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Differential Expression Analysis of Candidate Genes Related with Growth according to Dietary Supplementation of *Curcuma longa* in Chickens

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ABSTRACT

This experiment was conducted to investigate the genetic effects of candidate genes on the growth of spleen and liver tissues using dietary *Curcuma longa* (*C. longa*) supplementation. Expression analyses of candidate genes regarding animal growth was performed in order to determine the factors affecting the growth related to immune components of Curucumin, Turmerone, and Zingiberene as the bile secretion *Paratolyl methyl carbinol* (*PTMC*). The animals were divided into four groups of five chicks supplied with experimental diets of *C. longa* at 0.25, 0.5 and 1% and controls. The 19 growth-related genes were known to cell maturation, differentiation significant expression patterns in this analysis. Expression of growth response-related genes in chicks supplemented with 1% of *C. longa* showed better growth performance than chicks with 0.25 and 0.5% in spleen ($p<0.05$). The IGF1, MSTN, POU1F1, ADCYAP1 gene were known to central roles in mediating gonadotropin function, regulating steroidogenesis and promoting oocyte growth and maturation. Sex steroids, androgen and estrogen can affect sex differentiation and also can affect muscle development. On the other hand, GHSR and FABP3 gene showed significant expression patterns in this analysis. The results would be used as basic information for the variation of growth-related genes expression on the cell growth, sex cell growth, and sex hormones according to dietary supplementation with *C. longa* in chickens.

(Key words : *Curcuma longa*, Differential expression analysis, Genes related growth, Chicken)

INTRODUCTION

Several quantitative traits for production such as growth, egg-laying, feed conversion, carcass weight and body weight at different day-ages are important in domestic animals.

Several chemical compounds and antibiotics have been identified in herbaceous plants by researchers, which play a key role in human and animal health. The medicinal plant *Curcuma longa* is commonly used as a spice in human food. *Curcuma longa* (*C. longa*), a perennial herb which is known as tumeric, is a member of Zingiberaceae. The plant grows to height of 3 to 5 feet and has oblong pointed leaves, which bears funnel shaped yellow flowers (Durrani *et al*., 2006). The rhizome is the portion of the plant used medicinally; it is usually boiled, cleaned, and dried, yielding a yellow powder, tumeric, the ingredient that gives curry powder its characteristic yellow color (AL-Sultan, 2003). *C. longa* is medicinal plant widely used cultivated in tropical regions. Plant extracts were found to have antifungal, imunomodulatory, anti-oxidative and anti-mutagenic (Soni *et al*., 1997) activities. Some of pharmacological activities of *C. longa* as nematocidal (Kiuchi *et al*., 1993) and anti-inflammatory (Ammon *et al*., 1993) were demonstrated. Furthermore, the plant was used predominantly for endoparasites as well as internal and external injuries (Lans and Brown, 1998). More over (Soni *et al*., 1997) proved the protective effects of *C. longa* as food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity(Durrani *et al*., 2006). Keeping in view the significant importance of *C. longa*, this research study was conducted to investigate the effect of *C. longa* on the growth of broiler chicks.

The experiment was designed to investigate the pos-

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sible effect of *C. longa* feed additive on the growth performance of 20 broiler chickens. And the experiment was designed to select of 19 growth-related gene associated cell growth and sex cell growth.

MATERIALS AND METHODS

Experimental Design

The experimental chicks were divided at age of day-old into 4 groups, each consisted of 20 chicks and assigned as group 1-4. Birds group 1 were fed basal diet, while groups 2, 3, and 4 were fed basal diet supplemented with 0.25 (A group), 0.5 (B group) and 1% (C group) *C. longa.*

Sample Collection

On 21 days, all birds were weighed and feed consumption was records for each pen. Average feed intake and BW gain were determined. Liver and spleen tissue was collected, with at least 1 bird representing each pen. Collected liver and spleen tissue samples were snap-frozen in liquid nitrogen and stored at −80 ℃ for real-time PCR analysis.

Statistical Analysis

The normal distribution of data was confirmed by the χ^2 goodness of fit test. Data were subjected to repeated-measures ANOVA using the R program. Data in the same tissue sampling time were also compared using student's *t*-test. The threshold of significance was 0.05.

RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

Ribonucleic acid was extracted from the liver and spleen samples using an TRIzol Reagent (ambion, USA) and Chloroform (Merk, USA), and concentrated using RNase free water. The quality and integrity of the purified RNA was checked through agarose gel electrophoresis and the quantity was measured using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE; Flanagan, 2005). The purified RNA samples were preserved at −80℃ until used. Two-step quantitative real-time PCR was used to measure expression patterns of genes involved in growth. From each chick liver and spleen, 10ug of total RNA was reverse-transcribed using Super Script Ⅱ Reverse Transcriptase (invitrogen, Carlsbad, CA, USA) with oligo dT (200 U/ul, Promega, USA) and random primer (200 U/ul, Promega, USA). Forward and reverse primer final concentrations were 100 nM in the SYBR green assay (Applied Biosystems, Foster City, CA). Primers were designed using the Primer 3 program (Rozen and Skaletsky, 2000) with an annealing temperature of 60℃ and amplification size of less than 200 bp (Table 1). Glyceraldehyde phosphated dehydrogenase (GAPDH) was used as the endogenous control gene in the RT-PCR experiments.

RESULTS AND DISCUSSION

Expression of growth response-related genes in chicken supplemented with 1% of *C. longa* showed better growth performance than chicks with 0.25 and 0.5% in spleen ($p<0.05$). The 19 growth-related genes were known to cell maturation, differentiation significant expression patterns in this analysis.

That dietary *C. longa* supplementation modulates the growth response partly through changes in the expression growth-related genes such as TGFBR1, IGF1, IGF-2, MSTN, GHSR, TGFBR2, PDGFB, IGF1R, CTGF, FGF-2, GH1, POU1F1, GHRH, FABP3, ADCYAP1, BMPR2, CAPN3, IL1B and SST in liver and spleen. As shown in the above experiments dietary *C. longa* supplementation modulates the growth response partly through changes in the expression growth-related genes. Fig. 1 summary the data on mRNA expression of substances related to growth responses in the liver in experiment. The highest expression of IGF1, MSTN, POUF1 and ADCYAP1 gene was observed 0.25, 0.5 and 1.0% that of fed *C. longa* ($p<0.05$). Especially more highly growth-related genes was observed 0.25% group in liver ($p<0.05$). And GHSR, FABP3, IL1B gene was very low observed 0.5% group in liver ($p<0.01$). Fig. 2 summary the data on mRNA expression of substances related to inflammatory responses in the spleen in experiment. The highest expression of ADCYAP1, IL1B and SST gene was observed 0.25, 0.5 and 1.0% that of fed *C. longa.* Especially more highly growth-related genes were observed 1.0% group in spleen. But growth gene expression in spleen was never significant. The IGF1, MSTN, POU1F1 and ADCYAP1 gene were known as follows. The IGF1 (insulin-like growth factors), isolated from plasma, are structurally and functionally related to insulin but have a much higher growth-promoting activity (provided by RefSeq, Mar 2009). IGF genes also regulate development (Silha *et al*., 2005) and male sex determination (Nef *et al*., 2003). At the level of the gonad, IGFs play central roles in mediating gonadotropin function (Yu *et al*., 2003), regulating steroidogenesis (Mukhejee *et al*., 2006) and promoting oocyte growth and maturation (Santos *et al*., 2006). The protein encoded by MSTN (myostatin) gene is a member of the bone morphogenetic protein (BMP) family and the TGF-beta su-

