

## A COMPARATIVE STUDY OF ADIPOSE LIPID METABOLISM AND EGG YIELDS OF CHICKENS

C. H. Wang<sup>1</sup>, C. F. Chen<sup>2</sup>, Y. P. Lee<sup>2</sup>, and C. L. Chen<sup>1\*</sup>

<sup>1</sup> Department of Animal Science and Biotechnology, Tunghai University, Taichung 407

<sup>2</sup> Department of Animal Science, National Chungshing University, Taichung, 402

\* Corresponding author, e-mail: [clchen@thu.edu.tw](mailto:clchen@thu.edu.tw)

### ABSTRACT

The energy cost of egg production is significant. The objective of this study was to examine the variation of lipid metabolism in adipose tissue between chickens with different egg yields. Two selected strains of Taiwan country chickens originated from a common population with relatively low (B strain) and high (L2 strain) egg yields were used. Higher levels of glycerol-3-phosphate dehydrogenase (GPDH) activity were detected in the L2 than B hens, suggesting the L2 hens have higher fat storage capacity. The amounts of glycerol released to the medium from the adipocytes isolated from the L2 hens were significantly higher than those from the B hens, reflecting the mobilization of lipid stores from adipose tissue is more effective in the L2 than B hens. Results of reverse transcription polymerase chain reaction (RT-PCR) analysis showed that the mRNA levels of perilipin and glucose transporter 1 (GLUT1) were significantly higher in the L2 than B hens, while the B hens expressed higher mRNA levels of lipoprotein lipase (LPL) than the L2 hens. Higher levels of genes involved in lipid metabolism were expressed in the high egg-yield strain, although expression of the LPL gene did not correlate with egg yields. Taken together, the results imply that the genes involved in the regulation of the adipose lipid metabolism may contribute to the differences in reproduction performance as observed between the B and L2 hens.

**KEY WORDS:** Adipose, Chicken, Egg yields, GPDH activity, Lipolysis.

### INTRODUCTION

Reproduction is believed to be both energetically and nutritionally costly. Therefore, animals have evolved to be able to sense the energy supply and adjust their reproduction accordingly (Willams, 2005). Excess energy is stored mainly in the adipose tissue in the form of triacylglycerol (TAG), which is mobilized at times of increased energy demand. The metabolic processes of fat storage and mobilization in adipose tissue were once thought to be regulated by ex-adipose signals to maintain energy homeostasis. However, it has become increasingly apparent that the adipocyte functions as a major endocrine source, secreting a variety of biologically active molecules (such as adipocytokines) in a manner dependent upon its metabolic state. These molecules subsequently affect other metabolically active tissues, including reproductive organs, to maintain energy homeostasis.

A substantial proportion of the nutrients invested in eggs should come from body reserves. Therefore, we intended to investigate the role of adipose tissue, in terms of metabolic

functions, in egg yields of chickens. Two selected strains originated from a common population of chickens were applied in this study (Chao and Lee, 2001). The average eggs produced to 40-wk of age by the L2 hens ( $85.7 \pm 0.7$ ) were significantly greater than those by the B hens ( $56.8 \pm 1.7$ ) over 20 generations of selection. With the genetic homogeneity and distinguished phenotypes, these two strains can be applied in the search of biochemical and/or genetic parameters for performance of reproduction.

## MATERIALS AND METHODS

### *Animals*

The chicks were reared in floor pens. At 17 wk of age, the pullets were moved into individual cages of a 2-tier system. The light cycle in the laying house was set to 16L:8D from the day of housing. Layer mash containing 2,930 kcal/kg ME and 16.9% CP was provided for *ad libitum* consumption.

### *Glycerol-3-phosphate dehydrogenase (GPDH) activity assay*

Adipose tissue samples were homogenized and centrifuged. GPDH activity in the supernatant fraction was determined by following the disappearance of NADH during enzyme-catalyzed dihydroxyacetone phosphate reduction. Data were normalized to protein contents.

