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Effects of caponization on growth, carcass, and meat characteristics and the mRNA expression of genes related to lipid metabolism in roosters of a Chinese indigenous breed

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ABSTRACT: The effects of caponization on growth, body measurements, carcass traits, meat quality, and the mRNA expression of three genes related to lipid metabolism in Guang-xi Yellow roosters were evaluated. Thirty roosters (25 days) of similar weight were randomly divided equally into the experimental (capons, $n = 15$) and control (intact males, $n = 15$) groups. Caponization was conducted at 28 days of age, and birds were slaughtered at 140 days of age. Capons were significantly heavier ($P < 0.05$) than intact males and also had longer keel length and chest depth ($P < 0.05$ or $P < 0.01$). There were no significant differences in the percentage of eviscerated weight with giblet and eviscerated weight between the two groups. However, caponization increased breast muscle yield and decreased leg muscle yield ($P < 0.05$). The capons exhibited higher lightness values for thigh meat and lower redness values for both breast and thigh meat. Moreover, caponization up-regulated the fatty acid synthase (*FAS*) mRNA level in the liver, indicating enhanced hepatic lipogenesis, and also up-regulated the lipoprotein lipase (*LPL*) and peroxisome proliferator-activated receptor γ (*PPARγ*) mRNA levels in abdominal adipose tissue, which indicates increased lipid deposition and adipocyte differentiation. Therefore, caponization is associated with heavier body weight, higher breast meat yield, and higher abdominal fat percentage in Guang-xi Yellow roosters; the higher abdominal fat percentage may be associated with the increase in the mRNA expression of *FAS*, *LPL*, and *PPARγ*.

Keywords: capon; body weight; carcass composition; gene expression

INTRODUCTION

Capons are male chickens whose testes have been surgically removed. Surgical removal of the testes affects chicken growth (Cason et al. 1988; Lin and Hsu 2002; Symeon et al. 2012), carcass composition (Tor et al. 2002; Miguel et al. 2008), meat quality (Miguel et al. 2008; Sirri et al. 2009), abdominal adipose tissue lipid content, and fatty acid profile (Sinanoglou et al. 2011). However, the reported effects of caponization on these traits are not completely consistent.

Reportedly caponization significantly increased abdominal fat weight (Lin and Hsu 2003; Shao et al. 2009; Chen et al. 2014) and intramuscular fat in roosters (Tor et al. 2002; Symeon et al. 2010). However, the mechanism by which caponization affects lipogenesis remains unclear.

Fatty acid synthase (FAS) is a member of a multienzyme complex that plays a key role in endogenous lipogenesis. FAS catalyzes the synthesis of saturated fatty acids, myristate, palmitate, and stearate from the substrates acetyl-CoA, malonyl-CoA, and nicotinamide adenine dinucleotide phosphate (NADPH) (Smith et al. 2003). FAS also plays an important role in energy homeostasis by converting the excess food consumed into lipids for storage (Chirala and Wakil 2004).

Like FAS, lipoprotein lipase (LPL) also plays a key role in lipid metabolism, in particular, triglyceride

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(TG) metabolism. LPL hydrolyzes the circulating TG-rich lipoproteins (TGRLs)-chylomicrons and very-low-density lipoprotein (VLDL), resulting in the formation of chylomicron remnants, intermediate density lipoproteins (IDL), VLDL remnants, and low-density lipoproteins (LDL) (Talmud et al. 2004). The fatty acids and monoacylglycerol released from the hydrolysis can be stored in adipose tissue and be utilized for energy production by muscles (Bonnet et al. 2000). It is well known that increased adipose tissue mass depends on both the number and size of adipocytes, and that the increase in the number and size of adipocytes depends on the differentiation from preadipocytes and lipid accumulation (Zhao et al. 2011).

Peroxisome proliferator-activated receptor γ (PPARγ), which is a member of the nuclear hormone receptor subfamily of transcription factors, is abundantly expressed in adipose tissue, and it controls adipocyte differentiation, lipid uptake, transport, and storage (Schoonjans et al. 1996). In particular, PPARγ is a key regulator of chicken preadipocyte differentiation (Wang et al. 2008); its expression increases with differentiation of adipocytes, and reaches a peak when adipocytes get mature (Fan et al. 2011).

