

Long-term divergent selection for residual feed intake in Chinese broiler chickens

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ABSTRACT This study aimed to assess the effect of inbreeding on production traits using a long-term closed-line population recorded for residual feed intake (**RFI**). The study first used data from a previously reported population to determine the appropriate period of divergent selection for RFI. The results showed that RFI had similar moderate heritability estimates (0.28–0.34) during the fast-growing period (7–12 wk), and RFI at 7 to 10 wk had the highest heritability (0.34). Therefore, divergent selection was performed in a Chinese broiler population for RFI at 7 to 10 wk; the total sample size from generations zero (G0) to 13 was 9050. The divergence between the 2 lines increased steadily throughout generations, resulting in G13 with average RFI values of 304.55 in high RFI (**HRFI**) males, –160.31 in low RFI (**LRFI**) males, 296.30 in HRFI females and –157.55 in LRFI females. The feed intake (**FI**) and feed conversion ratio were almost higher in

HRFI broilers than in LRFI broilers, and the magnitude of the difference in FI increased from approximately 4% for both sexes in G1 to approximately 33% in G13. Body weight gain was irregular from G1 to G13 and higher in LRFI broilers than in HRFI broilers after G10. Indeed, the HRFI broilers consumed more food, but they were lighter than LRFI broilers. In G13, LRFI males had heavier slaughter weight, longer cecum length, more white blood cells (**WBC**), red blood cells (**RBC**) and hemoglobin (**HGB**), but triglycerides, lower dressed percentage, percentage of half eviscerated yield, and eviscerated yield than HRFI males. LRFI females had a higher percentage of breast muscle and gizzard yield, longer cecum length, and more WBCs, RBCs and HGB but less abdominal fat and serum total cholesterol than HRFI females. This study was the first to verify that long-term divergent selection for RFI in Chinese broiler chickens is positive and beneficial.

Key words: broiler chickens, residual feed intake, divergent selection, production traits

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INTRODUCTION

Poultry feed is estimated to account for up to 70% of the total cost of poultry production (Willems et al., 2013). Therefore, improving feed efficiency is a primary goal in poultry breeding strategies. Feed efficiency is related to the feed intake (**FI**) and the growth of an animal and has been represented by several traits, such as the feed conversion ratio (**FCR**) and residual feed intake (**RFI**). Among these, RFI was defined in 1963 as the difference between actual and expected feed intake required for animal maintenance and growth (Koch

et al., 1963). Additionally, the FCR was difficult to improve without a direct statistical effect on growth because it was a ratio trait with non-normality (Atchley et al., 1976) and no real mean and variance. However, RFI is not a ratio trait. RFI has evolved over the decades and is widely used in the genetic improvement of feed efficiency in livestock since it has the advantage of reducing FI without sacrificing body weight or body weight gain (**BWG**) and increasing feed efficiency (Kelly et al., 2010; Smith et al., 2011; Le Naou et al., 2012). Furthermore, the results from the divergent selection experiment on RFI in cattle and pigs for more than 10 generations were unique and positive (Kayser et al., 2015; Gilbert et al., 2017; Baldassini et al., 2018).

In poultry, the first divergent selection experiment on RFI was from Rhode Island Red egg-laying chickens (Bordas et al., 1992; Zerjal et al., 2021). After 30 generations of selection for the same body weight and egg production, high RFI (**HRFI**) birds consume 50% more food, but they are leaner than their counterpart low RFI

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(**LRFI**) birds (Sintubin et al., 2014). In slow-growing broilers, the RFI is more closely related to feeding behaviors than FCR is, and selection with improved RFI may result in fewer visits, shorter duration and faster feeding rate; therefore, RFI is a more suitable index to improve feed efficiency in slower-growing broilers (Wen et al., 2018; Yi et al., 2018; Yan et al., 2019; Li et al., 2020). Furthermore, strategies to incorporate the RFI trait into selection schemes in slow-growing broilers may yield more obvious results by comparing with egg-laying chickens and pigs because of the slow-growing broilers have the lowest feed efficiency.

There are more than 100 native chicken breeds in China, and their market share is roughly equal to that of imported commercial lines in China (Liu et al., 2018). The commercial broiler and native chicken industries in China both face the feed efficiency dilemma, which has restricted the further expansion of the poultry industry. Huiyang bearded (**HB**) chickens are a local Chinese breed with a muff and beard phenotype and a high meat quality tailored to Chinese tastes. Meat from HB chickens is popular in South China. To evaluate the potential of RFI for the improvement of feed efficiency in native chickens, the present study first reported growth traits and their correlation with RFI in a previously reported population (Luo et al., 2013, 2015), which may provide evidence for the appropriate period of divergent selection for RFI, and an experiment to examine RFI based on HB populations selected lines that had been developed in the past 12 yr. The main objective of the current study was to compare production traits between low- and high-RFI HB chickens after 14 generations of divergent selection and examine the correlations between these changes and performance, carcass characteristics, and hematological parameters in generation 13.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

All birds used in the current study were cared for and used according to the humane requirements of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. The protocol for the animal trial was approved by the Institutional Animal Care and Use Committee of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Approval No. 2022001), Guangzhou, China.

Animals, Rearing System and Source of Data

- **F₂** population. The **F₂** resource population was developed using the intercross of 2 divergent lines, which were high-quality chicken line A from Guangdong Wiz Agricultural Science & Technology Co. Ltd. (**HQLA**) and HB. HQLA was a closed population that had undergone more than 10 generations of selection for fast growing and maintaining meat

quality. The **F₂** population had 800 individuals from 6 hatches.