### *Preparation of adipocytes and lipolysis assay*

Adipocytes were obtained by collagenase digestion of the abdominal fat pads of 25 week-old B or L2 laying hens. After the isolated adipocytes were incubated for 60 min by shaking at 37 °C, the amounts of glycerol released from adipocytes to the medium was determined using a commercial kit (Tungyao, Taoyuan, Taiwan) to reflect the intensity of lipolysis. Data were normalized to protein contents.

### *Extraction of total RNA and RT-PCR*

Total RNA was extracted from isolated adipocytes by the TRIzol isolation method (Invitrogen, Carlsbad, CA). First-strand cDNA was generated from RNA and amplified with primers specific for chicken perilipin, LPL, and GLUT1. The primers for perilipin are: sense,  $5' \text{ATCCAGACGACCAGTTCCTG}^3'$ , and antisense,  $5' \text{AAGCTT-}$

$\text{GCCTCCAAACTGA-A}^3'$ ; for LPL are: sense,  $5' \text{GCCTGTTGGACACATTGATA}^3'$ , and antisense,  $5' \text{AGCGT-GAAAGGAATGTTCT-C}^3'$ , and for GLUT1 are: sense,  $5' \text{AAGAT-}$

$\text{GACAGCTCGCCTGAT}^3'$ , and antisense,  $5' \text{C-ACATACATGGGCACAAAGC}^3'$ . 18S rRNA was used as the internal control (QuantumRNA™ 18S Internal Standards, Ambion, TX, USA). Levels of mRNA were expressed as the ratio of signal intensity of the target genes relative to that of 18S rRNA.

### *Statistics*

All data were expressed as the mean  $\pm$  S.E. of three samples. Statistical significance was determined using the Student's *t* test with two-tailed *p* values. The threshold of significance used in all studies was  $p < 0.05$ .

## RESULTS AND DISCUSSION

### *GPDH activity and adipocyte lipolysis*

Playing central roles in the TAG synthesis, GPDH is a useful marker to characterize the process of lipid accumulation. The L2 hens had higher GPDH activity in adipose tissue than the B hens (Fig. 1A), suggesting the L2 hens had higher fat storage capacity. The amounts of glycerol released to the medium from the adipocytes isolated from the L2 hens during incubation were significantly higher than those from the B hens (Fig. 1B), reflecting the mobilization of lipid stores from adipose tissue was more effective in the L2 than B hens. The results implied that the L2 hens had higher basal lipolytic activity than the B hens.

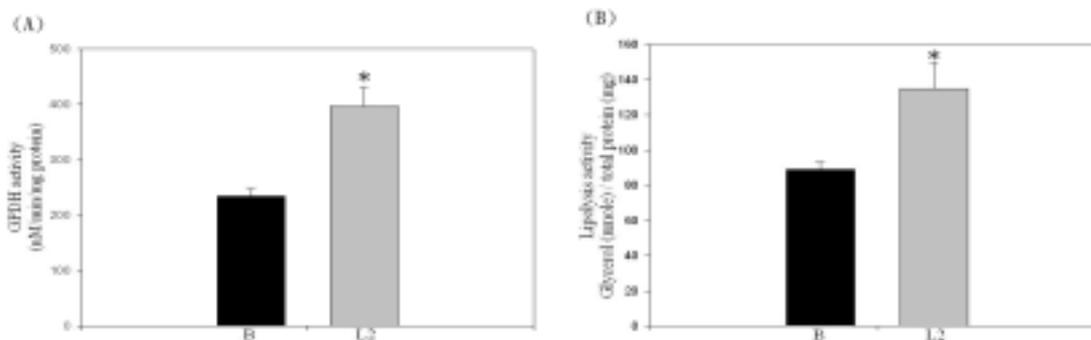


Figure 1. GPDH activity assay and lipolysis activity assay of adipose tissues from the B and L2 hens. (A) Extracts of adipose tissues derived from the B and L2 hens were subjected to GPDH activity assay. (B) The amounts of glycerol released from adipocytes isolated from the B and L2 hens were determined. Data were normalized against protein contents (mg). \*, Statistically significant differences compared to the level at B ( $p < 0.05$ ).