Table 1. Primer sequences used in real-time PCR

Gene	Primer sequence		Product size(bp)
	Forward(5'-3')	Reverse(3'-5')	
TGFBR1	AGCTGCGGACAACAAAGACAATGG	CAATTGCTGGTTTGCCTTGTGTGC	200
IGF1	TGGATGAGTGCTGCTTCCAGAGTT	GAGCGTGCAGATTTAGGTGGCTTT	85
IGF2	ATAACAGGAGGATCAACCGTGGCA	ATGGCTTCTGGAAGCTCTCCTTGT	168
IGFBP2	TGAGAGCATCCTTGCTGAGAACCA	TTTGAGTCCTCGTGGTTGTGATGG	180
MSTN	ACGGTGTTTGTGCAGATCCTGAGA	ATACCAGTGCCTGGGTTCATGTCA	104
GHSR	ACATGAGGACCACCACCAACTTCT	AACTGGAAGAGCTTGCAGAGGAGA	145
TGFBR2	ATGCCTACCGCACTCACAAGAAGA	TGTGGTTGATGTTGTTGGCACAGG	156
PDGFB	TCTAGCCTGGACCTGAATGCAACT	TGTCACGGGAGATCTCAAAGACCA	151
IGF1R	ACAACGAGTACAACTACCGCTGCT	ATTCTGGTCTGTGCAGGCTCTCTT	92
CTGF	TCGACCAGGGTCACCAACGATAAT	ACAGCTCAAACTTGATGGGCTTGG	160
FGF2	AAGAGCGATCCGCACATCAAACTG	AGCCAGAAAGCGGTTTGCACTTAC	90
GH1	TCAGACATGGAGCTGCTTCGGTTT	TCAAACACTCTGTCTGAGGTGCCA	122
POU1F1	TGTGCCCTCCTGTCACTATGGAAA	ATAGCATCTCTGGAGTTGCAGGCT	83
GHRH	TTGGCAAACGGCTCAGAAACAGTG	TCCCAAGAAGTCCCTCAGTACCAT	134
FABP3	AGCTGGATGAGGAGTTCGATGAGA	TGCCCATGGTGAGAGTCAGAATCA	168
ADCYAP1	ACATAGACGGCATCTTCACGGACA	ATACGCTACTCGGCGTCCTTTGTT	131
BMPR2	ACACAAGTGACTGGGTGAGCTCTT	CATTGCGGCTGTTCAAGTCACGAT	135
CAPN3	ATGCCGGGAAGAACTCAGGAATGT	AGCCGTCTGTATCCATCAAAGCGA	122
IL1B	AAAGTGAGGCTCAACATTGCGCTG	TGTAGAGCTTGTAGCCCTTGATGC	106
SST	TCTCAGAGCCAAGCCAGACAGAAA	AGAAGTTCTTGCAGCCCGCTTT	156

IGF1 : Insulin-like growth factor 1, IGF2 : Insulin-like growth factor 2, IGFBP2 : Inculin-like growth factor-binding protein 2, MSTN : Myostatin, GHSR : Growth hormone secretagogue receptor, CAPN3 : Calpain 3, TGFBR2 : Transforming growth factor beta receptor Ⅱ, PDGFB : Platelet-derived growth factor subunit B, IGF1R : Insulin-like growth factor 1 receptor, CTGF : Connective tissue growth factor, FGF2 : Fibroblast growth factor 2, GH1 : Growth hormone 2, POU1F1 : POU class 1 homeobox 1, GHRH : Growth hormone releasing hormone, FABP3 : Fatty acid binding protein 3, ADCYAP1 : Adenylate cyclase activating polypeptide 1, BMPR2 : Bone morphogenetic protein receptor, type Ⅱ, IL1B : interleukin 1 beta, and SST : Somatostatin.

perfamily. This group of proteins is characterized by a polybasic proteolytic processing site which is cleaved to produce a mature protein containing seven conserved cysteine residues. The members of this family are regulators of cell growth and differentiation in both embryonic and adult tissues. This gene is thought to encode a secreted protein which negatively regulates skeletal muscle growth (provided by RefSeq, Jul 2008). In addition, the expression of MSTN can be regulated by hormone and environmental factors. Sex steroids, androgen and estrogen can affect sex differentiation and also can affect muscle development (Yuhui *et al*., 2012). The POU1F1 (POU class 1 homeobox 1) gene encode by a member of the POU family of transcription factors that regulate mammalian development. The protein regulates expression of several genes involved in pituitary development and hormone expression (provided by RefSeq, Jul 2008). The genes of the POU1F1 pathway play a crucial role in embryonic survival rate (khatib *et al*., 2009). In bovine embryos the GH transcript has been detected in oocytes and all pre-attachment stages including blastocysts (Kolle *et al*., 1998; Joudrey *et al*., 2003). The ADCYAP1 (adenylate cyclase activating polypeptide 1) gene encodes a secreted proprotein that is further processed into multiple mature peptides. These peptides stimulate adenylate cyclase and increase

