Introduction

Capons are male chickens whose testes have been surgically incised. Capons show a significant increase in fat accumulation compared to intact male chickens. However, while caponization leads to a significant reduction in androgen levels in roosters, little is known about the molecular mechanisms through which androgen status affects lipogenesis in avian species. Therefore, investigation of the influence of androgens on fat accumulation in the chicken will provide insights into this process. In this study, Affymetrix microarray technology was used to analyze the gene expression profiles of livers from capons and intact male chickens because the liver is the major site of lipogenesis in avian species. Through gene ontology, we found that genes involved in hepatic lipogenic biosynthesis were the most highly enriched. Interestingly, among the upregulated genes, the cytosolic form of the phosphoenolpyruvate carboxykinase (PCK1) gene showed the greatest fold change. Additionally, in conjunction with quantitative real-time PCR data, our results suggested that androgen status negatively regulated the PCK1 gene in male chickens.

Microarray technology is a powerful method for profiling gene expression patterns of thousands of genes in a single experiment, and is therefore widely applied to identify the tissue- and disease-specific conditions under which genes are expressed [15–20]. In many respects, the 2 types of labeled targets, i.e., cRNA and cDNA, are considered to be equivalent for microarray analysis. However, cRNA has proven to be advantageous for experiments with small amounts of starting RNA [21,22] and is required for Affymetrix microarray analysis. In this study, Affymetrix microarray technology was employed to analyze the gene expression profiles of capons and intact male chickens. We performed gene expression profiling using livers from these 2 types of roosters and explored the possible molecular mechanisms governing lipid accumulation after caponization. From our results, we suggest that the gene encoding phosphoenolpyruvate carboxykinase 1 (PCK1) plays an important role in fat accumulation and is negatively regulated by androgen status in male chickens.

Materials and Methods

Animals

Healthy male single-comb White Leghorn chickens were selected from the Experimental Poultry Genetic Resource and Breeding Chicken Farm of China Agricultural University. All chickens were housed in a modern, nationally certified animal facility under the supervision of board-certified veterinarians. This study was carried out in strict accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals of...
Liver Microarray Analysis

Hormone Concentrations

Experimental Design

Sixty male chickens of the same age (ages 9 or 17 weeks) and with similar body weights were randomly divided into 3 groups (20 individuals per group per age). One group was taken as the control and the other 2 groups were caponized.

The caponization procedure was performed according to previously described methods [25,23]. Before the surgical operation, male chickens were prohibited from feeding for 12 h.

The incision site was sterilized with veterinary external disinfectants. A 2–3-cm lateral incision was made at the second or third last rib, and the chicken’s 2 testes were removed. Veterinary external disinfectant was applied again to the incision site, which was closed with surgical sutures. After caponization, there was a 4-week recovery, followed by a 10-week feeding trial.

After caponization, 1 of the 2 caponized groups was used for implanting testosterone because the removal of testes leads to a greater decrease in body testosterone content. Therefore, implantation of testosterone would allow us to investigate the effects changes in body androgen status on fat biosynthesis. The testosterone implantation procedure was performed according to previously reported methods [25], with modifications, and the testosterone amount implanted was based on a previously described method [5]. Exogenous testosterone was purchased from Sigma (USA) and formed into pellets (every pellet contained 18 mg testosterone). Using implantation guns from the Animal Reproduction Laboratory of China Agricultural University (Beijing), testosterone was implanted subcutaneously at the back of the chicken’s neck during the 10-week feeding trial (10 mg per individual dose with a total of 3 doses over 10 weeks; the “embed group”). The other 2 groups were called the control group (including only intact male chickens) and the capon group (including only caponized male chickens who were not given testosterone implants), respectively. All chickens were sacrificed by qualified technicians in a clean slaughterhouse by having their carotid arteries severed with clean neck cutters under anesthesia.

Determination of Abdominal Fat Content and Serum Hormone Concentrations

Blood samples were taken from the brachial veins of chickens following 12-h fasting from food and water prior to slaughter and were then stored in anticoagulant blood vessels at 4°C until use. Determination of sex hormone content using these blood samples was completed at the Sino-UK Institute of Biological Technology (Beijing). Serum testosterone and estradiol concentrations were determined by a previously described method [24] using a γ-counter (GC-911) with a radioimmunoassay kit.

