Expression of Genes Involved in the Somatotropic, Thyrotropic, and Corticotropic Axes During Development of Langshan and Arbor Acres Chickens

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ABSTRACT We investigated changes in mRNA expression of the somatotropic, thyrotropic, and corticotropic axes of Langshan (LS) and Arbor Acres (AA) broiler chickens during embryonic and postnatal development. We found an inverse expression profile between pituitary growth hormone (GH) and hepatic GH receptor mRNA [postnatal d (P)28 to P42], insulin-like growth factor (IGF)-I, and IGF-IR (P0 to P42), respectively. Hepatic IGF-I was a major point of control in the GH-IGF axis from P0 to P28. Pituitary GH-releasing hormone receptor may serve an autocrine-paracrine function from P0 to P28, and hypothalamic ghrelin may affect growth by stimulating the release of hepatic IGF-I from embryonic d (E)8 to P28. Hypothalamic ghrelin might interact with corticotropin-releasing hormone (CRH) from P0 to P28. Hepatic IGF-binding protein-2 regulated growth by regulating hepatic IGF-II bioavailability from P0 to P42. Hepatic IGF-binding protein-5 was an important IGF mediator. A coexpression profile was found between hypothalamic GH-releasing hormone (E10 to E16 and P0 to P42), somatostatin (SS; P0 to P28), thyrotropin-releasing hormone (E10 to E16 and P0 to P28), ghrelin (P0 to P42), and pituitary GH mRNA, hypothalamic SS (P0 to P28), corticotropin-releasing hormone (P0 to P42), thyrotropin-releasing hormone (E10 to E18 and P0-P42), and thyroid-stimulating hormone-β mRNA, respectively. Moreover, AA chickens were fed a nutrient-rich AA diet (as a control group) and LS chickens were fed either a less nutritious LS diet or the AA diet. Langshan and AA chickens fed the same AA diet showed no differences in pituitary GH, hypothalamic SS, ghrelin, hepatic IGF-I, or GH receptor mRNA. Our data indicate that select genes may show parallel expression during certain periods of development, and that differences in BW and gene expression respond differently to nutrient intake in LS and AA chickens. Our findings may help improve the molecular breeding of chickens.

Key words: gene expression, coexpression, nutrition

INTRODUCTION

In the developing pituitary gland, somatotrophs secrete growth hormone (GH; Porter et al., 1995) and thyrotrophs produce thyroid-stimulating hormone (TSH; Nakamura et al., 2004). Growth hormone secretion in the chicken is under the control of hypothalamic factors [GH-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH), and somatostatin (SS)], among which SS has an inhibitory effect (Tannenbaum et al., 1990; Harvey et al., 1991). Hypothalamic factors known to influence TSH release in the chicken include TRH, corticotropin-releasing hormone (CRH), and SS (Geris et al., 2002, 2003; De Groef et al., 2003; Kawasaki et al., 2003).

The biological actions of GHRH are mediated by GHRH receptor (GHRHR), a G-protein-coupled receptor specifically localized in the somatroph of the pituitary (Morel et al., 1999). Data on the expression of GHRHR mRNA in the embryonic pituitary is limited in vertebrates, including birds. In the liver of chickens, GH receptor (GHR) mRNA is detected transitorily between embryonic d (E)15 and E17 (Burnside and Cogburn, 1992), and the young of chicken strains that grow rapidly exhibit greater hepatic GHR binding (Vanderpooten et al., 1993).

Ghrelin, which is produced mainly by the stomach, displays strong GH-releasing activity, mediated by the activation of the GH secretagogue (GHS) receptor type 1a, which is specific for synthetic, peptidyl, and nonpeptidyl GHS (Kojima et al., 1999, 2001). Chicken ghrelin and GHS receptor type 1a mRNA is also expressed in the hypothalamus (Howard et al., 1996; Richards et al., 2006). Ghrelin also stimulates CRH release from hypothalamic explants in rodents (Wren et al., 2002).
In birds, insulin-like growth factor (IGF)-I and IGF-II act as endocrine or paracrine-autocrine signals whose physiological effects are mediated mainly by their binding to a common receptor (IGF type I receptor; McMurtry et al., 1997). The activity of IGF is regulated by IGF-binding proteins (IGFBP), a family of 6 or more related proteins that bind IGF with a high affinity (Mohan and Baylink, 2002).