- **RFI** lines. The base population consisting of 40 lines was the HB. In 2010, two divergent lines were established from the common foundation stock from 279 male and 390 female candidates. An average selection pressure of approximately 10% was applied across generations on males, and approximately 50% was applied on females. In each generation, 106 to 420 male or female candidates were tested per line, and approximately 20 males or 100 females with the lowest (LRFI line) or highest (HRFI line) RFI were retained to produce the next generation, respectively. All birds were offered ad libitum access to water and maize-soybean diets that met the requirements for broilers (NRC 1994). The ambient temperature was decreased regularly from 30°C at hatch to 25°C at 4 wk and to 22°C at 6 wk. The house was supplied with 24 h of light per day.

Traits Recorded on the F₂ Population

Individual body weight (**BW**) and feed consumption were measured every 2 wk (wk) from 0 to 12 wk and from 6 to 12 wk, respectively. The average daily gain (**ADG**) in each period was calculated as the BW gain in the period divided by the corresponding number of days. The feed conversion ratio (**FCR**) was defined as the ratio of feed intake (**FI**) to BW gain over the period. The RFI was calculated for each individual as the difference between observed and expected feed intake in each period (6–8 wk, 8–10 wk, 10–12 wk, 6–10 wk, 8–12 wk, or 6–12 wk).

Traits Recorded on Generation 0 (G₀)–G₁₃ Birds With LRFI and HRFI Lines

Birds were tagged with wing bands after birth. Before 6 wk of age, each hatch was kept by a group in a large cage. From 6 to 13 wk of age, all chickens were reared in individual cages. Birds and feed weights were measured on d 1 and wk 6, 8, and 10. BW, ADG, FI, and daily feed intake (**ADFI**) were determined for each individual. The feed conversion ratio (**FCR**) was defined as the ratio of FI to BW gain at 6 to 10 wk. The RFI was calculated for each individual as the expected feed intake in 6 to 10 wk and then averaged based on the LRFI or HRFI.

The Statistical Model of RFI

Expected feed intake was derived from multiple linear regression of direct feed intake on the mid-test metabolic body weight (mid-test MBW^{0.75}) and ADG (Van Eerden et al., 2004). The statistical model for the regression analysis was as follows:

$$Y = \beta + \text{gender} + \text{batch} + \text{house} + \text{cage} + aX_1 + bX_2 + e$$

where Y is direct feed intake (g), β is the equation intercept (g), gender, batch, house, and cage are fixed effects, a and b are partial regression coefficients, X_1 is the mid-test metabolic body weight ($MBW^{0.75}$), X_2 is ADG (g/d), and e is the residual (g) (i.e., RFI). Thus,

$$RFI = Y - (\beta + \text{gender} + \text{batch} + \text{house} + \text{cage} + aX_1 + bX_2)$$

Measures of Inbreeding

Inbreeding coefficients (F) were estimated using pedigree data. Pedigree inbreeding (F_{PED}) was calculated for each individual using the following formula (R1, 1976):

$$A_{ii} = \sum_{j=1}^i L_{ij}^2 D_{jj}$$

where A_{ii} is the i^{th} diagonal element of the A matrix (pedigree relationship matrix), which is equal to the inbreeding coefficient of the i^{th} animal plus 1. L is a lower triangular matrix containing the fraction of the genes that animals derive from their ancestors, and D is a diagonal matrix containing the within-family additive genetic variances of animals (Meuwissen The and Z, 1992). The computation for matrix elements L_{ij} and D_{jj} follows the rule of computation of the A matrix (Meuwissen The and Z, 1992). The analysis was conducted using the DMU package (Madsen et al., 2006).

Carcass Composition in G13

In G13, after the birth of the next generation from the chosen selection lines, 115 birds from the low and high RFI groups were randomly chosen to be euthanized by exsanguination, among which 66 (16 males and 40 females) were classified as having a low RFI and 49 (26 males and 36 females) were classified as having a high RFI. The carcass weight (including skin and wings, excluding giblets), deboned breast meat yield, and femoral muscle weight were measured. Deboned breast meat yield and femoral muscle weight were additionally evaluated relative to body weight to obtain information on the proportion of carcass parts. The length of the small intestine extending from the pylorus to the ileocecal junction was measured. In addition, the length of the cecum was measured, and the weights of the liver, gizzard, small intestine, cecum, and abdominal fat were recorded and expressed as a percentage of eviscerated weight according to the <Performance terminology and measurements for poultry> (NY/T 823—2020) as follows.

Abdominal fat (%) = Abdominal fat weight/(Eviscerated weight + Abdominal fat weight) \times 100%

Gizzard (%) = Gizzard weight/(Eviscerated weight + Gizzard weight) \times 100%

Liver (%) = Liver weight/(Eviscerated weight + Liver weight) \times 100%

Small intestine (%) = Small intestine weight/(Eviscerated weight + Small intestine weight) \times 100%

Cecum (%) = Cecum weight/(Eviscerated weight + Cecum weight) \times 100%

Assay of Hematological Parameters in G13

Whole blood (K2EDTA tube) was collected from the wing vein before slaughter for routine hematological parameters using an automatic hematology analyzer (Sysmex, Japan), including white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), and platelets (PLTs). Serum was isolated from non-anticoagulant blood via centrifugation at 3,000 rpm/min for 10 min at 4°C for serum biochemical indicator determination. Serum total cholesterol (TC) and triglyceride (TG) levels were analyzed using a Fully Automatic Biochemical Analyzer (Hitachi, Japan).