After slaughter, the abdominal fat and liver tissues were removed immediately. Abdominal fat was weighed, and liver tissues were frozen in liquid nitrogen and stored at −80°C for further analysis.

Liver Microarray Analysis

We randomly selected 9 individuals from the control chickens and capons at 23 weeks of age. After slaughter, total RNA was isolated from liver tissues using Trizol (Invitrogen, Paisley, UK). We used a pooled design in order to obtain a sufficient amount of RNA to run an array. Nine RNA samples from control chickens or capons were randomly divided into 3 pools to give 3 RNA samples per pool. RNA integrity was electrophoretically verified by ethidium bromide staining and by OD260/OD280 nm absorption ratio (>1.95). Next, we prepared the cRNA and microarray chips following the technical manual for GeneChip expression analysis provided by Affymetrix (File S2). All liver microarray analyses were performed at the Bioassay Laboratory of CapitalBio Corporation (10 Life Science Parkway, Changping District, Beijing, China; http://www.capitalbio.com).

First- and second-strand cDNA were synthesized from total RNA (~1 µg) using the SuperScript II system (Invitrogen, CA, USA). After a clean up and quality check of the double-stranded cDNA, an in vitro transcription reaction was conducted with the Enzo RNA Transcript Labeling Kit (Affymetrix, Santa Clara, CA) to produce biotin-labeled cRNA from the cDNA. The cRNA was then purified with the RNasy Mini Kit (Qiagen, Valencia, CA) and fragmented for hybridization analysis. Finally, the fragmented cRNA was hybridized with the Chicken Liver Microarray (Affymetrix) in a hybridization cocktail. Hybridization took place overnight (16 h) at 45°C in a GeneChip Hybridization Oven 640 at 60 rpm (Affymetrix), followed by washing and staining with streptavidin-phycocery-thrin (SAPE, Molecular Probes, Eugene, OR) as described in the Affymetrix GeneChip Expression Analysis Technical Manual (File S2). The distribution of fluorescent material on the array was imaged using the GeneArray Scanner 3000 (Affymetrix), Microarray Suite (MAS) Version 5.0 and GeneChip Operating Software (GCOS), supplied by Affymetrix, were used for gene expression analysis. High-density oligonucleotide array probe level data were normalized using previously described methods [25].

Significance analysis of microarrays (SAM) is a method that can be used to identify differentially expressed genes. Each gene was assigned a score on the basis of its change in gene expression relative to the standard deviation of repeated measurements. Genes with scores that are significantly higher than the expected score were termed differentially expressed. The expected score was calculated by permuting the measurements and then taking the average score for all the permuted scores as the expected score. To control the type I error rate for multiple-hypothesis testing, SAM was used to fix the rejection region and then estimate its corresponding error rate. SAM applies the methodology to both the positive false discovery rate and q-value as presented in previous studies [26]. To identify significantly differentially expressed genes, we used the following criteria: fold change, ≥2 or ≤0.5; q-value, ≤5%. Gene ontology analysis was conducted at http://www.geneontology.org, and the pathway analysis was performed by KEGG (http://www.genome.jp/kegg).

Quantitative Real-time PCR (qRT-PCR)

Twelve chicken livers were randomly selected from each of the 3 groups. Total RNA was extracted from the livers. cDNA was synthesized from 1 µg total RNA with M-MLV Reverse Transcriptase (Promega). Aliquots of cDNA were used as a template for real-time PCR. Reactions contained primers and probes for PCK1 or ME1 genes or primers and a probe for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which served as a reference gene. Each reaction contained cDNA derived from 20 ng total RNA. Three replicates were performed for each reaction.

qRT-PCR was carried out using a CFX Connect Real-Time PCR Detection System (Bio-R, Hercules, California, USA) in a Bio-Rad Real Time-PCR9600. Relative expression of target genes was calculated by a previously described method [27]. First, 12 livers from each of the control and capon groups were selected for carrying out qRT-PCR. Then, if significant differences were found between the 2 groups, irrespective of age, 12 livers from the embed group were selected for qRT-PCR as well.
Primer sets for PCK1 (forward, 5’-GCAGGGTTATGAT-GAGAAGT-3’; and reverse, 5’-ACGGATACAGTTGTGAA-GAC-3’); ME1 (forward, 5’-CGGAGTGGCCTGTGT-3’; and reverse, 5’-TCCTGAGGGTTTCTTCGC-3’), and the housekeeping internal control gene GAPDH (forward, 5’-GAACCCAGCGATATGAT-3’; and reverse, 5’-ACCATG-GAAATCGAGGAG-3’) were designed based on the sequences published in GenBank and using Primer Premier 5.0 software.

