INTRODUCTION

Excess body fat deposition in broiler is now the concern of both producers and consumers. The fat represents as a waste to consumers who are concerned about nutritional and health aspects of their food. High body fat deposition in broiler chickens may represent an economic loss to producers, as it is inefficient in terms of energy metabolism and overall feed utilisation (Gaya et al., 2005). Moreover, obesity in birds increases the incidence of reproductive failure and death due to heart failure (Zubair and Leeson, 1996).

Very low density lipoprotein (VLDL) is a heterogeneous lipid-protein macromolecule complex that circulate in plasma and lymph. It is a globular micelle like particles that consist of a non-polar core of triacylglycerol (TAG) and cholesteryl ester (CE) coated with a layer of amphiphilic compounds of phospholipid (PL), free cholesterol (FC) and protein that is called apolipoprotein (apo) (Smith et al., 1978). Very low density lipoprotein is one of the major lipoproteins transporting TAG from the liver to extrahepatic tissues, such as the adipose tissue, heart and lung (Cryer, 1981). Very low density lipoprotein metabolism plays an important role in fattening of poultry. It involves the process of synthesis, secretion and catabolism intravascularly. These processes lead to lipid uptake and storage by adipose tissue (Hermier, 1997). About 70-80% of VLDL TAG secreted into circulation is taken up by adipose tissue (Griffin et al., 1992). Liver is the main organ responsible for de novo lipogenesis in chicken (Saadoun and Leclercq, 1987). Griffin et al. (1991) reported that body fat content was highly correlated with rate of secretion of plasma TAG-rich lipoproteins. The difference in rate of VLDL secretion between lines appears to be caused by differences in both rates of hepatic lipogenesis and in the proportions of fatty acids directed towards lipoprotein synthesis and oxidation (Griffin et al., 1991).

Apolipoprotein of VLDL in chicken plasma and serum is different from that in human. The information of human apolipoprotein is well established (Elovson et al., 1988; Evans et al., 1989; Young, 1990), however the exact role of individual avian apolipoprotein is not known. It has been reported that VLDL of commercial broiler and crossed village chicken can be purified and subfractionated using fast protein liquid chromatography (Tan et al., 2005). The
characteristics of apolipoprotein were studied in the subfractions of VLDL, however the relationships between abdominal fat and apolipoprotein are not well documented. Thus, the objectives of the present study were to study the relationships between plasma TAG, VLDL TAG, VLDL apolipoprotein and fat deposition in two breeds of chicken.

MATERIALS AND METHODS

Animals and husbandry

Two breeds of chicken used in this study were crossbred village chicken (Sasso crossed) (AK) and commercial broiler (Avian) (CB). The CB is considered as fat line chicken and AK as lean line chicken. They were used in order to study their differences in VLDL metabolism. One hundred and eighty day-old females and 180 day-old males from each breed were used. Crossbred village chickens were purchased from Pusat Kemajuan Peladang Selangor (PKPS), Puchong and commercial broiler were purchased from Pusat Kemajuan Peladang Selangor (PKPS), Puchong and commercial broiler were purchased from Sin Mah, Melaka, respectively. The birds were housed

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Sampling

All the birds were tagged individually. Blood samples were collected at every three week intervals. The birds were fasted for 18 hours prior to blood withdrawal in order to ensure portomicros were cleared from the circulation (Bachorik, 1982; Hermier, 1997). Three birds from both sexes from each pen were slaughtered and the blood samples were collected into vacutainer tubes (Beckton Dickinson, UK) containing disodium EDTA as anticoagulant to give a final concentration of 1 mg/ml of blood. The blood sample was mixed by inverting the collection tube gently in order to avoid hemolysis. The total abdominal adipose tissues were collected, weighed and kept in the polystyrene box with ice and then kept under -70°C.

Preparation of plasma samples

The blood samples were pooled in a clean glass test tube and centrifuged at 1,500×g for 30 minutes under 4°C. Plasma was transferred to a clean storage test tube by using a Pasteur pipette equipped with a rubber bulb. The plasma was then kept under -20°C before VLDL separation and composition analysis.