We used real-time quantitative PCR (Q-PCR) to assess gene expression profiles in the hypothalamus, pituitary, and liver of slow-growing Langshan (LS) and fast-growing Arbor Acres (AA) broiler chickens. Very few studies have assessed the effect of diet on differences in BW and mRNA expression of genes involved in the somatotropic, thyrotropic, and corticotropic axes during postnatal development of different chicken strains. Therefore, we fed AA chickens a nutrient-rich AA diet, and fed LS chickens either a less nutritious LS standard diet (LL) or the AA standard diet (LA) under the same environmental conditions. We investigated the potential effects of diet on differences in BW and mRNA expression between the 2 strains during the postnatal development period, and quantified the expression changes during the embryonic and postnatal development periods.

**MATERIALS AND METHODS**

**Animals and Tissues Sampling**

Fertile LS and AA chicken eggs were incubated in a standard commercial incubator. On incubation d 8, 10, 12, 14, 16, and 18, 8 eggs were removed, embryos were weighed, and samples of the hypothalamus, pituitary, and liver were collected. The LS and AA poults were reared from hatching to 7 wk of age under normal conditions at the Nanjing Agricultural University Animal Farm. The AA chickens were fed the AA standard diet, and LS chickens were divided into 2 groups and fed either the LL diet or the LA diet. The differences in nutritional value between the LL diet and AA diets are shown in Table 1. Diets and water were available ad libitum.

**Table 1. Ingredients and analysis of the diets**

<table>
<thead>
<tr>
<th>Item</th>
<th>Lansheng diet</th>
<th>Arbor Acres diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow cornmeal</td>
<td>55</td>
<td>56</td>
</tr>
<tr>
<td>Soybean meal (dehulled)</td>
<td>11</td>
<td>22.5</td>
</tr>
<tr>
<td>Soybean powder (expanded)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<td>1.7</td>
</tr>
<tr>
<td>Limestone powder</td>
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<td>1.5</td>
</tr>
<tr>
<td>Salt</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Corn gluten feed</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Calculated nutrient composition

| ME (MJ/kg) | 12.09 | 12.99 |
| CP (%)     | 17.54 | 22.23 |
| Amino acids (%) |
| Lysine     | 0.85  | 1.27  |
| Methionine | 0.29  | 0.36  |
| Methionine + cystine | 0.58 | 0.70 |
| Total P (%) | 0.78  | 0.78  |
| Calcium (%) | 1.13  | 1.22  |

1Supplied (mg/kg diet) niacin, 48; folic acid, 1.2; calcium pantothenate, 12; roxarsone, 50; biotin, 0.06; salinomycin, 90; vitamin A, 5,000 IU; vitamin K₂, 2.1; vitamin B₁, 2.4; vitamin B₂, 9; vitamin B₆, 5.1; vitamin B₁₂, 0.02; vitamin D₃, 3,000 IU; vitamin E, 25.5; Fe, 69, as FeSO₄·H₂O; Mn, 98.6, as MnSO₄·H₂O; Cu, 8, as CuSO₄·5H₂O; I, 1.14, as KIO₃; Zn, 84, as ZnSO₄·H₂O; Se, 0.30, as NaSeO₃·5H₂O.

Establishment of Standards of Chicken Genes for Real-Time Q-PCR

Recombinant plasmids containing a specific fragment of each target chicken gene and the endogenous reference gene (β-actin) were constructed to establish the real-time Q-PCR standards. Briefly, specific fragments of respective genes were amplified by using the same primers as in subsequent real-time Q-PCR (Table 2). The PCR products were excised after being confirmed by electrophoresis on 2% Tris-acetate-EDTA agarose gel and purified by a V-gene DNA Purification Kit (V-gene Biotechnology Ltd., Beijing, China) protocol. The first mix was prepared with a final volume of 10 μL by using 2 μg of total RNA, 1 μg of Oligo(dT)₁₅ (Promega), and 0.1% diethyl-pyrocarbonate solution (Promega). This mix was heat-denatured at 70°C for 5 min. Samples were then placed on ice and briefly centrifuged. The second mix was prepared with a final volume of 15 μL by using 0.5 mM dNTP, 25 U of RNase inhibitor (N2511, Promega), 200 U of M-MLV (Moloney murine leukemia virus) transcriptase (M1701, Promega), 5x RT buffer, and 0.1% diethyl-pyrocarbonate solution. The second mix was then added to each sample to form a 25-μL reaction mix. The mixtures were incubated at 42°C for 1 h and then at 95°C for 5 min. The cDNA (RT products) were obtained and stored at −20°C for quantification by real-time Q-PCR.