Statistical Analysis
All statistical analyses were performed with the GLM procedure in SAS 9.1 software (SAS Institute, 1990). Tests of differences were carried out using Duncan’s new multiple range method [28] and values are presented as the mean ± standard error (SE).

Results
Abdominal Fat Content and Serum Sex Hormone Content
In previous observations, abdominal fat content and blood sex hormone content were shown to exhibit significant differences in capons compared with intact male chickens [2–5]. We therefore compared the 2 indexes among the different groups. As shown in Table 1, in White Leghorn male chickens aged 23 or 31 weeks, the capon group exhibited a greater increase in abdominal fat content than the control and control groups, and there was no significant difference in abdominal fat between the control and control groups (P>0.05). This result indicated that caponization enhanced abdominal fat deposition and that implantation of testosterone significantly inhibited abdominal fat deposition.

As shown in Figure 1, after caponization, serum testosterone content decreased dramatically in the capon group compared with the control and control groups, irrespective of age, but showed no significant difference between the control and control groups (P>0.05). The results also indicated that implantation of testosterone resulted in a significant recovery in serum testosterone content in capons. In contrast, serum estradiol levels did not differ among all groups, irrespective of age (P>0.05; Figure 2).

Liver Microarray Analysis
Affymetrix microarray technology was used to analyze the gene expression profiles of chicken livers from the capon and control groups. Of the 30336 probes analyzed (File S5), 79 genes were upregulated by at least 2-fold, and 42 genes were downregulated by at least 2-fold in the livers of capons (File S1, Table 2). Gene ontology enrichment analysis indicated that the largest proportion of upregulated genes was involved in metabolic pathways, whereas genes involved in lipid metabolism were the most highly enriched (Table 3). Furthermore, pathway analysis by KEGG showed that genes with differential expression were mainly involved in lipid metabolism and Jak-STAT signaling pathways (Table 3).

Expression Patterns of the PCK1 and ME1 Genes
Among the upregulated genes, the largest fold change was observed in the cytosolic form of PCK1, while only a 2-fold change was found in ME1 (Table 2). In a previous study, the ME1 gene was suggested to play a key role in fat biosynthesis in capons [14]. At the same time, microarray analysis showed that the PCK1 gene exhibited the largest fold increase in capons. Therefore, qRT-PCR was carried out to analyze the expression patterns of the ME1 and PCK1 genes. Interestingly, we found that the expression of the ME1 gene differed significantly between the capon and control groups only at 23 weeks of age, but not at 31 weeks of age (Figure 3). Thus, our results showed that expression of the ME1 gene was associated with caponization age but not androgen status in male chickens.

In contrast, there was a significant difference in the expression of the PCK1 gene between the control and capon groups and between the capon and control groups, irrespective of age. At the same time, the expression of the PCK1 gene showed no significant difference between the control and control groups, irrespective of age (Figure 4). These results indicated that the expression of the PCK1 gene was negatively regulated by androgen status.

Discussion
Androgen Status Negatively Affected Fat Accumulation in Male Chickens
Castration has been reported to result in an increase in fat accumulation over the longissimus muscle in male bulls or rams, more backfat in male boars [29], and an obvious increase in subcutaneous, intercellular, and abdominal fat in male chickens [2–5], which is consistent with the findings of our present study. Additionally, an increase in proliferation capacity and a loss of differentiation capacity were observed in epididymal pre-adipocytes in castrated rats [7].

