Effect of age on hepatic cytochrome P450 of Ross 708 broiler chickens

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ABSTRACT Age has significant impact on hepatic cytochrome P450 (CYP450) systems in animals. Ross 708 broiler chicken is a breed of chicken with fast growth characteristics. Cytochrome P450 in the livers of Ross 708 broiler chicken of different ages has been investigated. The birds were raised under standard husbandry conditions. A certain number of chickens was randomly sampled weekly for liver collection from d 1 to 56 posthatch. The chicken body and liver weights were recorded. The chicken livers were processed for liver microsomes through a multiple-step procedure at low temperature. Total CYP450 content in chicken liver homogenates and liver microsomes was measured using a UV/visible spectroscopic method. The enzymatic activities of CYP450 in the chicken liver microsomes were determined through incubation of CYP450 isoform substrates followed by measurement of formation of their metabolites. The chicken showed an opposite age pattern in hepatic CYP450 content and activities compared with most mammals. The hepatic CYP450 content and activities of chicken at d 1 posthatch were higher than at other ages. The total hepatic CYP450 content in chickens at d 1 posthatch was more than twice the average hepatic value of the chickens at d 7 to 28. This high CYP450 fell quickly in the first week posthatch and slightly rose from d 28 to 56. Hepatic CYP450 activities of CYP1A, 3A, 2C, 2D, and 2H were much higher in the chicken at d 1 posthatch. The differences of these enzymatic activities between d 1 and other ages of chicken were CYP450 isoform dependent. This result suggests that embryonic development of chicken livers has a significant impact on the age profile of hepatic CYP450 content and activities of posthatch chickens.

Key words: hepatic CYP450, chicken liver, age effect, Ross 708 broiler

INTRODUCTION

Cytochrome P450 (CYP450) monooxygenases are important in the metabolism of many therapeutic agents and endogenous metabolic reactions. It has long been recognized that human and animals of different ages display marked differences in hepatic CYP450 content and activities. In humans, the age effect on liver CYP450 has been broadly investigated. The CYP450 activities in humans, such as CYP1A1/2, are typically low immediately after birth, increase and peak at the young/mature adult level, and then finally decrease in old age (Tanaka, 1998; Cotreau et al., 2005). Some exceptions are identified, such as that CYP2E1 activity increases rapidly after birth to reach a level equivalent to that in the young/mature adult, then gradually decreases (Tanaka, 1998), and that CYP3A7 is the most abundant CYP iso-enzyme at birth with a subsequent decrease during the first or 2 yr of life (Gow et al., 2001; Hines, 2007; Allegaert et al., 2007). Age-related changes in hepatic CYP450 activities have also been observed in dogs (Kawalek and el Said, 1990; Tanaka et al., 1998), horses (Nebbia et al., 2004), mice (Warrington et al., 2000; Hart et al., 2009) and rats (Warrington et al., 2003; Warrington et al., 2004; Yun et al., 2010).

Livestock or food-producing animals are important protein sources for humans. Those animals have comparable hepatic CYP450 systems as humans (Antonovic and Martinez, 2011). The age-related differences of CYP450 in food-producing animals have also been reported, such as in sheep (Kaddouri et al., 1990; Fløyen and Jensen, 1991; Galtier and Alvinerie, 1996; Pretheeban et al., 2012), cattle (Kawalek and el Said, 1994), and pigs (Short and Davis, 1970; Peggins et al., 1984). Chickens are different livestock compared with mammals. Chicken are hatched from fertile hatching eggs in 21 d and grow rapidly posthatch. There are significant physiological and biochemical changes in the birds prior to hatch and posthatch. The chick embryo of White Leghorns has been shown to have hepatic aryl hydrocarbon hydroxylase activity. This activity in the chicken embryo at 3 d of incubation is comparable with its adult level and this activity in chick liver increases about 3-fold from 20 d of incubation through d 1.
posthatch and returns to adult level at about 7 d of age. This pattern of development of aryl hydrocarbon hydroxylase activity can be significantly magnified by different inducers, such as 3,4,3′,4′-tetrachlorobiphenyl (Hamilton and Bloom, 1983; Hamilton et al., 1983). Both hepatic microsomal enzymatic activities and CYP450 content peak around hatch in White Leghorn and Cornell K-MRO line chickens (Lorr and Bloom, 1987; Lorr et al., 1989). This phenomenon has also been observed in testosterone hydroxylase activity (Paolini et al., 1997). The hepatic microsomal CYP450 content slightly increases from 3 to 9 wk posthatch and then decreases in 12 to 20 wk in White Leghorns and Sasso broilers (Coulet et al., 1996; Blevins et al., 2012).

