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The Expression of Lipoprotein Lipase Gene with Fat Accumulations and Serum Biochemical Levels in Betong (KU Line) and Broiler Chickens

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***Abstract*—**Betong chicken is a slow growing and a lean strain of chicken, while the rapid growth of broiler is accompanied by increased fat. We investigated the growth performance, fat accumulations, lipid serum biochemical levels and lipoprotein lipase (LPL) gene expression of female Betong (KU line) at the age of 4 and 6 weeks. A total of 80 female Betong chickens (KU line) and 80 female broiler chickens were reared under open system (each group had 4 replicates of 20 chicks per pen). The results showed that feed intake and average daily gain (ADG) of broiler chicken were significantly higher than Betong (KU line) ($P < 0.01$), while feed conversion ratio (FCR) of Betong (KU line) at week 6 were significantly lower than broiler chicken ($P < 0.01$) at 6 weeks. At 4 and 6 weeks, two birds per replicate were randomly selected and slaughtered. Carcass weight did not significantly differ between treatments; the percentage of abdominal fat and subcutaneous fat yield was higher in the broiler ($P < 0.01$) at 4 and 6 week. Total cholesterol and LDL level of broiler were higher than Betong (KU line) at 4 and 6 weeks ($P < 0.05$). Abdominal fat samples were collected for total RNA extraction. The cDNA was amplified using primers specific for LPL gene expression and analysed using real-time PCR. The results showed that the expression of LPL gene was not different when compared between Betong (KU line) and broiler chickens at the age of 4 and 6 weeks ($P > 0.05$). Our results indicated that broiler chickens had high growth rate and fat accumulation when compared with Betong (KU line) chickens, whereas LPL gene expression did not differ between breeds.

***Keywords*—**Lipoprotein lipase gene, Betong (KU line), broiler, abdominal fat, gene expression.

I. Introduction

IN Thailand, Thai native chickens have become increasingly popular because of their taste and very lean meat, while the rapid growth of broiler increased fat. Betong chicken (KU line), a slow growing chicken, is one of the native chickens and is most popular in the southern part of Thailand due to the high quality of the meat and low carcass fat compared to broiler chickens and other Thai breeds. A pprevious study has reported that the Betong chickens (KU line) (requires a diet with 17 %CP and an energy level of 3,000–3,200 ME Kcal/kg, while high levels of dietary protein) 23, 21 and 19 %CP (did not alter growth performance at 0–42 days of age)[1]. The suitable protein and energy level of chickens were 18% CP and 3,000 ME Kcal/kg at 4–16 weeks of age, and 16% CP and 2,800 ME Kcal/kg at 16–20 weeks of age [2]. The data also showed the analysis of investment and return of chickens 0–20 weeks of age to gain a final body weight of 1,700–2,400 kg. Moreover, Betong chicken (KU line) was reared under open house system, while broiler chickens seems to be particularly sensitive to temperature-associated environmental challenges, especially heat stress. Therefore, broiler chickens were reared under evaporative cooling system, which was controlling temperature and humidity. In chickens, lipogenic activity takes place primarily in the liver and most of the fats accumulated in the adipose tissue result from incorporation of triacylglycerols from plasma lipoproteins that are synthesized in the liver or provided from dietary fats [3], [4].

LPL-catalyzed hydrolysis of triacylglycerols in peripheral tissues is a rate-limiting step in lipoprotein metabolism [5]. LPL plays a crucial role in fat accumulation in adipose tissue [6]. In the present work, we therefore further investigate whether breeds of chicken, can be used to modulate adipose LPL activity or mRNA expression, growth performance, fat accumulation and serum biochemical levels in genetically Betong chicken (KU line) and broiler chickens.

II. Materials and Methods

A. Experimental Design and Diets

A total of 80 female Betong chickens (KU-Line) and 80 female broiler chickens were reared under open system. The birds were distributed for the treatments according to a completely randomized block design (CRBD) (4 replicates of 20 birds per treatments). One day-old chicks from the same hatch were conventionally raised under the same conditions. The composition of the experimental diet is shown in Table I.

B. Growth Performance and Carcass Characteristics

In order to determine weight gain, all birds were weighed in the beginning, every 2 weeks, and the end of the experimental period. These data were used to measure BW, ADG and feed intake (FI).

At 4 and 6 weeks of age, two chickens from each pen were randomly selected and euthanized by CO_2 inhalation. Immediately after euthanasia, these birds were killed and slaughtered. Abdominal fat was reported as a percentage of live weight. Samples of the abdominal fat were collected and

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stored in RNAlater Solution (Ambion, USA) at -20 °C until total RNA extraction.