**RNA Extraction and cDNA Synthesis**

Total RNA was isolated from the hypothalamus, pituitary, and liver by using TRIzol reagent (15596-026, Invitrogen, Beijing, China) according to the manufacturer’s protocol. The integrity of RNA was verified electrophoretically by ethidium bromide staining, and its purity was examined by UV spectrophotometry [optical density (OD) 260 to OD280]. Reverse transcription (RT) was carried out following the Promega (Shanghai, China) protocol. The first mix was prepared with a final volume of 10 μL by using 2 μg of total RNA, 1 μg of Oligo(dT)₁₅ (Promega), and 0.1% diethyl-pyrocarbonate solution (Promega). This mix was heat-denatured at 70°C for 5 min. Samples were then placed on ice and briefly centrifuged. The second mix was prepared with a final volume of 15 μL by using 0.5 mM dNTP, 25 U of RNase inhibitor (N2511, Promega), 200 U of M-MLV (Moloney murine leukemia virus) transcriptase (M1701, Promega), 5x RT buffer, and 0.1% diethyl-pyrocarbonate solution. The second mix was then added to each sample to form a 25-μL reaction mix. The mixtures were incubated at 42°C for 1 h and then at 95°C for 5 min. The cDNA (RT products) were obtained and stored at −20°C for quantification by real-time Q-PCR.

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9-T vector (TakaRa, Dalian, China) and subsequently transformed into competent *Escherichia coli* DH5α cells. The recombinant plasmids were purified with a plasmid Maxi Kit (Generay Biotech Co. Ltd., Shanghai, China) according to the manufacturer's instructions and solubilized in sterile water as stocks. The concentrations of the stocks were determined by measuring at OD260 with a spectrophotometer (WFZ800-D3B, Rarleigh Analytical Instrument Corp., Beijing, China). For a standard curve, serial 10-fold dilutions of these stocks were made to obtain batches of standards in the range of 10<sup>1</sup> to 10<sup>6</sup> copies/μL for real-time Q-PCR.

### Real-Time Q-PCR Assay

Quantitative analysis of PCR was performed in the DNA Engine Opticon (MJ Research, Beijing, China) by using SYBR Green I (RS0976, Generay, Shanghai, China). Primer sequences, annealing temperature, and the approximate sizes of the amplified fragments are shown in Table 2. For each amplification, 2-μL standards or test RT products or water was added to a premix of 18 μL that contained 10 μL of SYBR Premix Ex Taq (2x; DRR041A, Takara), 1 μL of primer mix (0.2 μM final concentration each; Table 2), and 7 μL of sterile water. The optimized thermal cycling programs were 95°C for 1 min, followed by 45 cycles of 95°C for 10 s, annealing temperature (Table 2) for 20 s, and 72°C for 20 s. Specificities of amplification products were confirmed by melting curve analysis (60 to 95°C in 0.2°C/s increments).