The Ross 708 broiler is one of the fast-growth broiler chickens produced worldwide (Kidd et al., 2005; Dozier et al., 2006; Rosebrough and Mitchell, 2007). This breed has higher feed conversion efficiency and higher breast muscle yield (Schmidt et al., 2009). These birds can grow from about 40 g at d 1 posthatch to more than 4 kg in 8 wk and are typically slaughtered for meat within 8 wk in the poultry industry. The objective of this study was to evaluate the development of hepatic CYP450 in Ross 708 broiler chickens from d 1 to 56 posthatch.

### MATERIALS AND METHODS

#### Chemicals

Phenacetin, 4-acetamidophenol, midazolam, 1′-hydroxymidazolam, temazepam, oxazepam, bufuralol, hydroxybufuralol, tolbutamide, 4-hydroxy tolbutamide, and β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate were purchased from Sigma-Aldrich (St. Louis, MO). The Pierce BCA protein assay kit was purchased from Thermo Scientific (Rockford, IL).

#### Bird Care

The chickens were raised under standard husbandry conditions, and all in vivo work was conducted under the oversight of the Institutional Animal Care and Use Committee and according to local, state, and national regulations. Newly hatched Ross 708 chicks were distributed into 2 pens with 130 birds (65 males and 65 females) in each pen. The chickens were housed in the concrete floor pens (~1.20 m × 3.05 m) within an environmentally controlled facility (22 to 36°C). The housing density was ~0.07 m²/bird at placement. All birds were placed in the clean pens with pine wood shavings as bedding. Lighting was via incandescent lights, and a commercial lighting program was applied. Hours of light for every 24-h period are described in Table 1. Standard corn-soybean diets were prepared for starter (0 to 21 d), grower (22 to 35 d), and finisher (36 to 58 d) as shown in Table 2. The starter diet was fed as crumbles. The grower and finisher diets were fed as pellets. Water was provided ad libitum throughout the study via one automatic Plasson bell drinker per pen. The birds were vaccinated for Marek’s at the hatchery. Newcastle and infectious bronchitis vaccines were administered using a spray cabinet before distributing chicks to their pens. No other vaccinations or treatments were applied during the study.

Equal numbers of male and female chickens were randomly selected for liver collection at d 1, 7, 14, 21, 28, 35, 42, 49, and 56 posthatch from both pens. The number of chickens selected at each sampling day were 64, 32, 16, 8, 8, 8, 6, 6, and 6, with half males and half females. The chickens were euthanized via complete cranial decapitation followed by exsanguination. Both body and liver weights were recorded. The chicken livers were stored in a −80°C freezer before processing.

### Table 1. Lighting program in chicken pens of this study

<table>
<thead>
<tr>
<th>Approximate bird age (d)</th>
<th>Approximate hours of continuous light per 24-h period</th>
<th>Light intensity (lx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 4</td>
<td>24</td>
<td>10.8 to 14.0</td>
</tr>
<tr>
<td>5 to 10</td>
<td>10</td>
<td>10.8 to 14.0</td>
</tr>
<tr>
<td>11 to 14</td>
<td>12</td>
<td>2.15 to 3.23</td>
</tr>
<tr>
<td>19 to 56</td>
<td>16</td>
<td>2.15 to 3.23</td>
</tr>
</tbody>
</table>

### Table 2. Composition of the corn-soybean meals for starter, grower, and finisher chickens

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter (%)</th>
<th>Grower (%)</th>
<th>Finisher (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn (yellow)</td>
<td>61.140</td>
<td>64.410</td>
<td>70.124</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>30.096</td>
<td>26.463</td>
<td>20.671</td>
</tr>
<tr>
<td>Meat and bone</td>
<td>5.000</td>
<td>5.000</td>
<td>5.693</td>
</tr>
<tr>
<td>Soy oil</td>
<td>1.749</td>
<td>2.318</td>
<td>2.282</td>
</tr>
<tr>
<td>Phosphorus, defluorinated</td>
<td>0.488</td>
<td>0.349</td>
<td>0</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.451</td>
<td>0.410</td>
<td>0.314</td>
</tr>
<tr>
<td>Salt</td>
<td>0.411</td>
<td>0.431</td>
<td>0.467</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>0.240</td>
<td>0.232</td>
<td>0.175</td>
</tr>
<tr>
<td>Choline chloride-60</td>
<td>0.103</td>
<td>0.087</td>
<td>0.057</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>0.078</td>
<td>0.059</td>
<td>0.017</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.100</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Trace mineral premix</td>
<td>0.100</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Biocox 60</td>
<td>0.042</td>
<td>0.041</td>
<td>0</td>
</tr>
</tbody>
</table>
**Chicken Liver Processing**