Statistical Analyses

In the present study, variance components were estimated using a univariate model. The model used in the analyses was as follows:

$$y = Xb + Za + e$$

where y is the vector of RFI, carcass and meat quality traits; b is the vector of fixed effects, including sex (2 levels) and hatch (6 levels); a is the vector of animal additive genetic effects; e is the vector of random residuals; and X and Z are the corresponding incidence matrices of b and a . Fixed effects are not considered in analyzing RFI because the fixed effects are already corrected when calculating RFI. The distributions for the random effects in the univariate model were assumed as follows:

$$a \sim N(0, G) \text{ and } e \sim N(0, R).$$

It is defined that

$$G = A\sigma_a^2, R = I\sigma_e^2,$$

where A is the matrix of the genetic relationship between individuals in the pedigree of all animals; σ_a^2 is animal additive genetic variance; I is an identity matrix; and σ_e^2 is the residual variance.

Variance and covariance components are estimated by applying the average information restricted maximum likelihood algorithm (Gilmour et al., 1995) and using the DMU package (Madsen et al., 2006). The standard errors of estimates of (co) variances were obtained from an approximation of the Hessian matrix, and standard errors of estimates of heritability and genetic correlation were calculated using an expansion of the Taylor series.

RESULTS

RFI in Various Periods in the F_2 Population

The estimated phenotypic and genetic parameters for RFI in various periods are shown in Table 1. Heritabilities of RFI in different periods ranged from 0.28 to 0.34. Genetic correlations between different periods ranged

Table 1. Estimates of heritability (the diagonal) for RFI in various periods, along with estimates of genetic (below the diagonal) and phenotypic (above the diagonal) correlations between every two RFI of different periods, and their standard errors (in parentheses).

Traits ¹	RFI1	RFI2	RFI3	RFI4	RFI5	RFI6
RFI1	0.33 (0.10)**	0.52	0.32	0.86	0.46	0.71
RFI2	0.79 (0.11)**	0.29 (0.09)**	0.62	0.86	0.87	0.87
RFI3	0.38 (0.22)*	0.86 (0.08)**	0.28 (0.09)**	0.51	0.93	0.83
RFI4	0.95 (0.03)**	0.93 (0.04)**	0.64 (0.15)**	0.34 (0.10)**	0.74	0.91
RFI5	0.58 (0.17)**	0.96 (0.03)**	0.97 (0.02)**	0.79 (0.09)**	0.32 (0.10)**	0.94
RFI6	0.79 (0.09)**	0.99 (0.01)**	0.86 (0.07)**	0.94 (0.03)**	0.95 (0.02)**	0.34 (0.10)**

¹Traits: RFI1, RFI2, RFI3, RFI4, RFI5, and RFI6 represented RFI in 7–8 wk, 9–10 wk, 11–12 wk, 7–10 wk, 9–12 wk, and 7–12 wk, respectively.

*Significantly different from zero at $P < 0.05$.

**Significantly different from zero at $P < 0.01$.

from 0.38 to 0.99. RFI4 and RFI6 had the highest heritability (0.34) and the highest phenotypic and genetic correlations with the other RFIs. Therefore, RFI4 could be considered an indicator trait of RFI during the growth period in the practical breeding of Chinese native chickens. Only the correlations between RFI4 and the other economically important traits are demonstrated below.

Growth Traits and Their Correlation With RFI in the F₂ Population

The heritability estimate of RFI was greater than that of growth traits in the same period (Table 2). The heritability estimates of BW decreased with increasing age. ADG and FCR both had low heritability estimates (0.08 and 0.03, respectively). Phenotypic correlations of RFI with ADG, FCR, and BW were very weak, but phenotypic correlation between RFI and FI was moderate (0.49). On the other hand, RFI had a moderate negative genetic correlation with BW and ADG but a moderate positive genetic correlation with FI (0.40) and FCR (0.58).

Selection Experiment for RFI

In Generation 0 (G0), the 279 males that were retained displayed a large variation in feed efficiency, with RFI values ranging from -78.5 to 108.0 (Figure 1A), and the 390 females ranged from -92.7 to 62.4 (Figure 1G). We selected 20 and 20 males with the most extreme RFI values to form ‘high’ and ‘low’ RFI

groups (Figure 1B), while 78 and 79 females with the most extreme RFI values formed ‘high’ and ‘low’ RFI groups (Figure 1H). In males, the HRFI and LRFI groups indeed showed a significant difference in RFI ($P < 0.01$), with average RFI values of 77.5 and -53.7 , respectively (Figure 1B). As expected, there was no difference in the tenth week BW ($P = 0.193$; Figure 1C) or ADG at 7 to 10 wk ($P = 0.334$) (Figure 1D), but significant differences in ADFI ($P < 0.01$; Figure 1E) and FCR ($P < 0.01$; Figure 1F) were observed between the HRFI and LRFI groups. Although the number of chickens in females we selected was eight times that in males, the RFI, in the tenth week BW, ADG, ADFI, and FCR in females were consistent with the trend in males (Figures 1H–1L).

The numbers of male and female birds recorded by line and generation are reported in Table 3. The total numbers of male and female candidates for selection in generations 0 to 13 were 4,615 and 4,435, respectively.

Inbreeding From G0 to G13

The inbreeding coefficient is commonly used to describe the proportion of autozygosity in the genome of an individual, and it can be estimated from the pedigree. The inbreeding levels estimated from the pedigree per generation are shown in Table 4 and Figure S1. In G0 and G1, the inbreeding level was zero. As expected, the inbreeding level gradually increased over the course of selection for the 2 lines. In G13, the average inbreeding level was 0.16 in the LRFI line and 0.26 in the HRFI line, at a higher rate in the HRFI line.

Genetics of Residual Feed Intake From G0 to G13

Estimates of genetic (r_a) and phenotypic (r_p) correlations between RFI and growth traits from G0 to G13 are shown in Table 5. RFI had a low negative genetic correlation as well as phenotypic correlations with BWG but a moderate positive correlation with FCR.

