

# GENETICS AND GENOMICS

## Construction of multiple linear regression models using blood biomarkers for selecting against abdominal fat traits in broilers

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**ABSTRACT** Plasma very low-density lipoprotein (VLDL) can be used to select for low body fat or abdominal fat (AF) in broilers, but its correlation with AF is limited. We investigated whether any other biochemical indicator can be used in combination with VLDL for a better selective effect. Nineteen plasma biochemical indicators were measured in male chickens from the Northeast Agricultural University broiler lines divergently selected for AF content (NEAUHLF) in the fed state at 46 and 48 d of age. The average concentration of every parameter for the 2 d was used for statistical analysis. Levels of these 19 plasma biochemical parameters were compared between the lean and fat lines. The phenotypic correlations between these plasma biochemical indicators and AF traits were analyzed. Then, multiple linear regression models were constructed to select the best model used for

selecting against AF content. and the heritabilities of plasma indicators contained in the best models were estimated. The results showed that 11 plasma biochemical indicators (triglycerides, total bile acid, total protein, globulin, albumin/globulin, aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, uric acid, creatinine, and VLDL) differed significantly between the lean and fat lines ( $P < 0.01$ ), and correlated significantly with AF traits ( $P < 0.05$ ). The best multiple linear regression models based on albumin/globulin, VLDL, triglycerides, globulin, total bile acid, and uric acid, had higher  $R^2$  (0.73) than the model based only on VLDL (0.21). The plasma parameters included in the best models had moderate heritability estimates ( $0.21 \leq h^2 \leq 0.43$ ). These results indicate that these multiple linear regression models can be used to select for lean broiler chickens.

**Key words:** Abdominal fat, chicken, multiple linear regression, plasma biochemical parameter, very low-density lipoprotein

2018 Poultry Science 97:17–23  
<http://dx.doi.org/10.3382/ps/pex319>

## INTRODUCTION

For the past 60 years, substantial advances have been made in improving the body weights of chickens. However, a rapid growth rate has been accompanied by an increase in the deposition of body fat in broilers, and fat is considered a by-product of very little commercial value. Moreover, excessive fat deposition can reduce feed conversion efficiency.

Body fat content and composition in chickens can be influenced by diet, including the energy/protein ratio and density in the diet (De Los Mozos et al., 2017).

However, a genetic approach seems to be the most efficient way to modify carcass fat in chickens (Ricard and Rouvier, 1970; Thiruvengadan et al., 2011). Therefore, many selection programs have been undertaken to directly or indirectly reduce fat deposition in chickens.

The most effective way to produce lean chicken lines is to directly select against abdominal fat (AF) with sib-test procedures (Leclercq et al., 1980; Cahaner and Nitsan, 1985; Leenstra and Pit, 1987). Lilburn et al. (1982) established lean and obese lines of chickens by genetically selecting for mature AF pad size. Leclercq (1988) constructed lean and fat chicken lines by selecting against the proportion of AF and live weight. However, one of the main problems associated with direct selection for AF is that the birds must be slaughtered to measure the AF weight (AFW) or AF percentage (AFP) directly and precisely, which is costly and

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Received December 2, 2016.

Accepted September 24, 2017.

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time-consuming. Therefore, it may be better to select against AF indirectly by selecting against traits that are strongly associated with AF. The results of previous studies have indicated that selection based on the feed conversion ratio can reduce AF in chickens (Pym and Solvyns, 1979; Leenstra and Pit, 1987). Plasma very low-density lipoprotein (VLDL) in the fed state was shown to be significantly associated with AF and can be used in commercial broiler breeding programs (Griffin and Whitehead, 1982). Whitehead and Griffin (1984) established 2 lines divergently selected for AF for 3 years, using the plasma VLDL concentration as the selection criterion. These 2 lines were selected over 10 generations until 1992, and the lean and fat lines differed significantly in AF (Hocking et al., 1992).

Since 1996, we have developed the Northeast Agricultural University broiler lines divergently selected for AF content (NEAUHLF), using the plasma VLDL concentration in the fed state and AFP as the selection criteria (Guo et al., 2011). Our previous study reported high phenotypic correlations between plasma VLDL concentrations and AFW or AFP (0.56 to 0.65), but these correlations were not detectable after the chickens were fasted for 6 h, indicating that VLDL levels should be measured in the fed state (Li et al., 1999). Although the associations between VLDL and these AF traits were significant, we wondered whether a combination of other plasma biochemical indicators with VLDL would exert a better selective effect against AF than VLDL used alone. Therefore, the objective of this study was to construct multiple linear regression models using various plasma biochemical indicators (including VLDL) in the fed state to achieve a better selection effect against AF content.