### Table 2. Primer sequences and PCR conditions<sup>1</sup>

<table>
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<tr>
<th>Item</th>
<th>Accession no.</th>
<th>Primer sequence (5′→3′)</th>
<th>Size (bp)</th>
<th>AT&lt;sup&gt;2&lt;/sup&gt; (°C)</th>
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<tr>
<td>β-actin</td>
<td>NM_205518</td>
<td>F&lt;sup&gt;3&lt;/sup&gt; 5′-TGCGTGACATCAAGGAGAAG-3′</td>
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<td>IGF-I</td>
<td>NM_001004384</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; 5′-TGCCAGGTATCATTGTTGTA-3′</td>
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<td>59</td>
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<tr>
<td>IGF-II</td>
<td>XM_421026</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; 5′-TGCCAGGTATCATTGTTGTA-3′</td>
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<td>66</td>
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<tr>
<td>IGF-IR</td>
<td>AJ223164</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; 5′-TGCCAGGTATCATTGTTGTA-3′</td>
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<td>IGFBP-2</td>
<td>NM_205359</td>
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<td>299</td>
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<tr>
<td>IGFBP-5</td>
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<tr>
<td>GHR</td>
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<tr>
<td>TSHβ</td>
<td>BI392271</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; 5′-TGCCAGGTATCATTGTTGTA-3′</td>
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<tr>
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<tr>
<td>SS</td>
<td>X60191</td>
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<tr>
<td>GHRH</td>
<td>AY956323</td>
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<td>52</td>
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<tr>
<td>GHRHR</td>
<td>DQ230840</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; 5′-TGCCAGGTATCATTGTTGTA-3′</td>
<td>93</td>
<td>64.5</td>
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<td>Ghrelin</td>
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<td>195</td>
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<td>CRH</td>
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<td>92</td>
<td>62</td>
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</table>

<sup>1</sup>IGF = insulin-like growth factor; IGFBP = IGF-binding protein; GH = growth hormone; GHR = GH receptor; GHRH = GH-releasing hormone; GHRHR = GHRH receptor; TSHβ = thyroid-stimulating hormone β-subunit; SS = somatostatin; TRH = thyrotropin-releasing hormone; CRH = corticotropin-releasing hormone.

<sup>2</sup>AT = annealing temperature.

<sup>3</sup>F = forward; R = reverse.

### Real-Time Q-PCR Data Calculation

The method used for quantification of expression was the relative standard curve method. The quantification was normalized to an endogenous control (β-actin), and standard curves were prepared for each target gene and the endogenous reference. For each experimental sample, the amount of mRNA of each target gene and β-actin was determined from their respective standard curves. The exported Excel (Microsoft Corporation, Redmond, WA) files contained the threshold cycle values (Ct, which was defined as the cycle at which fluorescence rose above the background for each sample during the log-linear phase of the reaction) and the copy concentrations of the respective gene in test samples. The mRNA relative expression level of each target gene in test samples was expressed as the copy concentration of each target gene divided by that of the β-actin gene (target gene/β-actin).
Statistical Analysis

All data were analyzed with SPSS statistical software (Version 13.0, SPSS Inc., Chicago, IL). Data on ontogenic changes of gene expression were subjected to a one-way ANOVA, followed by Duncan's multiple range tests for comparison of the means. For evaluating the effects of the diet on gene expression in general at postnatal development on P14, P28, and P42, the GLM procedure (with developmental age and strain as fixed factors) of the SPSS software was used, in which the option of Duncan's multiple range test was selected. Results are expressed as the means ± SEM. Significance for the mean differences was set at \( P \leq 0.05 \). A Pearson correlation coefficient was used to characterize the relationship between BW and gene mRNA expression.

RESULTS AND DISCUSSION

Developmental Changes in BW of LS and AA Chickens

Body weight increased rapidly after hatching in both strains (Figure 1). The GLM analysis revealed a difference between the strains at P14, P28, and P42 when the data were pooled \( F(1, 42) = 104.17 \) (BW); \( P \leq 0.05 \) (Duncan's multiple range test). The more rapidly growing AA chickens displayed significantly greater BW than the slower growing LL chickens (\( P \leq 0.05 \)), although the difference between the strains was not significant for some age groups. A difference in BW \( F(1, 42) = 39.17; P \leq 0.05 \) still existed between the LA and AA chickens.

Ontogenic Changes of mRNA Expression of Genes

In the hypothalamo-pituitary-liver axis, GH combines with GHR at the surface of liver cells and promotes the synthesis and secretion of IGF, which acts on the target tissues and regulates cellular proliferation and differentiation through the endocrine or paracrine-autoparacrine systems. We assessed the expression profiles of GH, GHR, and IGF at the transcription level. Pituitary GH mRNA (McCann-Levorse et al., 1993) typically has a different expression profile than hepatic GHR mRNA (Mao et al., 1998) in chickens. Growth hormone mRNA peaked at P28 and then decreased through P42, whereas hepatic GHR mRNA continued to increase (Figure 1). These data indicate that GH and GHR mRNA expression profiles were inversely related between P28 and P42 in LS and AA chickens. In addition, hepatic GHR mRNA (P0 to P42) was positively correlated with BW (Table 3), consistent with previous reports that hepatic GHR gene expression helps control rapid growth in young chickens (Zhao et al., 2004) and that GH depresses GHR mRNA levels in the liver cells of chickens (Hull and Harvey, 1998). The inverse