RFI and Growth Traits From G0 to G13

Figure S2 demonstrates the changes in individual RFI values at 7 to 10 wk for males and females from G1 to

Table 2. Estimates of heritability (h^2) for growth traits, estimates of genetic (r_a) and phenotypic (r_p) correlations between RFI4 and growth traits, and their standard errors (SE).

Traits ¹	Means (SE)	h^2 (SE)	r_a (SE)	r_p
BW6, g	803 (4.60)	0.24 (0.09)**	-0.31 (0.27)	-0.01
BW10, g	1663 (9.88)	0.14 (0.07)*	-0.36 (0.29)	0.08
FI4, g	2700 (14.6)	0.09 (0.06)	0.40 (0.20)*	0.49
ADG4, g/d	123 (0.90)	0.08 (0.05)	-0.34 (0.34)	0.13
FCR4	3.22 (0.03)	0.03 (0.03)	0.58 (0.40)	0.08

¹Traits: BW6 and BW10 represented body weight at 6 wk and 10 wk, respectively. FI4 represented the feed intake in 6–10 wk. ADG4 represented the average daily gain in 6–10 wk. FCR4 represented the feed conversion ratio in 6–10 wk.

*Significantly different from zero at $P < 0.05$.

**Significantly different from zero at $P < 0.01$.

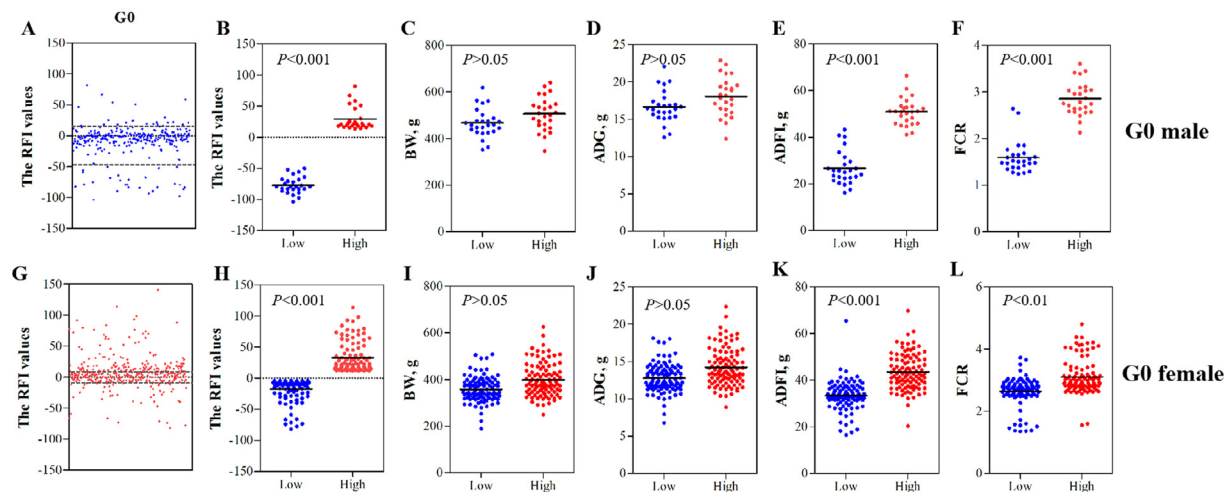


Figure 1. Production performance of the chickens with extremely high and low RFI values. The Huiyang bearded chickens were individually housed from wk 7 to 10. Residual feed intake (RFI) was calculated individually for 279 male and 390 female apparently healthy chickens, respectively. In male, 20 and 20 chickens with extremely high and low RFI values were selected (as shown by dashed lines), respectively (A). In female, 78 and 79 chickens were selected (G). RFI (B and H), the tenth week body weight (BW) (C and I), average daily gain (ADG) (D and J), average daily feed intake (ADFI) (E and K), and feed conversion ratio (FCR) (F and L) were calculated for the HRFI and LRFI groups of selected chickens in male or female, respectively. Statistical significance was determined using Student's *t* test.

Table 3. Distribution by line and generation of the birds recorded for residual feed intake (RFI).

line ¹	Sex	Male			Female		
		F	L	H	F	L	H
Generation	G0	279	-	-	390	-	-
	G1	-	203	185	-	189	184
	G2	-	199	201	-	184	218
	G3	-	229	160	-	251	157
	G4	-	161	202	-	156	219
	G5	-	173	194	-	218	191
	G6	-	142	161	-	57	49
	G7	-	126	159	-	65	70
	G8	-	181	211	-	172	201
	G9	-	201	219	-	192	188
	G10	-	171	200	-	183	196
	G11	-	135	142	-	149	128
	G12	-	93	128	-	89	144
	G13	-	73	87	-	85	110

¹F, foundation population; L, low-RFI line; and H, high-RFI line.

G13. Accordingly, the average RFI values were altered considerably over the course of selection (Figure 2). The responses to selection were significant since G1 on the

average RFI values. Divergence of the selected lines, although gradual, resulted in an extremely significant difference in LRFI compared to HRFI males and females for the selected RFI trait in G13 ($P < 0.01$). In males of G13, the average RFI values were -160.31 and 304.55 between the LRFI and HRFI lines, respectively, and in females, the average RFI values were -157.55 and 296.30 between them, respectively (Figure 2A). A similar situation was observed for FI and FCR (Figures 2B and 2D). Interestingly, the average values of BWG at 7 to 10 wk were first higher in HRFI males and females than in LRFI males and females; then, this trend became slow and appeared to reverse in G10 (Figure 2C). In other words, the average BWG values of the HRFI line in males and females reached an ascending plateau between G2 and G7 and then decreased both; however, the LRFI line in both males and females reached a descending plateau between G2 and G4, then an ascending plateau between G6 and G8, and another ascending plateau after G9.

