Comparison of serum biochemical parameters between two broiler chicken lines divergently selected for abdominal fat content[1]

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ABSTRACT: In humans, obesity is associated with increased or decreased levels of serum biochemical indicators. However, the relationship is not as well understood in chickens. Due to long-term intense selection for fast growth rate, modern broilers have the problem of excessive fat deposition, exhibiting biochemical or metabolic changes. In the current study, the Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF) were used to identify differences in serum biochemical parameters between the 2 lines. A total of 18 serum biochemical indicators were investigated in the 16th, 17th, and 18th generation populations of NEAUHLF, and the genetic parameters of these serum biochemical indicators were estimated. After analyzing the data from these 3 generations together, the results showed that the levels of 16 of the tested serum biochemical parameters were significantly different between the lean and fat birds. In the fat birds, serum concentrations of high-density lipoprotein cholesterol (HDL-C), HDL-C:low-density lipoprotein cholesterol (LDL-C), total bile acid, total protein, albumin, globulin, aspartate transaminase (AST):alanine transaminase (ALT), γ-glutamyl transpeptidase (GGT), uric acid, and creatinine were very significantly higher ($P < 0.01$), whereas LDL-C, albumin:globulin, glucose, AST, ALT, and free fatty acids concentrations in serum were very significantly lower than those in the lean birds ($P < 0.01$). Of these 16 serum biochemical parameters, 5 (LDL-C, HDL-C:LDL-C, total bile acid, albumin, and albumin:globulin) had high heritabilities ($0.58 \leq h^2 \leq 0.89$), 6 (HDL-C, total protein, globulin, AST:ALT, GGT, and creatinine) had moderate heritabilities ($0.29 \leq h^2 \leq 0.48$), and the remaining 5 had low heritabilities ($h^2 < 0.20$). Serum HDL-C, HDL-C:LDL-C, and glucose had higher positive genetic correlation coefficients (r_g) with abdominal fat traits ($0.30 \leq r_g \leq 0.80$), whereas serum globulin, AST, and uric acid showed higher negative genetic correlations with abdominal fat traits ($-0.62 \leq r_g \leq -0.30$). The remaining 10 serum biochemical parameters had lower genetic correlations with abdominal fat traits ($-0.30 < r_g < 0.30$). In conclusion, we identified serum HDL-C and HDL-C:LDL-C levels as potential biomarkers for selection of lean birds. These findings will also be useful in future studies for investigating obesity and lipid metabolism in humans as well as in other animal species.

HDL-C高遗传力，且与AF正遗传相关。

HDL-C:LDL-C中等遗传力，且与AF正遗传相关。

INTRODUCTION

In humans, obesity has become a growing public health problem and is a major risk factor for certain metabolic syndromes (Powell, 2007). Obesity-related metabolic syndrome is associated with increased or decreased levels of several serum biochemical indicators (Xu et al., 2006; Kelishadi et al., 2009), and the detection of some of these parameters has been used to estimate the risk of developing these obesity-related diseases (Yang et al., 2002; Kelishadi et al., 2009). The relationships between serum biochemical parameters and obesity have also been analyzed in some animal models (He et al., 2012; Baéza and Le Bihan-Duval, 2013; Kurhe et al., 2014).

For more than 60 yr, broiler chicken breeders have focused on the selection of important economic traits and made great genetic improvements (Hill and Dansky, 1954; Bedford and Classen, 1992; Demeure et al., 2013). However, long-term intense selection for fast juvenile growth in broiler chickens has increased their abdominal fat deposition and resulted in metabolic changes (Pym, 1987; Emmerson, 1997; Scheele, 1997; Julian, 2005). Excessive deposition of abdominal fat has negative impacts on feed efficiency and carcass quality (Demeure et al., 2013; Ramiah et al., 2014). Exploring the metabolic mechanisms of fat deposition in broilers is helpful for optimal breeding, and the results of such studies will also be beneficial to improve our understanding of obesity in humans and other animal species.

Detection of serum biochemical parameters will be helpful for understanding fat metabolism in chickens. This study had 3 main objectives. We aimed to investigate whether there were differences in 18 serum biochemical indicators between lean and fat broiler chicken lines that had been selected for abdominal fat content. Next, we sought to estimate the genetic parameters of these serum biochemical indicators. Lastly, we attempted to identify which serum biochemical indicators have the potential to be used as markers for the selection of lean birds in the future.

