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Expression of IGFs during postnatal development of *Longissimus dorsi* muscle and its relationship with meat quality traits in Nanyang cattle

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ABSTRACT

The IGFs pathway plays important roles in body energy metabolism and muscle mass regulation. The expressions of *IGFs* in *Longissimus dorsi* muscle were analyzed by qRT-PCR at postnatal 3, 6, 12, 18 and 24 month-old. The expression of *IGF-I*, *IGF-II*, *IGF-IR*, *IGF-IIR*, *IGFBP1* and *IGFBP3* showed significant decreasing tendency during the postnatal *Longissimus dorsi* muscle development, while the expression of *IGFBP2*, *IGFBP4*, *IGFBP5* and *IGFBP6* had no significant changes from postnatal 3 to 24 month-old in Nanyang cattle. The expression of *IGF-IIR*, *IGFBP5* and *IGFBP6* had significant positive correlation with IMF and expression of *IGF-II* had significant negative correlation with meat flavor trait of *Longissimus dorsi* muscle in 24 month-old Nanyang cattle. This study indicated that endogenous IGFs pathway played critical roles in regulation of skeletal muscle development, IMF accumulation and meat sensory traits.

Key words: IGFs, IMF, Muscle development, Nanyang cattle

The development of muscle is mainly regulated by endocrine and nutrition via the GH (growth hormone)/IGFs (insulin-like growth factors) axis in a body. GH is a peptide hormone produced and secreted mainly by the somatotroph cells of anterior hypophysis (pituitary gland), which stimulates synthesis of IGF-I in most tissues (Gostelli *et al*. 1994). The serum IGF-I is mainly secreted by liver, but is not required for postnatal muscle growth (Sjogren *et al*. 1999). The effects of IGF-I are mediated mainly by IGF-IR, which has tyrosine kinase activity and signals through the Akt/PKB pathway. The activated Akt inhibits protein degradation and thus represses the transcription factors of FoxO family (Forkhead box class O family), and stimulates protein synthesis (Manning *et al*. 2007). FoxO factors are required for the activation of MAFbx (muscle atrophy F-box) and MuRF1 (muscle ring finger 1) and lead to the ubiquitylation of myosin and other muscle proteins via the proteasome system. In addition to IGF ligands and receptors, IGF-binding protein (IGFBPs) is another component in the IGFs signaling pathway. IGFBPs function as carrier proteins in circulation and regulate IGFs turnover, transport, and half-life of circulating IGFs (Jones *et al*. 1995). The IGF/IGFBP complexes also help to prevent potential hypoglycemic effect by preventing possible cross-binding of IGFs with the insulin receptor (Rajaram *et al*. 1997). There are 6 IGFBPs and most serum IGF-I is found in a tripartite complex with IGFBP3.

The muscle mass and meat quality are important economic traits for beef cattle and lots of reports had demonstrated that the IGFs pathway had predominant effects on the adipogenesis in mammalian (Baxter *et al*. 2009). The IMF (intramuscular fat) has an important effect on the sensory quality of culinary meat (Moeller *et al*. 2010). So in this paper, expressions of *IGFs* were analyzed during the postnatal *Longissimus dorsi* muscle development and the correlation of *IGFs* expression with IMF, tenderness, flavour and juiciness were tested in Nanyang cattle. The results of this study indicated that the IGFs pathway played important roles in regulation of postnatal skeletal muscle development and meat quality traits in cattle.

MATERIALS AND METHODS

Tissue collection, RNA isolation and cDNA synthesis: Postnatal *Longissimus dorsi* muscle samples (4 animals at each 3, 6, 12, 18 and 24 month old, respectively) were collected in experimental abattoir. Total of 24 Nanyang bulls at 24-month-old were selected to analyze the correlation of meat quality traits with *IGFs* expression. The *Longissimus dorsi* muscle was excised from each animal within 30 min post-mortem in experimental abattoir. After dissection samples were quickly frozen in liquid nitrogen and stored at –80°C for later analysis.

