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Developmental changes of *GHR* and *IGF-I* mRNA expression in lamb rumen

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Abstract

Lambs from birth (0-day-old) to 56-day-old were selected in present study to investigate developmental changes of growth hormone receptor (*GHR*) and insulin-like growth factor I (*IGF-I*) mRNA expression in their rumen tissue. Forty-five lambs (5 lambs per group) were slaughtered at 0, 7, 14, 21, 28, 35, 42, 49, 56 days of age respectively for sampling the tissue of the rumen dorsal sac. The abundance of *GHR* and *IGF-I* mRNA were detected through real-time quantitative PCR method. The results indicated that the expression levels of *GHR* and *IGF-I* mRNA had similar change tendency in rumen tissue that the *GHR* and *IGF-I* mRNA abundance decreased with age from birth to 56-day-old. There was significant positive correlation between the two gene mRNA expression levels. The results suggested that *GHR* and *IGF-I* gene expression levels had the specific developmental pattern in rumen tissue.

Keywords: developmental change, growth hormone receptor (*GHR*), insulin-like growth factor (*IGF-I*), rumen, lamb

Introduction

As a central link of regulating growth, GH-IGF axis plays a very important role during the growth and development of animals (Florini *et al.* 1996). Growth hormone (GH) must combine with its corresponding receptor to play biological effects, and it has mainly two role ways for growth and development of tissues and organs in animals:

- Insulin-like growth factor-I (*IGF-I*) is generated by GH combining with the *GHR* in liver tissues, in the form of endocrine into the blood, after binding insulin-like growth factor binding protein (IGFBPs) reaches target organs, acts on insulin-like growth factor receptor and then regulates the growth of the tissues and organs;
- GH directly acts on liver tissue, in where it combines with the *GHR*, and generates *IGF-I* which promotes the tissues growth and development through the way of paracrine and autocrine (Lupu *et al.* 2001).

Many researches had shown that almost all of the tissue, such as the liver, stomach, kidney and skeletal muscles were available as a target organ providing *GHR* for GH. However, there were significantly tissue specificity and breed differences in developmental changes of *GHR*, GH-binding protein (GHBP), IGF and IGF binding protein genes (Schnoebelen-Combes *et al.* 1996, Peng *et al.* 1996, Peng *et al.* 1998, Xia *et al.* 2002). As we all know, GH, IGFs and insulin

influence postnatal gastrointestinal development and function, but, the current studies on the development changes of *GHR* and *IGF-I* gene expression in animal tissue were infrequence, which mainly focused on liver (Xu *et al.* 2003) and gastrointestinal tract (Xu & Wang 1996, Shen & Xu 2000, Xia *et al.* 2002) in pig, and there were no literature report in gastric tissue of sheep. So, this study explored the developmental changes of *GHR* and *IGF-I* mRNA expression in rumen tissue by real-time quantitative PCR (RT-PCR) method in order to provide a foundation for further study changing patterns of GH-IGF axis on lamb rumen tissues during the early.

Material and methods

Animal, supplement feed, experimental design and tissue collection

Forty-five male lambs of Gansu mutton sheep, five for each different age group (0, 7, 14, 21, 35, 42 and 56 days of age), were selected from the Yongchang sheep farm in Gansu province, P.R. China for the present study. They were health, similar birth weight and born of the oestrus-treated Gansu mutton ewe in February to April 2009. The lambs timely were fed colostrum after birth and were induced to feed the supplement and high quality forages on day 7. Subsequently, the lambs had a free choice of the supplement and alfalfa hay twice a day at 9:00 and 17:00 in addition to sucking schedule. Animals were slaughtered for sampling tissue of the rumen dorsal sac on the corresponding day of age. The removed samples were snap-frozen in liquid nitrogen immediately and then stored at −80 °C for total RNA analysis later.