![Graph showing BW and relative expression levels of pituitary growth hormone (GH), hepatic GH receptor (GHR), insulin-like growth factor (IGF)-I, and IGF-II mRNA in Langshan (LS) and Arbor Acres (AA) chickens. Values represent means ± SEM of 8 observations. LL = LS chickens fed the LS diet; LA = LS chickens fed the AA diet; AA = AA chickens fed the AA diet. The GLM analysis revealed differences between the strains when data from P14, P28, and P42 were pooled \( [F(1, 42) = 104.17 \) (BW); \( 7.78 \) (GH), \( 11.47 \) (GHR), \( 7.18 \) (IGF-I); \( P \leq 0.05 \) (Duncan's multiple range test)]. Arbor Acres chickens exhibited greater BW, GHR, and IGF-I mRNA expression compared with LL chickens, whereas the opposite was true for GH mRNA, although differences between the strains for some age groups were not significant. Differences in GH, GHR, and IGF-I mRNA disappeared \( [F(1, 42) = 1.88 \) (GH), \( 1.85 \) (GHR), \( 2.81 \) (IGF-I); \( P \geq 0.05 \)], whereas differences in BW \( [F(1, 42) = 39.17; P \leq 0.05 \) remained. a–k Different letters above each bar denote statistically significant \( (P \leq 0.05 \) differences for mean comparisons.)
profile of GH and GHR mRNA expression from P28 to P42 suggests that these 2 genes play a sensory role, relaying the reversed expression profile signal to cellular mechanisms of growth regulation. Insulin-like growth factor-I mRNA tended to increase from hatching to P28, where it peaked, whereas IGF-II mRNA expression displayed a greater level only through E16 and E18 just before hatching (Figure 1). These results support previous reports that IGF-I plays a larger role in mediating the growth-promoting effects of GH on tissue growth during postnatal development, whereas IGF-II is more influential during embryonic growth and development (Dupont and Holzenberger, 2003). Rosselot et al. (1995) suggested that IGF-I is expressed in the liver in a GH-dependent manner only after hatching. We found that hepatic IGF-I mRNA had a positive correlation with GH mRNA (P0 to P28; Table 3), which suggests that IGF-I and GH mRNA expression might show parallel expression from P0 to P28 in both chicken strains. Liver production of IGF-I is a major point of control in the GH-IGF axis, the endocrine system that regulates body growth in fish and other vertebrates (Pierce et al., 2005). The parallel expression of IGF-I and GHR suggests that IGF-I is a major point of control in the GH-IGF axis from P0 to P28 in chickens. Furthermore, IGF-II appears not to regulate growth after hatching in chickens (Spencer et al., 1996).

Hypothalamic GHRH, SS, TRH, and ghrelin control the secretion of GH. The parallel expression effect of genes has not been studied during either embryonic or postnatal development. We found that GHRH mRNA was comparatively high through stage E16, tended to increase during the first 4 postnatal weeks, and declined through P42 (Figure 2). However, reports on the expression profile of GHRH mRNA in the hypothalamus of birds are limited. We found that SS mRNA increased sharply through E18, decreased through P0, tended to increase from P0 to P28, and slightly decreased through P42 (Figure 2); the TRH mRNA level increased progressively during the last week of incubation and continued to increase after hatching, and ghrelin mRNA peaked on P28 (Figure 2). These results agree with earlier reports on the expression of SS (Geris et al., 1998; Zhao et al., 2004), TRH (Geris et al., 1998, 1999), and ghrelin (Chen et al., 2007) mRNA in chickens. Correlation analysis showed that GH mRNA was positively correlated with hypothalamic GHRH (E10 to E16 and P0 to P42), SS (P0 to P28), TRH (E10 to E16 and P0 to P28), and ghrelin (P0 to P42) mRNA (Table 3). These results suggest that GH, GHRH, and TRH mRNA may exhibit parallel expression from E10 to E16, and GH, GHRH, SS, TRH, and ghrelin mRNA might exhibit parallel expression from P0 to P28 in LS and AA chickens. Growth hormone-releasing hormone stimulates somatotroph differentiation and GH synthesis and secretion, whereas SS suppresses GH release in response to GHRH. In mammals, TRH is somatotropic and exhibits equal or somewhat less potent release
of GH than GHRH, and thyroid hormones provide an inhibitory feedback for this TRH-induced GH release (Scanes and Harvey, 1989). In addition, ghrelin, which binds to and activates the GHS receptor, thereby potentially stimulating GH secretion (Kojima et al., 1999), links the metabolic status of an animal directly to GH release. The concurrent expression of GHRH (E10 to E16 and P0 to P42), SS (P0 to P28), TRH (E10 to E16 and P0 to P28), ghrelin (P0 to P42), and GH suggests that primary hypothalamic control of GH production is both stimulatory and inhibitory during the corresponding developmental period.