## 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 was approved by the Laboratory Animal Management Committee of Northeast Agricultural University (Harbin, China).

### Experimental Populations, Blood Sample Collection, and Measurement of AF Traits

The lean and fat lines of NEAUHLF used in this study have been selected since 1996 using AFP and plasma VLDL concentration as the selection criteria (Guo et al., 2011; Dong et al., 2015). AF traits were significantly different between these 2 lines from the 4th generation, whereas their body weight at 7 wk of age (BW7) had no significant difference through generations (Zhang et al., 2017). All birds were kept in similar environmental conditions and had free access to

**Table 1.** Feed composition and nutrient content of the basal diets.

Ingredients	0 to 4 wk of age	5 to 7 wk of age
Crude protein %	19	15
Metabolic energy kcal/kg	2800	2800
Lysine %	0.90	0.66
Methionine + Cystine %	0.70	0.55
Methionine %	0.35	0.27
Threonine %	0.62	0.49
Valine %	0.70	0.55
Isoleucine %	0.61	0.50
Arginine %	0.97	0.76
Tryptophan %	0.14	0.15
Calcium %	1.00	0.90
Effective phosphorus %	0.45	0.42
Sodium %	0.18	0.16
Chlorine %	0.18	0.16
Potassium %	0.40	0.40
Copper mg/kg	16	16
Iodine mg/kg	1.25	1.25
Iron mg/kg	40	40
Manganese mg/kg	120	120
Selenium mg/kg	0.30	0.30
Zinc mg/kg	100	100

feed and water. Commercial corn-soybean-based diets that met all National Research Council (1994) requirements were provided in the study (Table 1).

On d 46 and 48 of age, blood was collected from the male birds after they were fed. Ethylenediaminetetraacetic acid (EDTA) was used as the anticoagulant. The plasma was separated from the whole blood by centrifugation at  $3,000 \times g$  for 10 min at room temperature. The plasma samples were stored in 1.5 mL tubes at  $-20^{\circ}\text{C}$  until analysis. BW7 and AFW of the male birds were measured, and AFP was calculated as the ratio AFW/BW7.

### Selection of Experiment Individuals

Male birds from the 18<sup>th</sup> generation of the lean and fat lines were used in this study. The AFP of every bird was calculated, and the birds were sorted according to their AFPs. In total, 176 individuals from the 18<sup>th</sup> generation (88 birds with the lowest AFP in the lean line and 88 birds with the highest AFP in the fat line) were selected (Dong et al., 2015).

### Measurement of Plasma Biochemical Parameters

Eighteen plasma biochemical indicators were investigated in the fed state. The plasma concentrations of 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), gamma-glutamyl transpeptidase (GGT), uric acid, creatinine, and free fatty acids were measured with standard commercial kits (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions, 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). The globulin concentration in the plasma was calculated as the difference between the total protein and albumin. The ratios HDL-C/LDL-C, albumin/globulin, and AST/ALT were calculated.

The concentration of plasma VLDL in the fed state was measured with the rapid turbidimetric method described by Griffin and Whitehead (1982).

### Statistical Analysis

Our previous results showed that the VLDL concentration should be calculated as the average of measurements made on 2 d (Li et al., 1999). Therefore, in this study, 19 plasma biochemical indicators levels were calculated in the fed state in birds from NEAUHLF at 46 and 48 d of age, and the average concentration of every plasma biochemical indicator on these 2 d was used for the statistical analysis.

Statistical differences in the AF traits (AFW and AFP) and the plasma biochemical parameters between the lean and fat bird lines were calculated with JMP version 7.0 (SAS Inst. Inc., Cary, NC), with the following model:

$$y_{ijkl} = \mu + L_i + F_j(L) + D_k(F, L) + BW7 + e_{ijkl} \quad (1)$$

in which  $y_{ijkl}$  is the phenotypic value of the AF traits or the plasma biochemical parameters,  $\mu$  is the population mean,  $L_i$  is the fixed effect of the line,  $F_j(L)$  is the random effect of the family within the line,  $D_k(F, L)$  is the random effect of the dam within the family and line, BW7 is treated as a covariate (BW7 was not used as the covariate when analyzing AFP), and  $e_{ijkl}$  is the random residual effect. The significance of the differences between the least squares means of the phenotypes of the lean and fat lines was calculated.

