The Expression Characterization of Chicken Uncoupling Protein Gene

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ABSTRACT : The UCPs are members of the mitochondrial inner membrane transporter family, present in the mitochondrial inner membrane. Their main function is increasing the energy expenditure via diminishing the resulting production of ATP from mitochondrial oxidative phosphorylation instead of yielding dissipative heat. They are associated with the metabolism of fat and regulation of energy expenditure. The UCP gene can be viewed as the candidate gene for chicken fatness. In the present study, RT-PCR and Northern Blot methods were developed to investigate the expression of the UCP gene in ten tissues including heart, liver, spleen, lung, kidney, gizzard, intestine, brain, breast muscle and abdominal fat of chicken. The results of both RT-PCR and Northern Blot methods showed that the UCP gene expressed specific in breast muscle. The expression levels of UCP gene in breast muscles from egg-type and meat-type chickens of hatching, 2, 4, 6 and 8 wk of age were detected by RT-PCR assay and results showed that the expression levels of UCP gene were related to breeds. Expression level of UCP gene in layers was higher than that in broilers at various weeks of age except at 6 wk. The UCP gene's expression was higher at 6 wk and had no significant difference among other weeks of age in broilers; in layers the expression level of UCP gene had no significant difference among weeks of age. The experiment results also showed that insulin could increase the expression level of UCP gene by 40% compared with control group. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1552-1556)***

Key Words : Chicken, UCP Gene, RT-PCR, Northern Blot, Insulin

INTRODUCTION

Obesity has increased at an alarming rate in recent years and is now a worldwide health problem. The obese people suffer poor health as well as increase risk of illness such as hypertension and heart disease (Trisha, 1998; Friedman, 2000). As for the agricultural animals, more deposition of fat could decrease the feed conversion rate, meat quality, and reduce the edible portion. Fat deposition in broiler chicken has commanded a great deal of interest in recent years because of its prominent issue in chicken breeding (Ikeobi et al., 2002). The application of molecular genetic skills in chicken such as candidate gene analysis (Rothschild and Soller, 1997) and DDRT-PCR (Wang et al., 2005) will be helpful to improve these problem.

Some studies showed that decrease in the energy expenditure was the main reason caused by obesity (Ravussin et al., 1988; Roberts et al., 1988). Great progresses have been made in identifying the components of the homeostatic system that regulates the body weight, including several genes responsible for animal and human obesity (Friedman, 2000; Meng et al., 2005). The new discoveried genes that codes for novel uncoupling protein play an important role in energy metabolism in human and mouse (Trisha. 1998; Richard, 1998). Furthermore, these genes mapped to regions of human chromosome 11 and mouse chromosome 7 were reported to been linked to hyperinsulinaemia and obesity (Fleury et al., 1997).

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Received December 16, 2004; Accepted May 27, 2005

Uncoupling proteins are members of the mitochondrial membrane transporter family, present in the mitochondrial inner membrane that mediate discharge of the proton gradient that is generated by the respiratory chain and diminish the resulting production of ATP instead of yielding dissipative heat (Trisha, 1998; Ricquier et al., 2000). This energy-dissipater mechanism can be associated with the metabolism of fat and regulation of the energy expenditure. It has been reported that UCP gene was partially involved in the obesity (Fleury et al., 1997; Zhou et al., 1997; Trisha et al., 1998; Ricquier et al., 2000). In addition, UCP gene also can be regulated by Leptin, the production of the ob gene, which plays an important role in the energy intake and expenditure (Kim and Baik, 2004). This inspires us to view UCP gene as the candidate gene that impacts chicken growth and body composition traits.

In mammals, uncoupling proteins gene family has four members that named UCP1, UCP2, UCP3 and UCP4 (Ricquier et al., 2000). Chicken uncoupling protein gene has been cloned and its mRNA length is 1,550 bp (Ricquier et al., 2001). The predicted 307 amino-acid sequence of chicken UCP is 55, 70 and 70% identical with mammalian UCP1, UCP2 and UCP3. Therefore we deduce the chicken UCP gene plays the same role with mammalian UCP2 and UCP3 that related to energy metabolism. The study in our lab also showed that SNPs in the 3'- UTR region of chicken UCP gene was associated with variation of weight as well as percentage of abdominal fat (Zhao et al., 2002).