Castration primarily decreases androgen levels in male animals due to the removal of the male testes. The primary and most well-known androgen is testosterone, which is an important determinant of body composition in male mammals [30,31]. In men, abdominal obesity is usually associated with low serum testosterone levels [32–34]. At the same time, testosterone supplementation in healthy, young, hypogonadal men can result in a decrease in fat mass [35–39]. Likewise, testosterone supplementation increases skeletal muscle mass and decreases fat mass in mice [11]. In our study, we also found that serum testosterone levels were negatively correlated with abdominal fat accumulation in male chickens, irrespective of age while testosterone implantation resulted in a significant decrease in abdominal fat, which is consistent with a previous observation [14]. The results suggested that androgen status negatively affected fat accumulation in male chickens.

Liver Microarray Analysis
Capons can accumulate lipids in the body, which enhances flavor and meat juiciness when compared with intact cockerels [1,3,40]. Therefore, it was expected that the largest proportion of upregulated genes would be involved in lipid metabolism. Additionally, previous studies have indicated that the Jak-STAT signaling pathway plays a key role in innate immunity [41–43]. Thus, our results suggested that caponization increases the

Table 1. Abdominal fat content of White Leghorn male chickens at different ages for the 3 groups.

| Group      | Abdominal fat content (g) | 23 weeks of age* | 31 weeks of age* |
|------------|--------------------------|------------------|------------------|
| Capon      | 10.55±1.53 (n = 20)      | 4.25±0.78 (n = 20) |
| Embedded   | 0.00±0.00 (n = 20)       | 1.44±0.75 (n = 20) |
| Control    | 0.00±0.00 (n = 20)       | 0.00±0.00 (n = 20) |

Note: All values are depicted as means ± SE.
*p<0.01; +p<0.05;
*age at the end of the experiment.
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immune response in male chickens, which is consistent with another previous study [44].

Androgen Status Negatively Influenced PCK1 Gene Expression in Male Chickens

PCK1 catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, the rate-limiting step in hepatic and renal gluconeogenesis and adipose glyceroneogenesis, and is expressed at high levels in liver, kidney, and adipose tissue [45]. In the liver, expression of the PCK1 gene at the transcriptional level is stimulated by a number of hormones, including glucagon, cAMP, glucocorticoids, and thyroid hormone [46–50], but is inhibited by insulin [48,51,52]. However, the mechanism through which testosterone regulates expression of the PCK1 gene has not been reported. Importantly, the results of our current study suggested that testosterone negatively regulates PCK1 mRNA.

An increasing number of studies have shown that the PCK1 gene plays a crucial role in multiple physiological processes in mammalian species and is involved in obesity, insulin resistance.
Table 2. Genes differentially expressed (q <0.05) in capons’ livers compared to control livers of male White Leghorn chickens.