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Total RNA was isolated from all samples using RNAiso puls kit. The RNA quality was analyzed by 1.2% agarose gel electrophoresis and the spectrophotometric absorption at 260 nm in a nanodrop ND-1000 spectrophotometer. After Dnase I treatment, total RNA was reverse transcribed to cDNA using PrimeScript RT reagent Kit following the manufacturer's instructions.

qRT-PCR analysis: qRT-PCR was performed on an iQ Real Time PCR Detection Systems using SYBR Premix Ex Taq II. Thermal cycling consisted of an initial step at 95°C for 4 min followed by 42 cycles at 95°C for 30 sec, annealing for 30 sec and extension/fluorescence acquisition at 72°C for 30 sec. *GAPDH* was chosen as the reference gene for normalization of all data because it was expressed more stably in muscle tissues. Each qRT-PCR reaction (in 20 µl) contained 10 µl of SYBR Premix Ex Taq II, 0.7 µl of each primer (Table1), 1 µl of normalized template cDNA, and 7.6 µl ultrapure water. The qRT-PCR measurements were performed in triplicate on each cDNA sample, and gene expression was quantified relative to *GAPDH* expression using the $2^{-\Delta\Delta Ct}$ method (Livak *et al*. 2001).

Meat quality traits measurement: Meat tenderness measurements were conducted using a muscle tenderness device with a Warner-Bratzler attachment in accordance with methodology given by Agnieszka (Agnieszka *et al*. 2011). Prior to value measurements of the shear force, samples were cut into slices 25–30 mm thick, vacuum-packed and heated in a water-bath at the temperature of 80° to reach inside temperature of 72° and kept at the above temperature for 90 min. Following their thermal treatment, samples were cooled to room temperature and cuboids were excised measuring 10 mm×10 mm×40 mm which were subsequently cut perpendicularly to muscle fibres. Additionally, the sensory tenderness assessment according to linear scale was carried out where the score of 10 points corresponded to very tender and 1 point very tough meat. IMF content of *Longissimus dorsi* muscles was determined by extraction with petroleum ether in a Soxhlet apparatus and expressed as percentages of the dry weight of the muscle.

Flavour and juiciness measures were carried out by sensory analysis and total of 10 trained panelists were used in each session. Participants were instructed not to eat or drink for 1 h prior to the test and rinse their mouths with water before tasting began. Panellists graded flavour and juiciness in a scale of 1–8, as follows: flavour: 1 = very poor, 8 = extremely good; juiciness: 1 = very dry, 8 = extremely juicy (Ozlem *et al*. 2010).

Statistic analysis: The expression of *IGFs* at different stages were analyzed by one-way ANOVA followed by *Bonferroni test* for pair wise comparison. The relationship of meat quality traits with *IGFs* expression were analyzed by *pearson* correlation at the significant level of 0.05.

Table 1. Information of primers used in this study

Gene name	Template sequence	Primer sequence	Anneal temperature (°C)	Product length (bp)
IGF-I	NM_001077828.1	5' TCAGCAGTCTTCCAACCC 3'	53	88
		5' AGATGCGAGGAGGATGTG 3'		
IGF-II	NM_174087.3	5' TTCGCCTCGTGCTGCTAT 3'	56	127
		5' TTATGCGGCTGGATGGTC 3'		
IGF-IR	NM_001244612.1	5' CCCATTGCGGTTCTGTTG 3'	57	114
		5' GGGTTCACAGAGGCATACAG 3'		
IGF-IIR	NM_174352.2	5' ACCAGTGACAAGTCCAAGG 3'	55	126
		5' TAGTTCATCTTCAGCACCCC 3'		
IGFBP1	NM_174554.2	5' GATACAAAAGACACCACCAG 3'	51	229
		5' CTAATCTGTCCAGCACTTTG 3'		
IGFBP2	NM_174555.1	5' ATGGGCAAGGGTGGCAAAC 3'	63	126
		5' AAGGCGCATGGTGGAGATC 3'		
IGFBP3	NM_174556.1	5' GAACTCTGGAAACCGAC 3'	47	129
		5' TACTTGTGATGCCTCTGG 3'		
IGFBP4	NM_174557.3	5' AGGTCTGAGTCCTGGCTTTG 3'	57	170
		5' GAAACATACCAGGGCTCTCC 3'		
IGFBP5	NM_001105327.1	5' GTGACCGCAAAGGGTTCTAC 3'	58	99
		5' GCAGCTTCATCCCGTACTTG 3'		
IGFBP6	NM_001040495.1	5' CCTGCCGCAAACATCTGG 3'	61	105
		5' GGTAGAAGCCCCTTTGGTCAC 3'		
GAPDH	NM_001034034.1	5' ATGCTGGTGCTGAGTATGTG 3'	54	320
		5' GAAGTTACGGCTATCTGTCG 3'		