The current study compared the difference of chicken UCP gene expression at various raising stages and tissues in broiler and layer by the methods of RT-PCR and Northern

Blot. The change of UCP gene's expression affected by the insulin that injected intramuscularly was also investigated. The objectives of the study were to discover the expression rule of chicken UCP gene in broiler and layer and effects of insulin in order to elucidate the function of chicken UCP gene in fat metabolism and energy expenditure in chicken.

MATERIALS AND METHODS

Experimental animals and tissue samples collection

Ten tissues samples, including heart, liver, spleen, lung, kidney, gizzard, intestine, brain, breast muscle and abdominal fat of two Arbor Acres broiler (AA broiler) and two Hyline layers (one is male another is female for each breed) at day of birth, 2, 4, 6 and 8 wk of age were collected. All tissues were snap-frozen in liquid nitrogen after slaughtered.

Six AA broilers at 7 wk of age and live weight around 2kg were divided into two groups. In the experiments group, 7-wk-old AA broilers were injected intramuscularly with porcine insulin (Sigma) 200 μg kg body wt^{-1} day^{-1} (n = 3) for a period of 4 days. Insulin was dissolved in saline. Sham-injected controls (n = 3) were treated with an equal volume of saline. All birds were killed 24 h after the final injection (day 5), and the breast muscle was frozen in liquid nitrogen and then stored at -80°C until processed.

Primer design

Gene specific primers were designed according to the mRNA sequence of the chicken UCP gene (GenBank accession No.: AF287144) and the GAPDH gene (GenBank accession No.: K01458) by using the software of primer premier 5.0. *UCP* gene: Forward： 5' -GGCAGT ACCGC AATGTGC-3'; Reverse: 5'-TTTGCTGTCTCCCTTCCCT-3'; *GAPDH* gene: Forward: 5'-TGACGTGCAGCAGG AACAC-3' Reverse: 5'-CAGTTGGTGGTGCACGATG -3'. These two pairs of primers were used for the semi-quantitative RT-PCR and Northern blotting.

Tissue expression pattern of the UCP gene

Total RNA extraction and Semi-quantitative RT-PCR : Ten tissues of AA broiler at 6 wk were used to investigate the tissue expression pattern. RNA was extracted with a TRIzol reagent kit (Life Technologies, Grand Island, USA) according to the standard protocols. Two RNA samples of each tissue were mixed equally as template for RT-PCR. Reverse transcription (RT) was performed as Wang described (Wang et al., 2004). The PCR reactions were performed in a volume of 25 μl of 1×PCR buffer (Promega), containing of 50 ng of template cDNA, 0.1 μM of each primer, 800 μM of each dNTPs, 1.5 mM $MgCl_2$ and 1.0 Units *Taq* DNA Polymerase (Promega). Conditions for amplification were 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, 40 s at 72°C, and a final extension of 10 min at 72°C °C. Amplification of one fragment of GAPDH cDNA was performed as an internal control. For semi-quantitative comparisons, PCR reactions for UCP and GAPDH gene were restricted to the linear range of amplification by limiting the cycle number to 28 and 24, respectively. PCR primers were designed to flank known introns, preventing amplification of any contaminating genomic DNA. The two PCR-amplified fragments were run beside molecular weight markers on the same 2% agarose gels stained with ethidium bromide. Gels were photographed using the electrophoresis gel imaging system (UVP). The semi-quantitative measure of gene expression was using the ratios of UCP/GAPDH absorption density of bands on a gel (Meng et al., 2004).