The chicken livers collected at d 1, 7, 14, 28, and 56 were processed for liver microsomes. Every 8 male or female livers collected at d 1 and every 2 male or female livers collected at d 7 were randomly combined as one sample due to weights of the livers. The chicken livers collected from other days were processed individually. Three liver samples of each sex collected at those days were selected to be processed for liver microsomes. The chicken livers were weighed and prehomogenized using a SPEX SamplePrep 6870 Freezer/Mill (Metuchen, NJ). In the Freezer/Mill, the liver samples were pre-cooled in liquid nitrogen for 3 min, ground at 5 cycles per second for 3 min, and cooled an additional 3 min in liquid nitrogen. Weighed powders were transferred to a Dounce tissue grinder with homogenizing buffer (50 mM Tris buffer with 150 mM KCl, pH 7.4) and homogenized with a grinding tube for 10 strokes. An aliquot of homogenized liver suspension was taken for total CYP450 measurement. The rest of the liver suspensions were centrifuged at 9,000 × g and 4°C for 30 min in a Beckman Coulter Optima L-90K ultracentrifuge (Brea, CA). The pellets were resuspended in cold homogenizing buffer and centrifuged at the same conditions. Both supernatants were combined and centrifuged at 100,000 × g and 4°C for 60 min. The pellets were suspended in cold wash buffer (150 mM KCl, 10 mM EDTA, pH 7.4) and centrifuged at 100,000 × g and 4°C for 60 min. The pellets were washed one more time using the wash buffer as before. The final pellets (microsomes) were resuspended in cold storage buffer (50 mM phosphate buffer, pH 7.4), and the protein contents and total CYP450 were measured.

**Total CYP450 Measurement**

Total CYP450 in both chicken liver homogenates and microsomes was determined based on the method of Omura and Sato (1964, a, b) and further described by Schenkman and Jansson (2006). Debris and fibrous tissue of liver homogenates were settled in a 4°C refrigerator for about 4 h, and aliquots of the top layer were taken for assay (Joly et al., 1975). The chicken liver homogenates were diluted to 30 to 50 times with 100 mM phosphate buffer (pH 7.4). Liver microsomes were diluted to 100 times with 100 mM phosphate buffer (pH 7.4). The liver homogenate suspensions or liver microsome samples were added to 1-cm light path quartz sample and reference cuvettes. The suspensions were saturated with oxygen free carbon monoxide. A baseline spectrum was measured using an Agilent Cary 300 Bio UV-Visible spectrophotometer (Santa Clara, CA). A few crystals of solid sodium dithionite were added to the sample cuvette. The spectrum was then recorded after about 1 min. The CYP450 concentration in the cuvette was calculated from the absorbance at 450 nm relative to the absorbance at 490 nm using the Beer’s law equation and the extinction coefficient ε_{450–490} = 91 mM⁻¹cm⁻¹.

**Protein Content Measurement**

Protein content in chicken liver microsomes was measured using the Thermo Scientific Pierce BCA protein assay kit (Rockford, IL) and a BMG Labtech PH-ERAstar FS plate reader (Ortenberg, Germany). The samples were prepared in several dilutions with 3 replicates of each dilution. The diluted samples (25 μL) were mixed with 200 μL of BCA protein assay working reagent and incubated at 37°C for 30 min. The protein concentrations were determined at absorbance of 562 nm against standard solutions.

**CYP450 Activity Measurement**

Phenacetin dealkylation, midazolam 1'-hydroxylation, temazepam demethylation, tolbutamide 4-hydroxylation, and bufuralol hydroxylation were determined. A series of varied concentrations of those probe molecules were preincubated with chicken liver microsomes of final concentration 0.1 mg of protein/mL in pH 7.4 phosphate (with 10 μM MgCl₂) buffer in 96-well plates on an Eppendorf thermomixer at 42°C for 3 min. Then the reactions started with addition of the reduced form of NAD phosphate (NADPH, final concentration 1 mM) and lasted for 10 min at 700 rpm and 42°C. The reactions were terminated with cold acetonitrile including internal standard. The mixtures were centrifuged at 4°C and 20,000 × g in an Eppendorf 5417R centrifuge (Hauppauge, NY) for 20 min. The supernatants of those samples were dried and redissolved to a half of the initial volumes with 1:1 methanol:water for analysis.