Pituitary GHRHR plays an important role in the functional differentiation or proliferation of GH-producing cells in mammals (Godfrey et al., 1993; Lin et al., 1993). Activation of GHRHR by GHRH stimulates G-protein and increases intracellular calcium, which causes the release of premade GH and an increase in intracellular cyclic adenosine monophosphate; this in turn activates protein kinase A and stimulates the transcription of the GH gene (Muller et al., 1999). In agreement with previous findings (Ellestad et al., 2006; Wang et al., 2006), abundant expression of GHRHR mRNA already appeared by E12 and continued to show strong expression before hatching (Figure 3). Rare somatotroph cells are detected during E12 (Porter et al., 1995; Porter, 2005). Hence, the high expression of GHRHR mRNA before somatotroph differentiation suggests that GHRHR may be actively involved in the rapid expansion of these undifferentiated progenitor cells. As shown in Figures 1 and 3, GHRHR and GH mRNA simultaneously showed high expression levels during later embryonic stages, suggesting that GHRHR might have a conserved role in GH cell differentiation in late embryonic stages in the chicken. In addition, GHRHR mRNA showed a positive correlation with GH mRNA during postnatal development (Table 3). The coexpression of GHRHR and GH suggests that pituitary-produced GHRHR might play an autocrine-paracrine role from P0 to P28 of the chicken pituitary. Our results strongly suggest that GHRHR plays a key role in the hypothalamo-pituitary (HP) GH axis.

The action of ghrelin on cell proliferation and apoptosis could be due to its stimulatory effects on the release of IGF-I, a growth factor known to affect these functions (Gimpl and Fahrenholz, 2001; Schams and Berisha, 2002, 2004). We found that ghrelin mRNA (E8 to P28) was correlated with hepatic IGF-I mRNA (Table 3), suggesting that hypothalamic ghrelin and hepatic IGF-I mRNA might show parallel expression from E8 to P28 in LS and AA chickens. Ghrelin may also affect cell proliferation and apoptosis by stimulating the release of IGF-I from E8 to P28. Moreover, in mammals, ghrelin may activate the hypothalamo-pituitary-adrenal (HPA) axis via CRH (Schleim et al., 1999; Asakawa et al., 2001). In neonatal chicks, the inhibitory effect of central ghrelin on food intake is caused by the activation of the endogenous CRH system (Saito et al., 2005). As shown previously in rats (Grino et al., 1989) and chickens (Vandenborne et al., 2005), we found that CRH mRNA decreased in E16, remained low through P0, and then increased after hatching (Figure 3). Corticotropin-releasing hormone plays a major role in behavioral responses to stressors and in the activation of the HPA axis (Inui, 1999). We found that ghrelin mRNA was positively correlated with CRH mRNA (P0 to P28; Table 3), suggesting that hypothalamic ghrelin and CRH mRNA might show parallel expression be-
between P0 and P28 in LS and AA chickens. That is, hypothalamic ghrelin might interact with CRH, and subsequently the HPA axis, in chickens from P0 to P28.

