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RT-PCR Analysis of LDL Receptor MRNA Expression in Different Tissues of Genetically Fat and Lean Chickens

[1,2]H.H. Musa, [1]J.H. Cheng, [1]W.B. Bao, [1]J.T. Shu, B.C. [1]Li and [1]G.H. Chen
[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China
[2]Department of Animal Production, Faculty of Veterinary Science, University of Nyala, Sudan

Abstract: Tissues specimens include (liver, kidney, small intestine and abdominal fat) were excised from lean (Rugao) and fat (Anka) chickens, both breeds were raised under the same conditions. The expression of LDL receptor mRNA in various tissues of genetically fat and lean chickens were determined by semi-quantitative RT-PCR. The level of gene expression was determined as the ratio of integrated peak area for each individual gene PCR product relative to that of the co-amplified ß-actin internal standard. The results shows that the expression of low density lipoprotein receptor mRNA was significantly ($p<0.05$) different between abdominal fat and liver tissues, while they were non significantly different between the other tissues. The levels of LDLR1 mRNA expression in intestine, kidney and abdominal fat tissues were none significantly different between genetically fat and lean, while it was significantly ($p<0.05$) different in liver. In addition, the levels of LDLR5 mRNA expression in various tissues were non significantly different between genetically fat and lean chickens.

Key words: RT-PCR, LDL receptor, mRNA expression, chicken, tissue, abdominal fat

INTRODUCTION

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is a highly sensitive and specific method useful for the detection of rare transcripts or for the analysis of samples available in limiting amounts (Carding and Bottomly, 1992). Three methods were available for the measurement of RT-PCR products such as semi-quantitative RT-PCR, competitive RT-PCR and Real-time RT-PCR. The major drawback of semi-quantitative RT-PCR and competitive RT-PCR is the use of hazardous chemical like ethedium bromide. In addition, results should be transformed into numbers by image analysis, which is depends on the software quality. Therefore, real-time RT-PCR was technically preferred because does not require post-PCR methods. The physiological role of the LDL receptor is to transport cholesterol carrying lipoprotein particles into cells. It is plays an important role in cholesterol homeostasis. Receptor lipoprotein complexes enter cells by endocytosis via clathrin-coated pits, where the receptor molecules cluster on the cell surface (Anderson *et al*., 1977). The complexes are then delivered to endosomes, where the low pH environment triggers release of the bound lipoprotein particles (Brown *et al*., 1981). The receptors are subsequently returned to the cell surface in a process called receptor recycling (Brown and Goldstein, 1986). Previous results on the expression of lipoprotein receptors in the laying hen and related studies by others Reagan *et al*. (1990) in pigeons revealed low levels of LDL receptor expression in cultured avian cells, even when grown under sterol-depleted conditions (Hayashi *et al*., 1989). Also low levels of chicken LDL receptor expression in somatic cells in vivo would be expected (Susanna *et al*., 2003). Thus, when a tissue survey was performed by Northern blot analysis using 20ug total RNA and a full length cDNA probe, transcript was undetectable in heart, kidney, lung, spleen, liver and muscle, despite testing a wide range of conditions. However, with 5ug poly A RNA, a 4.5kb transcript was detected in the adrenal gland (Aoki *et al*., 1993). The objective of this study was to quantify the expression of LDL receptor mRNA in different tissues of genetically lean and fat chickens using semi-quantitative RT-PCR techniques.

MATERIALS AND METHODS

Animals and tissue collection: Tissues specimens include (liver, kidney, small intestine and abdominal fat) were excised from lean (Rugao) and fat (Anka) types of chicken in Jiangsu Poultry Institute (Yangzhou China), both breeds were raised under similar conditions (Musa *et al*., 2006). Approximately 1.5 g of tissues was collected from each breed in 1.5 mL microfuge tube after cervical

Corresponding Author: G.H. Chen, College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China

dislocation. The excised specimens were immediately snap frozen in liquid nitrogen and transferred to laboratory and stored at -80C until RNA extraction.

RNA extraction: Total RNA was extracted from tissues using the standard Trizol methods according to the manufacturer's protocol (Invitrogen /Life Technologies, Carlsbad, CA), RNA samples were dissolved in 100% formamide deionzed (Chomcznski, 1993). Samples were then quantified by fluorimeter at 260nm and the purity was determined by the OD_{260}/OD_{280} ratio. The RNA quality or integrity was also checked by 1% agarose gel electrophoresis, stained with 1 μg mL^{-1} gold view.