The RT-PCR products were purified with Wizard PCR Preps DNA Purification System (Promega, Madison,WI, USA) and cloned into the pGEM T-easy vector (Promega), then sequenced using a commercial service. BLAST search program (http://www.ncbi.nlm.nih.gov/blast/nr) was used to find if the amplified product is the expected gene.

Northern blotting : The probe for Northern blotting analysis is the RT-PCR product of the UCP gene. Probes were labeled with [α-^{32}P]dCTP by a Prime-a-Gene labelling system (Promega, USA). Hybridization was carried out according to the manufacturer's recommendations.

Quantitative expression level of UCP gene in boilers and layers at the different weeks of age

Breast muscle of broilers and layers at the different weeks of age were used in this study. Total RNA extraction and semi-quantitative RT-PCR were performed as described above at various weeks of age.

Effect of the insulin-treated on the UCP gene expression

Insulin-treated group and the control group of AA broilers at 7 wk were used to investigate the effect of the insulin on the UCP gene expression. RNAs isolation from breast muscles and semi-quantitive RT-PCR were carried out as described above.

RESULTS

RT-PCR and tissue expression pattern of the UCP gene

Sequence analysis revealed that RT-PCR product of UCP gene was 260 bp and homologous analysis showed that this gene was 92% identical to the gene in the database (Acc. No. AF287144), which indicated that the sequence were from the gene expected.

RT-PCR was performed to detect the broilers UCP gene expression pattern in ten tissues and the PCR products of UCP were normalized assuming that the expression of GAPDH is the same level in the entire sample. Broiler

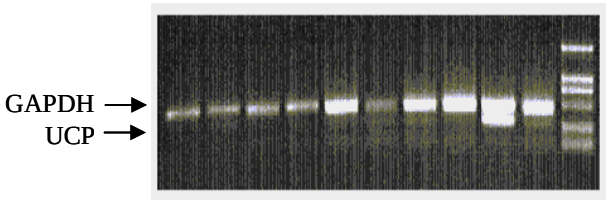

Figure 1(A). The detection of UCP gene expression in different tissues with RT-PCR method (The lanes from left to right represent heart, liver, spleen, lung, kidney, gizzard, intestine, brain, breast, abdominal fat and marker, respectively).

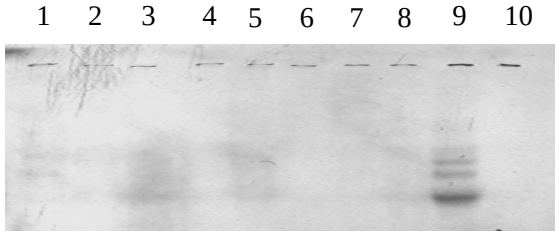

Figure 1(B). Northern Blot result of UCP gene in ten tissues (The lanes from l to10 represent heart, liver, spleen, lung, kidney, gizzard, intestine, brain, breast and abdominal fat, respectively).

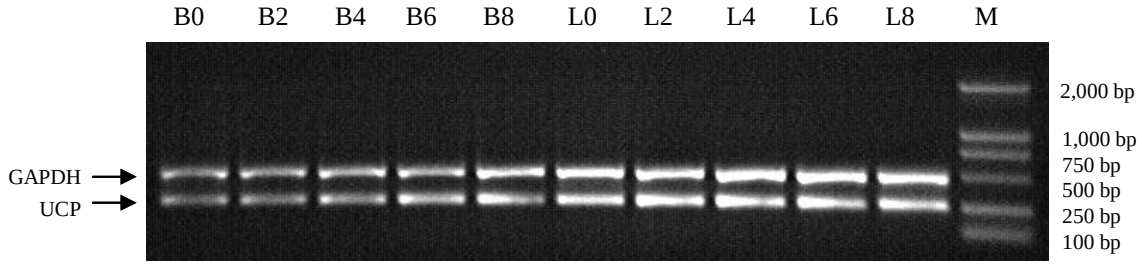

Figure 2. UCP gene express level in the breast muscle of broiler and layer at various weeks of age (B0-B8 and L0-L8 represent broiler and layer, respectively, at various weeks of age).