**Liquid Chromatography Mass Spectrometry Analysis**

Metabolites formed from phenacetin, midazolam, temazepam, tolbutamide, and bufuralol were analyzed by a liquid chromatography mass spectrometry system consisting of a Waters Acquity UPLC and a Applied Biosystem API4000 Q trap mass spectrometer. Separation of the metabolites was accomplished using an Acquity UPLC BEH C18 reversed phase column (1.7 μm, 2.1 × 50 mm) eluted with 5 or 10 min gradient using acetonitrile with 0.1% formic acid and water with 0.1% formic acid as mobile phases at a flow rate of 0.4 mL/min. The MRM transitions of metabolites in mass spectrometer are listed in Table 3.

**Data Processing**

The enzymatic reaction parameters Vmax and Km were obtained from nonlinear regression fitting of metabolite formation rates against incubation concentra-
tions of probe molecules to Michaelis-Menten kinetics using SigmaPlot v 9.0 (Systat Software Inc., San Jose, CA). All CYP450 activities were expressed using the ratio of enzymatic parameters Vmax/Km with microliters per minute per milligram of protein.

RESULTS

Ross 708 male broiler chickens grew faster than the females. Both liver weights and BW in the chickens followed the familiar exponential growth curves that started to level off at the later stage of growth in this study (Figure 1a and 1b). The liver weights of new hatched chicks increased faster than their BW in the first week. Therefore, the liver to BW ratio increased during the week. Then this tendency reversed quickly due to accelerated BW increase. The liver to BW ratio peaked at d 7 (Figure 1c). There were no significant differences of liver to BW ratio between the male and female chickens across growth during the study.

Chicken liver microsomes were prepared through a multiple-step procedure at low temperature. Recovery of total CYP450 in the process was evaluated by comparing with spectrophotometric measurement of CYP450 in initial liver homogenates and final liver microsomes. The average recovery was 24.6% in the liver microsome preparation across different ages of chickens (Figure 2).

Total CYP450 in both chicken liver homogenates and specific CYP450 in those chicken liver microsomes varied with age of chickens (Figure 3). The chickens at d 1 posthatch showed the highest content of total hepatic and microsomal CYP450. The total CYP450 content in the liver homogenate of d 1 was 2.7-fold higher than the average of value from d 7 to 28. The specific CYP450 in the liver microsomes of chickens of d 1 posthatch were about 2.2-fold higher than the average value from the chickens of d 7 to 28. The CYP450 declined rapidly in a week and tended to rise slightly from wk 4 to 8 on average. No significant sex difference was observed in those spectrometric measurements of CYP450 at the same ages.

The activities of CYP450 were evaluated using intrinsic clearance of Vmax/Km of phenacetin, midazolam, temazepam, tolbutamide, and bufuralol. Metabolism of those probe molecules was characterized for CYP1A, CYP3A, CYP2H, CYP2C, and CYP2D, respectively. Age effects on enzymatic activities in chicken livers expressed by intrinsic clearance of metabolism of all these CYP450 substrates are shown in Figure 4. The high hepatic enzymatic activities in the liver of Ross 708 broiler chickens were also observed at d 1. The differences between d 1 and other ages of chicken were much more obvious. The age pattern of the activities was similar to that of total CYP450 content. Although the intrinsic clearance (Vmax/Km) varied with age of chicken, the Km values of the metabolites were consistent across the different ages (Table 3).

DISCUSSION

The age patterns of liver to BW ratio are similar between chicken and mammals. The observation that liver to BW ratios peak at neonatal ages has also been noted for rats, dogs, and humans (Tavoloni et al., 1985; Vollmar et al., 2002; Walthall et al., 2005). However, liver maturation and development is quite different between chicken and mammals. The metabolic enzyme activities in fetal livers of mammals are minimal. When neonates of mammals loose umbilical blood supplies and experience dramatic changes in liver blood flow and hepatic oxygenation at birth, hepatic CYP450 isoforms are developed at different rates in the postnatal period (Gow et al., 2001). Therefore, hepatic CYP450 activities of mammals are typically low at birth.