The phenotypic correlations between the 19 plasma biochemical parameters and AF traits were analyzed with JMP version 7.0.

The multiple linear regression analyses were also performed with JMP version 7.0. The multiple linear relationships between the plasma biochemical parameters and the AF traits were estimated with mixed stepwise regression, and the best multiple linear regression models were selected.

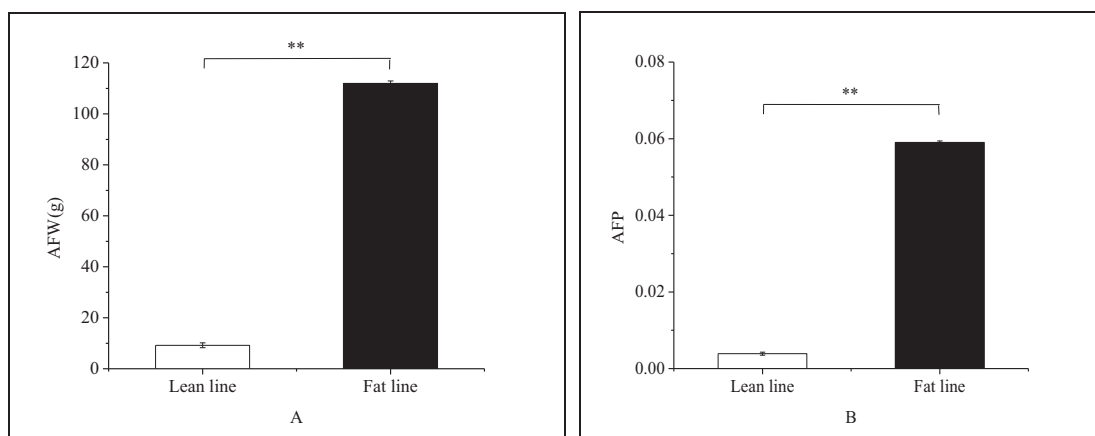
Heritabilities of plasma biochemical parameters were estimated using MTDFREML software (Boldman et al., 1995), with line treated as a fixed effect, and the SE of heritability estimates were approximated as described in Falconer and Mackay (1996).

## RESULTS

### Differences in Plasma Biochemical Parameters between the Lean and Fat Birds

To confirm that the experimental birds were appropriate for use in this study, AFW and AFP were measured and compared in the lean and fat lines to determine the differences in these AF traits between the lean and fat individuals. As expected, AFW ( $111.94 \pm 0.97$  g vs.  $9.20 \pm 0.96$  g) and AFP ( $0.0589 \pm 0.0004$  vs.  $0.0041 \pm 0.0004$ ) were significantly higher in the fat birds than in the lean birds ( $P < 0.0001$ ; Figure 1).

Nineteen plasma biochemical parameters were measured at 46 and 48 d of age in the male chickens in the fed state, and the average value for each indicator on these 2 d was used to investigate which indicator(s) differed significantly between the lean and fat chickens. Each of the plasma biochemical parameters was compared between the lean and fat birds using analytical Model 1, and the results are given in Table 2. The levels of 11 biochemical indicators differed significantly between the lean and fat birds. The plasma concentrations of triglycerides, total bile acid, total protein, globulin, GGT, uric acid, creatinine, and VLDL were very higher in the fat birds than in the lean birds ( $P < 0.01$ ), whereas the plasma concentrations of albumin/globulin, AST, and ALT were very lower in the



**Figure 1.** Comparisons of abdominal fat traits between the lean and fat lines of male chickens. A) The comparisons of abdominal fat weight (AFW) between the lean and fat lines. B) The comparisons of abdominal fat percentage (AFP) between the lean and fat lines. \*\*Means very significantly different between the lean and fat lines ( $P < 0.01$ ).