MATERIALS AND METHODS

Ethics Statement

All animal work was conducted according to the guidelines for the Care and Use of Experimental Animals established by the Ministry of Science and Technology of the People's Republic of China (approval number 2006-398) and approved by the Laboratory Animal Management Committee of Northeast Agricultural University.

Experimental Populations, Measurement of Abdominal Fat Traits, and Serum Collection

The broilers used in this study were derived from the Northeast Agricultural University broiler lines divergently selected for abdominal fat content (**NEAUHLF**). The NEAUHLF lines have been selected since 1996, using abdominal fat percentage (**AFP**) and plasma very low-density lipoprotein (**VLDL**) concentration as selection criteria. Abdominal fat percentage was calculated as the ratio of abdominal fat weight (**AFW**) to BW at 7 wk of age (**BW7**). A previously published study from our group had described the breeding scheme in detail (Guo et al., 2011). Briefly, the base population (G_0) of NEAUHLF came from the same grandsire line originating from the Arbor Acres broiler, which was then divided into 2 lines (lean and fat) according to their VLDL concentration at 7 wk of age. From the first generation to 18th generation, birds from each line were raised in 2 hatches, with free access to feed and water. Plasma VLDL concentration was measured for all male birds at 7 wk, and AFP of the male birds in the first hatch was measured after slaughter at 7 wk. Sibling birds from the families with lower (lean line) or higher (fat line) AFP than the average value of the population were selected as candidates for breeding, considering plasma VLDL concentration and BW7 of male birds in the second hatch and egg production of female birds in both hatches.

In this study, male birds from the first hatch of the 16th (G_{16}), 17th (G_{17}), and 18th (G_{18}) generations of NEAUHLF were slaughtered at 7 wk of age after 12 h of fasting, and BW7 and AFW were measured. The blood was collected from birds without using an anticoagulant, and the serum was separated from the whole blood by centrifugation at $3,000 \times g$ for 10 min at room temperature.

Selection of Experiment Individuals

In G_{16}, the AFP mean value of every family was calculated and all families were sorted by the mean value of AFP. A total of 206 individuals in G_{16} (103 birds from the lowest families in the lean line and 103 birds from the highest families in the fat line) were selected. In G_{17}, the AFP of every bird was calculated, and the birds were sorted by AFP. A total of 176 individuals in G_{17} (88 birds with the lowest AFP in the lean line and 88 birds with the highest AFP in the fat line) were selected. In G_{18}, a total of 176 individuals (88 birds from the lean line and 88 birds from the fat line) were selected using the same method as in the G_{17} population.

Measurement of Serum Biochemical Parameters

Serum biochemical parameters (triglycerides, total cholesterol, high-density lipoprotein cholesterol [**HDL-C**], low-density lipoprotein cholesterol [**LDL-C**], total bile acid, total protein, albumin, glucose, aspartate transaminase [**AST**], alanine transaminase [**ALT**], γ-glutamyl transpeptidase [**GGT**], uric acid, creatinine, and free fatty acids) were measured with standard commercial kits (Roche Diagnostics GmbH, Sandhofer Strasse, Germany), following the manufacturer's instructions by using an Architect C8000 Automatic Biochemical Analyzer (Abbott, Inc., Chicago, IL) in the clinical laboratory of the Fourth Hospital of Harbin Medical University (Harbin, China). Globulin concentration in the serum was calculated as the difference between total protein and albumin. The ratios of HDL-C:LDL-C, albumin:globulin, and AST:ALT were also calculated.

Statistical Analysis

We combined data from all 3 generations for the statistical analysis.