Very low density lipoprotein separation

Very low density lipoprotein was separated from the plasma by using Fast Protein Liquid Chromatography (Äkta-FPLC) (Amersham Pharmacia Biotech, Sweden) as described by Tan et al. (2005). After FPLC separation, the fractions were freeze-dried for 18 hours and kept under -20°C for composition analysis and subfractioning.

Subfractionation of VLDL by heparin-sepharose affinity chromatography

The VLDL was dialysed with 0.05 M NaCl, 5 mM Tris and 0.02% NaN₃, pH 7.4. MnCl₂ were added to not more than 1 mg of VLDL protein to a final concentration of 0.025 M. Subfractionation was carried out by using Fast Protein Liquid Chromatography (Äkta-FPLC). The heparin-Sepharose column (Amersham Pharmacia Biotech, Sweden) was equilibrated with three column volume (CV) of Buffer A (0.05 M NaCl, 5 mM Tris, 25 mM MnCl₂ and 0.02% NaN₃, pH 7.4). A three CV of Buffer A was passed through the system to wash out the unbound protein followed by five CV of Buffer B (0.2 M NaCl, 5 mM Tris and 0.02% NaN₃, pH 7.4) and four CV of Buffer C (2.8 M NaCl, 5 mM Tris and 0.02% NaN₃, pH 7.4). Then, the system was washed by three CV of Buffer D (0.5 M NaCl, 0.01 M glycine and 0.02% NaN₃, pH 5.5) and Buffer E (0.5 M NaCl, 0.01 M glycine and 0.02% NaN₃, pH 8.5), respectively. Fractions were collected at 0.5 ml/min and absorbance was monitored at 280 nm. The fractions were dialysed against 0.15 M NaCl, 0.01% Na₂EDTA, 0.02% NaN₃, pH 7.4 for 24 h. The collected fractions were freeze-dried and studied for their apolipoprotein profiles.

Chemical composition analysis

The chemical compositions of the whole plasma and VLDL were determined. Plasma protein concentration was determined by Biuret method (Gornall et al., 1949), whereas VLDL protein concentration by the method of Lowry et al. (1951), using diagnostic kits provided by Sigma Diagnostics, Sigma Chemical Co. Ltd., Poole, Dorset, UK. The TAG and cholesterol concentrations were determined by an enzymatic method, using diagnostic kits provided by Randox Laboratories Ltd., UK. Free cholesterol and PL concentrations were determined by using kits provided by Wako Pure Chemical Industries, Ltd., Japan. The CE concentration was calculated as the difference between total cholesterol and free cholesterol concentration. The particle size was calculated according to Fungwee et al. (1992) by calculating the ratio of the sum of PL and FC over the sum of TAG and CE.
Postheparin plasma LPL activity assay

Postheparin plasma LPL activity assays were carried out using a modified method of Nozaki et al. (1984). One female and male bird from each pen was injected with 0.5 ml of 100 IU heparin/kg body weight. After 15 minutes, the birds were slaughtered. The blood was collected into clean test tubes placed in a polystyrene ice box. Plasma was isolated by centrifuging at 1,500 g for 30 minutes. The free fatty acids were measured by using non-esterified fatty acid kit purchased from Roche, Germany at 546 nm.

Statistical analysis

The trial was set up in a randomised complete block design. Lipid parameters were analysed with breed, sex and their interactions as the main effects. Data were analysed by analysis of variance (ANOVA) using the general linear model (SAS, 1989). However, no significant difference (p>0.05) was found for the interactions of main effects. Relationships among adipose tissues deposited, lipid parameters and VLDL subfractions were analysed by Pearson’s correlation coefficient using linear regression (Minitab, 1995). Male and female chickens were used in the analyses. A total of 24 birds from AK and CB were used to study the relationships.

