Genetic and microbiome analysis of feed efficiency in laying hens

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ABSTRACT Improving feed efficiency is an important target for poultry breeding. Feed efficiency is affected by host genetics and the gut microbiota, but many of the mechanisms remain elusive in laying hens, especially in the late laying period. In this study, we measured feed intake, body weight, and egg mass of 714 hens from a pedigreed line from 69 to 72 wk of age and calculated the residual feed intake (**RFI**) and feed conversion ratio (**FCR**). In addition, fecal samples were also collected for 16S ribosomal RNA gene sequencing (V4 region). Genetic analysis was then conducted in DMU packages by using AI-REML with animal model. Moderate heritability estimates for FCR ($h^2 = 0.31$) and RFI ($h^2 = 0.52$) were observed, suggesting that proper selection programs can directly improve feed efficiency. Genetically, RFI was less correlated with body weight and egg mass than that of FCR. The phenotypic variance explained by gut microbial variance is defined as the microbiability ($\mathbf{m^2}$). The microbiability estimates for FCR ($m^2 = 0.03$) and RFI ($m^2 = 0.16$) suggested the gut microbiota was also involved in the regulation of feed efficiency. In addition, our results showed that the effect of host genetics on fecal microbiota was minor in three aspects: 1) microbial diversity indexes had low heritability estimates, and genera with heritability estimates more than 0.1 accounted for only 1.07% of the tested fecal microbiota; 2) the genetic relationship correlations between host genetics and different microbial distance were very weak, ranging from −0.0057 to −0.0003; 3) the microbial distance between different kinships showed no significant difference. Since the RFI has the highest microbiability, we further screened out three genera, including *Anaerosporobacter, Candidatus Stoquefichus*, and *Fournierella,* which were negatively correlated with RFI and played positive roles in improving the feed efficiency. These findings contribute to a great understanding of the genetic background and microbial influences on feed efficiency.

Key words: feed efficiency, heritability, microbiability, layer chicken, fecal microbiota

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INTRODUCTION

Feed costs account for 60 to 70% of the total production costs in poultry industry. Improving feed efficiency is one of the important targets in poultry breeding and contributes to reducing feed costs (Yang et al., 2020). The feed conversion ratio (**FCR**) and residual feed intake (**RFI**) are pivotal indicators routinely used to evaluate feed efficiency. The most commonly used measure of feed efficiency is FCR, which is defined as the ratio of feed intake (**FI**) to egg mass in layers (Yuan et al., 2015a). As a sensitive and accurate indicator of feed efficiency, RFI was first proposed by Koch et al. (Koch et al., 1963) and is defined as the feed intake above or below what is predicted for production and maintenance.

The moderate heritability estimated previously (van Kaam et al., 1999; Yuan et al., 2015b; Sell-Kubiak et al., 2017) suggested that host genetics substantially affect feed efficiency, which can be directly improved by proper selection programs. Moreover, considerable research has focused on the molecular regulation mechanisms of feed efficiency and the identification of reliable molecular markers for feed efficiency in chickens (Yuan et al., 2015b).

The gastrointestinal tract (**GIT**) is densely populated with microorganisms (Pan and Yu, 2014). The resident intestinal microbiota, specifically its diversity, composition, and function, is likely to influence the feed efficiency of chickens (Singh et al., 2014; Stanley et al., 2016; Yan et al., 2017). Elucidating the host effect on

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the gut microbiome is essential to help design strategies to modulate its composition to improve production (Siegerstetter et al., 2017; Yan et al., 2017) and health (Fouad and El-Senousey, 2014; Wen et al., 2019); however, the specific contribution of host genetics remains elusive and debated in various studies. Zoetendal et al. (2001) found that human monozygotic twins possess a more similar microbiome than marital partners or unrelated individuals. Several heritable bacterial taxa were identified in a cohort of 416 twin pairs, indicating that host genetics influences the composition of the human gut microbiome (Goodrich et al., 2014). Similar results were found in a larger population of 1,126 twin pairs (Goodrich et al., 2016). Combined with whole-genome analysis, researchers have also found associations between host single nucleotide polymorphisms (**SNP**s) and individual bacterial taxa or pathways (Blekhman et al., 2015; Bonder et al., 2016a; Li et al., 2019). In contrast, the gut microbiome and host genetics are largely independent in humans with several distinct ancestral origins (Rothschild et al., 2018) and similar results were found in the fat deposition (Wen et al., 2019) and feed efficiency (Wen et al., 2021) of broiler chickens, suggesting a complex relationship between host phenotypes and the gut microbiome. Therefore, the effect of the interplay between host genetics and microbiota on phenotypes (such as feed efficiency) needs to be explored in different species.