In the present study, we tried to corroborate the already reported results on the effect of caponization on the growth, carcass, and meat parameters in a Chinese indigenous chicken breed, and determined the mRNA expression of the *FAS*, *LPL*, and *PPARγ* genes in capons and intact males, in order to gain insight into the effect of caponization on lipid metabolism.

MATERIAL AND METHODS

Experimental animals and management. Guang-xi Yellow chicken, a Chinese indigenous chicken breed (medium-growth broilers), was used in this experiment. Thirty roosters (age 25 days) with similar body weight were purchased from Xingmu Poultry Breeding Co. Ltd., China, and randomly divided into two groups (n = 15 in each group), experimental (capons) and control (intact males). Castration was performed at 28 days of age. The caponization procedure was performed according to the method described by Chen et al. (2005). Before the surgical operation, male chickens were not given feed or water for 12 h. A 1.0-cm incision was made between the two last ribs and the bilaterally testes were removed from the right side. Iodine-alcohol was applied to the incision site. Shank rings were used in all experimental birds. All birds were kept in a group reared under the same house, with a density of six animals per square meter indoor, and the chickens could easily access the outdoor area (free-range conditions). All chickens were raised up to 20 weeks of age. The diet contained 11.3 MJ/kg of metabolizable energy (ME) and 19% crude protein (CP) in weeks 4–7 of age, and 11.5 MJ/kg of ME and 15% CP thereafter. Feed and water were provided *ad libitum*. The ambient temperature in weeks 4–20 of age was 10–28°C.

All experimental procedures were approved by the Animal Care and Use Committee of the Anhui Agricultural University.

Measurement and sampling

Growth parameters. At 140 days of age, the live weight and body measurements were recorded individually. Body width, body depth, shank length, and shank circumference were measured using the method of Chambers and Fortin (1984). Body length and keel length were determined according to the method of Ojedapo et al. (2012). Comb length (maximum length of comb), height (from the top of comb to head), Wattle width (maximum width of the wattle), wattle length (distance between the top and bottom of the wattle), comb and wattle thickness (maximum thickness of the comb and wattle), comb and wattle weight and pelvis width (the distance between the two hip bones) were also measured.

Carcass parameters. Before slaughter, 5–10 ml blood samples were collected via the wing vein. After blood sample collection, the animals were electrically stunned (120 V, 200 Hz) and then bled to death by a single cut made to the neck by a qualified technician. After bleeding for 2 min, abdominal fat and liver were sampled and immediately frozen in liquid nitrogen, and the birds were scalded in water at 60°C for 2 min for defeathering. Carcass parameters were measured according to the method of Sarsenbek et al. (2013).

Meat quality parameters. Relevant meat quality traits of the left *Pectoralis major* and thigh muscle were assessed. The pH at 45 min was measured using a PHB-4 pH meter (Rex, Shanghai, China). After skin removal, lightness (L^*), redness (a^*), and yellowness (b^*) values of the external surface of the muscle were measured with an ADCI-WSI

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chromameter (Chen Tai Ke, Beijing, China). Drip loss was determined by suspending one intact fillet (1 × 2 × 0.5 cm) from each of the birds in a sealed pocket at 4°C for 24 h; it was calculated as the percentage of weight loss during storage.