Development of liver and metabolic activities in chickens occurs in the early embryonic stage (Hamilton and Bloom, 1983; Hamilton et al., 1983; Lorr and Bloom, 1987; Lorr et al., 1989; Paolini et al., 1997). During the development of the chick embryo, it has been estimated that more than 90% of the total energy requirement is derived from oxidation of yolk lipid fatty acids. Over the 21-d development of the chick embryo, the last several days are notable as an intense period of lipid metabolism and rapid embryonic growth. About 80% of the entire lipid content of the yolk is mobilized and absorbed into embryonic tissues over this period of time (Noble and Cocchi, 1990). The liver is not only constructed through the lipid metabolism, but the metabolites of lipids also activate particular liver receptors, which then back-regulate expression of CYP450 (Hafner et al., 2011). The assimilation of the large amount of lipid results in rapid increase of hepatic metabolic enzyme activity at late embryonic development.

### Table 3. Cytochrome P450 (CYP450) probe substrates, metabolites, transitions used in multiple reaction monitoring (MRM) and the mean Km values

<table>
<thead>
<tr>
<th>P450 isoform implicated</th>
<th>Substrate</th>
<th>Metabolite</th>
<th>Q1 to Q3</th>
<th>Apparent Km (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Phenacetin</td>
<td>4-Acetamidophenol</td>
<td>152 to 110</td>
<td>98.1 ± 18.6</td>
</tr>
<tr>
<td>2H</td>
<td>Temazepam</td>
<td>Oxazepam</td>
<td>287 to 241</td>
<td>19.5 ± 7.7</td>
</tr>
<tr>
<td>2C</td>
<td>Tolbutamine</td>
<td>Hydroxytolbutamine</td>
<td>287 to 171</td>
<td>678 ± 137</td>
</tr>
<tr>
<td>2D</td>
<td>Bufuralol</td>
<td>1′-OH bufuralol</td>
<td>278 to 186</td>
<td>11.8 ± 5.5</td>
</tr>
<tr>
<td>3A</td>
<td>Midazolam</td>
<td>1′-OH midazolam</td>
<td>342 to 203</td>
<td>1.30 ± 0.67</td>
</tr>
</tbody>
</table>

1 Mean values and SD of liver microsomes of male (n = 3) and female (n = 3) chickens from d 1, 7, 14, 28, and 56.
and early posthatch phases. This embryonic development significantly and rapidly increases chicken hepatic CYP450 level at hatch. This makes the age pattern of chicken hepatic CYP450 opposite to mammals: higher immediately posthatch, reduced in a week, and slightly increased several weeks later.

Figure 1. Ross 708 broiler (a) growth curve, (b) liver weight curve, and (c) liver to BW ratio from d 1 to 56.

Figure 2. Recovery of total cytochrome P450 (CYP450) in the liver microsomes from the liver homogenates of different ages of chickens (n = 6, 3 males and 3 females).

Figure 3. Effect of age on hepatic cytochrome P450 (CYP450) in chicken livers. (a) Total CYP450 in chicken liver homogenates; (b) total CYP450 in chicken liver microsomes.
The hepatic CYP450 contents are similar among different breeds of chickens (Table 4). This hepatic microsomal content is also similar among avian species but lower than most livestock species (Dalvi et al., 1987; Dalvi, 1988; Cottright and Craigmill, 2006), rats (Gay and Ehrich, 1990), and dogs (Khalil et al., 2001). Although overall hepatic CYP450 content in chickens is lower, the catalytic efficiency of CYP450 in chickens is much higher than several other species including cattle, pigs, rats, and horses when expressed as CYP450 turnover number in metabolism of several compounds (Nebbia et al., 2001).

Several cytochrome P450 subfamilies have been found in chicken livers. The CYP1A subfamily is one of these subfamilies identified early in chickens. Both CYP1A4 and CYP1A5 in chick embryo have about 60% amino acid similarities in cDNA sequences as CYP1A of other species (Gilday et al., 1996). A partial gene sequence of CYP1A5 indicates that the intron/exon organization of this gene is identical to that of the CYP1A1 and CY-

![Figure 4. Effect of chicken age on liver microsomal metabolism of (a) phenacetin de-ethylation, (b) midazolam 1-hydroxylation, (c) bufuralol hydroxylation, (d) tolbutamide hydroxylation, and (e) temazepam N-demethylation.](https://academic.oup.com/ps/article-abstract/92/5/1283/1574485/Effect-of-age-on-hepatic-cytochrome-P450-of-Ross?download=true)
P1A2 mammalian genes (Bentivegna et al., 1998; Gannon et al., 2000). The CYP1A1 activity has been evaluated in chicken embryonic tissues and liver through 7-ethoxyresorufin-O-deethylation (Heinrich-Hirsch et al., 1990; Goeger and Anderson, 1992). The maximum hepatic 7-ethoxyresorufin-O-deethylase activity has been found at d 11 of incubation. This CYP1A activity is also identified in chicken liver microsomes posthatch (Nebbia et al., 2003). Using phenacetin de-ethylation as a marker for CYP1A activity in this study, the CYP1A activity in the chicken liver microsomes at d 1 posthatch is about 4 times higher than those observed at later posthatch intervals.