**Table 2.** Comparisons of plasma biochemical parameters between the lean and fat lines in the fed state of male chickens.<sup>1</sup>

Parameter <sup>2</sup>	Lean line (n = 88)	Fat line (n = 88)	P-value
Triglycerides (mmol/L)	0.70 ± 0.05	1.29 ± 0.05	<0.0001**
Total cholesterol (mmol/L)	3.21 ± 0.07	3.12 ± 0.07	0.3212
HDL-C (mmol/L)	1.85 ± 0.04	1.86 ± 0.04	0.7909
LDL-C (mmol/L)	0.65 ± 0.02	0.64 ± 0.02	0.7023
HDL-C/LDL-C	3.05 ± 0.09	3.15 ± 0.10	0.4568
Total bile acid (μmol/L)	10.82 ± 0.60	14.44 ± 0.60	<0.0001**
Total protein (g/L)	37.22 ± 0.73	42.38 ± 0.74	<0.0001**
Albumin (g/L)	15.35 ± 0.25	15.42 ± 0.25	0.8615
Globulin (g/L)	21.85 ± 0.55	26.96 ± 0.56	<0.0001**
Albumin/Globulin	0.71 ± 0.01	0.58 ± 0.01	<0.0001**
Glucose (mmol/L)	13.98 ± 0.32	14.61 ± 0.33	0.1864
AST (U/L)	186.21 ± 6.15	152.88 ± 6.13	0.0005**
ALT (U/L)	2.61 ± 0.09	2.26 ± 0.09	0.0087**
AST/ALT	73.24 ± 1.33	71.28 ± 1.37	0.3184
GGT (U/L)	18.72 ± 0.46	21.12 ± 0.47	0.0007**
Uric acid (μmol/L)	187.99 ± 7.67	329.53 ± 7.88	<0.0001**
Creatinine (μmol/L)	3.60 ± 0.09	4.10 ± 0.10	0.0007**
Free fatty acids (mmol/L)	0.24 ± 0.01	0.21 ± 0.01	0.0861
VLDL (mg/mL)	0.15 ± 0.01	0.21 ± 0.01	0.0002**

<sup>1</sup>Data are least squares means ± SE.

<sup>2</sup>HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; AST, Aspartate transaminase; ALT, Alanine transaminase; GGT, Gamma-glutamyl transpeptidase; VLDL, Very low-density lipoprotein.

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

fat birds than in the lean birds ( $P < 0.01$ ). There were no significant differences in the levels of the remaining 8 plasma biochemical indicators (total cholesterol, HDL-C, LDL-C, HDL-C/LDL-C, albumin, glucose, AST/ALT, and free fatty acids) between the lean and fat birds ( $P > 0.05$ ).

## Phenotypic Correlations between Plasma Biochemical Parameters and AF Traits (AFW and AFP)

After we determined which of the tested plasma biochemical parameters were expressed at significantly different levels in the lean and fat birds in the fed state, we investigated the correlations between these 19 plasma indicators and the AF traits (AFW and AFP) with phenotypic correlation coefficients. The results are shown in Table 3. Eleven plasma biochemical parameters were significantly associated with both AFW and AFP, and among these, plasma triglycerides, total bile acid, total protein, globulin, GGT, uric acid, creatinine, and VLDL correlated positively with the AF traits ( $P < 0.05$ ), whereas plasma albumin/globulin, AST, and ALT correlated negatively with the AF traits ( $P < 0.01$ ). There were no significant phenotypic correlations between the AF traits and the remaining 8 plasma biochemical indicators (total cholesterol, HDL-C, LDL-C, HDL-C/LDL-C, albumin, glucose, AST/ALT, or free fatty acids;  $P > 0.05$ ).

## Multiple Linear Regression Models for Selecting against AF Traits (AFW and AFP)

Based on the results described above, we screened the 11 plasma biochemical indicators (triglycerides, total bile acid, total protein, globulin, albumin/globulin, AST, ALT, GGT, uric acid, creatinine, and VLDL) that differed significantly between the lean and fat bird lines

**Table 3.** Phenotypic correlations between plasma biochemical parameters and abdominal fat traits (AFW and AFP) in the fed state of male chickens.<sup>1</sup>