All the primers were designed with Premier 5.0 software and synthesized in commercial companies

RESULTS AND DISCUSSION

Expression of IGFs in postnatal development of Longissimus dorsi *muscle:* The expression of *IGF-I* and *IGF-II* were showed decreasing tendency during the postnatal development in the *Longissimus dorsi* muscle of Nanyang cattle (Fig. 1). The expression level of *IGF-I* at 3 month-old was significantly higher than 12, 18 and 24 month-old ($P<0.01$). The expression of *IGF-II* at 3 month-old was significantly higher than 6, 12, 18 and 24 month-old ($P<0.01$). It was demonstrated that GH administration increased skeletal muscle *IGF-I* mRNA production in hypophysectomized rats 20-fold (Gostelli *et al*. 1994) and direct infusion of IGF-I into rat muscle resulted in increased muscle mass. In adult rats, plasmid-mediated *IGF-I* gene transfer prevented corticosteroid-induced muscle atrophy (Schakman *et al*. 2005) and resulted in muscle hypertrophy (Barton *et al*. 2010). The previous studies provided evidences that the local autocrine/paracrine rather than systemic endocrine had important effects on the muscle hypertrophy (Adams *et al*. 1998). This was consistent with the expression patterns of *IGF-I* and *IGF-II* in the postnatal development of *Longissimus dorsi* muscle in this study.

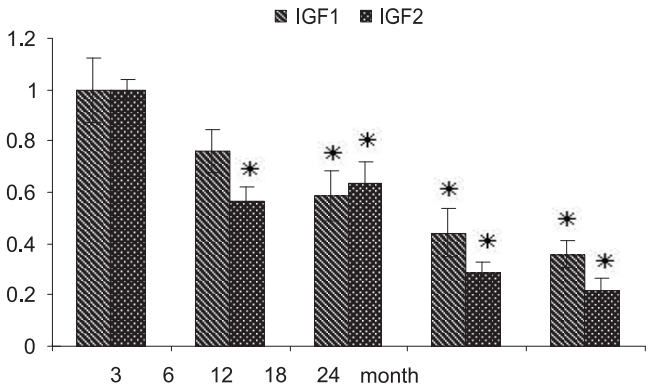

Fig. 1. Expression of *IGF-I* and *IGF-II* in the *Longissimus dorsi muscle* of Nanyang cattle.

The expression of *IGF-IR* and *IGF-IIR* also showed decreasing tendency, except a slight increase in 12 month old, during the whole postnatal development in the *Longissimus dorsi* muscle of Nanyang cattle (Fig. 2). The expression of *IGF-IR* at 3-month-old was significantly higher than 18- and 24-month-old ($P<0.05$). The expression of *IGF-IIR* at 3-month-old was significant higher than 6, 18 and 24 month-old ($P<0.05$). The *IGF-IR* and *IGF-IIR* were widely expressed in all tissues and knockout of the *IGF-IIR* gene or loss of the imprinted *IGF-IIR* resulted in fetal overgrowth and perinatal lethality (Wylie *et al*. 2003). *IGF-II* over-expression or exogenously added IGF-I or IGF-II accelerated myoblast differentiation (Ren *et al*. 2008, Ren *et al*. 2010). There was a growing interest in the specific importance of IGFs pathway mediated actions on skeletal muscle satellite