As a predominantly domesticated animal worldwide, chicken is not only a superior protein source but also a preferable experimental animal model, which can be easily handled to obtain numerous full- and half-sib individuals with pedigree information and samples. With the improvement of laying persistency and stability, extending the laying period is an important goal in breeding companies. A decline in feed efficiency has been found in the later laying periods of chickens (Yuan et al., 2015a). To our knowledge, few studies have focused on the feed efficiency of laying hens, especially in the late laying period, resulting in the lack of an accurate and reliable theoretical foundation for the selection of target traits in breeding programs to improve feed efficiency. The purpose of our study was to 1) estimate genetic parameters for feed efficiency in the late laying period and investigate the contribution of host genetics and fecal microbiota to feed efficiency, 2) evaluate the contribution of host genetics to variation in microbial composition, and 3) further identify microorganisms considerably associated with feed efficiency. The findings would help better improving feed efficiency in chickens by favoring more efficient microbiota and selective breeding.

MATERIALS AND METHODS

Animals and Samples Collection

The complete procedure was performed according to the regulations and guidelines established by the Animal Care and Use Committee of the China Agricultural University.

A total of 714 hens from a pedigreed line of Rhode Island Red were used from Beijing Huadu Yukou Poultry Breeding Co., Ltd., China. Birds were generated from two hatches and housed in individual cages with free access to feed (Table S1) and water. We then collected fecal samples owing to its convenience and non-invasiveness (Yan et al., 2019). Since the adult chickens have fully developed microbiota and the microbial communities are more stable (Videnska et al., 2014; Ngunjiri et al., 2019), fecal samples were collected at 60 wk of age once the excreta were discharged and placed in sterile plastic bags on dry ice using forceps. All samples were stored at −80°C immediately after sample collection.

Calculation of Feed Efficiency

Feed consumption, body weight, and egg mass were measured from 69 to 72 wk of age. The body weight of each bird was measured using an electronic scale at the beginning and end of the feeding trial. Feed intake was calculated weekly and the egg weight and egg number were recorded every day. An individual metal feed trough was used to provide mash feed for each hen. Feed was added daily by hand after weighing the troughs. The remaining feed weight was recorded seven days later, and the individual feed intake in this interval was calculated. This process was repeated for 28 consecutive days. The sum of feed intake at each interval and the daily feed intake (**DFI**) of each hen was calculated. The daily egg mass (**DEM**) was calculated as the product of the average egg weight and the total egg number over the test days. The metabolic body weight (**MBW**), feed conversion ratio, and residual feed intake were calculated. The FCR was calculated as the ratio of DFI and DEM. The RFI was estimated based on the following formula first proposed by Luiting and Urff (1991):

$$RFI = FI - (b_0 + b_1MBW + b_2DEM + b_3BWG)$$

where b_0 is the intercept, and b1 and b2 are partial regression coefficients. All phenotype data lying outside 3 SD of the mean were regarded as outliers and excluded from the analysis.

Genetic Parameters Estimation

The phenotypes were normalized through rank-based inverse normal transformations using the GenABEL package in R (Aulchenko et al., 2007). We used AI-REML with an animal model to calculate genetic parameters (Yuan et al., 2015a) and performed it using the DMU software (Madsen et al., 2018). We constructed a univariate animal model to obtain estimates of the heritability for each trait as follows:

$$y = XB + Za + e$$

y is the vector under observation, X and Z are the incidence matrix of fixed effects and random additive effects, respectively, b is a vector of fixed effects, a is a

vector of random additive effect, and e is the random residual effect.

DNA Extractions and 16S rRNA Gene Sequencing of Fecal Microbiota

Total DNA of the fecal microbiota in each bird was extracted using the QIAamp Stool Mini Kit (QIAGEN, D4015-01) according to the manufacturer's recommendations. PCR amplification of the V4 region (515F-806R) of the bacterial 16S rRNA gene was performed.

The PCR reactions were performed in a 30 μL system containing 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM forward primer, 0.2 μM reverse primer, and 10 ng template DNA. The optimum PCR program was as follows: 98°C for 1 min, 30 cycles of 98°C for 10 s, 50°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min.

Equal volumes of 1 × loading buffer (containing SYBR green) were mixed with PCR products and electrophoresed on a 2% agarose gel for detection. The PCR products were mixed at equidensity ratios. Then, the PCR products were purified using the GeneJETTM Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using the Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) following the manufacturer's recommendations. Library quality was assessed on a Qubit@ 2.0 Fluorometer (Thermo Scientific). The library was sequenced on an Ion S5TM XL platform, and 400 bp single-end reads were generated. Single-end reads were assigned to the samples based on their unique barcodes and truncated by cutting off the barcode and primer sequences.

Analysis of 16S rRNA Sequencing Data

The 16S rRNA sequencing data were processed using Quantitative Insights Into Microbial Ecology (QIIME2, version 2019.10) (Bolyen et al., 2019). The preliminary quality screening was performed for the original high-throughput sequencing data using the QIIME2 plugin DADA2 (Callahan et al., 2016). The chimeric sequences were filtered and the remaining sequences were trimmed to a final length of 252 bp. The remaining high-quality sequences were merged and classified by amplicon sequence variants (**ASVs**), and the representative sequence of each ASV was used to identify the classification status and for phylogenetic analysis. Subsequently, ASVs with an average relative abundance $<10^{-6}$ and detection rate <1% were removed from the analysis because they were generated mainly by sequencing errors. The identified taxonomies were aligned using the Silva database (Release 132) (Quast et al., 2013). The ASV abundance of each sample and the six-level taxonomic classification from phylum to species were obtained. The alpha and beta diversity were calculated by using the vegan package (Philip, 2003).