Statistical differences of abdominal fat traits (AFW and AFP) and serum biochemical parameters between the lean and fat birds for the 3 generations were analyzed using JMP version 7.0 (SAS Inst. Inc., Cary, NC) with the following model:

$$y_{ijklm} = \mu + L_i + G_j + (L_i \times G_j)$$
$$+ F_k(L, G) + D_l(F, L, G)$$
$$+ BW7 + e_{ijklm}, \qquad [1]$$

in which y_{ijklm} is the phenotypic value of abdominal fat traits and serum biochemical parameters; μ is the population mean; L_i is the fixed effect of the line; G_j is the fixed effect of the generation; $L_i \times G_j$ is the interaction effect of line × generation; $F_k(L, G)$ is the random effect of family within line and generation; $D_l(F, L, G)$ is the random effect of dam within family, line, and generation; BW7 is treated as a covariate (BW7 was not used as the covariate when analyzing AFP); and e_{ijklm} is the random residual effect. Significant differences between the least squares means of phenotypes of the lean and fat lines were calculated.

The genetic parameters, including the heritabilities of serum biochemical parameters and the genetic and phenotypic correlations between these serum biochemical parameters and abdominal fat traits (AFW and AFP), were estimated using MTDFREML software (Boldman et al., 1995), with generation and line treated as 2 fixed effects. Heritabilities of these traits were estimated using a single-variate model, and the

bivariate model was used to calculate the genetic and phenotypic correlations. The SE of heritability estimates and correlations between serum biochemical parameters and abdominal fat traits were approximated as described in Falconer and Mackay (1996).

The genetic model used for parameter estimations is described as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Zu} + \mathbf{e}, \qquad [2]$$

in which **y** is an n-dimensional vector of observed values for the traits, **X** is an $n \times p$ matrix of the fixed effects, β is a p-dimensional vector of the fixed effects, **Z** is an $n \times q$ matrix of the random effects, **u** is a q-dimensional vector of the random genetic effects, and **e** is an n-dimensional vector of the random residual effects.

The random effects **u** and **e** were assumed to follow the normal distributions with mean 0, that is, Expectation [**y**] = **Xβ**. The variances of **u** and **e** were assumed to be Var(**u**) = Ag and Var(**e**) = Ir, respectively, in which **A** is the numerator relationship matrix of all animals in the pedigree file, g is the additive genetic variance for the single-variate and the additive genetic variance–covariance matrix between traits for the bivariate model analysis, **I** is the identity matrix of order equal to the number of animals with phenotypes, and r is the residual variance for the single-variate and the variance–covariance matrix between residuals on the same animal when performing the bivariate model analysis, where residual covariance equal to 0 (Buchanan et al., 2015).

RESULTS

Differences in Abdominal Fat Content between Lean and Fat Birds

To determine if the lean and fat birds were appropriate for use in this study, we first needed to establish that differences exist for abdominal fat traits between the experimental birds, which were selected from the lean and fat lines, respectively (selection criteria described as in Materials and Methods). Therefore, the AFW and AFP in the G_{16}, G_{17}, and G_{18} populations were measured, and the values from the lean and fat birds were compared. As expected, the results showed that the fat birds had very significantly higher AFW and AFP than the lean birds (Table 1).

Differences in Serum Biochemical Parameters between Lean and Fat Birds

Eighteen serum biochemical parameters were measured in the 3 generations of chickens to investigate

Table 1. Comparisons of abdominal fat traits between the lean and fat lines[1]

Trait[2]	Lean line (n = 279)	Fat line (n = 279)	P-value
AFW, g	12.83 ± 0.72	117.95 ± 0.72	<0.0001**
AFP	0.0058 ± 0.0003	0.0589 ± 0.0003	<0.0001**

**Means very significantly different between the lean and fat lines (P < 0.01).

[1]Values are expressed as least squares means ± SE.

[2]AFW = abdominal fat weight; AFP = abdominal fat percentage.

which ones had significantly different levels between the lean and fat chickens. The comparisons of each of the serum biochemical indicator levels between lean and fat birds were performed using Model [1], and the results are shown in Table 2. Sixteen serum biochemical indicator levels were significantly different between the lean and fat birds. Serum concentrations of HDL-C, HDL-C:LDL-C, total bile acid, total protein, albumin, globulin, AST:ALT, GGT, uric acid, and creatinine in the fat birds were very significantly higher than those in the lean birds (P < 0.01), whereas serum LDL-C, albumin:globulin, glucose, ALT, AST, and free fatty acids concentrations in the fat birds were very significantly lower than those in the lean birds (P < 0.01). There were no significant differences of the levels of the remaining 2 serum biochemical indicators (triglycerides and total cholesterol) between the lean and fat birds.

