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Study on the Changes of IGF-I mRNA Expression Levels in the Developmental Muscles of the Geese

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Abstract: In this study, researchers detected the expression of IGF-I mRNA at 1 (day 1), 30 (day 30), 60 (day 60th) and 90 (day 90th) days of age in the breast muscle and leg muscle of geese from Jilin White goose (meat type, egg type) and Hortobagyi goose. Researchers found that the expression of IGF-I mRNA in skeletal muscle tissues differed based on developmental age. The expression of IGF-I mRNA exhibited a trend that ascended firstly and then descended after birth. IGF-I mRNA was expressed in all breast muscle tissues during each stage with higher levels in meat type goose and Hortobagyi goose than that in Jilin egg type goose ($p<0.05$). The expression pattern of IGF-I mRNA in breast muscle of meat type goose was similar with this in and Hortobagyi goose and in leg muscle, IGF-I mRNA expression patterns were all down-regulated from day 30. Similarity in IGF-I mRNA expression pattern were found in breast among Jilin meat type goose, egg type goose and Hortobagyi goose which indicates that IGF-I may be related to skeletal muscle differentiation and growth and suggests IGF-I mRNA maybe tissue-specific expression and individual difference.

Key words: Insulin-like growth factor-I, goose, muscle, real-time PCR, expression

INTRODUCTION

Growth Hormone (GH) is located in the center of the growth axis and regulates animal growth and development mainly though binding to the Growth Hormone Receptor (GHR) of target organ (Etherton and Bauman, 1998). GH plays a role *in vivo* mainly in two ways: GH binds to the GHR of liver and other target organs. Then, Insulin-like Growth Factor (IGF) is produced and subsequently enters the blood in endocrine way. After binding to Insulin-like Growth Factor Binding Protein (IGFBP), the complex arrives at the target organ and plays a role on Insulin-like Growth Factor Receptor (IGFR) to regulate the growth and development of animals. GH binds to the GHR of target organs and thus regulates the growth and development or directly affects cell metabolism through paracrine or autocrine IGF. In addition to the liver, skeletal muscle is also a very important target organ of GH. These results suggest that the expression level of IGF-I in muscle may have an important physiological role in controlling the growth and development of neonatal animals. In the experiment, the expression of *IGF-I* gene was detected in breast and leg muscle of jilin meat type goose, Jilin egg type goose and hortobagyi goose. IGF-I mRNA determined by real time PCR aiming at providing basic information for the study of geese growth.

MATERIALS AND METHODS

Experimental animals and sampling: All experiments were performed in accordance with the principles and procedures of Animal Ethics Committee of Jilin Agricultural University. The animals that Jilin meat type goose, Jilin egg type goose and hortobagyi goose were fed in the same condition in poultry farm of Jilin Agricultural University, all of them were normal development and health. For each species, tissue samples were collected from twenty animals at 1, 30, 60 and 90 days old (n = 20) and put them in liquid nitrogen immediately then kept at -80°C.

RNA extraction and cDNA synthesis: For all animals, RNAs were extracted using TRIZOL reagent (Invitrogen Life Technologies Inc., USA) at the stages described above and then treated with Rnase-free Dnase I (TIANGEN) to eliminate genomic DNA. To assess the integrity and the amount of RNA extracted, agarose gel electrophoresis and spectrophotometric A260/280 readings were performed. Total RNA (2 μg) was retrotranscribed into cDNA. First-strand cDNAs were synthesised by using the 1st Strand cDNA Synthesis kit (Takara, Japan). The resultant cDNA was frozen at -20°C until use.

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Quantitative real-time PCR: Relative quantification assays were performed to detect the relative expression of IGF-I among different developmental stages. The expression analysis was performed by SYBR Green I dye chemistry detection with an ABI StepOne Real Time PCR System (Applied Biosystems) and data were collect with ABI's StepOne Software Ver. 2.1. For IGF-I and β-actin assays, 10 folds serial-dilution of cDNA samples was amplified to assess standard curves and PCR efficiency. Standard curves created on the basis of the linear relationship between the Ct value and the logarithm of cDNA content showed acceptable slope values. To demonstrate that efficiencies of targets and reference gene were approximately equal, a validation experiment was performed calculating the ΔΔCt = Ct (target gene)-Ct (reference gene) value. The ΔΔCt was plotted against the logarithm of the dilution factors; the absolute value of the slope from the efficiency plot should be <0.1. The experiments were made from SYBR® Premix Ex Taq™ (Takara, Japan) with the IGF-I specific oligonucleotides, the amplification reactions were performed in a 20 μL final volume containing 100 ng of cDNA. The basic PCR protocol for amplification was composed of 30 sec at 95°C followed by 40 cycles of 5 sec at 95°C, 34 sec at 60°C. To normalize the amount of expressed IGF-I mRNAs, the internal housekeeping gene *β-actin* was used. Each cDNA product was tested in triplicates. A standard curve was generated and used to evaluate the relative expression levels of the *IGF-I* gene in terms of the ratio of the target gene expression to the control gene expression. Specific primers used for IGF-I were IGF-I-FP: 5'-TGAGGAGGCTGGAGATGTACTG-3', IGF-I-RP: 5'-TTCCTTCTGTGCTTTTGGCATA-3'.