Phenotype Prediction Based on Host Genetics and Gut Microbial Communities

We calculated the effect of the microbial communities on feed efficiency. The phenotypic variance explained by gut microbial variance is defined as microbiability ($\mathbf{m^2}$) in animals (Camarinha-Silva et al., 2017; Difford et al., 2018). The construction of the microbial relationship matrix (**MRM**) follows equation described in our previous study (Wen et al., 2019):

$$m_{ij} = \frac{1}{N}\sum_{o=1}^{N}\frac{(x_{io} - \overline{x_o})\ (x_{jo} - \overline{x_o})}{\sigma_o^2}$$

where m_{ij} represents the microbial relationship in feces between birds i and j; x_{io} and x_{jo} represent the relative abundances of ASV o in birds i and j, respectively; $\overline{x_o}$ is the average relative abundance of the ASV o for the whole population; σ^2o is the variance of the abundance of ASV o; and N is the total ASV number.

The microbiability was calculated as follows:

$$y = Kc + m + e$$

where y is a vector of the phenotype; c is a vector of fixed covariates, including batch effect; K is the corresponding matrix for c; and m is a vector of microbial effects following the multinomial distribution $m \sim N(0, M\sigma^2 m)$. M represents the MRM. The microbiability was estimated with GCTA software (Yang et al., 2011).

Contribution of Host Genetics to the Variation of Microbial Composition

To explore the effects of host genetics on gut microbiota, the heritabilities of fecal microbes were estimated, and taxa that were detected in less than 30% of the samples were excluded from this analysis. The relative abundance of qualified taxa was normalized by using the GenABEL package in R and the following heritability calculation was performed using the DMU package, as mentioned above. In addition, the heritability estimates of community phenotypes (including Observed amplicon sequence variants (**ASV**s), Shannon index, Simpson index, and Chao l index) were also calculated.

In addition to the heritability of microbial taxa and community phenotypes, the correlation between host genetics and gut microbial distance were also calculated for determining the influence of genetics on microbial composition. The host genetic relationship was calculated based on pedigree information using the nadiv package in R (Wolak, 2012). We then calculated the Pearson's correlation between host genetic relationships and microbial distance (Bray−Curtis dissimilarity, unweighted UniFrac distance, and weighted UniFrac distance). We further compared the difference in microbial distance among full sibs, half sibs, first cousins, and genetically unrelated individuals by one-way ANOVA.

Identification of Feed Efficiency-associated Microbiota

Since taxa at lower detection rates are less informative for difference analysis and association analysis, we retained only genera with a detection rate of more than 30%. Pearson's and Spearman's correlations were calculated among the microbes and target traits using the psych package in R.

All birds were then sorted by RFI value; the lowest 10% (n = 71) and the highest 10% (n = 71) of the ranked individuals were considered two distinct groups, and 4 differential methods were used to find specific genera. First, we used corncob's differential test function (Martin et al., 2020) to find RFI-associated genera between two distinct groups. We chose Wald tests (with the default non-bootstrap setting) to perform significance testing, and we obtained BH FDR-corrected *P*-values as output. Then, DESeq2 (Love et al., 2014) were performed as follows: 1) estimate size factors; 2) estimate of dispersions from the negative binomial likelihood for each feature, and subsequent shrinkage of each dispersion estimate toward the parametric (default) trendline by empirical Bayes; 3) fitting each feature to the specified class groupings with negative binomial generalized linear models and performing hypothesis testing, for which we chose the default Wald test. We obtained the resulting BH FDR-corrected *P*-values for output. Linear discriminant analysis effect size (**LEfSe**) was performed to identify bacteria enriched in two distinct groups (Segata et al., 2011). Relative abundance was converted to percentages for this analysis. Differences in the features were identified in the genus. The LEfSe analysis conditions were as follows: 1) the alpha value for the factorial Kruskal-Wallis test among classes was less than 0.05; 2) the alpha value for the pairwise Wilcoxon test among subclasses was less than 0.05; 3) the threshold on the logarithmic LDA score for discriminative features was less than 2.0; 4) multiclass analysis was set as all-against-all. Furthermore, we calculated Pearson's correlations between the feed efficiency-associated microbiota and phenotypes. Subsequently, Pearson's correlations between feed efficiency-associated microbiota and host phenotypes were performed using the psych package in R.

In addition, the associations between fecal genera and RFI were analyzed using a two-part model (Fu et al., 2015). The binary model is described as: $y = \beta 1b + e$, where y is the RFI value, b is a binary feature (0 for absent or 1 for present for each sample), $\beta 1$ is the regression coefficients for the binary model, and e represents the residuals. If p value from the binary model was less than 0.05, the presence or absence of microorganisms could influence feed efficiency. The quantitative model is written as: $y = \beta 2q + e$, where q is the log10-transformed abundance of a microbe, $\beta 2$ is the regression coefficients for the quantitative model, and e represents the residuals. The analysis was only for the samples in which the specific microorganism was present. If p value from the quantitative model was less than 0.05, the relative abundances of microorganisms was significantly associated with feed efficiency.