Table 1
Composition and nutrition level of the supplement (air-dry basis, %)

Items	Percentage
Ingredients	
Corn	48.15
Soybean meal	20.00
Beet pulp	10.00
Full fat soybean	8.00
Corn gluten meal	3.50
Cottonseed meal	3.00
Rapeseed meal	2.00
Vegetable oil	1.50
Calcium hydrophosphate	1.40
Premix[1]	1.00
Limestone meal	0.82
Salt	0.36
Lysine	0.27
Total	100
Nutrient level	
DE, MJ/kg	13.80
CP, %	21.00
CF, %	3.34
Ca, %	0.76
P, %	0.59
Ca/P	1.29

[1]The premix provided following per kg of air-dry the supplement: S 200 mg, Fe 25 mg, Zn 40 mg, Cu 8 mg, Mn 40 mg, I 0.3 mg, Se 0.2 mg, Co 0.1 mg, VA 940 IU, VE 20 IU

The lamb supplement (pellet, granule diameter 2.5 mm, length 10.0 mm) was prepared according to the requirement of lamb moderate growth rate (daily gain 200 g) described in nutrient requirement standard of National Research Council USA (1992). Feed formulation and nutritional level were shown in Table 1.

Quantitative RT-PCR

Total RNA was extracted using Trizol reagent kit (Invitrogen, Carlsbad, CA, USA). RNA concentration and OD260: 280 ratio (between 1.9 and 2.1) of the samples were measured with the NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), respectively. Aliquots of total RNA were reverse transcribed through Primescript RT reagent Kit (Takara, Dalian, China).

Table 2
The primer sequences of *GHR*, *IGF-I* and *GAPDH* genes

Target gene	GenBank accession	Primer sequence	Product Size, bp	Annealing temperature, °C
GHR	NM_001009323	F: AAC CAC CAC CCA ATA CAG	134	50.1
		R: CAA CGA GTA CAT CGG AAC		48.1
IGF-I	NM_001009774	F: AGC AGT CTT CCA ACC CAA	85	52.9
		R: ACA TCT CCA GCC TCC TCA		53.6
GAPDH	AF035421	F: GCA AGT TCC ACG GCA CAG	118	57.6
		R: TCA GCA CCA GCA TCA CCC		57.5

Oligomucleotide primer sets for the three genes were designed using Primer premier 5.0 software (PREMIER Biosoft Int, Palo Alto, CA, USA). GAPDH was used as an internal standard. Primers were synthesized by Takara Bio Inc (Dalian, China).

The cDNA obtained from rumen tissue was subjected to real-time quantitative PCR analysis using the specific primer pairs (Table 2). A total volume of 20 µL contained 0.5 µL cDNA template, SYBR premix EX taq (2×) 10 µL, specific 0.4 µL forward and reverse primers, each at a final concentration of 20 µmol/L, and 8.7 µL ultrapure water. According to the manufacturer instructions of RT-PCR reagent Kit (TaKaRa, Dalian, China), for each target, an initial denaturation step of 30 s at 95 °C was followed by 40 cycles of 5 s at 95 °C, an annealing of 20 s at 58 °C.

Statistical analysis

Every relative expression abundance was calculated according to $2^{-\triangle\triangle}$Ct method of Livak & Schmittgen (2001). Data of all genes expression were subjected to analysis using SPSS 11.5 (SPSS Inc., Chicago, IL, USA) statistical software for single factor analysis of variance and significant test.

Results and discussion

From Figure 1, *GHR* and *IGF-I* mRNA expression abundances were high at the time of birth in lamb rumen tissue, no significant change ($P>0.05$) in 0~7 days (*IGF-I* in 0~14 days), then started a significant decline ($P<0.05$), appeared a small rise from 28 days to 35 days (*IGF-I* mRNA from 21 days to 35 days), subsequently showed a fall and the lowest on 49 days of age,

and increased later. Overall, developmental change trends of *GHR* and *IGF-I* mRNA in lamb rumen tissue were similar from birth to 56 days of age, which kept decline during 0~49 days and rose during 49~56 days of age. There were positive line correlation between *GHR* and *IGF-I* mRNA expressions (Figure 2), and correlation coefficient was 0.928 (P=0.000, P<0.01).