Table 4. Estimated minimum, maximum and mean of pedigree-based inbreeding coefficient.

Item	LRFI				HRFI			
	Minimum	Maximum	Mean	SD	Minimum	Maximum	Mean	SD
G0	0	0	0	-	0	0	0	-
G1	0	0	0	-	0	0	0	-
G2	0	0.2500	0.0075	0.0371	0	0.2500	0.0206	0.0640
G3	0	0.2500	0.0302	0.0505	0	0.3750	0.0394	0.0725
G4	0	0.3125	0.0648	0.0481	0	0.1875	0.0589	0.0494
G5	0	0.2891	0.0808	0.0534	0.0020	0.3125	0.0775	0.0645
G6	0	0.2539	0.0945	0.0468	0	0.3867	0.1155	0.0795
G7	0	0.3345	0.1087	0.0504	0	0.3538	0.1248	0.0585
G8	0.0809	0.3524	0.1334	0.0535	0	0.4056	0.1338	0.0596
G9	0	0.3274	0.1342	0.0382	0	0.3919	0.1301	0.0597
G10	0	0.3325	0.1423	0.0398	0	0.3750	0.1579	0.0637
G11	0	0.2848	0.1572	0.0430	0.1368	0.3421	0.2117	0.0540
G12	0	0.3637	0.1666	0.0582	0	0.3999	0.2414	0.0739
G13	0	0.3626	0.1567	0.0871	0	0.4518	0.2605	0.0620

Abbreviation: SD, Standard Deviation.

Table 5. Estimates of genetic (r_a) and phenotypic (r_p) correlations between RFI and growth traits, and their standard errors (SE) from G0 to G13.

Traits ¹	Means (SE)	r_a (SE)	r_p
BWG, g	324.80 (0.06)	-0.11 (0.02)	-0.04 (0.02)
FCR	10.86 (0.04)	0.56 (0.01)	0.57 (0.01)

¹Traits: BWG represented body weight gain in 7–10 wk. FCR represented the feed conversion ratio in 7–10 wk.

Performance and Carcass Characteristics in the 13th Generation

The growth performance and carcass characteristics of broiler chickens from both lines are shown in Table 6. The offspring for both sexes from the LRFI line and HRFI line in the 12th generation showed that there was no difference in the 1-d BW and 6-wk BW between these 2 lines. However, the 10-wk BW in LRFI male offspring was significantly higher than that in HRFI male offspring ($P = 0.0176$). There was no significant difference among females ($P = 0.9005$).

After RFI calculation, birds were divided into the LRFI group and HRFI group in the 13th generation. At 7 to 10 wk, the ADFI and FCR for both sexes were significantly higher in HRFI chickens than in LRFI chickens ($P < 0.01$), whereas the ADG was significantly lower in HRFI chickens than in LRFI chickens ($P = 0.0044$).

After the birth of the next generation from both lines in G13, chickens were randomly chosen to be slaughtered. In males, slaughter weight in HRFI chickens was significantly lower than that in LRFI chickens ($P = 0.0042$), whereas the dressed percentage, percentage of half eviscerated yield, and percentage of eviscerated yield were all significantly higher in HRFI chickens than in LRFI chickens ($P < 0.05$). In females, no

difference was observed in these trials. The percentage of breast muscle, liver yield, small intestine yield, small intestine length (cm), and cecum yield were not different between the 2 lines in both sexes. In females, the percentage of leg muscle and gizzard yield were both higher in the LRFI line than in the HRFI line ($P < 0.01$), and compared to the HRFI line, the LRFI line had a lower percentage of abdominal fat ($P < 0.01$). In males, no difference was observed in these 3 trials. Furthermore, the cecum length for both sexes was significantly longer in LRFI chickens than in HRFI chickens ($P < 0.01$).

Hematological Parameter Response to Divergently Selection for the 13th Generation

The data of routine blood parameters showed that the levels of WBC, RBC, and HBC for both sexes in the LRFI group were significantly higher than those in the HRFI group, and there were no significant differences in the level of PLT between the group (Table 7). In addition, we also assayed the serum lipid biochemical indicators TG and TC (Table 7). The results showed that the concentration of TG in LRFI male chickens was significantly lower than that in HRFI male chickens ($P = 0.0006$), whereas there was no obvious difference in females ($P = 0.1517$). In males, there were no significant differences in the concentration of TC, while in females, the LRFI chickens had a significantly lower concentration of TC than the HRFI chickens ($P < 0.01$).

DISCUSSION

A previous study showed that miR-1596 can be a candidate gene related to RFI by using the F₂ population (Luo et al., 2015), indicating that the F₂ population