The amount of targets, normalized to *β-actin* gene and relative to the calibrator sample, is given by the formula $2^{-\Delta\Delta Ct}$ and the calibrator sample is expressed as 1 arbitrary unit. Dissociation curves confirmed the specific amplification of the cDNA target and the absence of non-specific products.

Statistical analyses: Comparisons between groups were performed by one-way ANOVA. A value of $p<0.05$ was considered statistically significant and a value of $p<0.01$ was considered statistically highly significant.

RESULTS

***IGF-I* gene mRNA expression in the breast muscle of geese:** The expression patterns of *IGF-I* gene in the breast muscle of geese of Jilin meat type goose, Jilin egg type goose and Hortobagyi goose are shown in Fig. 1. IGF-I mRNA increased continuously from Day 1 (D1) and reached a peak at day 60 then dramatically decreased and remained at days 30 level at day 90 ($p<0.05$) in Jilin meat type goose and Hortobagyi goose. IGF-I remained at peak levels at day 30 in Jilin egg type goose and then dramatically decreased through days 30 and 90 ($p<0.05$) and IGF-I mRNA decreased down to day 1 level on days 90 (Fig. 1a). The expression of IGF-I mRNA was lower in Jilin egg type goose compared with Jilin meat type goose and Hortobagyi goose at days 1 and 90 ($p<0.05$) but there were no significant differences in IGF-I mRNA between Jilin meat type goose and Hortobagyi goose at days 1 and 90 ($p>0.05$). Jilin meat type goose expressed higher levels of IGF-I mRNA compared with Jilin meat type goose at days 60 but no differences with Hortobagyi goose. Furthermore, there were no significant differences in IGF-I mRNA among the three at day 30 ($p>0.05$) (Fig. 1b).

***IGF-I* gene mRNA expression in the leg muscle of geese:** The expression patterns of *IGF-I* gene had no significant differences among Jilin meat-type goose, Jilin egg-type goose and hortobagyi goose. IGF-I mRNA expression increased from day 1 and peaked at days 30 ($p<0.05$)

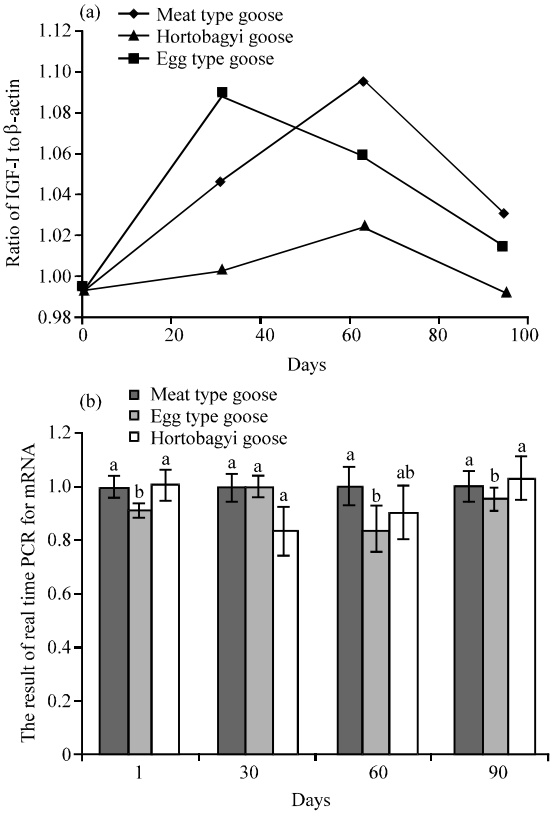

Fig. 1a, b): Developmental changes and relative abundance of IGF-I mRNA expression in the breast muscle of Jilin meat type goose, Jilin egg type goose and Hortobagyi goose