The CYP3A is the most abundantly expressed CYP and accounts for approximately 30 to 40% of the total CYP content in human liver and small intestine (de Wildt et al., 1999). This CYP subfamily mediates approximately 50% of the metabolism of all marketed human drugs (Liu et al., 2007). An isoform of CYP3A designated as CYP3A37 has been identified in chicken liver. The sequence of its cDNA shares 60% homology with CYP3A of other species. This cDNA produces a protein that is recognized by polyclonal rat CYP3A1 antibody (Ourlin et al., 2000). The CYP3A activity in chicken has been investigated using both CYP3A specific substrates (Nebbia et al., 2001; Cortright and Craigmill, 2006; Kawalek et al., 2006; Blevins et al., 2012) and antibody (Coulet et al., 1996; Diaz et al., 2010). Metabolism of midazolam is predominantly mediated by CYP3A in mammals. The intrinsic clearance of midazolam to 1-hydroxylation of midazolam in more than 17-wk-old male Japanese Leghorn chickens is about 6.7 ± 2.2 μL/min per mg of protein in midazolam 1-hydroxylation (Khalil et al., 2001) compared with the value in this work, 2.3 ± 2.2 μL/min per mg of protein of male and female Ross 708 broilers from d 7 to 28. The CYP2D activity is about 16 times higher in the liver collected from d 1 posthatch chickens than those from other ages.

The CYP2C family members are instrumental in the metabolism of 20 to 25% of commonly prescribed drugs in humans. A CYP2D enzyme has been characterized from chicken liver as CYP2D49. This enzyme cDNA contains an open reading frame of 502 amino acids that share 52 to 57% identities with other CYP2D. The gene structure and neighboring genes of CYP2D49 are conserved and similar to those of human CYP2D6 (Cai et al., 2012). Hydroxylation of bufuralol has been investigated as a CYP2D probe previously (Khalil et al., 2001). The intrinsic clearance of CYP2D identified in more than 17-wk-old male Japanese Leghorn broiler chicken is about 6.7 ± 2.2 μL/min per mg of protein in bufuralol 1-hydroxylation (Khalil et al., 2001) compared with the value in this work, 2.3 ± 2.2 μL/min per mg of protein of male and female Ross 708 broilers from d 7 to 28. The CYP2D activity is about 16 times higher in the liver collected from d 1 posthatch chickens than those from other ages.

The CYP2C is another important subfamily of cytochrome P450. Two proteins in the liver of the chick embryo have been found to interact with CYP2C11 antibody and one of them is inducible by phenobarbital (Machala et al., 1996). The CYP2C45 has been reported in chicken liver with its sequence sharing up to 58% amino acid identity of other CYP2C in other species (Baader et al., 2002). Hydroxylation of tolbutamide as CYP2C probe was found to have 50 times higher activity in chicken liver microsomes than in dog liver microsomes (Khalil et al., 2001). In this study, tolbutamide
hydroxylation activity is about 10 times higher in the liver of d-1 chicks than those of other ages of chickens. 

For all substrates tested in the study, the apparent Km does not change with the age of chicken (Table 3). The differences of intrinsic clearance at different ages of chicken are dependent on Vmax, the maximum metabolism rate. The Vmax is directly proportional to the amount of enzymes. Therefore, the differences of activities of those CYP reflect a different amount of those CYP enzymes at different ages of chicken. Some sex differences have been observed in CYP450 activities in this study. Although the higher hepatic CYP450 content and activities in males of 13-mo-old White Leghorn chickens has been reported previously (Pampori and Shapiro, 1993), further investigation will be needed.

In summary, age has significant impact on hepatic CYP450 content and activities in Ross 708 broiler chickens. The age pattern in hepatic CYP450 is almost opposite from most mammals. The hepatic CYP450 content and activities are higher at d 1 posthatch, reduced in a week, and slightly increased several weeks later. The CYP450 content is two times greater in the liver at d 1 posthatch than in other ages of chickens. The differences of hepatic CYP450 activities between d 1 and other ages of chickens are much larger, and these differences are CYP450 isoform dependent.

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