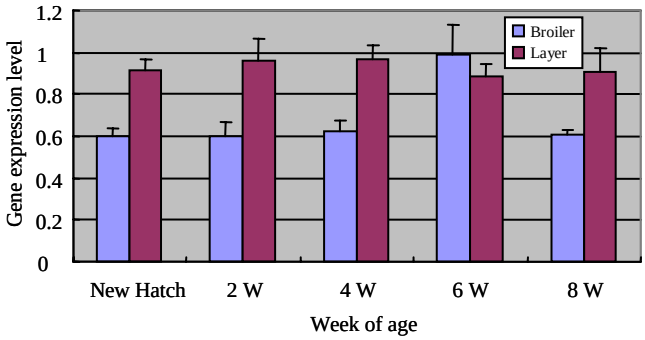

Figure 3. Comparison of UCP gene expression level in the breast muscle of broiler and layer at various weeks of age.

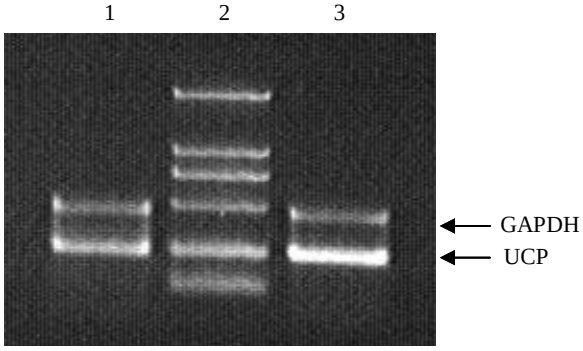

Figure 4. The expression level of UCP gene in the breast muscle of insulin treated chicken (Lane 1: the control group, lane 2: DL2000 marker, lane 3: insulin treatment group).

GAPDH gene was ubiquitously expressed and this indicated that the RNA isolation and the reverse transcription were successful. Results showed that broiler UCP gene was expressed only in breast muscle (Figure 1(A)).

Northern blotting result conformed that this gene only expressed in the breast muscle (Figure 1(B)).

Expression profile of UCP gene in boilers and layers at different weeks of age

Semi-quantitative RT-PCR was employed to investigate the UCP gene expression levels in the breast muscle of the broiler and layer at the day of birth, 2, 4, 6 and 8 wk of age. GAPDH was used as the internal control (Figure 2). According to their optical intensity value, the bar graph were constructed (Figure 3). From the Figure 3, we can observe that UCP gene expressed more highly in layers than in broilers at each stage, except for 6 wk. Highest expression level of UCP gene was found in broiler at 6 wk and the expression level was very similar at the other various stage. There is no significant diffience among expression levels of UCP gene in layers at the various weeks of age.

Effect of insulin-treated on the UCP gene expression

Semi-quantitative RT-PCR method also was used to study the effect of the insulin on the UCP gene expression in the chicken breast muscle, results showed in Figure 4 and 5. We can find that higher expression level was detected in the insulin-treated group than in the control group (Figure 4), insulin-treatment makes the UCP gene expression level improve 40% according to their optical intensity value (Figure 5).

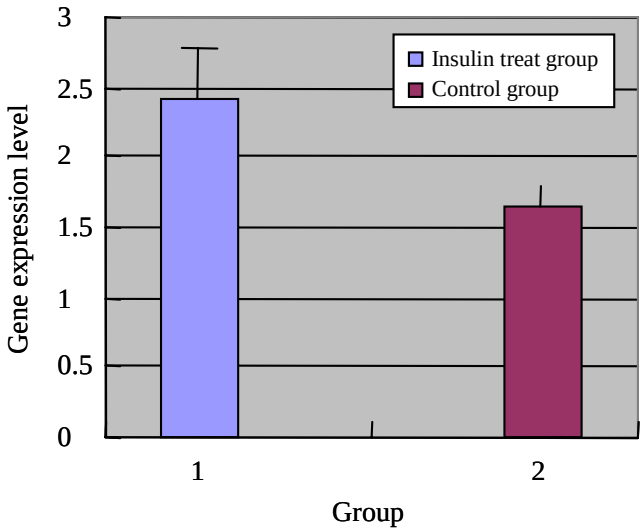

Figure 5. The influence of insulin treatment on UCP gene expression level in chicken breast muscle.