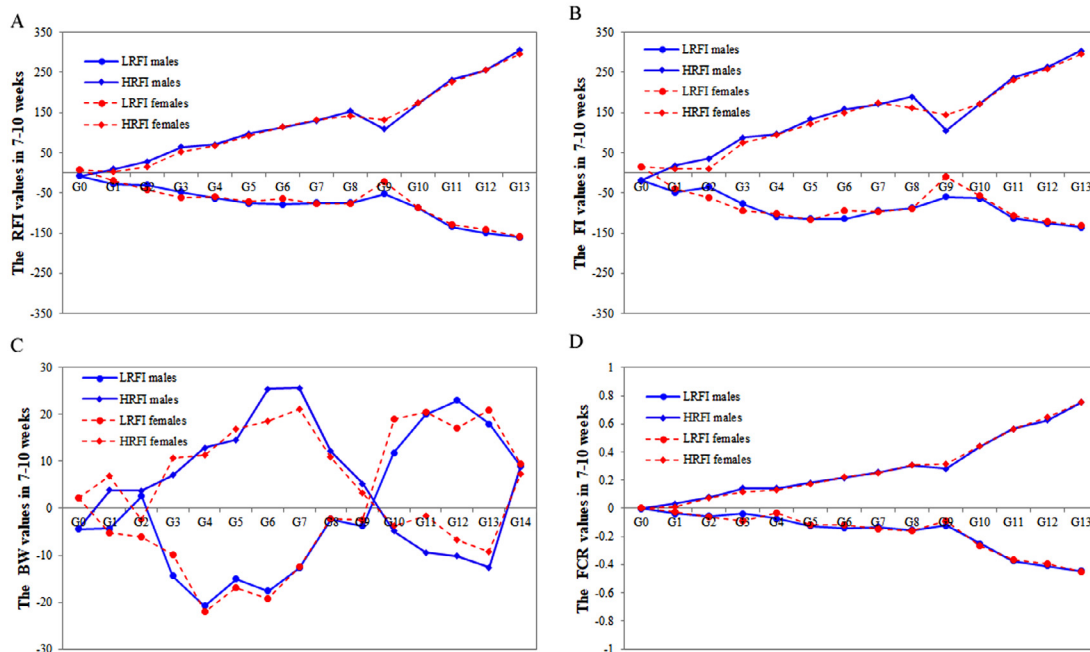


Figure 2. The average values of RFI, FI, BWG and FCR in wk 7–10 for lines of Huiyang bearded chickens selected for high or low RFI from 0 to 13 generations. The average values of RFI (A), FI (B), BWG (C), and FCR (D) from LRFI and HRFI groups according to male and female.

Table 6. Growth performance, carcass characteristics in Huiyang bearded chicken divergently selected for 13 generations on low RFI and on high RFI.

Item	Male ¹				Female ¹			
	LRFI	HRFI	SEM ²	P-value	LRFI	HRFI	SEM ²	P-value
Number of birds ³	72	73	-	-	82	101	-	-
One day BW (g) ³	25.19	25.01	0.1767	0.5974	24.78	24.78	0.1487	0.9881
Sixth week BW (g) ³	380.19	370.53	4.4428	0.2785	303.30	294.15	3.8841	0.2421
Tenth week BW (g) ³	871.67 ^a	840.06 ^b	6.6911	0.0176	649.80	647.62	8.6774	0.9005
RFI values ⁴	-160.31 ^B	304.55 ^A	18.5140	<0.0001	-157.55 ^B	296.30 ^A	16.3968	<0.0001
ADG (g) ⁴	17.74 ^A	16.86 ^B	0.1544	0.0044	13.50 ^A	12.74 ^B	0.1306	0.0035
ADFI (g) ⁴	50.07 ^B	66.16 ^A	0.7515	<0.0001	40.77 ^B	54.12 ^A	0.6415	<0.0001
FCR ⁴	2.83 ^B	3.96 ^A	0.0509	<0.0001	3.03 ^B	4.43 ^A	0.1158	<0.0001
Number of slaughter birds ⁴	16	26	-	-	40	36	-	-
Slaughter weight (g)	2,081.08 ^A	1,826.95 ^B	44.6156	0.0042	1473.25	1526.23	28.0861	0.8301
Dressed percentage (%)	90.17 ^b	91.40 ^a	0.2664	0.0274	81.52	83.83	0.6917	0.0958
Percentage of half eviscerated yield (%)	83.33 ^b	84.82 ^a	0.2730	0.0103	71.46	72.84	0.5878	0.2416
Percentage of eviscerated yield (%)	80.59 ^b	81.85 ^a	0.2753	0.0286	66.38	67.18	0.1625	0.4481
Percentage of breast muscle (%)	12.70	12.06	0.2207	0.1677	15.10 ^A	14.12 ^B	0.1625	0.0020
Percentage of leg muscle (%)	26.65	25.84	0.3501	0.2713	22.33	21.21	0.3182	0.0787
Percentage of abdominal fat (%)	0	0	-	-	2.72 ^B	4.22 ^A	0.2070	0.0002
Gizzard (%)	0.82	0.71	0.2765	0.1159	1.36 ^A	1.08 ^B	0.0378	0.0001
Liver (%)	1.62	1.86	0.0633	0.0650	1.84	1.78	0.0417	0.4998
Small intestine (%)	2.04	2.82	0.4053	0.3580	4.27	4.07	0.0962	0.2981
Small intestine length (cm)	111.41	109.02	2.8035	0.6874	101.94	104.58	1.1474	0.2511
Cecum (%)	0.83	1.02	0.1493	0.5473	1.14	1.05	0.0299	0.1597
Cecum length (cm)	19.52 ^A	17.01 ^B	0.3880	0.0009	17.41 ^A	15.87 ^B	0.2236	0.0004

¹Male and female were analyzed independently.

²Standard Error of Mean.

³The offspring from LRFI line or HRFI line in 12th generation.

⁴After calculating the RFI values in weeks seven to ten, birds were divided into LRFI group and HRFI group in 13th generation.