TABLE I
COMPOSITION AND NUTRIENT LEVELS OF THE BASAL DIET

Items	0 - 10 days	11 - 25 days	26 - 42 days
Ingredients (g kg^{-1}, as fed basis)			
Corn	509.6	544.6	589.3
Soybean meal	394.7	354.5	305.2
Palm oil	49.8	59.9	67.9
Monodicalciumphosphate	20.6	18.2	16.2
Calcium carbonate	13.1	12	11.1
L-lysine	2.8	2.2	2.2
L-threonine	1.2	0.9	0.6
DL-methionine	4.1	3.6	3.3
Salt	1.6	1.6	1.7
Premix[a]	2.5	2.5	2.5
Calculated nutritive values			
Metabolizable energy (Kcal kg^{-1})	3000	3100	3200
Crude protein (%)	23.22	21.5	19.5
Lysine	1.44	1.29	1.16
Met + Cys[b]	1.08	0.99	0.91
Methionine	0.74	0.67	0.62
Threonine	0.97	0.88	0.78
Valine	1.07	0.99	0.91
Iso-leucine	0.97	0.90	0.82
Arginine	1.52	1.39	1.25
Tryptophan	0.26	0.23	0.21
Calcium	0.96	0.87	0.79
Available phosphorus	0.48	0.44	0.39

[a] Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (all-rac-α-tocopherol), 30 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 4mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamin), 0.013 mg; Fe (fromferrous sulfate), 80 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.
[b] Methionine + Cystine

C. Serum Biochemical Levels

Total serum cholesterol, triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured by colorimetric enzymatic methods using commercial kits, HDL Cholesterol liquid color (Human, Germany) .

D. Gene Expression Analysis with Real-Time PCR

Total RNA was extracted using RNeasy® Fibrous Tissue Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Total RNA concentration and quality were measured spectrophotometrically at 260/280 nm. 400 nanograms of total RNA were used to prepare complementary DNA (cDNA). Real-time PCR reaction used the fluorescent dye SYBR GREEN (SYBRs GREEN PCR Master Mix, Bio-Rad, USA).

The primers used were: *β-actin* forward primer (5'- TGC GTG ACA TCA AGG AGA AG -3') and *β-actin* reverse primer (5'-GGG AAG CAG GAC CCT TG TT-3'), *LPL* forward primer (5'- GCA TTC ACC ATT CAG AGA GTC AG -3') and a *LPL* reverse primer (5'-TGC CAG GGT ACA TTG TGG TA -3'). It was initiated with a first denaturation step of 5 min at 95 °C, followed by 40 cycles of 94 °C for 2 min and 55 °C for 1 min. The *LPL* and *β-actin* transcripts were simultaneously quantified for each sample during each real-time PCR run, and negative controls consisted of samples with no template cDNA. A standard curve for quantitation of *LPL* and *β-actin* was constructed using serial dilutions of the PCR product containing *LPL* and *β-actin*, respectively. The amount of *LPL* transcripts was related to that of *β-actin* transcripts. The statistical analysis was carried out using SAS version 9.0 software, and a general linear model (GLM) at a 95% confidence level was used to evaluate the differential mRNA expression between the treatments.

E. Statistical Analysis

Data were evaluated with ANOVA in a completely randomised design. Computations employed the GLM procedure of the Statistical Analysis System (SAS: version 9; SAS Institute Inc., Cary, NC, USA). Pen means were used as the experimental unit for the analysis. Differences among treatments were tested for significance by using the Duncan's multiple range tests at 5% significance level.

III. RESULTS AND DISCUSSION

The results of growth performance and fat weight were reported in Table II. BW, ADG and FI of broiler chickens were higher ($p < 0.01$) than Betong chickens (KU Line) at 4 and 6 weeks of age. Moreover, the FCR of broiler chickens was better ($p < 0.01$) than Betong chickens (KU Line) through all experiment. Dressing percentage can be calculated by comparing to live weight of chicken. The highest values of abdominal fat percentages were found in the broiler chickens at 4 and 6 week ($p < 0.01$). These results are in agreement with the findings of [2] and [7], who reported a similar growth performance of a slow growing Betong chicken (KU Line). Moreover, we found the increasing of growth rate in broiler chickens has been associated with increased fat deposition [8].

TABLE II
GROWTH PERFORMANCE AND PERCENTAGE OF ABDOMINAL FAT COMPARED BETWEEN THE BREEDS

Items	BW (g)	ADG (g)	FI (g)	FCR	Abdominal fat (%)
0–4 weeks					
Betong	346.38[a]	12.27[a]	33.79[a]	2.46[a]	1.13[a]
Broilers	1,313.67[b]	46.92[b]	93.39[b]	1.54[b]	2.53[b]
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
4–6 weeks					
Betong	527.71[a]	13.42[a]	42.25[a]	2.54[a]	1.44[a]
Broilers	2,184.65[b]	52.02[b]	144.73[b]	1.67[b]	2.99[b]
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a, b] Means in the same row with different superscript are significantly difference (P < 0.05).