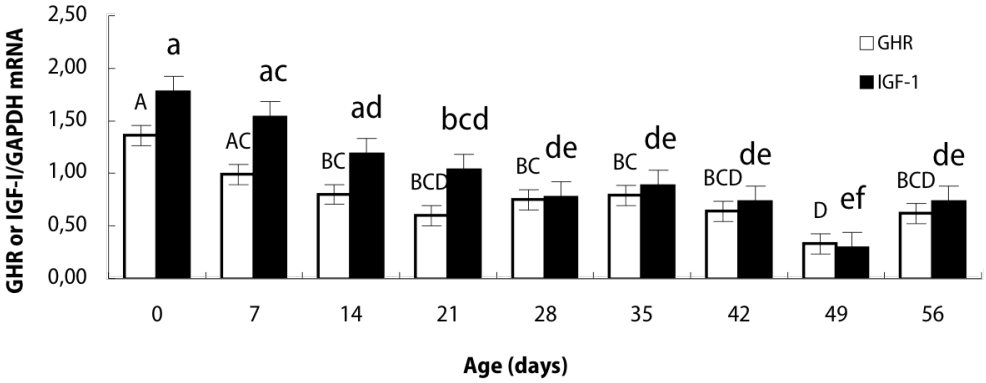

Figure 1
Expressions of *GHR* and *IGF-I* mRNA in lamb rumen tissue. Without common letter indicating differ significantly between ages within same gene (P<0.05).

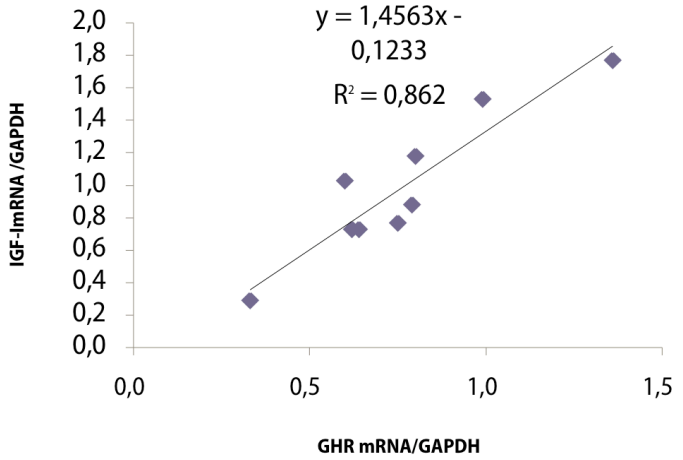

Figure 2
The correlation analysis of *GHR* and *IGF-I* mRNA in lamb rumen tissue

To a large extent, animal growth depended on the *GHR* and related gene expression of the target organ. Gastric growth was regulated by GH, and the experiments had been demonstrated that there was *GHR* gene expression in rats, rabbits, pigs and human gastric (Xia *et al.* 2002, Delehaye-Zervas *et al.* 1994, Nagano *et al.* 1995). But until now, there was no report about *GHR* and *IGF-I* mRNA expressions in sheep rumen. This study had detected expressions of *GHR* and *IGF-I* mRNA in lamb rumen tissue, and the expression abundances declined with lamb age as a whole. This may be related to the expression level of GH in pituitary decreased with age after animal birth (Matteri & Carrol 1997).

Many factors affecting the regulation of GH on tissue, such as the content of GH and soluble GHBP into the tissue, the content of *GHR* in tissue, and environmental factors, in which the *GHR* quantity of tissues was an important factor in determining GH to regulate tissue-specific (Hull & Harvey 1998). Ilkbahar *et al.* (1995) and Peng *et al.* (1998) found that *GHR* expression had a particular development pattern, at the same time there was interspecies differences and tissue specificity, regardless of mice or pigs. Schnoebelen-Combes *et al.* (1996) found that developmental changes of *GHR* expression not only displayed tissue specific, but also varieties differences between Large White and Meishan pig. Developmental change of *GHR* mRNA expression in lamb rumen in our study was different with liver (Huang & Xie 2009a), muscle (Huang & Xie 2009b) and skin (Jia *et al.* 2006) in Kazakhstan sheep and Xinjiang fine wool sheep, which indicated that *GHR* gene expression in lamb rumen tissue existed tissue specificity.

In the process of early rumen growth, the results of *GHR* and *IGF-I* mRNA expression patterns, and correlation between them suggested that *IGF-I* gene expression in lamb rumen may depend on GH that acted firstly on *GHR* of rumen tissues, and then the tissue growth and development were regulated by *IGF-I* from paracrine or /and secretion. But, *IGF-I* mRNA expression in rumen was whether affected by *IGF-I* from breast milk or not, and *GHR* mRNA expression was whether regulated by *IGF-I* negative feedback or not were both unclear, which also requires further study.

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