Blood lipid parameters. The serum concentrations of total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL)-C, and low-density lipoproteins (LDL)-C were measured by colorimetric enzymatic methods using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Quantitative real-time PCR. Total RNA of the abdominal fat and liver samples was extracted using the TRIzol reagent (Invitrogen, Carlsbad, USA). The purity and concentration of total RNA were determined with a UV spectrophotometer (Beckman Coulter, Fullerton, USA), and electrophoresis on 1.0% agarose gels was used to verify the integrity of the RNA. Then, 1 μg total RNA from the liver and 0.2 μg of total RNA from the abdominal adipose tissue in a final volume of 20 μl was used to generate cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, USA) in accordance with the manufacturer's instructions. The gene-specific primers for *LPL*, *FAS*, *PPARγ*, and *GAPDH* are listed in Table 1. qPCR was performed with the iTaq Universal SYBR Green Supermix (Bio-Rad) using 25-μl reaction mixtures with 0.3μM of each oligonucleotide primer on a Rotor-Gene 6000 real-time cycler (Corbett Research, Cambridge, UK). The PCR program consisted of a 10-min activation step at 95°C, followed by 40 cycles of 10 s at 95°C, 15 s at 60°C, and 20 s at 72°C. After cycling, the products were melted by increasing the temperature from 72 to 95°C. Each sample was run in triplicate, averaged and referenced to a capon (calibrator). Negative (without template) and positive control reactions were performed for each assay. The relative expression levels of the *LPL*, *FAS*, and *PPARγ* genes were analyzed according to the $2^{-\Delta\Delta Ct}$ method using the *GAPDH* gene for normalization (Livak and Schmittgen 2001). Therefore, all gene transcription results are reported as the *n*-fold difference relative to the calibrator.

Statistical analysis. Analyses were performed using the SAS software (Statistical Analysis System, Version 9.1, 2003). The data were expressed as means and standard error. Differences between means were tested using the Student's *t*-test. The *P*-values less than 0.05 were considered significant.

RESULTS

Body measurements. The live body weight and body size of capons and intact males are shown in Table 2. The live body weight of capons was higher ($P < 0.05$) by approximately 12% compared to the intact males at 140 days of age. The keel length was also greater ($P < 0.05$) in the capons than in the intact birds. However, the comb and wattle length, height, thickness, and weight of the capons were lower ($P < 0.001$). Moreover, caponization resulted in the increase ($P < 0.01$) in chest depth and in the decrease ($P < 0.05$) in pelvis width. There was no significant ($P > 0.05$) difference in body length, breast width, shank length, and shank circumference between the two groups.

Carcass composition. Table 3 presents a comparison of carcass traits between capons and intact birds. The percentage of abdominal fat weight was higher ($P < 0.001$) in capons than in intact birds, while the percent weight of the heart (calculated from live body weight) in the capons was lower ($P < 0.001$). The breast muscle percentage was

Table 1. qRT-PCR primers for the candidate genes

Genes	Primer sequences (5'→3')	Product size (bp)	Reference
FAS	F-AAGGAGGAAGTCAACGG R-TTGATGGTGAGGAGTCG	196	NM_205155
LPL	F-GGATTGCTGGAAGTTTAACCAAG R-AGAGATGGATGGATCGTTCATGA	327	NM_205282
PPARγ	F-GTGCAATCAAAATGGAGCC R-CTTACAACCTTCACATGCAT	170	NM_001001460
GAPDH	F-CTACACACGGACACTTCAAG R-ACAAACATGGGGGCATCAG	244	NM_204305

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Table 2. Comparison of body weights and body measurements between capons and intact males

	Capons (n = 15)	Intact males (n = 15)
Body weight at 25 days (g)	295.6 ± 5.0	296.4 ± 5.2
Body weight at 140 days (g)	2143.7 ± 78.5*	1915.5 ± 44.5
Body length (cm)	23.1 ± 0.3	22.5 ± 0.2
Keel length (cm)	12.2 ± 0.2*	11.6 ± 0.2
Chest depth (mm)	108.59 ± 1.92**	102.36 ± 1.08
Breast width (mm)	73.73 ± 1.84	75.72 ± 1.81
Shank length (mm)	80.12 ± 1.41	77.20 ± 1.11
Shank circumference (cm)	4.48 ± 0.06	4.38 ± 0.06
Pelvis width (mm)	61.88 ± 0.88	65.23 ± 1.22*
Comb length (mm)	42.26 ± 1.31	86.33 ± 2.11***
Comb height (mm)	15.69 ± 0.67	42.76 ± 1.28***
Comb thickness (mm)	4.10 ± 0.20	6.20 ± 0.23***
Comb weight (g)	1.14 ± 0.09	13.48 ± 1.33***
Wattle width (mm)	24.29 ± 0.90	41.33 ± 1.34***
Wattle length (mm)	14.76 ± 1.16	39.94 ± 2.00***
Wattle thickness (mm)	1.03 ± 0.06	1.88 ± 0.10***
Wattle weight (g)	1.02 ± 0.16	7.19 ± 0.80***