| Gene symbol | Gene or function | q-value | FC |
|-------------|-----------------|---------|----|
| Upregulated  |
| 1 | PCK1 | phosphoenolpyruvate carboxykinase 1 (soluble) | 0 | 28.87 |
| 2 | ABCB1 | ATP-binding cassette, sub-family B (MDR/TAP), member 1 | 0 | 6.26 |
| 3 | MOGAT1 | monoacylglycerol O-acyltransferase 1 | 0 | 5.80 |
| 4 | CDKN2B | cyclin-dependent kinase inhibitor 2B (melanoma, p16, inhibits CDK4) | 0.008 | 5.48 |
| 5 | FABP1 | fatty acid binding protein 1, liver | 0 | 4.94 |
| 6 | ABCG5 | ATP-binding cassette, sub-family G (WHITE), member 5 (sterolin 1) | 0 | 4.61 |
| 7 | RBM38 | RNA binding motif protein 38 | 0 | 4.29 |
| 8 | LOC428660 | similar to very large inducible GTPase-1 | 0 | 3.90 |
| 9 | CHAC1 | ChaC, cation transport regulator homolog 1 (E. coli) | 0.005 | 3.65 |
| 10 | GCLC | glutamate-cysteine ligase, catalytic subunit | 0.009 | 3.65 |
| 11 | RCJMB04_16d24 | ELOVL family member 6, elongation of long chain fatty acids | 0 | 3.37 |
| 12 | GALE | UDP-galactose-4-epimerase | 0 | 3.36 |
| 13 | NCAM1 | Neural cell adhesion molecule 1 | 0 | 3.30 |
| 14 | APOA4 | apolipoprotein A-IV | 0 | 3.27 |
| 15 | ADFP | Adipose differentiation-related protein | 0.010 | 3.24 |
| 16 | SIK1 | salt-inducible kinase 1 | 0.003 | 3.22 |
| 17 | TMCC2 | transmembrane and coiled-coil domain family 2 | 0 | 3.16 |
| 18 | BRP44L | brain protein 44-like | 0.005 | 3.13 |
| 19 | ELOVL2 | elongation of very long chain fatty acids-like 2 | 0 | 3.12 |
| 20 | ALDH18A1 | aldehyde dehydrogenase 18 family, member A1 | 0.006 | 2.99 |
| 21 | RCJMB04_5k4 | selenoprotein 1 | 0 | 2.93 |
| 22 | DBC1 | deleted in bladder cancer 1 | 0.019 | 2.91 |
| 23 | ELOVL1 | elongation of very long chain fatty acids | 0.007 | 2.87 |
| 24 | LOC420707 | hypothetical gene supported by CR386894 | 0 | 2.73 |
| 25 | WDR66 | WD repeat domain 66 | 0.011 | 2.73 |
| 26 | SEC22C | SEC22 vesicle trafficking protein homolog C (S. cerevisiae) | 0 | 2.70 |
| 27 | ABI3 | ABI gene family, member 3 | 0 | 2.68 |
| 28 | EREG | epiregulin | 0.026 | 2.68 |
| 29 | MFSD2 | major facilitator superfamily domain containing 2 | 0 | 2.66 |
| 30 | FICD | FIC domain containing | 0.014 | 2.64 |
| 31 | C7orf23 | chromosome 7 open reading frame 23 | 0.008 | 2.61 |
| 32 | FADS2 | Fatty acid desaturase 2 | 0.003 | 2.60 |
| 33 | SERPINA3 | serpin peptidase inhibitor, clade A, member 3 | 0.019 | 2.58 |
| 34 | SPG20 | spastic paraplegia 20 (Troyer syndrome) | 0.004 | 2.56 |
| 35 | IL10RB | interleukin 10 receptor, beta | 0.005 | 2.55 |
| 36 | SLC39A8 | solute carrier family 39 (zinc transporter), member 8 | 0.005 | 2.54 |
| 37 | SH3YL1 | SH3 domain containing, Ysc84-like 1 (S. cerevisiae) | 0 | 2.53 |
| 38 | CRAT | carnitine acetyltransferase | 0.011 | 2.52 |
| 39 | SEBOX | SEBOX homeobox | 0.012 | 2.49 |
| 40 | SOCS3 | suppressor of cytokine signaling 3 | 0.019 | 2.44 |
| 41 | LOC421910 | similar to Acp1 protein | 0.004 | 2.40 |
| 42 | SOCS1 | suppressor of cytokine signaling 1 | 0.006 | 2.35 |
| 43 | ATOH8 | Atonal homolog 8 (Drosophila) | 0.006 | 2.29 |
| 44 | IRG1 | immunoresponsive 1 homolog (M. musculus) | 0.020 | 2.28 |
| 45 | ECE1 | endothelin converting enzyme 1 | 0 | 2.25 |
| 46 | FADS1 | fatty acid desaturase 1 | 0.005 | 2.25 |
| 47 | ABCD3 | ATP-binding cassette, sub-family D (ALD), member 3 | 0.005 | 2.23 |
| 48 | SLC16A5 | solute carrier family 16, member 5 (monocarboxylic acid transporter 6) | 0 | 2.22 |
| 49 | THRSP | thyroid hormone responsive (SPOT14 homolog, R. norvegicus) | 0 | 2.22 |
| Gene symbol | Gene or function                                      | q-value | FC  |
|-------------|-------------------------------------------------------|---------|-----|
| GNPNAT1     | glucosamine-phosphate N-acetyltransferase 1           | 0       | 2.19|
| LOC768655   | similar to pim-3 protein                              | 0       | 2.19|
| PHOSPHO1    | phosphatase, orphan 1                                 | 0.004   | 2.19|
| RCJMB04_28i8| Fas (TNFRSF6) binding factor 1                        | 0.011   | 2.19|
| SLC41A1     | Solute carrier family 41, member 1                    | 0.015   | 2.19|
| LOC418109   | hypothetical LOC418109                                | 0.016   | 2.18|
| ME1         | malic enzyme 1, NADP(+) dependent, cytosolic         | 0.013   | 2.18|
| ZYG11B      | zyg-11 homolog 8 (C. elegans)                         | 0       | 2.18|
| LOC768655   | similar to pim-3 protein                              | 0       | 2.18|
| PHOSPHO1    | phosphatase, orphan 1                                 | 0.004   | 2.19|
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| LOC768655   | similar to pim-3 protein                              | 0       | 2.18|
| PHOSPHO1    | phosphatase, orphan 1                                 | 0.004   | 2.19|
| RCJMB04_28i8| Fas (TNFRSF6) binding factor 1                        | 0.011   | 2.19|
| SLC41A1     | Solute carrier family 41, member 1                    | 0.015   | 2.19|
| LOC418109   | hypothetical LOC418109                                | 0.016   | 2.18|
| ME1         | malic enzyme 1, NADP(+) dependent, cytosolic         | 0.013   | 2.18|
| ZYG11B      | zyg-11 homolog 8 (C. elegans)                         | 0       | 2.18|
| LOC768655   | similar to pim-3 protein                              | 0       | 2.18|
| PHOSPHO1    | phosphatase, orphan 1                                 | 0.004   | 2.19|
| RCJMB04_28i8| Fas (TNFRSF6) binding factor 1                        | 0.011   | 2.19|
| SLC41A1     | Solute carrier family 41, member 1                    | 0.015   | 2.19|
| LOC418109   | hypothetical LOC418109                                | 0.016   | 2.18|
| ME1         | malic enzyme 1, NADP(+) dependent, cytosolic         | 0.013   | 2.18|
| ZYG11B      | zyg-11 homolog 8 (C. elegans)                         | 0       | 2.18|
| LOC768655   | similar to pim-3 protein                              | 0       | 2.18|
| PHOSPHO1    | phosphatase, orphan 1                                 | 0.004   | 2.19|
| RCJMB04_28i8| Fas (TNFRSF6) binding factor 1                        | 0.011   | 2.19|
| SLC41A1     | Solute carrier family 41, member 1                    | 0.015   | 2.19|
| LOC418109   | hypothetical LOC418109                                | 0.016   | 2.18|
| ME1         | malic enzyme 1, NADP(+) dependent, cytosolic         | 0.013   | 2.18|
| ZYG11B      | zyg-11 homolog 8 (C. elegans)                         | 0       | 2.18|
and the mammary gland [53–65]. In chicken livers, the main form of PCK is mitochondrial PCK, also called PCK2; in contrast to PCK2, PCK1 plays an important role in gluconeogenesis in the kidney [66,67]. Presently, the PCK1 gene has not been reported to be involved in glyceroneogenesis in avian livers. In our study, the results