Genetic Parameter Estimations of Serum Biochemical Indicators

After determining which of the tested serum biochemical parameters were expressed at significantly different levels in the lean and fat birds, we wanted to know if the expression of these parameters was heritable. The h^2 estimates of the 18 tested serum biochemical parameters, and the genetic and phenotypic correlation coefficients between each of the serum biochemical parameters and abdominal fat traits (AFW and AFP) were estimated, and the results are given in Table 3.

The serum HDL-C:LDL-C (h^2 = 0.86) and albumin:globulin (h^2 = 0.89) ratios had the highest estimated heritabilities among all of the measured serum biochemical parameters followed by the total bile acid, LDL-C, and albumin ($0.58 \leq h^2 \leq 0.64$). The heritabilities of AST:ALT, total protein, triglycerides, globulin, HDL-C, GGT, and creatinine were moderate ($0.29 \leq h^2 \leq 0.48$), whereas the heritabilities of the remaining 6 measured serum biochemical parameters were low ($0.05 \leq h^2 \leq 0.18$; Table 3).

Higher positive genetic correlation coefficients (r_g) were found between serum HDL-C:LDL-C, HDL-C, and glucose levels and abdominal fat traits ($0.30 \leq r_g \leq 0.80$), respectively, whereas serum triglycerides, AST,

Table 2. Comparisons of serum biochemical parameters between the lean and fat lines[1]

Parameter[2]	Lean line (n = 279)	Fat line (n = 279)	P-value
Triglycerides, mmol/L	0.45 ± 0.01	0.45 ± 0.01	0.6324
Total cholesterol, mmol/L	3.51 ± 0.03	3.52 ± 0.03	0.9006
HDL-C, mmol/L	2.17 ± 0.02	2.43 ± 0.02	<0.0001**
LDL-C, mmol/L	0.94 ± 0.02	0.79 ± 0.02	<0.0001**
HDL-C:LDL-C	2.64 ± 0.08	3.66 ± 0.08	<0.0001**
Total bile acid, μmol/L	1.96 ± 0.26	4.18 ± 0.25	<0.0001**
Total protein, g/L	30.68 ± 0.38	36.21 ± 0.37	<0.0001**
Albumin, g/L	12.30 ± 0.18	13.00 ± 0.17	0.0053**
Globulin, g/L	18.47 ± 0.32	23.19 ± 0.31	<0.0001**
Albumin:globulin	0.69 ± 0.01	0.59 ± 0.01	<0.0001**
Glucose, mmol/L	11.78 ± 0.11	10.71 ± 0.11	<0.0001**
AST, units/L	238.80 ± 3.27	206.79 ± 3.27	<0.0001**
ALT, units/L	5.49 ± 0.14	4.73 ± 0.15	0.0004**
AST:ALT	52.51 ± 1.34	57.90 ± 1.35	0.0062**
GGT, units/L	12.43 ± 0.32	17.67 ± 0.31	<0.0001**
Uric acid, μmol/L	192.08 ± 6.20	249.78 ± 6.19	<0.0001**
Creatinine, μmol/L	4.28 ± 0.12	5.39 ± 0.12	<0.0001**
Free fatty acids, mmol/L	0.85 ± 0.02	0.71 ± 0.02	<0.0001**

**Means very significantly different between the lean and fat lines (P < 0.01).

[1]Values are expressed as least squares means ± SE.

[2]HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; AST = aspartate transaminase; ALT = alanine transaminase; GGT = gamma-glutamyl transpeptidase.

uric acid, and globulin levels showed higher negative genetic correlations with abdominal fat traits ($-0.84 \leq r_g \leq -0.30$), respectively (Table 3). The phenotypic correlation coefficients (r_p) between serum biochemical parameters and abdominal fat traits were found to be relatively lower ($-0.29 \leq r_p \leq 0.28$; Table 3).