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

^{A,B}Within a row, means without a common superscript differ ($P < 0.01$).

could be used to evaluate the potential of RFI for the improvement of feed efficiency in Chinese native chickens. In addition, the HB chicken, which was one of the 2 intercross populations used to build the F₂ population, came from the same base population as the HB RFI lines. Therefore, the data of the F₂ population were used to estimate the genetic parameters of RFI and other traits in the present study. The results showed that RFI had similar moderate heritability estimates (0.28–0.34) during the fast-growing period (42 d–84 d), and genetic correlations among RFI in different periods were very high (0.38–0.99; Table 1). This result coincided with the estimated range of heritability (0.2–0.6) for RFI in previous studies of laying hens and broilers (Luiting and Urff, 1991; Aggrey et al., 2010). The estimates of heritabilities for RFI in this study were lower than those reported by Aggrey et al. (2010), probably because the

stages (42 d–84 d) of measuring RFI in this study were later than theirs (28 d–42 d). In the present study, genetic correlations of RFI with FCR were moderately or highly positive, and genetic correlations of RFI with BW and ADG were moderately negative at d 42 to 70, notwithstanding very low phenotypic correlations. These results suggested that selection for chickens with low RFI at d 42 to 70 could improve not only feed efficiency but also growth rate. Some previous studies have supported this hypothesis (Kennedy et al., 1993; Arthur et al., 2001). Romero et al. (2009) reported that offspring of low RFI broiler breeders had greater BW at 38 d than broiler breeders with average or high RFI. Chen et al. (2021) reported that RFI had negative genetic correlations with BWG in Chinese native chickens, whereas it had a positive genetic correlation with BW at 4 wk and BW at 7 wk (Romero et al., 2009). Accordingly, our

Table 7. The routine blood parameters and serum biochemical parameters between LRFI and HRFI lines in the 13th generation.

Item	Male				Female			
	LRFI	HRFI	SEM ¹	P-value	LRFI	HRFI	SEM ¹	P-value
Number of slaughter birds	16	26	-	-	40	36	-	-
WBC (10 ⁹ /L)	23.13 ^a	16.95 ^b	1.3730	0.0370	11.68 ^a	6.50 ^b	1.1719	0.0243
RBC (10 ¹² /L)	1.08 ^a	0.92 ^b	0.0282	0.0122	0.83 ^A	0.53 ^B	0.0538	0.0028
PLT (10 ⁹ /L)	5.00	5.00	0.4082	1.0000	5.56	8.56	0.7795	0.0660
HGB (g/L)	72.40 ^a	62.40 ^b	1.9964	0.0221	53.11 ^A	34.67 ^B	3.1912	0.0020
TG (mmol/L)	0.89 ^B	2.65 ^A	0.2673	0.0006	18.49	22.41	1.3637	0.1517
TC (mmol/L)	3.76	3.45	0.1328	0.2516	6.01 ^B	22.14 ^A	1.4205	<0.0001

Abbreviations: HGB, hemoglobin; PLT, platelet; RBC, red blood cells (RBC); TC, serum total cholesterol; TG, triglycerides; WBC, White blood cells (WBC).

¹Standard Error of Mean.

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

^{A,B}Within a row, means without a common superscript differ ($P < 0.01$).

next establishment of experimental lines showed that BWG was first higher in HRFI than in LRFI lines, which were divergently selected for high or low RFI, and the trend increased from generation to generation before decreasing in G10 (Figure 1). These findings confirmed that RFI was phenotypically independent of BW, but genetically, it was not independent of production.

The establishment of experimental lines is a common strategy to evaluate the direct and correlated responses to a criterion for selection and to study the impact of the selection on animal physiology (Bordas et al., 1992).

Furthermore, RFI at 7 to 10 wk had the highest heritability (0.34) in the current study; therefore, it could be considered an indicator trait of RFI during the growth periods in the practical breeding of Chinese native chickens. Therefore, divergent selection was undertaken in a Huiyang bearded population for RFI at 7 to 10 wk of this study, measured in males and females, using mass selection.

In the present report, we compared the RFI, FI, BWG, and FCR at 7 to 10 wk from G0 to G13 between 2 HB chicken lines divergently selected for high or low