values are expressed as means ± SEM
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

higher and the leg muscle percentage was lower ($P < 0.05$) in capons than in intact birds. There were no significant differences in the percentage of dressing, eviscerated weight with giblet, eviscerated weight, liver weight, and gizzard weight between the two groups.

Table 3. Comparison of carcass traits between capons and intact males

	Capons (n = 15)	Intact males (n = 15)
Carcass weight (g)	1931.4 ± 80.3**	1662.5 ± 42.4
Percent weights (%)[1]		
Dressing	88.74 ± 0.92	86.98 ± 0.42
Eviscerated weight with giblet	82.60 ± 0.65	81.29 ± 0.44
Eviscerated weight	68.55 ± 0.70	68.23 ± 0.66
Breast muscle	11.31 ± 0.34*	10.31 ± 0.31
Leg muscle (thigh + drumstick)	14.86 ± 0.44	16.06 ± 0.21*
Abdominal fat	4.34 ± 0.31***	2.14 ± 0.34
Heart	0.43 ± 0.03	0.66 ± 0.04***
Liver	1.42 ± 0.04	1.49 ± 0.05
Gizzard	1.63 ± 0.07	1.58 ± 0.06

[1]based on live body weight of chickens
values are expressed as means ± SEM
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Table 4. Comparison of meat quality traits between capons and intact males

	Capons (n = 15)	Intact males (n = 15)
Breast meat		
pH_{45}	6.05 ± 0.06	5.97 ± 0.03
lightness (L^*)	40.82 ± 1.02	40.03 ± 0.97
redness (a^*)	12.45 ± 0.63	16.52 ± 0.47***
yellowness (b^*)	11.81 ± 0.36	11.87 ± 0.38
drip loss (%)	5.43 ± 0.75	3.92 ± 0.73
Thigh meat		
pH_{45}	5.70 ± 0.04	5.72 ± 0.03
lightness (L^*)	48.88 ± 1.47**	43.98 ± 0.83
redness (a^*)	7.72 ± 0.53	11.65 ± 0.48***
yellowness (b^*)	14.46 ± 0.79	13.29 ± 0.62
drip loss (%)	2.17 ± 0.30	1.71 ± 0.35

pH_{45} = pH at 45 min postmortem
values are expressed as means ± SEM
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Meat quality. As shown in Table 4, capons exhibited lower ($P < 0.001$) redness values than intact birds for both breast and thigh meat. Moreover, caponization significantly ($P = 0.01$) increased thigh meat lightness values. However, caponization had no effect on meat pH or drip loss.

Blood lipids. Table 5 presents the comparison of blood lipid values. Caponization resulted in increased plasma concentrations of LDL, HDL, and TC ($P < 0.05$ or $P < 0.01$). On the contrary, TG concentration was not affected by the testectomy ($P > 0.05$).

mRNA expression of FAS, LPL, and PPARγ genes. Compared with the intact birds, capons

Table 5. Comparison of blood lipid parameters between capons and intact males

Parameter (nmol/l)	Capons (n = 15)	Intact males (n = 15)
LDL	1.82 ± 0.09**	1.49 ± 0.08
HDL	1.63 ± 0.07*	1.37 ± 0.07
TC	3.78 ± 0.12**	3.17 ± 0.11
TG	0.70 ± 0.02	0.68 ± 0.02

LDL = low-density lipoprotein, HDL = high-density lipoprotein, TC = total cholesterol, TG = triglyceride
values are expressed as means ± SEM
*$P \leq 0.05$, **$P \leq 0.01$

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Table 6. Comparison of mRNA levels between capons and intact males