DISCUSSION

In this study, we investigated whether lean and fat broiler chickens have significantly different serum levels of biochemical indicators, and we also assessed the genetic parameters of these indicators. Considering the absolute values of serum levels of these biochemical indicators, the levels of all the serum biochemical indicators detected in the current study are similar to the results reported in previous studies in chickens (Bowes et al., 1989; Yeung et al., 2009; Renli et al., 2012; Amiridumari et al., 2013; Chikumba et al., 2013; Cao and Wang, 2014).

In chickens, the plasma VLDL concentration has been shown to be related to abdominal fat content, and when used by breeders as a selection criterion to decrease the amount of abdominal fat deposition, significant progresses have been made (Whitehead and Griffin, 1984). We also successfully used the plasma VLDL concentration and AFP as selection criteria to reduce the abdominal fat content of broilers (Guo et al., 2011). In this study, ad-

Table 3. The heritabilities of serum biochemical parameters, and the genetic and phenotypic correlations between serum biochemical parameters and abdominal fat traits (abdominal fat weight [AFW] and abdominal fat percentage [AFP])[1]

Parameter[2]	Heritability	Genetic correlation		Phenotypic correlation	
		AFW	AFP	AFW	AFP
Triglycerides	0.31 ± 0.07	−0.84 ± 0.04	−0.76 ± 0.06	0.01 ± 0.15	0.00 ± 0.15
Total cholesterol	0.17 ± 0.05	0.03 ± 0.17	0.04 ± 0.17	0.02 ± 0.17	0.17 ± 0.17
HDL-C	0.48 ± 0.09	0.61 ± 0.08	0.57 ± 0.09	0.20 ± 0.13	0.28 ± 0.12
LDL-C	0.61 ± 0.10	−0.10 ± 0.12	−0.01 ± 0.13	−0.12 ± 0.12	−0.03 ± 0.13
HDL-C:LDL-C	0.86 ± 0.12	0.32 ± 0.10	0.30 ± 0.11	0.22 ± 0.11	0.20 ± 0.11
Total bile acid	0.58 ± 0.10	0.03 ± 0.13	0.17 ± 0.12	0.05 ± 0.13	0.06 ± 0.13
Total protein	0.30 ± 0.07	−0.31 ± 0.14	−0.25 ± 0.14	−0.25 ± 0.14	0.01 ± 0.15
Albumin	0.64 ± 0.10	0.26 ± 0.12	0.21 ± 0.12	−0.02 ± 0.12	0.12 ± 0.12
Globulin	0.45 ± 0.09	−0.44 ± 0.11	−0.30 ± 0.12	−0.29 ± 0.12	−0.04 ± 0.14
Albumin:globulin	0.89 ± 0.12	0.29 ± 0.10	0.25 ± 0.11	0.19 ± 0.11	0.11 ± 0.11
Glucose	0.05 ± 0.03	0.80 ± 0.08	0.69 ± 0.12	0.14 ± 0.23	0.14 ± 0.23
AST	0.05 ± 0.05	−0.60 ± 0.18	−0.62 ± 0.16	0.03 ± 0.18	−0.05 ± 0.18
ALT	0.15 ± 0.03	0.05 ± 0.15	0.29 ± 0.15	0.02 ± 0.23	−0.03 ± 0.24
AST:ALT	0.29 ± 0.07	0.34 ± 0.13	0.15 ± 0.15	0.00 ± 0.15	0.01 ± 0.15
GGT	0.48 ± 0.09	0.24 ± 0.13	0.05 ± 0.13	−0.04 ± 0.13	−0.03 ± 0.13
Uric acid	0.12 ± 0.04	−0.48 ± 0.14	−0.50 ± 0.14	−0.03 ± 0.19	−0.02 ± 0.19
Creatinine	0.48 ± 0.09	0.06 ± 0.13	0.27 ± 0.12	−0.04 ± 0.13	0.02 ± 0.13
Free fatty acids	0.18 ± 0.07	0.02 ± 0.19	−0.11 ± 0.19	0.03 ± 0.19	−0.04 ± 0.19

[1]Values are expressed as estimates ± SE.