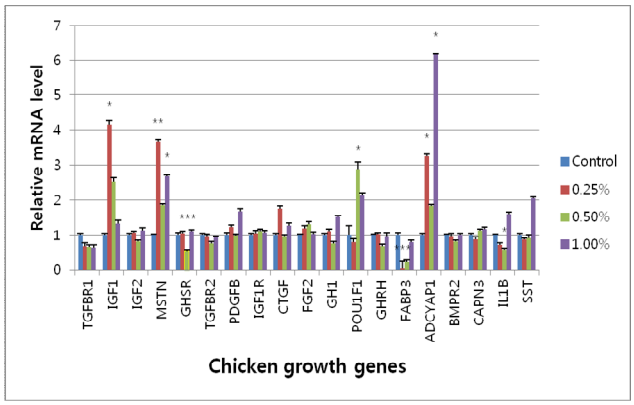

Fig. 1. Effect of dietary supplementation of *C. longa* on mRNA expression of growth response in liver of broiler chickens. * : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$.

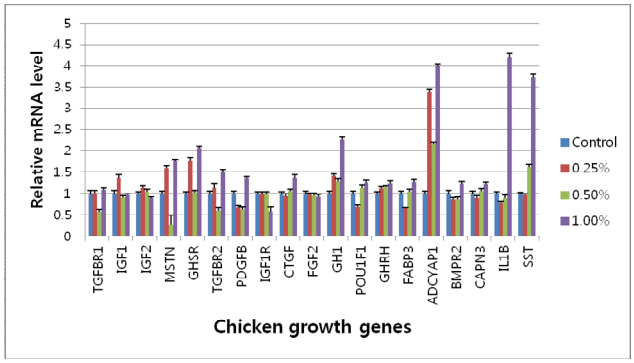

Fig. 2. Effect of dietary supplementation of *C. longa* on mRNA expression of growth response in spleen of broiler chickens. There was no statistically significant difference in the value.

cyclic adenosine monophosphate (cAMP) levels, resulting in the transcriptional activation of target genes (provided by RefSeq, Feb 2013). ADCYAP1 might regulate gonadotropin gene expression in the human fetal pituitary and might play a role in the differential secretion of LH (luteinizing hormone) and FSH (follicle-stimulating hormone) that occurs in normal adolescence, in hypogonadotropic hypogonadism including hyperprolactinemia, during fasting (Vertongen *et al*., 1995; Stephen *et al*., 2011).

On the other hand, GHSR and FABP3 gene showed significant expression patterns in this analysis. The GHSR (Growth hormone secretagogue receptor) gene encodes a member of the G-protein coupled receptor family. The encoded protein may play a role in energy homeostasis and regulation of body weight. Mutations in this gene are associated with autosomal idiopathic short stature (provided by RefSeq, Apr 2010). GHSR mRNAs were detected in morula or more advanced embryo stages (Kazuhiro *et al*., 2003). In addition, the GHSR is expressed in diverse periphera tissues (Mori *et al*., 2000; Papotti *et al*., 2000). In animals with insufficient nutrient intake, the fertility potential is suppressed, probably due to adaptive responses to evade the excess metabolic demands imposed by pregnancy (I'Anson *et al*,. 1991; Wade *et al*., 1996). The FABP3 (Fatty acid binding protein 3) may also be responsible in the modulation of cell growth and proliferation. Fatty acid-binding protein 3 gene contains four exons and its function is to arrest growth of mammary epithelial cells. This gene is a candidate tumor suppressor gene for human breast cancer (provided by RefSeq, Jul 2008). FABPs are needed for intracellular fatty acid transport and contribute to cell differentiation as shown in embryonic stem cells (Cornelia *et al*., 2012).

In conclusion, the present results suggest that dietary *C. longa* supplementation above levels recommended for growth increase. Also growth-related genes were known to central roles in mediating gonadotropin function, regulating steroidogenesis and promoting oocyte growth and maturation. The results would be used as basic information for the variation of growth-related genes expression on the cell growth, sex cell growth, and sex hormones according to dietary supplementation with *C. longa* in chickens.

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