Primer design: Primers of LDL receptor mRNA were designed by Oligo 6.0 software, according to chicken genomic sequence in Gen Bank database (accession number AJ515243). The following primers were used for LDLR1 forward was 5 CGC GTC CGG CTC CAT ATC3 and reverse was 5CTC GCA GCC CCA CTC ATC C3, whereas for LDLR5 forward was 5 GCG CTG AGC CCA AAG TGC3 and reverse was 5 ATT GGG GTC GGA GCT GTG G3. In addition one pair of chicken ß-actin primer was used, forward was 5TGC CAG GGT ACA TTG TGG TA3 and reverse was 5 TGC GTG ACA TCA AGG AGA AG3.

First strand cDNA synthesis: The cDNA was synthesis using first strand cDNA synthesis kits supplied by (TakaRa Biotechnology Dalian Co., Ltd.) according to the manufacturer recommendations. Briefly 100ng of total RNA was reverse transcribed using 10 pmol μL^{-1} of oligo(dT)18 primer. The reaction volume was 20 μL, containing 5×M-MLV buffer, 10mM of dNTP Mixture, 40U μL^{-1} of RNase inhibitor, 200U μL^{-1} RTase M-MLV (RNase H-) and RNase free water. cDNA was synthesis at 42C for 1 h and extended at 70°C for 15 min, thereafter cDNA stock was stored at -20°C.

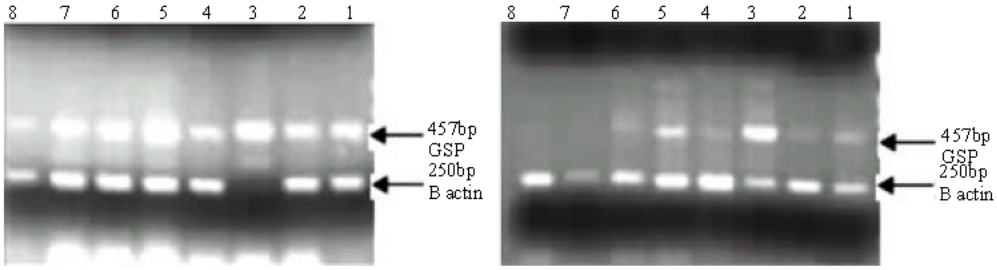
Semi-quantitative RT-PCR: Semi-quantitative RT-PCR with both primer sets and ß-actin were carried out in a total volume of 25 μL of solution containing 2.5 μL of 1xPCR reaction buffer, 2μL of (each 2.5 μmol) dNTP mix, 0.5U of Taq DNA polymerase, 1 μL of 5pmol gene specific primers and 1 μL of5pmol primer specific for ß-actin, 1.6 μL cDNA template and 14.6 μL sterilized water. The amplification was performed in Biometra PCR System (Applied Biosystems). Thermal cycling parameters were as follows: 1 cycle 94°C for 5 min, followed by 35 cycles, 94°C for 30s, 62 °C (LDLR5) 64.5°C (LDLR1) for 30s, 72°C for 1 min with a final extension at 72°C for 10 min., the samples were then run on the same agarose gel (1%) for quantitation.

Quantification and statistical analysis: Images of the RT-PCR gold view stained agarose gels were acquired with GENE- GENIUS (Bio-imaging system) and quantification of the bands was performed by Image Quant TL (Amersham Biosciences, USA). Band intensity was expressed as relative absorbance units. The ratio between the sample RNA to be determined and ß-actin was calculated to normalize for initial variations in sample concentration and as a control for reaction efficiency. Values were presented as the mean±SEM, significant differences were obtained using a paired Student's *t* test (Steel and Torrie, 1980) and analysis was performed by SPSS software.

RESULTS AND DISCUSSION

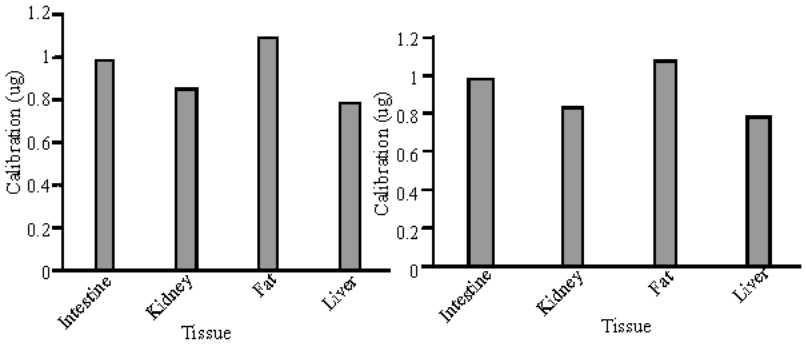
Quantitation of LDL receptor mRNA expression by semi-quantitative RT-PCR: Reliable methods for quantification of mRNA levels are necessary for investigation of gene expression. Recently by using semi-quantitative RT-PCR, we have determined the expression of LDL receptor mRNA in various tissues of genetically fat and lean chickens. The level of gene expression was determined as the ratio of integrated peak area for each individual gene PCR product relative to that of the co-amplified ß-actin internal standard (Fig. 1).