RESULTS AND DISCUSSIONS

Table 1 shows the total abdominal fat, plasma and VLDL protein and lipid concentrations, proportion of VLDL subfraction 2 and calculated VLDL particle size of female and male birds at weeks 3 and 6.

| Parameters | Week 3 | | | | | | Week 6 | | | |
|------------|--------| | | | | | | | | | |
| Abdominal fat | Female | Male | p | Female | Male | p |
| Protein | 9.53±3.70 | 10.11±2.56 | NS | 20.82±6.30 | 15.55±3.58 | ** |
| TAG | 42.74±3.44 | 42.72±6.45 | NS | 59.63±4.58 | 59.63±5.09 | NS |
| PL | 0.59±0.26 | 0.44±0.14 | NS | 0.55±0.25 | 0.51±0.24 | NS |
| Cholesterol | 1.28±0.60 | 1.28±0.71 | NS | 1.29±0.54 | 2.10±0.39 | NS |
| FC | 1.38±0.16 | 1.47±0.24 | NS | 1.29±0.22 | 1.42±0.19 | NS |
| VLDL Protein | 0.17±0.05 | 0.19±0.04 | NS | 0.09±0.04 | 0.11±0.04 | NS |
| TAG | 209.71±52.57 | 193.50±56.04 | NS | 45.02±28.36 | 29.58±26.40 | NS |
| PL | 86.84±52.59 | 81.31±44.19 | NS | 120.41±34.85 | 123.60±29.88 | NS |
| Cholesterol | 146.48±44.66 | 159.10±36.77 | NS | 117.05±30.82 | 114.07±22.32 | NS |
| FC | 45.12±10.68 | 45.93±10.95 | NS | 27.43±5.80 | 26.63±10.03 | NS |
| Subfraction 2 | 59.30±8.76 | 49.73±12.11 | * | 39.49±10.59 | 42.34±10.89 | NS |
| Particle sizea | 0.44±0.16 | 0.43±0.14 | NS | 1.13±0.29 | 1.30±0.29 | NS |

NS, p>0.05; * p<0.05; ** p<0.01.
The results are presented as mean±SD. Plasma composition concentrations and VLDL protein concentration are in mg/ml plasma. VLDL lipid concentrations are in μg/ml plasma. Fat is in g/kg BW and proportion of subfraction 2 is in %.

a Particle size = (PL+FC/TAG+CE) (Fungwee et al., 1992).
is a significant negative correlation (p<0.05) between abdominal fat and VLDL TAG concentration (Table 3). The results suggest that the uptake of TAG by adipose tissue of AK was lower compared to CB. This can be further supported by the lower postheparin plasma LPL activity in AK than CB (p<0.05). The LPL activities of AK and CB were 4.60±1.70 mM/h and 7.41±3.46 mM/h, respectively. The lower LPL activity in AK could have also accounted for the lower fat deposition. Hence, less TAG would be hydrolysed and little fatty acid would be released and deposited as fat. The result is in agreement with Whitehead (1988), who indicated that lean birds were expected to have lower LPL activities.

According to Gómez-Coronado et al. (1993), LPL prefer to act on larger, TAG-rich particles. The VLDL particle size of CB was bigger than AK at week three (p<0.01). The result is consistent with those reported by Gómez-Coronado et al. (1993). Beside this, from the correlation analysis, the particle size was positively correlated (p<0.05) with abdominal fat (Table 3). This suggests that CB with bigger particle size may lead to higher rates of LPL activity and higher hydrolysis of VLDL and more fat was deposited. This can be further supported by the correlation analysis between VLDL TAG concentration and particle size (Table 4). The VLDL TAG concentration of AK and CB was negatively correlated (p<0.05) with the particle size.