Data Availability

The datasets presented in this study can be found below: NCBI Sequence Read Archive under BioProject ID PRJNA861965.

RESULTS

Descriptive Statistics of Traits

The mean, standard deviation (**SD**), coefficient of variation (**CV**), and minimum and maximum values of each trait are summarized in Table 1. The mean values of DEM, DFI, FCR, RFI, MBW, and metabolic body weight (**MMBW**) were 52.90 g/d, 119.49 g/d, 2.37 g/g, −0.01 g/d, 2127.23 g, and 312.91 g, respectively. The CV of the traits in the population had a wide range, from 7.78% to 39.87%. The CVs of DFI and MMBW were less than 10%, whereas the CVs of FCR and DEM were greater than 15%, indicating a large phenotypic variation in these traits. The RFI was approximately equal to zero because it represented the residuals of the linear model.

Heritability of FCR, RFI, and Related Traits

As shown in Table 2, heritability estimates for DEM, MMBW, DFI, FCR, and RFI were 0.30 ± 0.09, 0.55 ± 0.10, 0.38 ± 0.10, 0.31 ± 0.09, and 0.52 ± 0.10, respectively. The high heritability of FCR and RFI in our study suggests that host genetics plays a substantial role in determining the feed efficiency.

Correlation of FCR and RFI with Other Traits

We calculated the genetic and phenotypic correlation coefficients among RFI, FCR, and other traits. A strong and positive genetic (r_a = 0.59) and phenotypic

Table 1. Descriptive statistics for phenotypes of pedigreed hens.

Traits	N	Mean	SD	CV (%)	Maximum	Minimum
BW69 (g)	714	2111.37	223.52	10.59	2799	1271
BW72 (g)	714	2143.09	224.56	10.48	2815	1437
MBW (g)	714	2127.23	220.41	10.36	2804	1416
MMBW (g)	714	312.91	24.36	7.78	385.33	230.83
DEM (g/d)	714	52.90	8.34	15.77	70.72	6.68
DFI (g/d)	714	119.49	11.40	9.54	160.57	77.13
FCR (g:g)	714	2.37	0.94	39.87	16.77	1.60
RFI (g/d)	714	−0.01	8.54	–	31.31	−20.68

N = Number of birds with phenotypic value.
SD = standard deviation.
CV = coefficient of variation.
BW69 = body weight at 69 wk of age.
BW72 = body weight at 72 wk of age.
MBW = mean body weight from 69 to 72 wk of age.
MMBW = metabolic body weight.
DEM = daily egg mass from 69 to 72 wk of age.
DFI = daily feed intake from 69 to 72 wk of age.
FCR = feed conversion ratio.
RFI = residual feed intake.

Table 2. Estimates of heritability (h^2) for feed efficiency and relevant traits along with estimates of genetic (r_a; the upper diagonal) and phenotypic (r_p; the lower diagonal) correlations among traits from 69 to 72 wk.

	BW69	BW72	MBW	MMBW	DEM	DFI	FCR	RFI
BW69	0.52 (0.10)	1.00 (0.01)	1.00 (0.00)	1.00 (0.00)	−0.11 (0.20)	0.46 (0.15)	0.48 (0.17)	0.07 (0.16)
BW72	0.94	0.56 (0.10)	1.00 (0.00)	1.00 (0.00)	−0.12 (0.20)	0.46 (0.14)	0.49 (0.16)	0.05 (0.16)
MBW	0.98	0.99	0.55 (0.10)	1.00 (0.00)	−0.11 (0.19)	0.47 (0.14)	0.48 (0.16)	0.07 (0.16)
MMBW	0.98	0.99	1.00	0.55 (0.10)	−0.11 (0.20)	0.47 (0.14)	0.48 (0.16)	0.07 (0.16)
DEM	0.07	0.07	0.07	0.07	0.30 (0.09)	0.31 (0.19)	−0.56 (0.15)	0.20 (0.19)
DFI	0.31	0.43	0.38	0.38	0.43	0.38 (0.10)	0.67 (0.16)	0.88 (0.05)
FCR	0.17	0.26	0.22	0.22	−0.68	0.32	0.31 (0.09)	0.59 (0.14)
RFI	0.01	0.01	0.01	0.01	0.12	0.78	0.50	0.52 (0.10)

(r_p = 0.50) (Table 2) correlation were found between RFI and FCR.

As expected, the RFI was phenotypically uncorrelated with the MMBW and DEM, and the genetic correlation coefficient between RFI and the two traits were 0.07 and 0.20. Compared to the RFI, the genetic and phenotypic associations between FCR and DEM were closer and all showed a strong negative correlation (r_a = −0.56, r_p = −0.68). This meant that selection for low FCR individuals had a greater impact on DEM.