| Gene symbol | Gene or function | q-value | FC  |
|-------------|-----------------|---------|-----|
| 19          | BUB1            | 0.006   | –2.32 |
| 20          | PTPN1           | 0       | –2.40 |
| 21          | GAL13           | 0.014   | –2.62 |
| 22          | LOC426431///OAT | 0.014   | –2.65 |
| 23          | FABP5           | 0       | –2.69 |
| 24          | ASAH2           | 0.042   | –2.71 |
| 25          | PPAT            | 0.013   | –2.71 |
| 26          | LPIN1           | 0.021   | –2.76 |
| 27          | HNF4A           | 0.013   | –2.84 |
| 28          | CYP1A4          | 0.009   | –2.89 |
| 29          | IL1R1           | 0.008   | –3.01 |
| 30          | LOC769659///OAT | 0.009   | –3.18 |
| 31          | CYP7A1          | 0.009   | –3.20 |
| 32          | LOC421091       | 0.019   | –3.25 |
| 33          | SOCS2           | 0       | –3.25 |
| 34          | GLDC            | 0       | –3.28 |
| 35          | FKBP5           | 0.008   | –3.44 |
| 36          | EPAS1           | 0       | –3.46 |
| 37          | CHIA            | 0       | –3.88 |
| 38          | GPT2            | 0       | –3.91 |
| 39          | IGFB2           | 0       | –4.64 |
| 40          | UPP2            | 0.005   | –6.37 |
| 41          | LOC770705       | 0.016   | –6.93 |
| 42          | CHIA/LOC768786  | 0       | –7.12 |

Note: Accession numbers of the genes are shown in File S1.