Genes	Capons (n = 15)	Intact males (n = 15)
LPL mRNA in abdominal adipose tissue	1.61 ± 0.26*	0.96 ± 0.16
PPARγ mRNA in abdominal adipose tissue	1.27 ± 0.13**	0.74 ± 0.11
FAS mRNA in liver	1.19 ± 0.33*	0.33 ± 0.06

values are expressed as means ± SEM
*$P \leq 0.05$, **$P \leq 0.01$

displayed higher ($P < 0.05$) mRNA levels of the *FAS* gene in the liver at 140 days of age. Caponization up-regulated ($P < 0.01$ or $P < 0.05$) the mRNA levels of *LPL* and *PPARγ* in abdominal adipose tissue at the age of 140 days (Table 6).

DISCUSSION

We found that the caponized birds had heavier live weight at 140 days of age; this is in agreement with the results of Lin and Hsu (2003), who reported that Taiwanese male chickens caponized at 10 weeks of age had heavier live weight at 28 weeks of age than intact birds of the same age. In contrast, Miguel et al. (2008) in their study on Castellana Negra chicken found that the body weight of castrated and uncastrated birds did not differ at 29 weeks; this result was supported by Symeon et al. (2012) in their study on Lohmann chicken. Another contrasting finding was reported by Cason et al. (1988), according to whom the live weight of capons was lower than that of intact males. The reasons for these discrepancies could be attributed to differences in breeds and slaughtering age.

We found an increase in the percent weight of the breast muscle in the caponized birds, which is in agreement with another report (Lin and Hsu 2003). The significantly heavier breast muscle weight observed could be attributed to longer keel length, as keel length has been reported to be significantly positively correlated with the weight of breast muscle (Johnson and Asmundson 1957). Moreover, our results showed that the percent weights of the gizzard and liver were not affected by caponization, while the percent weights of the heart and leg muscle were reduced, which is in agreement with the findings of Symeon et al. (2012).

Capons also had heavier carcass weight, which could be due to the increase in breast muscle and abdominal fat weight. The dressing percentage did not show any significant difference between the groups, which was in agreement with the results of Miguel et al. (2008). However, Symeon et al. (2012) showed that the dressing percentage of capons was lower than that of intact birds, while Chen et al. (2000) reported that capons had higher dressing percentage. These controversial results are probably attributable to difference in the breeds used.

Capons exhibited lower redness values for both breast and thigh meat and higher lightness values for thigh meat, which was in agreement with the results of Sirri et al. (2009). However, caponization had no effect on meat pH or drip loss.

It has been reported that caponization significantly increased abdominal fat weight (Lin and Hsu 2003; Chen et al. 2005; Shao et al. 2009). Statistical evidence for this increase was found in the present study. Compared to lipid metabolism in mammals, little or no fatty acid synthesis occurs in the adipose tissue of chicken, with the liver being the main organ of fatty acid synthesis in chickens (Leveille 1966). The liver is the main organ of lipogenesis and generates lipids containing VLDL for whole-body use (Chen et al. 2007). FAS is a key enzyme in *de novo* lipogenesis; therefore, in the present study, the up-regulated *FAS* mRNA levels in the capon liver samples indicated that caponization enhanced hepatic lipogenesis. Moreover, we found that adipose tissue from the capons had increased mRNA levels of another enzyme involved in lipid metabolism, LPL. Further, the increased LDL concentration indicated that large amounts of fat are transported out of the liver through VLDL and hydrolyzed by LPL, as remnants of hydrolyzed VLDL are found in the LDL form (Hermier 1997). We also found increased expression of *PPARγ*, which is a key regulator of chicken preadipocyte differentiation (Wang et al. 2008). The up-regulated mRNA level of *PPARγ* in capon adipose tissues implied that caponization enhanced adipocyte differentiation.

In conclusion, caponization in roosters enhanced growth, affected carcass composition by causing

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an increase in breast muscle yield and decrease in leg muscle yield, altered meat quality, increased abdominal fat percentage, and up-regulated the mRNA levels of *FAS*, *LPL*, and *PPARγ* genes.

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