DISCUSSION

Tissue expression characterization of the UCP gene

There are 4 members of the UCP gene family in mammalian animals, whereas only one member was found in chicken. Some researchers found that except expressing highly in the skeletal muscle, the chicken UCP gene also express in the heart, liver, kidney, fat, spleen, brain and lung (Christina et al., 2002). However, study from Ricquier (2001) revealed that chicken UCP gene expressed only in the skeletal muscle (Raimbault et al., 2001). Our data from the semi-quantitative and Northern blotting were consistent with the results from Ricquier. As we know, muscle is one of the major tissues involved in the energy metabolism; this special tissue expression characterization of the UCP gene maybe was related to its participation in the energy metabolism.

Comparison of the UCP gene expression levels in the breast muscle between the broiler and layer

Broiler and layer are two extremely breeds with large differences through the artificial selection. Compared with layers, broilers have fast growth rate. Great differences exist in the speed of fat deposition and energy metabolism between the two breeds. Studies of UCP gene expression level on the Isoland chicken lines of divergent selection for food conversion rate showed that lower food conversion rate Isoland lines presented 1.3 fold UCP gene expression level and consumed 30-40% more food compared with higher food conversion rate lines while own the same body weight as well as egg yields (Christina et al., 2002). Owing to the important role of the UCP gene played in the energy metabolism and regulation of the body temperature (Trisha et al., 1998; Ricquier et al., 2000), the mRNA expression levels of UCP gene were determined in broiler and layer in present study. The current study also showed the same results as Christina M (2002), that the layer presented higher expression level of UCP gene at each week of age than broiler when feed the same commercial broiler food. This maybe indicated that UCP gene participate in the energy metabolism of chicken and could be viewed as the candidate gene to study the body fatness traits of broiler in order to increase the food conversion rate.

Effect of the insulin-treated on the UCP gene expression

Some evidences reported that external insulin could up-regulate the expression of the rat UCP gene in the fat and skeletal muscle (Jin et al., 2000), but there is no related study in the livestock. As we all know that insulin is related to energy metabolism and could ingest the glucose as well as increase the fat anabolism (Shen and Wang, 1990), but the function of UCP could improve the fat catabolism and increase energy expenditure. Our results exhibited that external insulin injection make expression of the UCP gene in the broiler breast muscle at 7 wk increased by 40%. Insulin could up-regulate the mRNA expression of the *UCP* gene in skeletal muscle and. So it seems that insulin plays a positive role in the fat catabolism. But this was inconsistent with the classical theory that insulin could increase the capability of tissues to ingest the glucose and increase the fat anabolism, which hints us to further study the function of the UCP gene and the mechanism in which the insulin involves.

In conclusion, we report that the chicken UCP gene expressed only in breast muscle among ten tissues of the broilers at 7 wk of age; UCP gene expressed more highly in layers than in broilers at earlier stage; Insulin-treated could up-regulate the expression of the UCP gene. Combining the earlier study on UCP gene in our lab, we speculated this gene participated in the energy metabolism of the body and was an important candidate gene for studying the energy metabolism, growth and the body composition trait, especially the fatness traits variation of the broiler. The biological function of the UCP gene is still not very clear, knock-out gene chicken and the other animal model should be established to further study the function of this gene.

ACKNOWLEDGEMENTS

This research was financed by a grant from the National Natural Science Foundation of China (No. 30270950), the National High Technology Research and Development Program of China (863 Program) (No. 2002AA211021) and Education Ministry Foundation for the Scholars Returned from Abroad. We thank Dr. Yanfang Wang (Institute of Liver Cancer, Fudan University) for her useful comments.

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