The genetic and phenotypic correlation coefficients between RFI and DFI were 0.88 and 0.78, and that two correlation coefficients between FCR and DFI were 0.67 and 0.32. The genetic and phenotypic correlation coefficients of FCR and DFI were both much less than that of RFI and DFI. This suggested that choosing RFI was more beneficial for individual consumption than FCR. Overall, the FCR was primarily related to growth traits such as DEM and BW, whereas the RFI was related to energy metabolism traits such as DFI.

Microbiability of FCR, RFI, and Related Traits

In addition to host genetics, microbes have important influence on phenotypes, we then estimated the proportion of variation of feed efficiency and its relevant traits explained by microbiota.

We performed 16S rRNA sequencing to characterize the fecal microbial composition of 714 samples and obtained a total of 31,818,930 quality-filtered sequences with an average of 44,564 reads per sample. A total of 1,863 ASVs were identified to be clustered with 99% sequence identity and classified into 597 species, 376 genera, 159 families, 76 orders, 38 classes, and 22 phyla. At the phylum level, we identified 22 phyla, in which *Firmicutes* was the most abundant phylum (73.55%) followed by *Fusobacteria* (12.19%), *Bacteroidetes* (10.65%), and *Proteobacteria* (2.07%) (Fig S1A). At the genus level, *Lactobacillus, Romboutsia, Fusobacterium, Streptococcus, Turicibacter, Bacteroides, Enterococcus,* and *Clostridium sensu stricto 1* were identified as the dominant genus, and the detailed proportion are displayed in pie charts (Figure S1B).

Similar to heritability, the microbiability indicates the contribution of the microbial community to host phenotype. The microbiability estimated for RFI and FCR were 0.16 and 0.03 respectively, lower than that of the heritability. Other traits relevant to the feed efficiency had moderate microbiability (0.15 for DFI, 0.14 for BW69, 0.12 for BW72, 0.12 for MBW, and 0.12 for MMBW), whereas DEM was close to zero (Figure 1). Among all the feed efficiency-related traits, the microbiability of RFI was the highest.

Association between Host Genetics and the Gut Microbiota

Fecal microorganisms have an effect on host feed efficiency, and elucidate the host effect on the gut microbiome is essential to help design microbial strategies to improve production. We first estimated the heritability of alpha-diversity indexes and taxa at phylum, class, order, family, genus, and species levels. The a-diversity parameters including the Chao 1, Shannon index, Simpson index, and Observed ASVs were used as phenotypes to estimate the pedigree-based heritability (Table S2). Host genetics minimally determined the microbiota diversities in the feces.

A total of 343 taxa (binary:177; quantitative:166) were used for heritability estimation (Table S3). The average heritability estimate was 0.02 for all fecal taxa. However, as shown in Figure 2A, species such as *Lactobacillus vaginalis* (h^2 = 0.28), *Lactobacillus agilis* (h^2 = 0.22), *Lactobacillus aviaries* (h^2 = 0.18), and

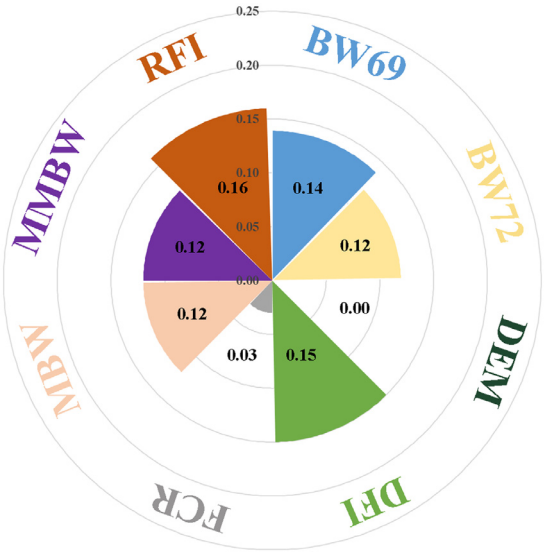

Figure 1. The contribution of the gut microbial community to host phenotypes.