Table 2. Total abdominal fat, plasma and VLDL protein and lipid concentrations, proportion of VLDL subfraction 2 and calculated VLDL particle size of AK and CB at weeks 3 and 6

| Parameters       | AK          | CB          | p     | AK          | CB          | p     |
|------------------|-------------|-------------|-------|-------------|-------------|-------|
| Abdominal fat    | 7.49±2.84   | 12.14±0.76  | **    | 14.85±3.77  | 21.52±5.41  | **    |
| Plasma Protein   | 42.83±4.36  | 42.63±5.87  | NS    | 57.60±4.79  | 61.67±3.86  | *     |
| TAG              | 0.59±0.14   | 0.44±0.25   | NS    | 0.64±0.29   | 0.42±0.08   | *     |
| PL               | 1.74±0.60   | 0.82±0.20   | NS    | 1.70±0.39   | 2.33±0.29   | **    |
| Cholesterol      | 1.37±0.22   | 1.47±0.19   | NS    | 1.21±0.12   | 1.51±0.17   | **    |
| FC               | 0.18±0.04   | 0.18±0.05   | NS    | 0.08±0.03   | 0.12±0.04   | **    |
| VLDL Protein     | 1.47±0.20   | 1.69±0.21   | *     | 1.17±0.08   | 1.21±0.10   | NS    |
| TAG              | 208.97±63.55| 194.24±43.47| NS    | 56.46±25.52 | 18.14±13.36| **    |
| PL               | 59.47±37.45 | 108.67±44.86| **    | 119.49±34.55| 124.51±30.10| NS    |
| Cholesterol      | 158.10±51.16| 147.47±27.48| NS    | 103.59±27.18| 127.53±20.04| *     |
| FC               | 48.35±9.07  | 42.69±11.60 | NS    | 28.12±8.37  | 25.94±7.87  | NS    |
| Subfraction 2    | 51.36±8.24  | 57.67±13.56 | NS    | 35.42±8.20  | 46.42±10.10| **    |
| Particle sizea   | 0.36±0.14   | 0.51±0.11   | **    | 1.15±0.26   | 1.29±0.32   | NS    |

NS, p>0.05; * p<0.05; ** p<0.01.

The results are presented as mean±SD. Plasma composition concentrations and VLDL protein concentration are in mg/ml plasma. VLDL lipid concentrations are in µg/ml plasma. Fat is in g/kg BW and proportion of subfraction 2 is in %.

a Particle size = (PL+FC/TAG+CE) (Fungwee et al., 1992).

Table 3. Relationships between abdominal fat and plasma TAG, VLDL TAG, VLDL particle size and proportion of subfraction 2 for AK and CB

| Week | Parameters | R     | p    |
|------|------------|-------|------|
| 3    | Plasma TAG | -0.138| NS   |
|      | VLDL TAG   | -0.499| *    |
|      | Particle size | 0.535 | **   |
|      | Subfraction 2 | 0.261 | NS   |
| 6    | Plasma TAG | -0.227| NS   |
|      | VLDL TAG   | -0.451| *    |
|      | Particle size | 0.199 | NS   |
|      | Subfraction 2 | 0.344 | NS   |

n = 24; NS, p>0.05; * p<0.05; ** p<0.01.

Table 4. Relationship between VLDL TAG concentration and VLDL particle size for AK and CB

| Week | R     | p    |
|------|-------|------|
| 3    | -0.422| *    |
| 6    | -0.566| **   |

n = 24; * p<0.05; ** p<0.01.
al., 1989; Wright et al., 1995; Loh et al., 2001). Table 5 shows the relative proportions of apo IV and apo E present in subfractions 1 and 2 of AK and CB. The proportion of apo E in subfraction 2 was numerically higher than subfraction 1. The apo E was believed to enhance the lipolysis process in human (Clark and Quarfordt, 1985). The apo E-poor subfraction or subfraction 1 contained higher TAG than subfraction 2 (Huff and Telford, 1984; Evans et al., 1989) and metabolised slower than subfraction 2 (Huff and Telford, 1984). Slow catabolism of subfraction 1 is due to the fact that it must first be converted to subfraction 2. The possibility remains that subfraction 1 is the precursor of subfraction 2; however, these two subfractions may be independently synthesised and catabolised (Huff and Telford, 1984).