RT-PCR analysis of LDL receptor mRNA expression in different tissues of genetically fat and lean chickens: LDL receptors are a cell surface transmembrane protein that mediates the uptake and lysosomal degradation of plasma LDL, thereby providing cholesterol to cells. An increase in LDLreceptor activity, especially in the liver can lower LDL levels and protect against atherosclerosis. Therefore, two approaches are widely used to lower steady-state hepatic cholesterol levels by reducing cholesterol synthesis and /or increasing the conversion of cholesterol into bile acid in the liver. In the present study we found that the expression of low density lipoprotein receptor mRNA was significantly ($p<0.05$) different between abdominal fat and liver tissues, while they were none significantly different between the other tissues (Fig. 2). Fong *et al.* (1995)indicated that the level of LDL receptor mRNA in intestinal epithelial cells was somewhat lower than in liver, his results suggest serum cholesterol level is not the prime determinant of LDL receptor level in intestine. However, the expression of LDL receptor on liver cells is regulated by cholesterol levels inside the cell (Daniel, 2001). The purification of LDL receptors from bovine adrenal cortex possesses the highest specific LDL-binding activity (Kovanen *et al*., 1979). Regulation of mRNA stability is a relatively common mechanism for controlling gene. In LDL



A. LDLR1 mRNA expression B. LDLR5 mRNA expression

Fig. 1: LDL receptor mRNA expression, 1 and2 kidney, 3 and4 intestine, 5 and6 liver and 7 and8 fat; 2, 4, 6 and 8 were samples of fat chicken and the remain were samples of lean chicken; GSP, Gene Specific Primer



A. LDLR1 mRNA expression B. LDLR5 mRNA expression

Fig. 2: LDL receptor mRNA expression in different tissues of genetically fat and lean chickens

Table 1: Quantification of LDL receptor mRNA expression in different tissues of genetically fat and lean chickens

Tissue	No	Fat chicken	No	Lean chicken	p value
LDLR1					
Intestine	11	0.952±0.119	8	1.001±0.201	0.937
Kidney	7	0.903±0.144	5	0.752±0.147	0.594
Fat	9	1.209±0.136	10	0.968±0.152	0.295
Liver	11	0.703±0.063	13	0.840±0.070	0.055
LDLR5					
Intestine	12	0.677±0.084	8	0.805±0.187	0.684
Kidney	9	0.756±0.084	8	0.710±0.095	0.724
Fat	9	0.992±0.158	8	0.845±0.076	0.895
Liver	12	0.724±0.067	13	0.919±0.108	0.180

receptors gene, promoter contains a Sterol Response Element (SRE) that is required for regulating transcription of the gene encoding LDL receptor in response to cellular sterol content (Hussain *et al*., 1999). Regulation of the hepatic expression of LDL receptors is regulates the level of LDL in the blood. In the other hand, intestinal cholesterol is transported in the blood in Chylomicrons (CM), which are converted to Chylomicron Remnants (CMR) and subsequently taken up by the liver via LDL receptors (Daniel, 2001). Argov *et al*. (2004) reported that LDL receptor mRNA expression was high in small antral follicles and decrease linearly with increasing follicular size.

The levels of LDLR1 mRNA expression in intestine, kidney and abdominal fat tissues were none significantly different between genetically fat and lean, while it was significantly ($p<0.05$) different in liver (Table 1). On the other hand, the levels of LDLR5 mRNA expression in various tissues were none significantly different between genetically fat and lean. Several genes involved in the fatty acid synthesis pathway are more expressed in fat birds than the lean ones. However, if differences in gene mRNA levels can be related to the observed difference in the resulting fatness, it does not mean that in heritable DNA variation responsible for phenotypic variability take place in these genes and it can not be concluded that these genes could be used to assist selection on fatness (Assaf *et al*., 2004). As previously mentioned over expression of specific transcription factors Steroid Regulatory Element-binding Proteins (SREBPs) in transgenic mice was increase LDL receptor (Harton and Shimomura, 1999).

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