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Pattern of GHR mRNA expression and body growth in the S2 line of sex-linked dwarf chickens

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ABSTRACT. Sex-linked dwarf (SLD) chickens have been widely used in cross breeding of broilers and laying hens. To study the molecular mechanisms underlying growth hormone receptor (GHR) in SLD chickens, the expression profiles of GHR were measured in three growth related tissues (liver, breast, and thigh) in male and female S2 SLD chickens at seven growth stages (1 day, 3 weeks, 7 weeks, 9 weeks, 11 weeks, 13 weeks, and 15 weeks). Growth curves of body weight were fitted using logistic and Gompertz models. The results show that the inflexion week and inflexion weight in male chickens was earlier than in female chickens. Regarding the expression profiles of GHR, there was no significant difference between tissues at hatching. The expression peaked at 7 weeks and dropped by degrees in muscle tissue; hepatic expression increased with age and was positively correlated with body

weight. Taken together, these results would provide a basis for further study on the molecular mechanisms underlying GHR regulation in SLD chickens.

Key words: Sex-linked dwarf chickens; GHR; Growth curve

INTRODUCTION

Dwarf chickens, characterized by low body weight (BW) and short bone growth (Guillaume, 1976), are an important genetic resource for breeding applications and biological study. The phenotype is caused by the sex-linked dwarfing (SLD) gene (Hutt, 1959). Previous studies confirmed that the dwarf gene is a growth hormone receptor (GHR) regulator. Leung et al. (1987) suggested that the lack of, or greatly reduced, GHR may be a major contributing factor to dwarfism. Other research indicates that point and deletion mutations, structural gene mutations, and mutations within the GHR regulatory region all resulted in the SLD phenotype (Burnside et al., 1991; Huang et al., 1993; Knízetová, 1993; Agarwal et al., 1994). Overall, the different SLD chicken lines may vary in the structure and expression of GHR. The SLD chickens, in recent years, have been widely used in cross breeding of broilers and laying hens. To further improve the production of dwarf chickens, it is crucial to determine the molecular mechanisms of GHR in different dwarf strains. This study used the S2 line of SLD chickens (male and female) caused by a 1773-bp deletion mutation in the 3' untranslated region (UTR) of GHR (Poultry Institute, Academy of Chinese Agricultural Science). The focus was on expression patterns of GHR in liver, breast, and thigh tissues at seven growth stages (1 day, 3 weeks, 7 weeks, 9 weeks, 11 weeks, 13 weeks, and 15 weeks), and established a BW growth curve from 1 week to 22 weeks. This research identified the critical growth period, provided a basis for further study of molecular mechanisms underlying GHR in SLD chickens, and provided further applications of the dwarf gene in broiler and layer chicken breeding.

MATERIAL AND METHODS

Animals and sample collection

All experimental procedures were performed according to the guidelines of the Animal Ethics Committee in the Academy of Chinese Agricultural Science. Day old hatchlings, 180 dwarf cocks and 196 dwarf hens of the same genetic background were studied in parallel under the same diet and living conditions. Feed and water were provided *ad libitum* during the experiment. BW was measured once per week up to 21 weeks for each chicken. At each age tested (day of hatching, 3, 7, 9, 11, 13, and 15 weeks), 12 birds (6 males and 6 females) with similar weight were killed by stunning and exsanguination following 12 h of fasting. The pectoralis major muscle, deboned thigh muscle, and liver were flash-frozen in liquid nitrogen and then stored at -80°C. Dwarf chickens had a 1773-bp deletion mutation at the end of exon 10 in the 3'-UTR of the GHR gene.

RNA extraction and standard curve construction

Total RNA was isolated from tissues using a commercially available kit according

to the manufacturer protocol (DP419, Tiangen, Beijing, China). The concentration and purity of RNAs was determined at A260 and A260:280 (A260:280 ≥ 1.8 and ≤ 2.0) using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA integrity was RIN ≥ 7 and 28S/18S ≥ 0.7. RNA samples were stored at -80°C until needed. An aliquot of 2 μg total RNA was reverse-transcribed in a final volume of 25 μL solution using a QuantScript RT Kit (KR103-04, Tiangen, Beijing, China). The primers (forward: 5'-ATGAGACAAAATGGAAGGAGTTAG-3' and reverse: 5'-ATCGGACTCGGATCTCATAAT-3') were designed and synthesized by TaKaRa Biotechnology Co. Ltd., Dalian, China. The purified PCR product was cloned into a pGEMT-Easy vector (VT302, Tiangen Biotech Co., Ltd., Beijing, China), and the plasmids were extracted using a Plasmid Maxprep Kit (Axygen, Inc., USA). Bacterial cultures were diluted twice and concentrations were plotted on a standard curve.