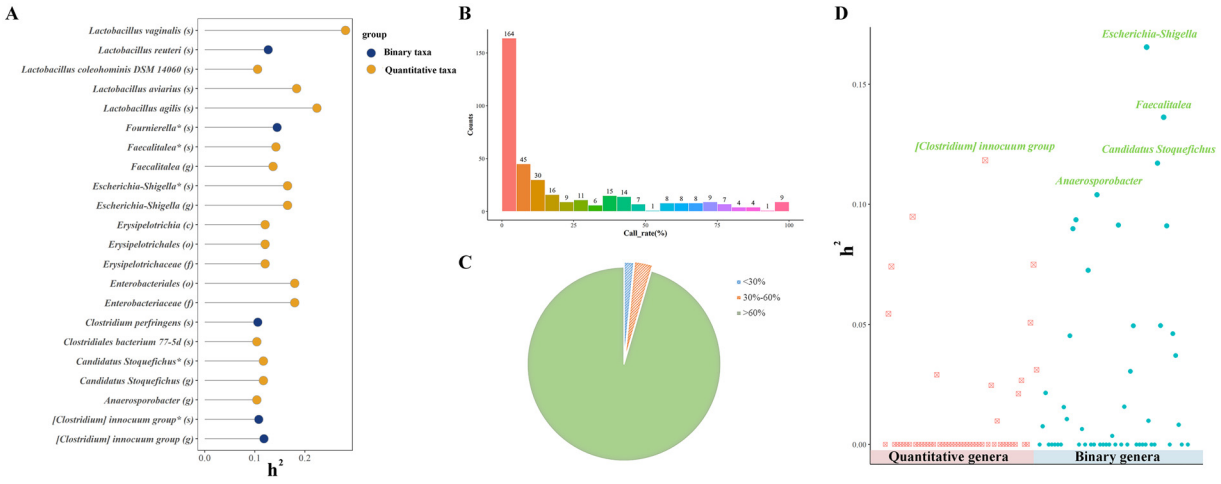

Figure 2. The heritability of each microorganism. (A) Heritability estimates for the taxa with a heritability of more than 0.1. * indicates taxa that are unclassified at the level, and p, c, o, f, g, and s represent phylum, class, order, family, genus, and species level, respectively. (B) Count distribution histogram of detection rates of identified microbial genera. (C) The sum of the relative abundances of microbial genera with different detection rates. (D) The heritability of each genus. Only genera with a heritability of more than 0.1 are exhibited with microbial names.

Lactobacillus reuteri ($h^2 = 0.13$), which belong to the genus *Lactobacillus,* were more heritable.

We then focused on the heritability of fecal genera to learn more about the role of host genetics played in fecal microbial community. Among 376 identified genera, 51 microbes possessed a detection rate between 30% and 60%, 50 had a detection rate greater than 60%, and the detection rate of the remaining genera was close to zero (Figure 2B). Notably, although most genera were detected with a low detection rate, genera with >30% detection rate accounted for 98.43% of the total community sequences (Figure 2C), indicating that these genera could represent the fecal community. Among 101 microbial genera, *Escherichia-Shigella* ($h^2 = 0.17$), *Faecalitalea* ($h^2 = 0.14$), *Candidatus Stoquefichus* ($h^2 = 0.12$), *Anaerosporobacter* ($h^2 = 0.10$), and *[Clostridium] innocuum group* ($h^2 = 0.12$) had a heritability estimate more than 0.1 (Figure 2D). The relative abundance of *Escherichia-Shigella, Faecalitalea, Candidatus Stoquefichus, Anaerosporobacter,* and *[Clostridium] innocuum group* was 0.31%, 0.14%, 0.23%, 0.38%, and 0.01%, respectively. These genera belonged to the phyla Firmicutes and Proteobacteria, accounting for 1.07% of the tested fecal microbiota (Table S4). The abovementioned results indicated that although several taxa with higher heritability and greater genetic influence, the effect of host genetics on the entire microorganisms is limited.

To further verify the limited influence of host genetics on microorganisms, we calculated the correlation of host genetics and microbial distance and difference of microbial β-diversity among different host genetic kinship pairs of individuals.

Most pairs of chickens showed no or a low degree of genetic relatedness, and the correlations between host genetics and different microbial distance were very weak ranging from −0.0057 to −0.0003 (Figure 3A). Since the genetic relationship was generally low, we further compared the difference in the beta diversity among full sibs, half sibs, first cousins, and genetically unrelated birds. Based on the pedigree, we obtained 494 full-sib pairs, 2,959 half-sib pairs, 2,964 first cousins and the remaining pairs were considered as unrelated pairs. Similar results were also observed in Figure 3B. Whether full-sib, half-sib pairs, first cousins, or unrelated birds, the microbial distance between different kinships showed no significant difference.

Correlation Analysis of the Screened Microbes and Host Phenotypes

To investigate the correlations between fecal microbes and host phenotypes, Pearson's and Spearman's correlations were performed. It is obvious that most taxa were not significantly associated with phenotypes, as shown in Figure 4A and B. A total of 416 ASVs were significantly correlated with the phenotypes (Pearson: 156; Spearsman: 260) and correlation coefficients ranged from -0.12 to 0.14 (Table S5). Similar to the results of microbiability, more taxa were related to body weight and RFI. The ASVs that were associated with host phenotypes in both methods belonged to the phyla Firmicutes (74.4%), Bacteroidetes (18.4%), Proteobacteria (4.3%), and Actinobacteria (2.8%; Figure 4C).

Identification of Genera Associated with Feed Efficiency

Identification of bacteria associated with host phenotypes in animals may offer a direct approach to the identification of probiotic bacteria for use in animal production. Since RFI had a relatively high microbiability, two-tailed tests (corncob, DESeq2, and LEfSe) and a two-part model association analysis were performed for the divergent RFI groups to detect the RFI-associated genera. We first selected individuals based on the 10% highest (**H**; n = 71) and 10% lowest (**L**; n = 71) RFI. As shown in Table S6, RFI significantly differed between the high-(16.80) and low-RFI (−13.31) groups.