The CB showed a higher proportion of subfraction 2 than AK at week six (Table 2). The results suggest that CB was having more apo E that lead to catabolism faster than AK. These results were further supported by the data shown in Table 5 that CB had more apo E than that of AK. Hence, more fatty acids were released for fat deposition. These findings were supported by the correlation analysis between abdominal fat and proportion of subfraction 2 (Table 3). The abdominal fat showed a positive correlation with proportion of subfraction 2. Gómez-Coronado et al. (1993) reported that the subfraction richest in apo E showed the lowest degree of TAG hydrolysis by LPL and lowest ability to deliver fatty acids to adipose tissue in rat. However, the actual role of apo E in VLDL metabolism in fat and lean lines chicken is not well understood.

In conclusions, CB had a bigger VLDL particle size and higher proportion of subfraction 2 than AK. All these lead to higher rate of VLDL TAG hydrolysis and more fat was deposited.

### REFERENCES

Bachorik, P. S. 1982. Collection of blood samples for lipoprotein analysis. Clin. Chem. 28/6:1375-1378.

Benson, J. D., V. Hearn, T. Boyd and A. Bensadoun. 1975. Triacylglycerol hydrolase of chicken and rat pre- and post-heparin plasma. Effects of fasting and comparison with adipose tissue lipoprotein lipase. Int. J. Biochem. 6:727-734.

Clark, A. B. and S. H. Quarfordt. 1985. Apolipoprotein effects on the lipolysis of perfused triglyceride by heparin-immobilised milk lipase. J. Biol. Chem. 260:4778-4783.

Cryer, A. 1981. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. Int. J. Biochem. 13: 525-541.

Eisenberg, S. and T. Olivecrona. 1979. Very low density lipoprotein. Fate of phospholipid, cholesterol and apolipoprotein C during lipolysis in vitro. J. Lipid Res. 20:614-623.

Elovson, J., J. E. Chatterton, G. T. Bell, V. N. Schumaker, M. A. Reuben, D. L. Puppione, J. R. Reese, Jr. and N. L. Young. 1988. Plasma very low density lipoprotein contain a single molecule of apolipoprotein B. J. Lipid Res. 29:1461-1473.

Evans, A. J., M. W. Huff and B. M. Wolfe. 1989. Accumulation of an apo-E poor subfraction of very low density lipoprotein in hypertriglyceridemic men. J. Lipid Res. 30:1691-1701.

Fungwée, T. V., L. Cagen, H. G. Wilcox and M. Heimberg. 1992. Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. J. Lipid Res. 33:179-191.

Gaya, L. G., G. B. Mourão, E. M. de Rezende, E. C. de Mattos, T. M. Filho, L. G. Figueiredo, J. B. Ferraz and J. P. Eler. 2005. Genetic trends of abdominal fat content in a male broiler chicken line. Genet. Mol. Res. 4:760-764.

Gibson, J. C. and W. V. Brown. 1988. Effect of lipoprotein lipase and hepatic triacylglycerol lipase activity on the distribution of apolipoprotein E among the plasma lipoprotein. Atherosclerosis 73:45-55.

Gómez-Coronado, D., G. T. Sáez, M. A. Lasunción and E. Herrera. 1993. Different hydrolytic efficiencies of adipose tissue lipoprotein lipase on very low density lipoprotein subfractions separated by heparin-Sepharose chromatography. Biochim. Biophys. Acta 1167:70-78.

Gornall, A. G., C. S. Bardawill and M. M. David. 1949. Determination of serum protein by means of Biuret reaction. J. Biol. Chem. 177:751.

Griffin, H. D., K. Guo, D. Windsor and S. C. Butterwith. 1992. Adipose tissue lipogenesis and fat deposition in leaner broiler chickens. J. Nutr. 122:363-368.

Griffin, H. D., D. Windsor and C. C. Whitehead. 1991. Changes in lipoprotein metabolism and body composition in chickens in response to divergent selection for plasma very low density lipoprotein concentration. Br. Poult. Sci. 32:195-201.

Hermier, D. 1997. Lipoprotein metabolism and fattening in poultry. J. Nutr. 127:805S-8085.

Huff, M. W. and D. E. Telford. 1984. Characterisation and metabolic fate of two very low density lipoprotein subfractions separated by heparin-Sepharose chromatography. Biochim. et Biophys. Acta 796:251-261.