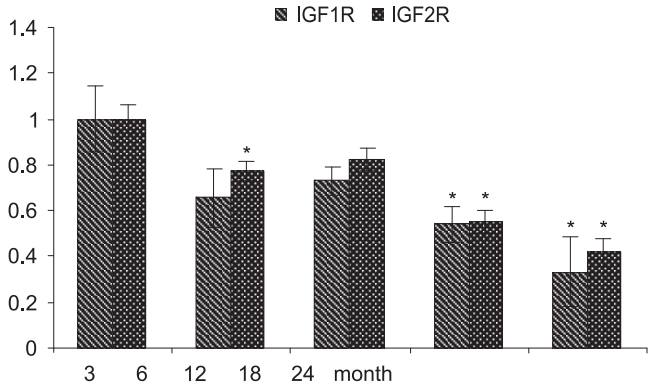

Fig. 2. Expression of *IGF-IR* and *IGF-IIR* in the *Longissimus dorsi* muscle of Nanyang cattle.

cells (Philippou *et al*. 2007). Studies established IGFs as critical factors for adult muscle regeneration and hypertrophy. Recent study showned that a myoblast cell decision to divide or differentiate is not only regulated by the hormonal or growth factor signals it receives, but also controlled by the availability of oxygen in its microenvironments (Ren *et al*. 2010). While IGFs pathway promoted myoblast differentiation under normoxia, while stimulated cell proliferation under hypoxia. In this study, the expression of *IGF-I*, *IGF-II*, *IGF-IR* and *IGF-IIR* showed decreasing tendency during the postnatal muscle development, which indicated that the reduced endogenous IGFs pathway had relationship with diminishing of myoblast differentiation after birth.

The expression of *IGFBP1* and *IGFBP3* also showed decreasing tendency-and the expression at 3-month-old was significantly higher than that at 6, 12, 18 and 24 month-old ($P<0.05$), while the expression of *IGFBP2*, *IGFBP4*, *IGFBP5* and *IGFBP6* had no significant changes during the whole postnatal development in the *Longissimus dorsi* muscle of Nanyang cattle (Fig. 3). IGFBPs regulate the half-life of circulating IGFs. In addition to their endocrine function, IGFBPs also modulate IGF availability and biological activity in local tissues. Ubiquitous over-expression of *IGFBP1*, *IGFBP2*, *IGFBP3* and *IGFBP5* in transgenic mice lead to various degrees of growth retardation (Salih *et al*. 2004). Mice null for *IGFBP3*, *IGFBP4*, and *IGFBP5* showed significantly diminished postnatal growth and enhanced glucose metabolism (Ning *et al*. 2006). These triple knockout mice also demonstrated significantly smaller quadriceps muscle. The *IGFBP5*, implicated in the regulation of cell growth and differentiation, was significantly down regulated in developing bovine muscle (Sigrid *et al*. 2007). This was consistent with *IGFBP5* expression pattern in our study. Although a large number of *in vitro* studies showed that IGFBP4 inhibits IGFBP and knockout of the *IGFBP4* gene in mice reduced rather than increased prenatal growth (Ning *et al*. 2008). IGFBP5 is mainly secreted by skeletal muscles. In cultured myoblast, *IGFBP5* expression levels increased

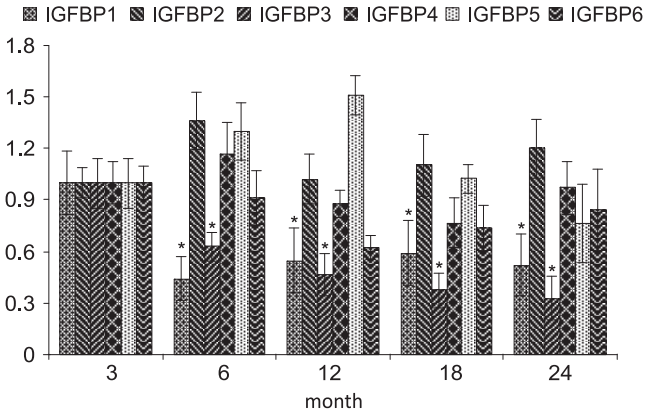

Fig. 3. Expression of *IGFBPs* in the *Longissimus dorsi* muscle of Nanyang cattle.