[2]HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; AST = aspartate transaminase; ALT = alanine transaminase; GGT = gamma-glutamyl transpeptidase.

ditional 18 serum biochemical indicators were compared between the lean and fat birds. Using Model [1], which included the generation and the line × generation interaction effects, we found that, interestingly, among the 18 tested serum biochemical indicators, 16 indices (HDL-C, LDL-C, HDL-C:LDL-C, total bile acid, total protein, albumin, globulin, albumin:globulin, glucose, AST, ALT, AST:ALT, GGT, uric acid, creatinine, and free fatty acids) were significantly different between the lean and fat birds (Table 2). The generation and line × generation interaction factors both had significant effects on abdominal fat traits and most of the serum biochemical indicators ($P <$ 0.05). However, for total bile acid and globulin, the generation factor had no significant effect, and for total bile acid, albumin, glucose, and AST, the line × generation interaction factor had no significant effect. For most of the traits we analyzed, both generation and line × generation interaction had significant effects, which indicated that the levels of biochemical indicators were significantly different between generations and that the differences between the 2 genetic lines could change also significantly over the generations. The statistical model considering all these factors could lead to a more accurate estimation of the genetic effects of lines. We were more interested in the differences of the serum biochemical indicators between the fat and lean chicken lines. As a result, 16 serum biochemical indicators of significant difference between the 2 lines may have the potential to be used as biomarkers to select lean birds in future breeding programs.

Previous studies in chickens found that fat line birds had higher plasma high-density lipoprotein (**HDL**) levels and significantly lower plasma low-density lipoprotein (**LDL**) levels than lean line birds (Leclercq, 1984; Hermier et al., 1991), and our findings agrees with these reports. It is known that HDL promotes the uptake of cholesterols from peripheral tissues and the transport of cholesterols to the liver for catabolism, whereas LDL plays the opposite role (Miller and Miller, 1975). In the present study, fat birds had a very significantly higher serum HDL level, a lower serum LDL level, and a higher HDL-C:LDL-C ratio than lean birds (Table 2). These results indicate that the transport ability of cholesterol from peripheral tissues to the liver in fat birds was much greater than in lean birds. Some of the cholesterols might be reassembled into lipoproteins in the liver and transport triglycerides to adipose tissue (Hermier, 1997), and some of these cholesterols might be used to synthesize bile acids, which could re-emulsify lipids from the diet in the small intestine (Yuan and Wang, 2010). Combining the results of the current study and our previous finding that fat birds had significantly higher plasma VLDL concentrations and liver fat content than lean birds (data not shown), we speculate that fat birds have a superior ability to synthesize and transport triglycerides than lean birds.

Bile acids are known to be essential in the emulsification of dietary lipids (Krogdahl, 1985; Yuan and Wang, 2010). Most of the bile acids are reabsorbed by an active bile salt reabsorption mechanism in the terminal

ileum and transported by portal circulation to the liver (Bernstein et al., 2005), and some of the bile acids enter the circulatory system. The serum bile acid level increases postprandially, suggesting that the serum bile acid level could be a diurnal hormonal signal reflecting food intake (Watanabe et al., 2006). In the current study, we found that fat birds had very significantly higher levels of total bile acid in their serum compared with lean birds (Table 2). This result indicates that fat birds might have a superior ability to emulsify dietary lipids compared with lean birds. To our knowledge, there are no previously published studies reporting a similar difference in the serum level of total bile acids between obese and normal groups in chickens, humans, or other animal species.

In the current study, we found that fat birds had very significantly higher serum ==total protein== and ==globulin== concentrations than lean birds (Table 2). This result is similar to previous studies in humans, rats, and pigs. In humans, obesity was significantly associated with higher serum total protein and globulin levels (Carroll et al., 2000). Likewise, the levels of serum total protein and globulin in the genetically obese Zucker rats were significantly higher than those in nonobese rats (Schirardin et al., 1979). Additionally, obese pigs had higher serum levels of total protein than normal ones (He et al., 2012), and the serum total protein level was shown to be the best biomarker for early estimation of fatness in pigs (Muñoz et al., 2012). Here, we also found that serum albumin concentrations in fat birds were very significantly higher than that in lean birds (Table 2). This result is consistent with previous reports that serum concentrations of albumin in the genetically obese Zucker rats were significantly higher than those in nonobese rats (Schirardin et al., 1979) and that obese individuals usually appeared to have decreased serum albumin levels after a weight loss operation (Bloomberg et al., 2005).