Loh, T. C., I. J. Lean and P. E. Dodds. 2001. Association of backfat thickness with postheparin lipoprotein lipase activity and very

### Table 5. Relative proportion (%) of apolipoprotein present in subfraction 1 and 2 of AK and CB

| Apo | Subfraction 1 | Subfraction 2 |
|-----|---------------|---------------|
|     | AIV E         | AIV E         |
| AK  | 57.4±8.84     | 50.8±0.64     |
| CB  | 52.8±1.84     | 49.8±0.71     |
| p   | NS            | NS            |

Percentage was determined by using Alphalmager™ 1220 Documentation and Analysis System.

NS, p>0.05. The results are presented as mean±SD.
low density lipoprotein subfractions. Asian-Aust. J. Anim. Sci. 14:1592-1597.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Maguire, G. F., M. Lee and P. W. Connelly. 1989. Sodium dodecyl sulfate-glycerol polyacrylamide slab gel electrophoresis for the resolution of apolipoproteins. J. Lipid Res. 30:757-761.

Minitab. 1995. Minitab reference manual release 10. Pennsylvania, Pennsylvania State College.

Nestel, P., T. Billington, N. Tada, P. Nugent and N. Fidge. 1983. Heterogeneity of very low density lipoprotein metabolism in hyperlipidemic subjects. Metabolism 32:810-817.

Nestel, P. J., W. E. Coonor, M. F. Reardon, S. Connor, S. Wong and R. Boston. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. J. Clin. Invest. 74:82-89.

Nozaki, S., M. Kubo, Y. Matsuzawa and S. Tarui. 1984. Sensitive non-radioisotopic method for measuring lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma. Clin. Chem. 30/5:748-751.

Saadoun, A. and B. Leclercq. 1987. In vivo lipogenesis of genetically lean and fat chickens: effects of nutritional state and dietary fat. J. Nutr. 117:428-435.

SAS. 1989. Statistical analysis systems. SAS/STAS User's Guide. Release 6.08 edition. SAS Institute INC., Cary, NC.

Smith, L. C., H. J. Pownall and A. M. Goto. 1978. The plasma lipoproteins: structure and metabolism. Ann. Rev. Biochem. 47:751-777.

Tan, B. K., H. L. Foo, T. C. Loh, A. Norhani and I. Zulkifli. 2005. Purification and characterisation of very low density lipoprotein in commercial broiler and crossbred village chickens by fast liquid chromatography. Asian-Aust. J. Anim. Sci. 18:1780-1785.

Tam, S. P., L. Dory and D. Rubinstein. 1981. Fate of apolipoproteins CII, CIII and E during lipolysis of human very low density lipoprotein in vitro. J. Lipid Res. 22:641-651.

Trevi, E., C. Calvi, P. Roma and A. L. Catapano. 1983. Subfractionation of human very low density lipoprotein by heparin-Sepharose chromatography. J. Lipid Res. 24:790-795.

Whitehead, C. C. 1988. Selection for leanness in broilers using plasma lipoprotein concentration as selection criterion. In Leanness in domestic birds. pp 41-57. London, UK. Butterworth & Co.

Whitehead, C. C. and H. D. Griffin. 1982. Plasma lipoprotein concentration as an indicator of fatness in broilers: effect of age and diet. Br. Poult. Sci. 23:299-305.

Wright, M. 1993. Metabolism of very low density lipoprotein of sow. PhD Thesis, Wye College, University of London.

Wright, M., I. J. Lean, E. Herrera and P. F. Dodds. 1995. Changes in the composition of plasma very low density lipoprotein during pregnancy and lactation in genetic lines of pigs. Anim. Sci. 61:361-365.

Young, S. G. 1990. Recent progress in understanding apolipoprotein B. Circulation 82/5:1574-1594.

Zubair, A. K. and S. Leeson. 1996. Compensatory growth in the broiler chicken: a review. World’s Poult. Sci. J. 52:189-201.