol; TES = testosterone; 5α-DHT = 5α-dihydrotestosterone; 19-NorT = 19-nortestosterone.

group than the CHOL group. At 48 h post-PHA-P injection, the response in the 19-NorT group was significantly higher ($P < 0.05$) than the TES group.

Implantation with different forms of TES in capons had no effect ($P > 0.05$) on γ-globulin and levels of IgM, IgA, and IgG, and the result was similar to trial 1. By 2 wk post-NDV injection, the titer of the 5α-DHT group was higher than that for the CHOL group ($P < 0.05$). The titer of the CHOL group was lower than those of other implanted groups ($P < 0.05$) by 3 wk post-NDV injection. In trial 1, the result showed that the HI titer of the intact male chickens was lower than that for the capons in trial 1 ($P < 0.05$). The difference from those of trial 1 capons was that capons were surgically implanted every 4 wk from the time of their caponization at 3 wk of age. Whether implantation surgery resulted in the discrepancy of NDV titers will require further investigation.

In trial 1, the PHA-P response at 48 h in capons was higher ($P < 0.05$) than that of the sham. In this trial, the TES group had a similar trend as trial 1 in that intact male chicks had a lower PHA-P response than capons. Among all groups, the 19-NorT resulted in a significantly higher ($P < 0.05$) response than the TES group. These results showed that different forms of TES exert different potencies on the PHA-P response of the cell-mediated immunity, and the inhibitory activity of 19-NorT was greater than 5α-DHT and TES.

There were no dramatic changes in HCT, erythrocyte population, and the differential leukocyte counts (lymphocytes and granulocytes). This was in agreement with trial 1 and Chen et al. (2009b), in which implanted TES in capons had led to no changes in these parameters. Although there was no difference in total leukocyte count among treatment groups ($P > 0.05$), the averages for the androgen implantation groups were slightly higher than that of the CHOL group. This might be the result of insufficient dosage of implanted androgen in achieving a significant result.

Changes of Lymphocyte Subsets in Peripheral Blood Mononuclear Cell. The effect of implanting different forms of androgen on peripheral blood mononuclear cells (**PBMC**) is shown in Table 6. Implanting different forms of androgen did not affect total lymphocytes (CD5) and T lymphocytes (CD3) percentages in the PBMC of capons ($P > 0.05$). In addition, the percentage of CD4 lymphocytes in TES was higher ($P < 0.05$) than 5α-DHT, and the CD8 lymphocytes in TES and 19-NorT were higher ($P < 0.05$) than 5α-DHT.

Chen et al. (2009b) found that 12-wk-old capons implanted with middle or high doses of TES promoted cell-mediated immunity in PHA-P response. This result

Table 6. Effects of different forms of androgen implantations on peripheral blood lymphocyte subpopulations in capons[1] (trial 2)

Item	CHOL	TES	5α-DHT	19-NorT
CD5, %	75.5 ± 3.68	74.1 ± 3.82	78.9 ± 1.23	78.9 ± 1.41
CD3, %	54.9 ± 3.98	56.6 ± 3.65	63.3 ± 3.23	65.8 ± 4.10
CD4, %	41.4 ± 5.63^{ab}	36.1 ± 4.45^{b}	55.8 ± 5.46^{a}	53.6 ± 5.68^{ab}
CD8, %	16.5 ± 1.69^{ab}	19.7 ± 2.73^{a}	11.3 ± 0.940^{b}	22.0 ± 2.27^{a}

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

[1]Means ± SE. CHOL = cholesterol; TES = testosterone; 5α-DHT = 5α-dihydrotestosterone; 19-NorT = 19-nortestosterone.

was similar to the current study using 3-wk-old capons. The coetaneous basophile response to PHA-P in birds was similar to the DTH in mammals that best represented the in vivo evaluation of cell-mediated immunity. It induced an immune response requiring the collaboration of T cells and antigen-presenting cells (Tizard, 2000; Coico et al., 2003). Hence, the objective of this study was to determine the effect of different forms of TES on the percentages of T cell subset populations in peripheral blood.

The percentages of CD5, CD3, CD4, and CD8 lymphocyte subsets of PBMC were analyzed with flow cytometry. The CD5 is the co-receptor on the cell membranes of lymphoid and bursa lymphocytes. Those of T cells expressed T cell receptor complex, CD3 molecules. The CD4 and CD8 T cells were T helper cells and T cytotoxic cells, respectively. Landsman et al. (2001) reported that low levels of TES (physiological dose, 10^{-9} *M*) and DHT had no effect on concanavalin A- or pokeweed mitogen-stimulated lymphocyte proliferations at 4, 6, and 9 wk of age in male chickens. Moreover, using concanavalin A to stimulate CD3, CD4, CD8, and B cells in 9-wk-old male chickens under a high level DHT (10^{-6} *M*) resulted in no change in lymphocyte proliferation. In this study, different forms of implanted TES had no effect on PBMC subpopulations in capons as compared with those in the CHOL group ($P > 0.05$). However, the 5α-DHT group had an increased CD4 T cell population percentage, and TES and 19-NorT had higher CD8 T cell percentages than that of the 5α-DHT group ($P < 0.05$). Liva and Voskuhl (2001) found androgen receptors expressed on CD4 and CD8 T lymphocytes. After a 2-wk implantation of 5α-DHT (5 mg) in female or male mice, interleukin-10 mRNA was upregulated in CD4 T lymphocytes. Yao et al. (2003) found that multiple injections of TES (0.5, 2.5, or 12.5 mg/kg of BW per 2 d) in male mice (50 to 80 g) for 2 wk would raise the ratio of CD8 T cell subset. Furthermore, Aboudkhil et al. (2003) indicated that TES injections (0.5 mg of depo-TES/100 g of BW per 2 d) for 2 wk in castrated 2-mo-old mice also increased the CD8 T cell subset ratio. These results showed that different forms of exogenous TES may have differentially altered the T cell subpopulation in the peripheral blood reservoir.

Male chicks caponized at 3 wk of age and fed to 16 wk of age (before sexual maturity) had deficient androgen, resulting in increased bursa weight and PHA-P response, whereas the implantation of different forms of exogenous androgen reverted the phenomena in an order of potency of 5α-DHT and 19-NorT > TES, and the PHA-P response was TES > 5α-DHT >19-NorT.

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