Table 2. The correlation coefficient of IGFs expression with meat quality traits in Nanyang cattle

Gene name	IMF	Tenderness	Juiciness	Flavor
IGF-I	0.348	0.031	-0.093	-0.067
IGF-II	0.147	-0.055	-0.381	-0.411*
IGF-IR	0.376	-0.135	-0.041	-0.106
IGF-IIR	0.633**	-0.214	-0.064	-0.187
IGFBP1	0.064	0.078	-0.100	-0.098
IGFBP2	0.315	-0.133	-0.052	-0.103
IGFBP3	0.265	-0.235	-0.102	-0.182
IGFBP4	0.288	-0.131	-0.019	-0.134
IGFBP5	0.413*	0.287	-0.009	-0.026
IGFBP6	0.495*	-0.047	-0.101	-0.123

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

dramatically during myogenic differentiation (Bayol *et al*. 2000) and knockdown of *IGFBP5* by siRNA impaired myogenesis and suppressed *IGF-II* gene expression. This indicated the local IGFBP5 played critical roles in muscle growth and differentiation.

Relationships of IGFs expression with meat quality traits in Nanyang cattle: The mean IMF content of *Longissimus dorsi* muscle sample was 1.932±0.612 (%), and the max value of IMF was 3.48% and the min value of IMF was 0.85%, respectively. The *pearson* correlation coefficient of *IGF-IIR*, *IGFBP5* and *IGFBP6* expression with IMF were 0.633 (P<0.01), 0.413 (P<0.05) and 0.495 (P<0.05) respectively, which indicated the *IGF-IIR*, *IGFBP5* and *IGFBP6* had significant positive effects on the IMF content in cattle (Table 2). The mean tenderness of *Longissimus dorsi* muscle sample was 4.94±1.12 kg, and the max tenderness and min tenderness were 7.10 kg and 3.11 kg, respectively. The expression of *IGFs* had no significant relationship with meat tenderness in cattle (Table 2). The juiciness and flavor were tested by sensory analysis and only expression of *IGF-II* had significant negative relationship with meat flavor in this study (Table 2).

IGF-II had variable degree influence on some meat quality traits, such as water holding capacity, colour and sensory quality (Lefaucheur 2010). The relationship of *IGF-II* gene with the IMF has been intensively studied in pigs. The effect of SNPs within the promoter region of *IGF-II* gene was associated with IMF (Aslan *et al*. 2012) and the significant differences in expression of *IGF-II* gene between the samples with high and low IMF content were observed (Burgos *et al*. 2012). The other IGFs, IGF-IR and IGFBP3, had been confirmed to regulate the differentiation and lipid accumulation, maintain cellular homeostasis (Kloting *et al*. 2008, Liu *et al*. 2012) of preadipocytes. This indicated that the IGFs pathway participate the regulation of muscle development and IMF accumulation in cattle.

The expression of *IGF-I*, *IGF-II*, *IGF-IR*, *IGF-IIR*, *IGFBP1* and *IGFBP3* showed significant decreasing tendency, while the expression of *IGFBP2*, *IGFBP4*, *IGFBP5* and *IGFBP6* had no significant changes during the postnatal *Longissimus dorsi* muscle development in Nanyang cattle. The expression of *IGF-IIR*, *IGFBP5* and *IGFBP6* had significant positive correlation with IMF and the expression of *IGF-II* had significant negative correlation with meat flavour trait of *Longissimus dorsi* muscle in 24 month-old Nanyang cattle.

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