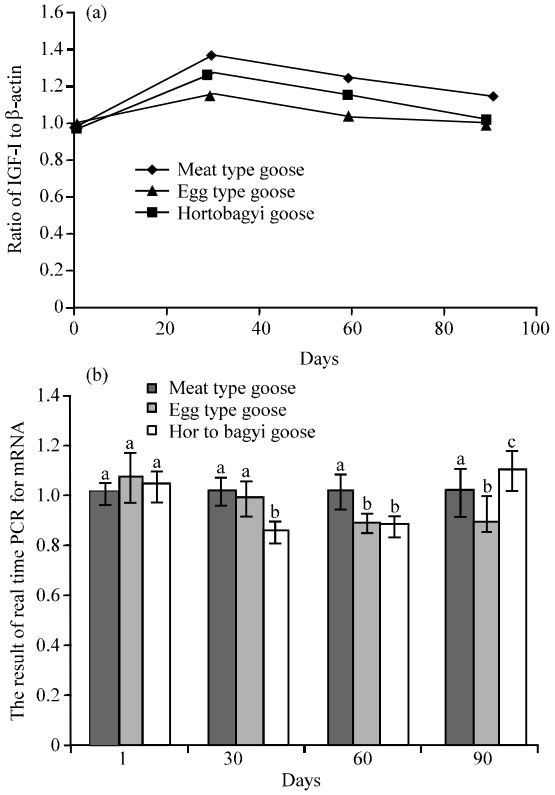

Fig. 2a, b): Developmental changes and relative abundance of IGF-I mRNA expression in the leg muscle of Jilin meat type goose, Jilin egg type goose and Hortobagyi goose

then from days 30-90. There were no significant decraesed differences in IGF-I mRNA among the three at days 1 and 90 (Fig. 2a). Hortobagyi goose expressed lower levels of IGF-I mRNA compared with Jilin meat type goose and Jilin egg type goose at day 30 ($p<0.05$) whereas the opposite was true at day 90 ($p<0.05$). The expression of IGF-I mRNA was higher in Jilin meat type goose compared with egg type Jilin goose at day 60 ($p<0.01$) and day 90 ($p<0.05$), IGF-I mRNA was lower in Hortobagyi goose compared with Jilin meat type goose at day 60 ($p<0.05$) but no significant differences in IGF-I mRNA between Jilin egg type goose and Hortobagyi goose at day 60 ($p>0.05$). Furthermore, there were no significant differences in IGF-I mRNA among the three at day 1 ($p>0.05$) (Fig. 2b).

DISCUSSION

IGFs play critical roles in skeletal muscle differentiation and growth (Florini and Ewton, 1992; Florini *et al*., 1993; McMurtry *et al*., 1997; Rosen *et al*., 1993) and are initially mitogenic and subsequently differentiation promoting in skeletal muscle (Rosenthal and Cheng, 1995). IGF-I has an important function in the regulation of muscle growth (Weller *et al*., 1993) and differentiation (Yang *et al*., 1999). The birth weight of IGF-I or IGF-II deficient mice is significantly lower than that of their normal littermates. IGF-I may mediate growth in muscle in response to variety of stimuli by autocrine/paracrine action or in response to certain stimuli possibly by endocrine action (Loughna *et al*., 1992).

In this study, researchers determined the expression patterns of *IGF-I* gene in the skeletal muscle during postnatal development. The *IGF-I mRNA* gene can express in breast muscle and leg muscle of the Jilin meat type goose, Jilin egg type goose and Hortobagyi goose. Earlier studies with RT-PCR assays reported that relative amounts of IGF-I mRNA peaked ($p<0.05$) at days 28-42 in breast muscle of Dagu chicken (Hu *et al*., 2004). It has been reported that IGF-I mRNA in the Beijing duck's breast muscle was lower at days 7, 14 and 21 compared with days 28, 35 and 42 (Wang and Huang, 2006). IGF-I mRNA in the breast of chicken at early stage expressed a higher level at weeks 4-6 after birth and then decreased (Guernec *et al*., 2003). The result of the current study was similar to that in previous studies. Of the whole growth period of goose, relative growth of the days 30-90 was decreasing slowly and then reaching a relatively stable state in the same time, IGF-I levels also showed the same changing trend. Moreover, IGF-I mRNA was expressed in all breast muscle tissues during each stage with higher levels in meat type goose and Hortobagyi goose than that in Jilin egg type goose which indicates that IGF-I may be related to skeletal muscle differentiation and growth.

Earlier studies have shown that *IGF-I* gene expressed different in liver and ileum and exhibited organization and individual differences (Pfaffl *et al*., 2002). Researchers found that the expression pattern of IGF-I mRNA in breast muscle of meat type goose was similar with this in and Hortobagyi goose and in leg muscle, IGF-I mRNA expression patterns were all down-regulated from days 30. Similarity in IGF-I mRNA expression pattern were found in breast muscle among Jilin meat type goose, Jilin egg type goose and Hortobagyi goose which suggests IGF-I mRNA maybe tissue-specific expression and individual difference.

CONCLUSION

IGF-I mRNA was expressed in goose breast muscle and leg muscle after birth, the results reveal ontogenetic changes in the expression of *IGF-I* genes during postnatal

development periods of Jilin meat type goose, Jilin egg type goose and Hortobagyi goose. These results imply that IGF-I may play critical roles in skeletal muscle differentiation and growth in geese.

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