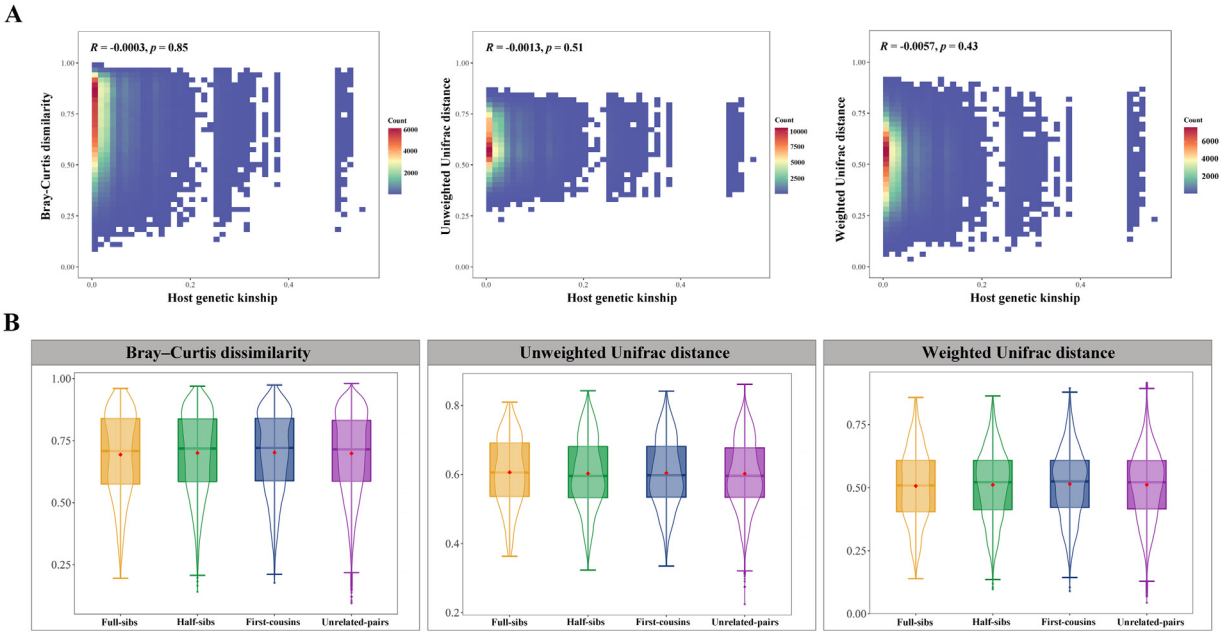

Figure 3. Effect of genetic kinship on fecal microbiota. (A) Scatter plot of the host genetic kinship of pairs of individuals (x axis) and their microbial distance (bray−Curtis dissimilarity, unweighted unifrac distance, and weighted unifrac distance) (y axis). The correlation coefficient and *p* value between host genetic relationships and microbial distance are exhibited. (B) Difference of microbial distance among full sibs, half sibs, first cousins, and genetically unrelated individuals. The central red point indicates the mean value in the corresponding group.

The FCR and DFI were significantly different in the H and L groups, with a difference of 0.83 for FCR and 31.07 g for DFI, which were consistent with the results of the correlation analysis above.

We then screened the microorganisms associated with RFI by different methods between high-and low-RFI birds and found 8, 6, and 4 significantly different genera by corncob, DESeq2, and LEfSe, respectively. The genera *Anaerosporobacter*, *Candidatus Stoquefichus*, *Fournierella*, and *Faecalitalea* were simultaneously identified by the three methods (Figure 5A; Figure S2; Table S7). Four associations were detected by quantitative analysis, and fifteen associations were identified by binary analysis (Figure 5B; Table S8). Notably, *Anaerosporobacter* was found both in the binary and quantitative models, suggesting that both the presence/absence