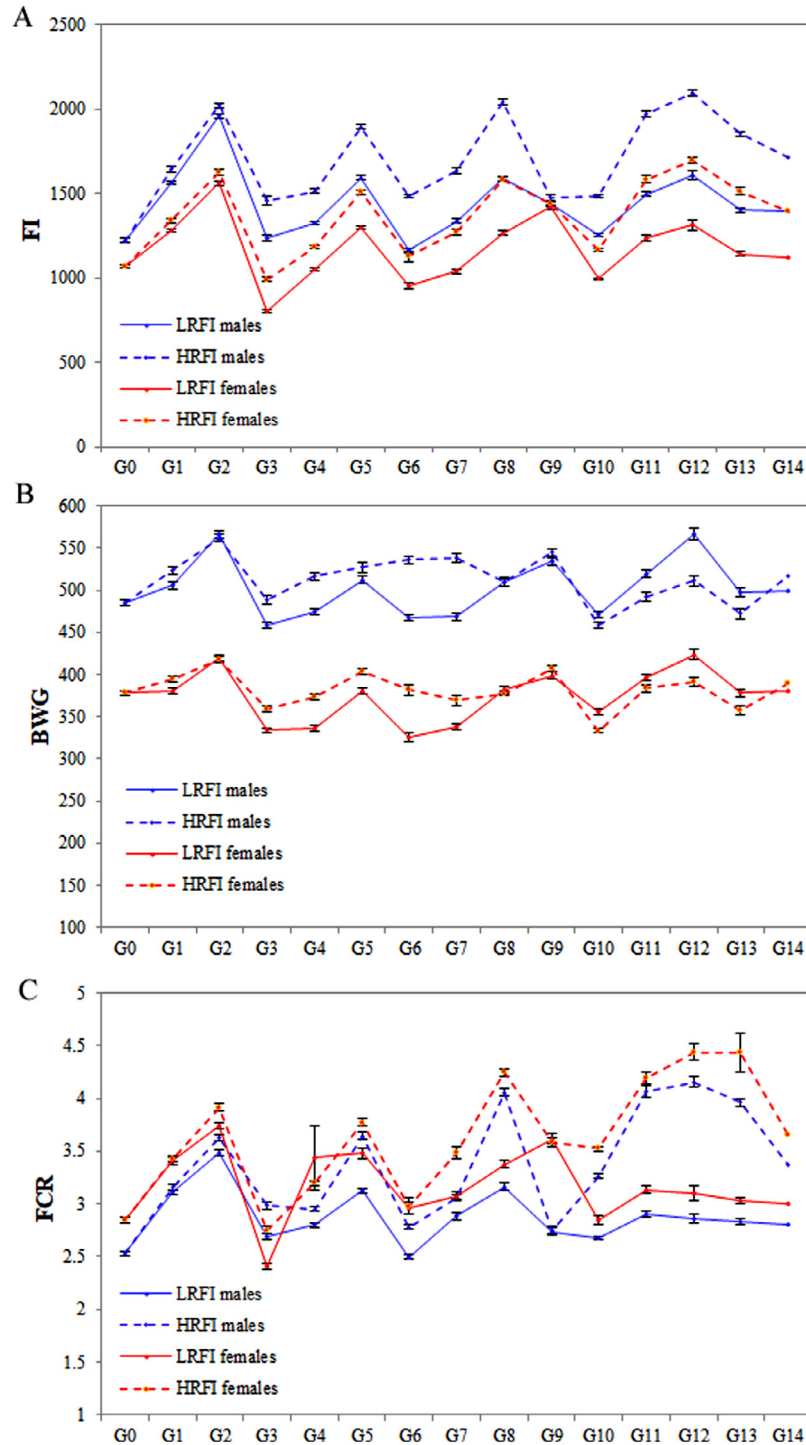


Figure 3. Mean of FI (A), BWG (B) and FCR (C) in 7–10 wk for lines of Huiyang bearded chickens selected for high or low RFI from 0 to 13 generations.

RFI. The average RFI, FI, and FCR values were higher in HRFI males than in LRFI males and females, and this difference increased from one generation to the next, except in G9 (Figures 2B and 2C). The correlated response to selection became significant very early for RFI (G1), FI (G3), and FCR (G3), although inconsistent fluctuations were found from generation to generation (Figure 3). The phenomenon of an irregular response (“waves of response”) also occurred in White Plymouth Rock chickens that underwent long-term (38 generations) divergent selection for 8-wk body weight (Dunnington and Siegel, 1996). There are 2 possible explanations for this phenomenon. 1) After so many generations of intense selection, genotypes were more sensitive to microenvironmental factors that facilitated irregular responses. Alternatively, 2) spontaneous mutations may have occurred periodically. Furthermore, there are more opportunities to observe the response in long-term selection experiments. In the final selection in G13 of our study, divergence between lines was observed with significantly higher ADFI and FCR in HRFI broilers in both sexes as well as significantly lower ADG (Table 6). Interestingly, there was no difference observed in the 1-d BW and 6-wk BW of each generation (data not shown). That is, after 13 generations of selection for the same body weight, the HRFI broilers consumed 33% more food, but they were lighter than their counterpart LRFI broilers (Table 6), which was partly consistent with previous results on laying hen lines divergently selected for high or low RFI (Bordas et al., 1992). In contrast, the LRFI broilers in G11 (3.25 in males and 3.41 in females) had significantly ($P < 0.05$) lower FCRs at 9 to 12 wk than those in the unselected HB chickens (4.14 in males and 4.23 in females, data not shown). These clear conformational differences give LRFI broilers high food efficiency. Other selection experiments on residual food intake also appear to be successful in improving food efficiency (Bordas et al., 1992; El-Kazzi et al., 1995; Zerjal et al., 2021). These results implied that the RFI could be considered for incorporation into selection schemes for improving feed efficiency in slow-growing broilers.

The percentage of abdominal fat in the present study was significantly higher ($P < 0.01$) in HRFI females (4.22%) than in LRFI females in G13 (2.72%) (Table 6), as was the weight of abdominal fat (data not shown). This is partly consistent with some previous reports that the weight and percentage of abdominal fat pads were lower in slow-growing birds by selecting for lower residual feed intake (Wen et al., 2018), and the percentage of abdominal fat was significantly reduced in low-RFI broilers according to the broiler lines divergent for high or low abdominal fat content for 19 generations (Chen et al., 2021). Similarly, many studies reported that a select line (LRFI) resulted in a leaner pig, including greater fat-free lean, greater loin depth, and less body fat content (Smith et al., 2011; Le Naou et al., 2012; Louveau et al., 2016). However, preliminary observations in females at generation 7 of the RFI selection experiment on laying hen lines indicated that LRFI hens

had more abdominal adipose tissue than HRFI females (El-Kazzi et al., 1995), probably because we did not use the same statistical model of RFI. The HRFI females had a significantly higher percentage of abdominal fat and a significantly lower ADG ($P = 0.0035$; 12.74 g) than the LRFI females (13.50 g). The explanation was provided by Mikulski et al. (2011) and Quentin et al. (2003), who stated that slow-growing birds provided with dietary energy and protein in excess of their nutritional requirements resulted in more abdominal fat (Quentin et al., 2003; Mikulski et al., 2011). Overall, this study provided a suitable population to study the RFI and examine the correlations of these changes after long-term divergent selection with growth, carcass composition, and meat quality traits.

The present study showed that RFI had similar moderate heritability estimates in Chinese broiler chickens. A selection experiment was conducted to evaluate the effects of selection for RFI in Chinese native broiler chickens over 13 generations, including both the selected trait and unselected characteristics. The direct response in RFI was found to be significant in both sexes; furthermore, compared with high RFI, low RFI was associated with lower feed intake, similar growth rate, and lower abdominal fat. Selection for LRFI in growing chickens as a measure of net feed efficiency is feasible with limited impacts on other production traits and no marked reduction in the chicken ability to face challenges. Our findings confirmed that RFI could be used in the genetic improvement of feed efficiency in broilers.

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DISCLOSURES

All authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

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