Parameter <sup>2</sup>	AFW (n = 176)		AFP (n = 176)	
	Coefficient	P-value	Coefficient	P-value
Triglycerides	0.65	$P < 0.0001$ **	0.65	<0.0001**
Total cholesterol	-0.12	0.1257	-0.10	0.1817
HDL-C	0.04	0.5783	0.05	0.5079
LDL-C	-0.11	0.1523	-0.09	0.2438
HDL-C/LDL-C	0.11	0.1308	0.10	0.1662
Total bile acid	0.29	0.0001**	0.30	<0.0001**
Total protein	0.26	0.0005**	0.31	<0.0001**
Albumin	-0.03	0.7012	0.00	0.9513
Globulin	0.35	$P < 0.0001$ **	0.40	<0.0001**
Albumin/Globulin	-0.49	$P < 0.0001$ **	-0.53	<0.0001**
Glucose	0.12	0.0999	0.10	0.1738
AST	-0.29	0.0001**	-0.30	0.0001**
ALT	-0.25	0.0010**	-0.25	0.0010**
AST/ALT	-0.02	0.7769	-0.03	0.7028
GGT	0.18	0.0155*	0.21	0.0062**
Uric acid	0.72	$P < 0.0001$ **	0.73	<0.0001**
Creatinine	0.36	$P < 0.0001$ **	0.34	<0.0001**
Free fatty acids	-0.14	0.0561	-0.13	0.0864
VLDL	0.46	$P < 0.0001$ **	0.45	<0.0001**

<sup>1</sup>AFW, Abdominal fat weight; AFP, Abdominal fat percentage.

<sup>2</sup>HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; AST, Aspartate transaminase; ALT, Alanine transaminase; GGT, Gamma-glutamyl transpeptidase; VLDL, Very low-density lipoprotein.

\*\*Means very significantly correlated between this plasma biochemical parameter and abdominal fat trait in fed state ( $P < 0.01$ ).

\*Means significantly correlated between this plasma biochemical parameter and abdominal fat trait in fed state ( $P < 0.05$ ).



**Table 4.** Parameter estimates of the best multiple linear regression models for AFW.<sup>1</sup>

Term <sup>2</sup>	Estimate	Std Error	t Ratio	Prob> t
Intercept	192.98	33.42	5.78	<0.0001
Triglycerides	34.36	7.05	4.87	<0.0001
Total bile acid	-1.25	0.45	-2.77	0.0062
Globulin	-4.17	0.67	-6.20	<0.0001
Albumin/Globulin	-244.88	30.91	-7.92	<0.0001
Uric acid	0.30	0.03	8.77	<0.0001
VLDL	176.75	30.56	5.78	<0.0001

<sup>1</sup>AFW, Abdominal fat weight.<sup>2</sup>VLDL, Very low-density lipoprotein.**Table 5.** Parameter estimates of the best multiple linear regression models for AFP.<sup>1</sup>

Term <sup>2</sup>	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.0915	0.0170	5.38	<0.0001
Triglycerides	0.0141	0.0036	3.92	0.0001
Total bile acid	-0.0005	0.0002	-2.26	0.0254
Globulin	-0.0018	0.0003	-5.18	<0.0001
Albumin/Globulin	-0.1242	0.0157	-7.89	<0.0001
Uric acid	0.0002	0.0000	9.13	<0.0001
VLDL	0.0833	0.0156	5.35	<0.0001

<sup>1</sup>AFP, Abdominal fat percentage.<sup>2</sup>VLDL, Very low-density lipoprotein.

in the fed state and were significantly associated with the AF traits. To explore the multiple linear regression relationships between these plasma parameters and the AF traits, multiple linear regression models were constructed. After a mixed stepwise regression analysis, we obtained the results of parameter estimates (Table 4 and 5), and the best multiple linear regression models for AFW and AFP were:

$$\begin{aligned} \text{AFW} = & 192.98 + 34.36 \times \text{triglycerides} \\ & - 1.25 \times \text{total bile acid} - 4.17 \times \text{globulin} \\ & - 244.88 \times \text{albumin/globulin} + 0.30 \times \text{uric acid} \\ & + 176.75 \times \text{VLDL} \quad (P < 0.0001) \end{aligned} \quad (2)$$

$$\begin{aligned} \text{AFP} = & -0.0915 + 0.0141 \times \text{triglycerides} \\ & - 0.0005 \times \text{total bile acid} - 0.0018 \times \text{globulin} \\ & - 0.1242 \times \text{albumin/globulin} + 0.0002 \\ & \times \text{uric acid} + 0.0833 \times \text{VLDL} \quad (P < 0.0001) \end{aligned} \quad (3)$$

According to the results, levels of total protein, AST, ALT, GGT, and creatinine do not appear to be significant factors for predicting AF traits.