We found that the serum concentrations of glucose in fat birds were very significantly lower than that in lean birds (Table 2). This result is consistent with previous studies in chickens that were similarly selected divergently for low or high abdominal fat deposition. These studies demonstrated that fat family birds consistently exhibited much lower plasma glucose levels than lean family birds over several generations (Simon and Leclercq, 1982; Leclercq et al., 1988b; Baéza and Le Bihan-Duval, 2013). The results of the present study also agree with a study using an obese pig model, in which obese pigs had significantly lower serum glucose levels than lean pigs (He et al., 2012). As glucose is the form of carbohydrate in the blood, it could be oxidized to provide energy and channeled into pathways for the synthesis of fatty acids (lipogenesis; Uyeda and Repa, 2006). The reduced glucose concentration in the serum of fat birds suggests a higher rate of glucose use in the fat birds compared with that in the lean birds (He et al., 2012).

Serum AST (Kelishadi et al., 2009), ALT (Tazawa et al., 1997), and GGT (Kong et al., 2013) are 3 major liver enzymes examined in liver function tests, and altered levels of these enzymes have been reported to be associated with human obesity. In the current study, we found that the serum AST concentrations in fat birds were very significantly lower than those in lean birds (Table 2). This result is similar to findings from a human study, in which Japanese girls had serum AST levels that were negatively associated with body mass index (**BMI**; Okuda et al., 2010). It is well known that BMI is closely related to body fat percentage and total body fat (Shah and Braverman, 2012). We found that the serum ALT concentrations in fat birds were very significantly lower than those in lean birds (Table 2), which is different from a human study in which elevated serum ALT levels were associated with high BMI (Okuda et al., 2010). Here, we also found that the serum levels of GGT in the fat birds were very significantly higher than those in the lean birds (Table 2). This result agrees with results reported in humans that obese individuals often have elevated serum levels of GGT compared with normal controls (Abdou et al., 2008; Okuda et al., 2010; Kong et al., 2013).

Uric acid is the main end product in nitrogen metabolism of birds, and its content could be expected to reflect the direction of protein metabolism in the birds (Okumura and Tasaki, 1969). Previous studies show that serum uric acid level was associated with human obesity (Krzystek-Korpacka et al., 2011; Kong et al., 2013). In this study, we found that the serum uric acid concentrations in the fat birds were very significantly higher than those in the lean birds (Table 2). The result is consistent with these reports in humans that serum uric acid level showed positive association with BMI (Bonora et al., 1996) and that obese individuals had significantly higher serum uric acid levels than lean ones (Krzystek-Korpacka et al., 2011). In addition, serum uric acid concentration even could be used as an obesity-related indicator in early adolescence (Oyama et al., 2006).

Creatinine is the metabolic product of creatine in muscle, and it is usually produced at a fairly constant rate by the body (Wyss and Kaddurah-Daouk, 2000). The results of previous human studies suggest that BMI is related to serum creatinine levels (Gelber et al., 2005; Bayoud et al., 2014). In the current study, we found that the serum creatinine levels in fat birds were very significantly higher than the levels in lean birds (Table 2). This result is similar to findings in human studies that serum creatinine levels significantly increased with increasing BMI (Siener et al., 2004; Gelber et al., 2005) and that elevated serum creatinine levels were more common in obese individuals than in normal people (Mahdi et al., 2015).

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一共讨论了8个血清学指标

开始讨论遗传参数，

In this study, we found that serum levels of free fatty acids in the fat birds were very significantly lower than those in the lean birds (Table 2). This result disagrees with a previous study in chickens, where no significant difference between lean and fat lines was found in plasma free fatty acids levels (Leclercq et al., 1988a). This inconsistency may be because of the different genetic backgrounds of the populations.