Thyroid-stimulating hormone is produced by a specific population of cells in the pituitary called thyrotropes. Thyrotropes first appear in the chick pituitary gland as early as E6.5, increase considerably on E11.5 (Thommes et al., 1983), and continue to increase through E17 (Muchow et al., 2005). Similar to previous studies on chickens (Gregory et al., 1998; Nakamura et al., 2004; Ellestad et al., 2006), we found that TSH β-subunit (TSHβ) mRNA increased before hatching, decreased markedly through P0, then increased through P14 (Figure 3). Hypothalamic factors known to influence TSH release in the chicken include TRH, CRH, and SS. Thyroid-stimulating hormone not only affects TSH secretion, but also affects TSH biosynthesis in birds (Kawasaki et al., 2003). Corticotropin-releasing hormone-induced TSH secretion is mediated by CRH receptor 2 expressed on thyrotropes (De Groef et al., 2003). Somatostatin inhibits the release of TSH by the pituitary gland by lowering the sensitivity of thyrotropes to TRH and CRH (Goris et al., 2002, 2003). We found that TSHβ mRNA had a positive correlation with SS (P0 to P28), CRH (P0 to P42), and TRH mRNA (E10 to E18 and P0 to P42; Table 3). The TSHβ and TRH mRNA might exhibit parallel expression between E10 and E18, and TSHβ, SS, CRH, and TRH mRNA might show parallel expression between P0 and P28. The concurrent expression of SS (P0 to P28), CRH (P0 to P42), TRH mRNA (E10 to E18 and P0 to P42), and TSHβ suggests that TRH, CRH, and SS play autocrine-paracrine roles in the TSH secretion mechanism in the HP axes during the corresponding developmental period of chickens.

Birds have both IGF-IR and IGF-IIR; however, the functions of IGF-I and IGF-II are mediated by the IGF-I receptor (McMurtry et al., 1997). As shown in Figure 4, there was steady expression of hepatic IGF-IR mRNA during embryonic development and high expression during the early postnatal period (P0 to P14). We found an inverse relationship between the expression levels of IGF-I and IGF type I receptor mRNA in the liver during the first 4 postnatal weeks (Table 3), similar to a study by Armstrong and Hogg (1992). This may indicate coordinated developmental regulation of these 2 genes in the chicken liver from P0 to P42. However, Richards et al. (2005) reported an inverse relationship between IGF-I and IGF-1R expression in the turkey brain, but not in the liver, through wk 3 posthatching. These conflicting findings suggest that the association of IGF-I and IGF-IR expression in poultry depends on the genetic strain of the animal, as well as age and tissue type.

In birds, IGFBP-2 is thought to be a main IGFBP (Yang et al., 1993). Insulin-like growth factor-binding protein-2 may act as an anabolic factor, particularly by increasing IGF-II bioavailability (Conover and Khosla, 2003). As shown in Figures 3 and 4, the expression of IGFBP-2 mRNA was similar to that of IGF-II, peaking just before hatching and showing a low level during postnatal development. We also found that IGFBP-2 mRNA was significantly related to IGF-II mRNA (E18 to P42; Table 3). These findings suggest that these 2 genes may show parallel expression between E18 and P42 in LS and AA chickens. In addition, IGFBP-2 mRNA (P0 to P42) had a negative correlation with BW (Table 3), supporting previous findings that IGFBP-2 regulates growth negatively during postnatal development in chickens (Kita et al., 2002). Insulin-like growth factor-binding protein-2 may have a high affinity for IGF-II and tightly regulate IGF-II availability during fetal and early neonatal growth (Gordon et al., 2005). Therefore, IGFBP-2 may regulate growth by regulating IGF-II bioavailability from P0 to P42 in chickens. Similar to a previous study on turkeys (Richards et al., 2005), we found that hepatic IGFBP-2 mRNA expression maintained a constant level throughout development in LS and AA chickens (Figure 4). Insulin-like growth factor-binding protein-5 may be an important
mediator of IGF paracrine action (Jones et al., 1993). The constant level of IGFBP-5 mRNA expression in our study also suggests that IGFBP-5 is an important IGF mediator.