### *Expression of genes involved in lipid metabolism*

Perilipin is an important determinant of hormone-sensitive lipase activity for the hydrolysis of TAG in adipocytes (Tansey *et al.*, 2004). The mRNA levels of perilipin were significantly higher in L2 than B (Fig. 2), suggesting the lipolytic activity differences observed between B and L2 hens was likely due to different expression levels of the genes involved in the lipolysis process. Furthermore, circulating TAG is catabolized by LPL to free fatty acids which can then be transported into adipocytes. The B hens appeared to express higher mRNA levels of LPL (Fig. 2), possibly to provide more substrates for lipid storage. Therefore, higher GPDH activity observed in the L2 hens may be due to other factors, such as the glucose uptake efficiency (Rumberger *et al.*, 2003). The mRNA levels of GLUT1, the main isoform of glucose transporter in adipose tissue of chickens (Kono *et al.*, 2005), were found to be higher in the L2 than B (Fig. 2). The L2 hens showed higher levels of glucose uptake and therefore higher GPDH activity to store lipids effectively. These results implied that any changes in GLUT1 expression and activity may modify glucose partitioning and, hence, lipid storage in adipose tissue.

In summary, the high egg-yield L2 hens showed higher dynamics in terms of storage and mobilization of lipids, and maybe more efficient in using lipids for both body energy demand and egg yields than the B hens after selection. This may be reflected by the differences in adipocytokine secretion and reproductive performance.

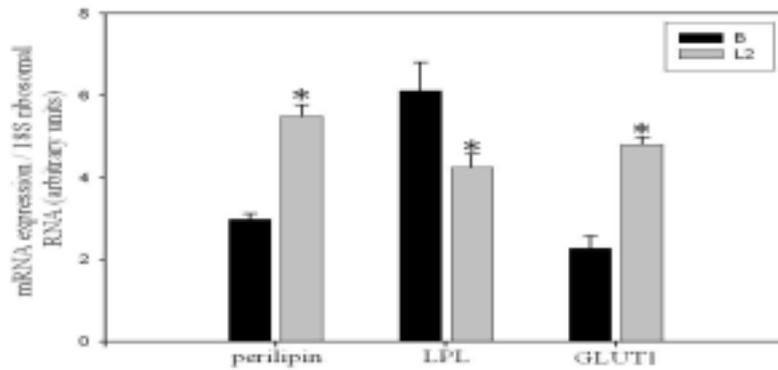


Figure 2. mRNA levels of lipid metabolism related-genes of adipocytes from the B and L2 hens. RNA prepared from adipocytes isolated from fat tissues was amplified by RT-PCR using primers specific to the individual marker genes, perilipin, LPL, and GLUT1. The results were expressed as ratios of the band intensities of the PCR products relative to those of the 18S ribosomal RNA, the internal control, in agarose gels. \*, Statistically significant differences compared to the level at B ( $p < 0.05$ ).

## REFERENCE

- Chao, C. H. and Y. P. Lee. 2001. Relationship between reproductive performance and immunity in Taiwan country chickens. *Poult Sci.* 80: 535-540.
- Kono, T., M. Nishida, Y. Nishiki, Y. Seki, K. Sato, and Y. Akiba. 2005. Characterisation of glucose transporter (GLUT) gene expression in broiler chickens. *Br. Poult. Sci.* 46: 510-515.
- Rumberger, J. M., T. Wu, M. A. Hering, and S. Marshall. 2003. Role of hexosamine biosynthesis in glucose-mediated up-regulation of lipogenic enzyme mRNA levels. *J. Biol. Chem.* 278: 28547-28552.
- Tansey, J. T., C. Sztalryd, E. M. Hlavin, A. R. Kimmel, and C. Londos. 2004. The central role of perilipin A in lipid metabolism and adipocyte lipolysis. *Life* 56: 379-385.
- Williams, T. D. 2005. Mechanism underlying the costs of egg production. *BioScience* 55: 39-48.