The genetic parameters of 18 serum biochemical indicators were estimated in the present study (Table 3). The heritability estimates of serum total cholesterol, total protein, albumin, glucose, uric acid, and free fatty acids levels were similar to those previously reported in chickens (Hollands et al., 1980; Abdel Latif, 2001; Amira et al., 2009; Demeure et al., 2013; Loyau et al., 2013). However, the heritability estimate of the serum globulin level in the current study ($h^2 = 0.45$) was smaller than in a previous chicken study ($h^2 = 0.71$; Amira et al., 2009), and the heritability estimate of serum triglyceride level in the current study ($h^2 = 0.31$) was much higher than in another study in the chicken ($h^2 = 0.02$; Loyau et al., 2013). To our knowledge, there have not been any reports concerning the heritability estimates of the remaining tested serum biochemical indicators in chickens, except that some of the serum biochemical indicators have been studied in humans. When we compared our results with those from human studies, we found that the heritability estimates of serum HDL-C, LDL-C, ALT, GGT, and creatinine levels were similar to each other (Pattaro et al., 2009; Pietiläinen et al., 2009; van Beek et al., 2013; Lin et al., 2014), whereas the heritability estimate of the serum AST level in the current study ($h^2 = 0.05$) was smaller than that reported in the human study ($h^2 = 0.22$; van Beek et al., 2013). The heritability estimates of the remaining 4 serum biochemical indicators, HDL-C:LDL-C, total bile acid, albumin:globulin, and AST:ALT, have not been previously reported in either chickens or humans. There have been few reports regarding the genetic correlations between the serum biochemical parameters tested in this study and body fat content. In the current study, the genetic correlations between glucose and abdominal fat traits (AFW and AFP) were 0.80 and 0.69, respectively. This result was inconsistent with a previous study in chickens, in which the genetic correlations between glucose and abdominal fat traits (AFW and AFP) were both –0.66 (Demeure et al., 2013). The genetic correlations between serum uric acid and abdominal fat traits (AFW and AFP) in the current study were –0.48 and –0.50, respectively, and this result was also inconsistent with a human study, in which the genetic correlation between serum uric acid and total body fat was 0.29 (Voruganti et al., 2009). These inconsistencies may be coming from a variety of factors, such as different breeds, species, genetic backgrounds, or environments.

开始提出四部曲。

第一个条件：浓度必须是差异的；

第二个条件：高的遗传相关；

第三个：遗传相关关系的方向；

第四个条件：高的遗传力。

The aim of this study was to identify serum biochemical indicators that could be used as biomarkers for selecting chickens with a lower abdominal fat content. Four criteria were considered to be essential in determining whether a given serum biochemical indicator could be successfully used as a marker. First, the average level of that serum biochemical indicator must be significantly different between the lean and fat birds. Second, the serum biochemical indicator must have a relatively high genetic correlation coefficient with both abdominal fat traits (AFW and AFP). Third, to select lean birds, the level of the serum biochemical indicator must be lower in the lean birds if its genetic correlation coefficients with abdominal fat traits are positive, and vice versa. Lastly, the heritability of the serum biochemical parameter should be high. We found 16 serum biochemical indicators that were significantly different between the lean and fat birds (HDL-C, LDL-C, HDL-C:LDL-C, total bile acid, total protein, albumin, globulin, albumin:globulin, glucose, AST, ALT, AST:ALT, GGT, uric acid, creatinine, and free fatty acids), meeting the first criterion described above. Among these 16 serum biochemical indicators, 6 (HDL-C, HDL-C:LDL-C, globulin, glucose, AST, and uric acid) had relatively high genetic correlations with abdominal fat traits ($r_g < -0.30$ or $r_g > 0.30$), meeting the second criterion described above. Of these 6 serum biochemical indicators, 3 (HDL-C, HDL-C:LDL-C, and AST) met the third criterion described above, and of these 3, HDL-C and HDL-C:LDL-C had relatively high heritabilities ($h^2 = 0.48$ and $h^2 = 0.86$, respectively; Tables 2 and 3), making them the only parameters to meet all 4 criteria. Therefore, we believe that out of the serum biochemical indicators tested in this study, HDL-C and HDL-C:LDL-C would be the best biomarkers for selecting chickens with a lower abdominal fat content.

In summary, we used lean and fat broiler chicken lines that had been divergently selected for abdominal fat content and compared their levels of 18 serum biochemical indicators. We also estimated the genetic parameters of these serum biochemical parameters. Taken together, and using a 4-faceted selection criterion, we conclude that serum HDL-C and HDL-C:LDL-C levels have the potential to be used as biomarkers for selecting lean broilers in the future.

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