Real-time PCR

The relative transcription abundances of GHR were detected in three tissues during different developmental stages using the Quantifast SYBR Green PCR Kit (FP204, Tiangen, Beijing, China). Controls were implemented to check for possible contamination from genomic DNA or the environment during reverse transcription and PCR amplification. The pooled sample, made by mixing equal quantities of total RNA from all samples, was used to optimize the PCR conditions and to standardize the curves. The final PCR products were verified with melting curves that showed a single peak specific for the target gene.

Statistical analysis

Data were analyzed using the general linear model procedure of SAS (Version 8.2; SAS Institute, Inc., Cary, NC, USA). Statistical differences in relative mRNA between tissues were assessed by ANOVA using the GLM model of the SAS program. All experimental data are reported as means ± SEM. Differences were considered statistically significant at $P < 0.05$.

Two nonlinear curve $(Logistic: y_t = A/(1+B_e - kt); Gompertz: y_t = Ae^{-B\exp(-kt)})$ models were used to fit the growth curve, where y_t was the BW at t week, A was the terminal BW, k was the growth parameters approximated the terminal BW, B was the adjustable parameters.

RESULTS

Growth curve of S2 strains

Weight gain every week did not exceed 50 g before week 2, weight gain exceeded 80 g between 6 and 12 weeks, and the highest weight gain in a week was 95.2 and 173.9 g in female and male chickens, respectively. The BW between male and female chickens had no significant differences before 5 weeks. The BW in male chickens was significantly higher than that of the females from 5 to 21 weeks ($P < 0.05$). The weight curves were fitted by two nonlinear curves models (Logistic and Gompertz); Results are shown in Table 1. Best fit curves are indicated in Figure 1.

Table 1. Parameter values and fitness of two nonlinear curves.

Sex	Model	A (g)	B	k	Fitness	Inflexion week	Inflexion body weight (g)
Female	Logistic	1415	19.9	0.3	0.9943	11.5	707.5
	Gompertz	1657	4.1	0.1	0.9981	10.1	609.6
Male	Logistic	1800	33.2	0.3	0.9945	11.3	900
	Gompertz	2029	5.4	0.2	0.9981	9.9	746.4

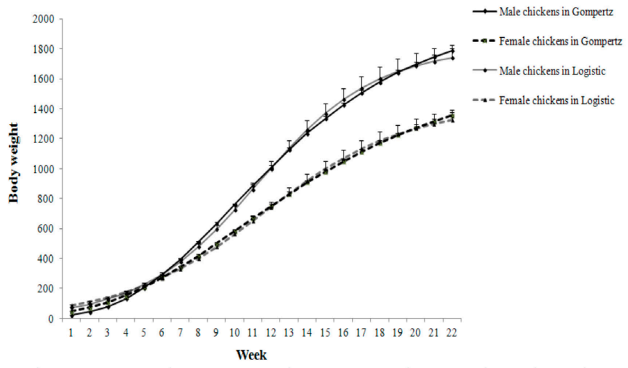

Figure 1. Weight gain curve in male and female SLD chickens using Gompertz and logistic models.

Expression patterns of GHR at different stages

Hepatic GHR mRNA expression (Figures 2 and 3) increased with age, but in muscle tissue, expression peaked at 7 weeks and dropped by degrees. There were no significant differences between tissues at hatching ($P > 0.05$), and the expression in muscle was higher than that of liver before 7 weeks. Starting at 9 weeks, expression in liver was significantly higher than that of muscle ($P < 0.05$). The expression in breast was significantly higher than that of thigh at 7 weeks of age ($P < 0.05$) and the expression in thigh was higher than the breast tissue after 9 weeks. There were no significant differences between female and male chickens, these data were not shown.

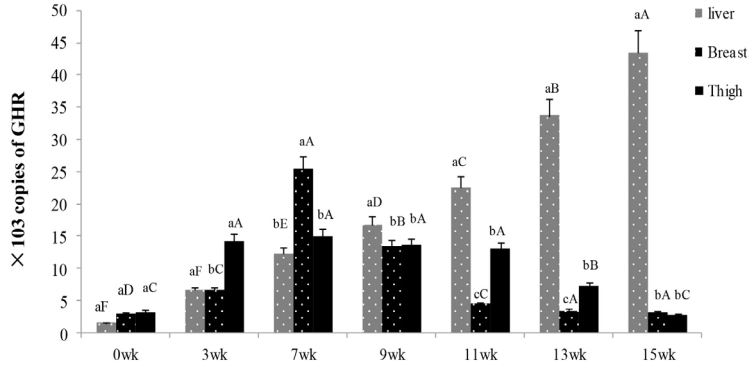

Figure 2. Expression pattern for GHR was analyzed by Q-PCRs for the S2 line of SLD female chickens. Capital letters indicate significant difference in the same tissue for different developmental stages. Lowercase letters indicate significant difference at the same developmental stages for different tissues.