**Effect of Diet on Growth and Gene Expression**

The effects of fasting, protein deficiency, and calcium deficiency on growth and endocrine status have been studied intensively in chickens (Scanes and Griminger, 1990), but the impact of diet has often been neglected or compromised, regardless of the fact that chickens selected for different traits have different nutritional requirements for optimal production. Therefore, we investigated the effects of diet in the 2 strains, and found that diet had different effects on BW and gene expression among chickens fed the LL, LA, and AA (control) diets. Langshan and AA chickens fed the same AA diet showed no differences in pituitary GH, hypothalamic SS, ghrelin, hepatic IGF-I, or GHR mRNA expression, but did show differences in BW and hepatic IGF-IR mRNA expression. All data shown in Figures 1 to 4 are in agreement with previous findings that differences among chicken strains in growth characteristics and somatotropic gene expression patterns are caused by both genotypic and nutritional factors (Zhao et al., 2004).

The effects of diet on gene expression in rodents, humans, and birds may be comprehensive, depending on the composition of the diet. For example, severe methionine deficiencies of 0.3 and 0.2% cause graded reductions in food intake and weight gain in chickens (Carew et al., 2003). In our study, the average BW of LS chickens fed a higher methionine AA diet was 17.1% greater at P42 than that of chickens fed the LL diet (Figure 1). Chickens that consume a low-protein diet exhibit depressed plasma IGF-I levels, which return to normal upon restoring dietary protein (Rosebrough and McMurtry, 1993). We found that the protein-rich AA diet up-regulated IGF-I mRNA by 23.5% in LS chickens at P28 (Figure 1). Matsumura et al. (1996) suggested a tissue- and development-specific response of chicken IGF-IR gene expression to nutritional status. We found that the AA diet, which had greater CP, lysine, and methionine contents, resulted in down-regulating IGF-IR mRNA by 18.2% in LS chickens at P28 (Figure 4). In rats, fasting for 24 and 48 h significantly decreased ghrelin mRNA expression in the hypothalamus to 24 and 28% of control values, respectively (Sato et al., 2005). In our study, the AA diet led to down-regulating ghrelin mRNA expression by 30.8% in LS chickens at P28 (Figure 2), suggesting that hypothalamic ghrelin mRNA expression varies with nutritional status in vertebrates, including birds. Further studies should investigate the link between diet and the mechanism regulating chicken gene expression.

In conclusion, we demonstrated the changes in gene mRNA expression involved in the somatotropic, thyrotropic, and corticotropic axes during embryonic and postnatal development of 2 strains of chickens (LS and AA), and found strong evidence for parallel expression among genes during certain developmental periods. We found a reversed profile for GH and GHR mRNA expression from P28 to P42, suggesting that these 2 genes play a sensory role, relaying the reversed expression profile signal to cellular mechanisms of growth regulation. The inverse relationship between the expression of IGF-I and IGF-IR could indicate possible coordinated developmental regulation of these 2 genes in chicken liver tissue from P0 to P42. The parallel expression of IGF-I and GH suggests that IGF-I is a major point of control in the GH-IGF axis from P0 to P28. The concurrent expression of GHRH (E10 to E16 and
P0 to P42), SS (P0 to P28), TRH (E10 to E16 and P0 to P28), ghrelin (P0 to P42), and GH mRNA suggests that the primary hypothalamic control for the production of GH is both stimulatory and inhibitory during the corresponding developmental period. The coexpression of GHRHR and GH suggests that pituitary-produced GHRHR might play an autocrine-paracrine role from P0 to P28 of the chicken pituitary. The concurrent expression of SS (P0 to P28), CRH (P0 to P42), TRH mRNA (E10 to E18 and P0 to P42), and TSHβ suggests that TRH, CRH, and SS play an autocrine-paracrine role in TSHβ secretion in the HP axes during the corresponding period of development. We also speculated that ghrelin affected cell proliferation and apoptosis by stimulating the release of IGF-I from E8 to P28. Hypothalamic ghrelin might interact with CRH, and subsequently the HPA axis, from P0 to P28. Insulin-like growth factor-binding protein-2 regulated growth by regulating IGF-II bioavailability from P0 to P42. The constant level of IGFBP-5 mRNA expression suggests that IGFBP-5 was an important IGF mediator.

Finally, LS and AA chickens fed the same AA diet showed no differences in pituitary GH, hypothalamic SS, ghrelin, hepatic IGF-I, or GHR mRNA expression, but did show differences in BW and hepatic IGF-IR mRNA expression. In addition, select genes may show parallel expression during certain periods of development. These findings may help improve the molecular breeding of chickens.

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