The R<sup>2</sup> values of Model 2 for AFW and Model 3 for AFP were both 0.73, which were much higher than those of the models based only on VLDL (Model 4 and 5 as followed), in which the R<sup>2</sup> values were 0.22 and 0.20, respectively.

$$\text{AFW} = 0.3438 + 331 \times \text{VLDL} \quad (P < 0.0001) \quad (4)$$

**Table 6.** Heritability estimates of plasma biochemical parameters in the fed state of male chickens.<sup>1</sup>

Parameter <sup>2</sup>	<i>h</i> <sup>2</sup>
Triglycerides	0.43 ± 0.13
Total bile acid	0.21 ± 0.06
Globulin	0.32 ± 0.10
Albumin/Globulin	0.29 ± 0.09
Uric acid	0.34 ± 0.10
VLDL	0.28 ± 0.09

<sup>1</sup>Data are estimates ± SE.<sup>2</sup>VLDL, Very low-density lipoprotein.

$$\text{AFP} = 0.0020 + 0.1612 \times \text{VLDL} \quad (P < 0.0001) \quad (5)$$

### Heritability Estimations of Plasma Biochemical Parameters

After the best multiple linear regression models were selected, the heritabilities of these 6 biochemical indicators (triglycerides, total bile acid, globulin, albumin/globulin, uric acid, and VLDL) that included in the best multiple linear regression models were estimated, to identify if the expression of these plasma parameters was heritable and was available for the selection of lean broilers. The results given in Table 6 demonstrated that the plasma triglycerides (*h*<sup>2</sup> = 0.43) had the highest estimated heritability among all these 6 measured biochemical indicators. The following were uric acid, globulin, albumin/globulin, and VLDL. Heritabilities of these 4 biochemical indicators were moderate (0.34 ≥ *h*<sup>2</sup> ≥ 0.28). Then, total bile acid had the lowest heritability of all 6 parameters with an estimate of 0.21.

## DISCUSSION

In this study, the plasma levels of 19 biochemical indicators were compared in lean and fat birds in the fed state, and the phenotypic correlations between these plasma indicators and the AF traits were estimated. We identified 11 biochemical indicators that differed significantly between the lean and fat birds and were significantly associated with the AF traits. Then we used these 11 plasma indicators to determine the best multiple linear regression models with which to select against AF in chickens.

Concentrations of 11 plasma indicators (triglycerides, total bile acid, total protein, globulin, albumin/globulin, AST, ALT, GGT, uric acid, creatinine, and VLDL) differed significantly between the lean and fat birds (Table 2). In this study, the fat birds had very significantly higher plasma triglycerides concentrations than the lean birds in the fed state (Table 2), which is consistent with previous studies in chickens and ducks. In chickens, Leclercq et al. (1984) demonstrated that fat family birds displayed higher plasma triglycerides than lean family birds in the fed state. In ducks, the

concentrations of plasma triglycerides were significantly higher after feeding in the fat birds than in the lean birds (Farhat and Chavez, 2001).

The plasma uric acid levels were very significantly higher in the fat birds than in the lean birds after feeding (Table 2), and our results are consistent with previous findings in chickens and ducks. In previous studies, a fat line of chickens had higher plasma uric acid levels than a lean line of chickens, and plasma uric acid was also used to select for high or low AF content (Leclercq, 1988). Fat ducks had significantly higher plasma uric acid concentrations than lean ducks after feeding (Farhat and Chavez, 2001).

The plasma VLDL concentration has been shown to be higher in fat birds than in lean birds, whether fed or starved (Leclercq, 1984), and the plasma VLDL concentration in the fed state is significantly positively associated with the AF traits (AFW and AFP) (Li et al., 1999). Our findings are consistent with these reports. In the present study, the plasma VLDL concentrations were very significantly higher in the fat birds than in the lean birds (Table 2), and correlated very significantly positively with both AFW and AFP, with phenotypic correlation coefficients of 0.46 and 0.45, respectively (Table 3). The heritability estimate of plasma VLDL in the current study ( $h^2 = 0.28$ ; Table 6) was similar to findings in our previous study, in which the heritability of plasma VLDL of female birds at 29 wk of age was 0.24 (Li and Yang, 1996). These results suggest that the plasma VLDL concentration in the fed state is a good criterion with which to select for lean broiler chickens with low AF. To our knowledge, few studies have reported the differences in the levels of biochemical parameters in obese and normal or lean individuals in the postprandial or fed state, either in chickens, humans, or other animal species.