Concentrations of plasma lipids and lipoproteins are indicative of the fat metabolic regulations, especially the basal adjustment of fatty acid circulation between the adipose tissue

and the liver [9]. Effects of chicken breeds on serum concentrations of lipoproteins in Betong and Broiler chickens after 4 and 6 weeks are shown in Table III. In broiler chickens, serum concentrations of cholesterol and LDL were significantly higher than in Betong chickens (KU Line) ($p < 0.01$), whereas resulted in a lower serum concentration of HDL ($p < 0.01$) at 4 and 6 weeks of age. No significant differences were observed on the serum TG through all experiments.

TABLE III
SERUM CONCENTRATION OF LIPOPROTEINS IN BETONG AND BROILER CHICKENS

Items	Cholesterol	TG	LDL	HDL
		0–4 weeks		
Betong	144.48[a]	91.71	61.75[a]	150.22[b]
Broilers	175.75[b]	89.44	76.79[b]	132.52[a]
P-value	< 0.01	0.211	< 0.01	< 0.01
		4–6 weeks		
Betong	146.17[a]	84.48	66.61[a]	182.59[b]
Broilers	186.89[b]	87.51	112.13[b]	140.26[a]
P-value	< 0.01	0.621	< 0.01	< 0.01

[a, b] Means in the same row with different superscript are significantly difference ($P < 0.05$).

Most chicken growth and fatness traits are controlled by multiple genes [10]. However, effect of each gene on body weight and fat weight differs between individuals with homozygous and heterozygous SNP genotypes and according to nutritional factors. Moreover, adipose tissue growth in chickens depends on the availability of TG transported by VLDL (very low density lipoprotein) [11]. In the present study, we found no significant differences in the serum TG, but found higher fat pad accumulation in broilers than Betong. In general, fat accumulation may be considered among dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism through lipolysis (β-oxidation). These data suggest that the lipid storage is more efficient in the adipose tissues in broiler chickens. Thus, LPL activity can control lipid accumulation in the liver and extra-hepatic tissues, and in chickens an increase in LPL activity produces strong peripheral accumulation of lipids [12], [13]. According to the results of the LPL gene expression (Fig. 1), the expression of LPL gene in the abdominal fat of Betong chickens (KU Line) and broiler chickens did not differ significantly at 4 and 6 weeks.

Reference [13] reported that LPL activity in broiler chicken adipose tissue was significantly increased up to 6 weeks of age and subsequently decreased at 8 weeks, while the growth rate of abdominal fat pads in broiler chickens increased rapidly between 1 and 4 weeks of age and remained high up to 10 weeks of age.

Additionally, in chickens it has been reported that the expression of lipogenic enzymes regulated fat deposition during adipogenesis [14], showing that dietary protein influences avian lipid metabolism [15]-[17]. Reference [18] reported that a combination of malic enzyme, fatty acid synthase or acetyl CoA carboxylase mRNA expression, mRNA stability and posttranscriptional events interact to regulate lipogenesis in the chicken.

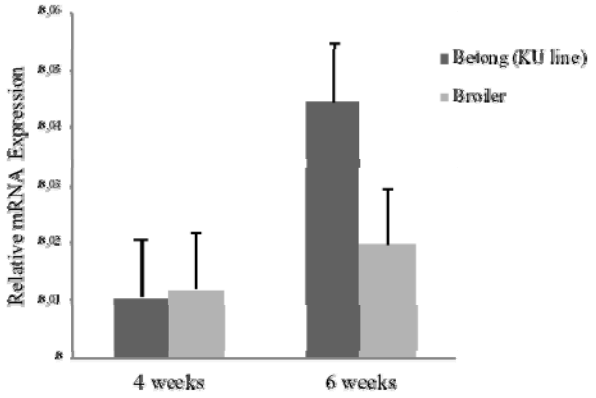

Fig. 1 Relative mRNA expression of LPL in the abdominal fat of Betong and broiler chickens at 4 and 6 weeks of age. The results are averages a posteriori and standard deviation represented by vertical bars

IV. CONCLUSION

In conclusion, we found that growth rate is slowing in Betong chickens (KU Line) compared with broiler chickens, whereas the lipogenic capacity of adipose tissue is reduced in Betong chickens (KU Line). Differences in the breeds of chicken alter some keys of serum lipids profiles, but not regulate TG circulating and LPL gene expression in adipose tissue.

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