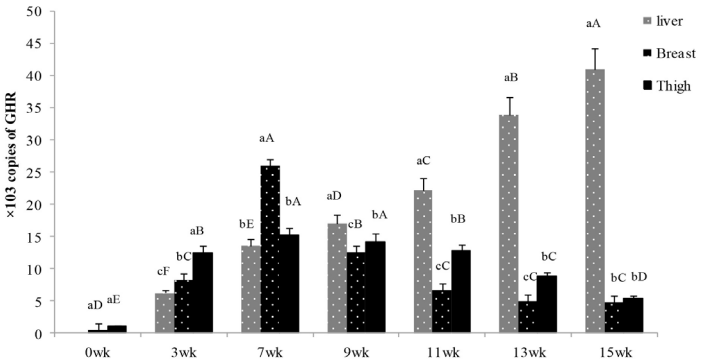

Figure 3. Expression pattern for GHR was analyzed by Q-PCRs for the S2 line of SLD male chickens. Capital letters indicate significant difference in the same tissue for different developmental stages. Lowercase letters indicate significant difference at the same developmental stages for different tissues.

Relationship between GHR expression and BW

As shown in Table 2, GHR mRNA expression in liver was positively correlated with BW in female and male chickens ($r = 0.82$, $P < 0.01$; $r = 0.96$, $P < 0.01$, respectively). There was no significant correlation between mRNA expression in breast and thigh tissues and BW in female and male chickens.

Table 2. Correlation coefficient between body weight and the expression levels of GHR mRNA in breast, thigh, and liver tissues.

Sex		Related coefficient	
	Liver	Breast	Thigh
Female	0.82**	-0.19	-0.03
Male	0.96**	-0.14	-0.01

**Extremely significant ($P < 0.01$) correlation ($N = 32$).

DISCUSSION

Effects of the dwarfing gene on BW was studied and the results show that the average BW of dwdw males was 30 and 40% lower than that of the homozygous (DwDw) normal chickens at 6 weeks and 11 weeks, respectively (Burnside et al., 1992). Hepatic GHR expression in SLD chickens was 3-fold higher than that of the normal chickens, but there was no significant difference in GH between SLD chickens and normal chickens. The transcription levels of IGF-1 decreased dramatically in SLD chickens, suggesting that the dwarf phenotype occurred independent of GH action resulting from a dysfunctional GHR (Wu et al., 2007). Another study suggested that dwarf chickens could increase carcass lipid content (Touchburn et al., 1980), but in laying hens, dwarfism reduced lipid mobilization of the adipose tissue, and likely also reduced *de novo* lipogenesis in the liver (Burghelle-Mayeur et al., 1989). However, the molecular mechanism underlying GHR in SLD chickens is not clear. The present study aimed to identify different GHR mRNA patterns in liver and skeletal muscle in relation to BW in female and male SLD S2 line chickens. These data can be used to study the molecular mechanisms governing GHR and have not been previously reported.

Gompertz and logistic models were used to obtain a smooth sigmoid curve of the fixed point of inflection (Darmani Kuhi et al., 2003). In this study, the Gompertz model, compared to the logistic model, was more suitable in determining the growth curves of S2 strain chickens because of its higher fitness and less bias toward predicted BW for each age. The inflexion week in male chickens was earlier than that of female chickens; the inflexion weight was also heavier than that of female chickens. These parameters may provide guidelines for practical production, such as monitoring chickens' growth conditions and identifying and correcting problems in a timely manner to improve chicken production.

GHR expression peaked at 7 weeks and dropped by degrees in breast and thigh tissue. Previous studies indicated that GHR mRNA expression peaked at 4 days in muscle tissue, but remained detectable up to 28 days (Halevy et al., 1996). However, our results suggest that different strains may have different muscle development profiles. Hepatic GHR mRNA expression increased with age, which is consistent with the previous studies (Hull et al., 1993; Halevy et al., 1996). Expression in liver was lower than that of muscle before 7 weeks, possibly due to a lower binding of GH to a hepatic-specific receptor during early development. These results can contribute to the knowledge of the developmental expression pattern of dw mRNA and facilitate further study on the molecular mechanisms of growth patterns in SLD chickens. Results could also guide further applications of the dwarf gene in broiler breeding.

Conflicts of interest

The authors declare no conflict of interest.

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