We used 3 procedures to select these biochemical indicators and to construct multiple linear regression models for predicting AF. First, the plasma biochemical indicators that differed significantly between the lean and fat birds were selected. Second, we selected from these plasma indicators that correlated significantly with both AF traits (AFW and AFP). Third, we used the plasma biochemical indicators selected in the 2 steps described previously to construct the best multiple linear regression models to use in selecting against AF. The levels of 11 plasma biochemical indicators (triglycerides, total bile acid, total protein, globulin, albumin/globulin, AST, ALT, GGT, uric acid, creatinine, and VLDL) differed significantly between the lean and fat birds (Table 2) in the first step described previously. All 11 plasma biochemical indicators correlated significantly with both AF traits (Table 3), and were therefore selected in the second step described previously. Using these 11 plasma biochemical parameters, we constructed the best multiple linear regression models (Model 2 and Model 3), which included 6 plasma parameters (albumin/globulin, VLDL, triglycerides, globulin, total bile acid, and uric acid). The  $R^2$

values for the best multiple linear regression models for AFW (Model 1) and AFP (Model 2) were both 0.73, which are much higher than the  $R^2$  values for the models based on VLDL alone, which were 0.22 and 0.20, respectively. These results indicate that Model 2 and Model 3 will allow better selection against AF than will VLDL alone. At last, we estimated the heritabilities of these 6 plasma biochemical indicators contained in the best multiple linear regression models. The results showed that the heritabilities of these 6 parameters were moderate ( $0.21 \leq h^2 \leq 0.43$ ; Table 6), indicating these plasma biochemical parameters had potential to be used for genetic selection.

Different conditions (fasting or fed) of blood biochemical indicators are measured for different study purposes. For example, most biochemical parameters are tested in the fasting state in human clinical context (Civilibal et al., 2014; Morita et al., 2014). However, total bile acid and glucose levels are detected in the fed state to study liver diseases and diabetes, respectively (Ferslew et al., 2015; Bibra et al., 2016). Because the components in foods, even the oxygen in water, both have different degrees influence on the concentrations of these biochemical parameters in blood (Armin et al., 2010; Yen et al., 2013; Shin et al., 2016). We sought to verify these biochemical indicators should be detected under which conditions when breeding lean broilers. In our previous study (2015), we investigated the differences in the biochemical parameters in the blood between lean and fat birds in the fasting state, and identified the parameters that could be used as biomarkers for selecting lean broilers (Dong et al., 2015). In the present study, we explored the levels of biochemical indicators in the fed state that could be combined with plasma VLDL to achieve a better selection. We also constructed multiple linear regression models and identified the best models for selecting against AF, which were better than the models based on VLDL alone. In these 2 studies, the blood biochemical parameters were efficiently measured with an automatic biochemical analyzer, an approach that is very convenient and effective and does not require the chickens to be slaughtered. This will accelerate the breeding process and shorten the generation interval in future broiler breeding programs. However, the biochemical indicators selected in these 2 studies should be used under different feeding conditions.

In summary, we used lean and fat broiler chicken lines that had been divergently selected for AF content and compared the levels of 19 plasma biochemical indicators in them in the fed state. We also analyzed the phenotypic correlations of these parameters with AF traits, and identified the best multiple linear regression models for predicting AF. Using 3 procedures, we constructed the best multiple linear regression models (Model 2 for AFW and Model 3 for AFP) based on these biochemical indicators (measured in the fed state) for use in predicting AF in chickens and selecting lean broilers, and these biochemical indicators were proved to be heritable by the estimation of heritability. However, another

selection criterion should be used when the experimental chickens are fasting (Dong et al., 2015).

## ACKNOWLEDGMENTS

The authors would like to thank the members of the poultry breeding group at Northeast Agricultural University for helping in managing the birds and collecting the data, and the members of clinical laboratory of the Fourth Hospital of Harbin Medical University for helping in testing the blood biochemical parameters. This research was supported by the National 863 Project of China (no. 2013AA102501), the China Agriculture Research System (no. CARS-42), the National Natural Science Foundation (no. 31301960), the Heilongjiang Postdoctoral Financial Assistance (no. LBH-TZ0612), and the University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (no. UNPYSCT-2015007).

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