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| Pathway                                | P-value  | Genes*               |
|----------------------------------------|----------|----------------------|
| Biosynthesis of unsaturated fatty acids| 1.50E–09 | ACAA1; RCJMB04_16d24; ELOVL2; FADS1; FADS2  |
| Bile acid biosynthesis                 | 2.24E–05 | ACAA1; AKR1D1; CYP7A1 |
| Jak-STAT signaling pathway             | 2.26E–05 | IL10RB;              |
| ABC transporters - General             | 8.78E–05 | ABCD3;               |
| Fatty acid metabolism                  | 1.08E–04 | CYP4B1;              |
| Glutamate metabolism                   | 0.0015   | PPAT;                |
| Aminosugars metabolism                 | 0.0017   | CHIA;                |
| Alanine and aspartate metabolism       | 0.0026   | CRAT;                |
| Retinol metabolism                     | 0.0029   | CYP4B1;              |
| Drug metabolism - other enzymes        | 0.0033   | UPP2;                |
| Pyruvate metabolism                    | 0.0043   | ME1;                 |
| Ubiquitin mediated proteolysis         | 0.0051   | SOCS3;               |
| Glycerophospholipid metabolism         | 0.0092   | PHOSPHO1;            |

*Genes in bold are upregulated.

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showed that \( PCK1 \) mRNA expression had a positive relationship with abdominal fat accumulation in male chickens, suggesting that the \( PCK1 \) gene plays a crucial role in lipogenesis in capons.

**The Mechanism through which Androgen Status Affects Adipogenesis in Male Chickens**

In castrated rats, androgen status is thought to affect adipogenesis from deep intra-abdominal pre-adipocytes through altered MAP kinase cascade/Fos signaling pathways [8]. In men, testosterone affects adipogenesis by regulation of the activities of lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) [12,68]. A previous study indicated that caponization increased \( ME1 \) mRNA expression at 26 weeks of age in male chickens [15]. This could be explained by the fact that ME1 catalyzes the oxidative decarboxylation of malate and simultaneously generates reduced NAPD, which is involved in the de novo synthesis of fatty acid. According to our results, however, the expression of the \( ME1 \) gene was mainly affected by the age at castration and not by androgen status, whereas the mRNA expression of the \( PCK1 \) gene was mainly regulated by androgen status and not by age at castration. Therefore, we suggest that androgen status affects fat accumulation in male chickens by negatively regulating the expression of \( PCK1 \) gene. Additionally, our microarray data found no differences in the expression levels of \( LPL \) and \( HSL \) genes between capons and intact male chickens, implying that avian species and mammals possess different mechanisms through which androgen status affects adipogenesis. This difference could be partly explained by the fact that the sites to lipogenesis are highly variable between avian species and mammals [13,69]. For example, in mammals, the liver and adipose tissue are the 2 major sites of fatty acid production, whereas in avian species the

**Figure 3. Relative mRNA expression of the \( ME1 \) gene in the different groups.** Twelve livers each from the control and capon groups (of chickens with different ages) were selected for carrying out qRT-PCR. Results are presented as means ± SE; *, \( P<0.05 \); **, \( P<0.01 \).

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**Figure 4. Relative mRNA expression of \( PCK1 \) in different groups.** Twelve livers each from the control and capon groups (at different ages) were selected for carrying out qRT-PCR. When significant differences were found between the 2 groups, 12 livers from the embed group were also subjected to qRT-PCR. Results are presented as means ± SE; *, \( P<0.05 \); **, \( P<0.01 \).

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liver is the main lipogenic site. Additionally, Human adipose tissue has a poor capacity to synthesize fatty acids de novo compared with that of the rat [70], which could explain the differences observed between humans and rats.

Supporting Information

File S1 capon_vs_control_Result: upregulated and downregulated genes.

File S2 Affymetrix GeneChip technical manual.

File S3 all_expression_signal.

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Author Contributions

Conceived and designed the experiments: HL CW JD FS. Performed the experiments: JD FS YS JL YL. Analyzed the data: JD FS YS. Contributed reagents/materials/analysis tools: JL KT. Wrote the paper: HL.

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