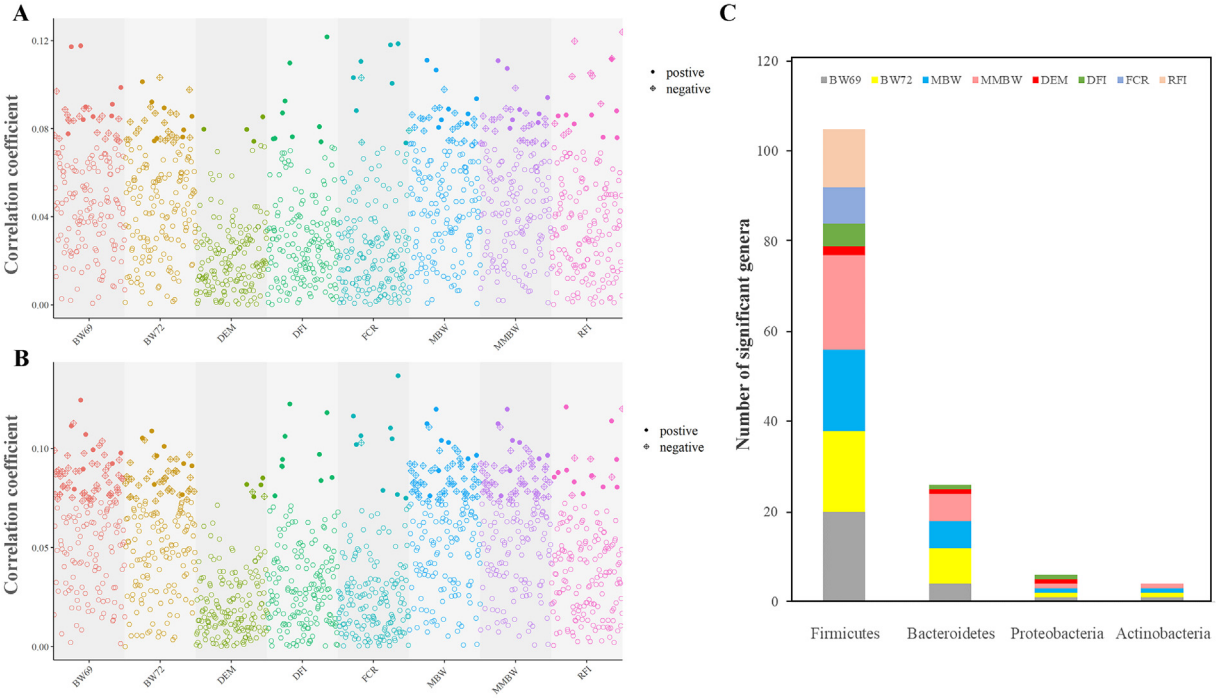

Figure 4. The association between fecal genera with host phenotypes by Pearson's (**A**) and Spearman's (**B**) correlations. Scatter plot of the adjusted *p* values. The plot indicates correlation coefficient (y-axis) plotted against taxonomic microbes (x-axis, detection rate >30%) (C) Distributions of significant microbes on phyla and traits.

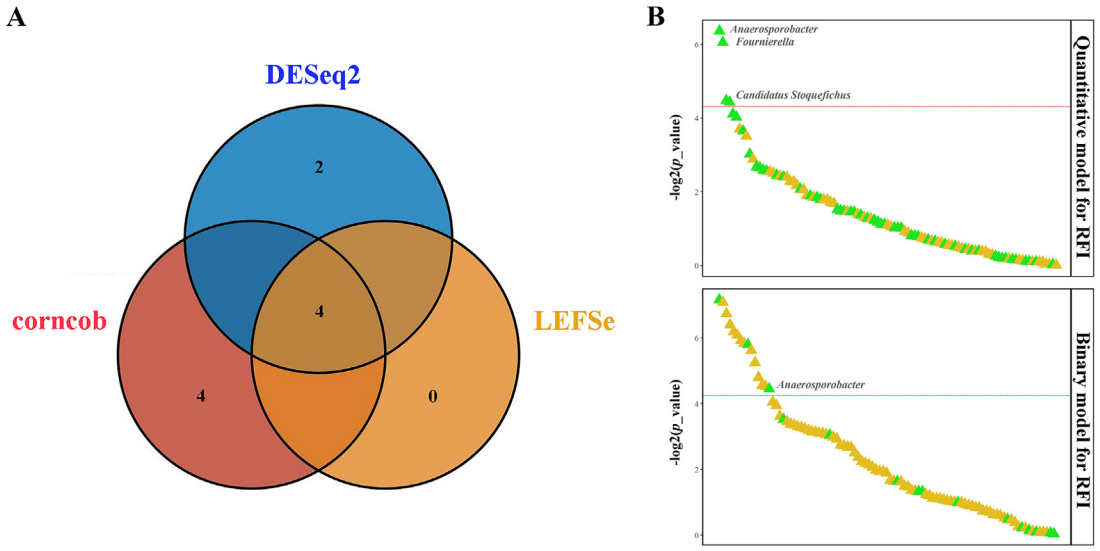

Figure 5. Identification of feed efficiency-associated microbiota (A) Number of genera associated with RFI detected by different methods and their overlaps. (B) Two-part model for association analysis between RFI and gut microbiota at genus level. Only genera that overlap with the two-tailed differential bacteria are exhibited with microbial names.

and abundances of the genus can affect RFI. Among these genera, *Anaerosporobacter*, C*andidatus Stoquefichus, and Fournierella* were observed in both the association analysis and 2-tailed tests (Figure 5B).

The detection rate of RFI-associated microorganisms in 714 hens ranged from 67.09% to 78.01% (Figure 6A). The average abundance of *Anaerosporobacter, Candidatus Stoquefichus*, and *Fournierella* was 0.38%, 0.23%, and 0.59%, respectively. The detection rate and average abundance of RFI-associated genera were 2-3 times higher in the L group than that in the H group (Table S9), indicating their positive roles in improving feed efficiency. Pearson's correlation coefficients were calculated for all birds to visualize the relationship between phenotypes and the associated microbes. As shown in Figure 6B, *Anaerosporobacter, Candidatus Stoquefichus,* and *Fournierella* were significantly correlated with RFI. These fecal genera were positively and moderately correlated with each other (Figure S3). Meanwhile, *Anaerosporobacter* was also correlated with FCR (Table S10). Moreover, based on the relatively high heritability estimates, *Anaerosporobacter* and *Candidatus Stoquefichus* were more susceptible to host genetics.

DISCUSSION

Feed efficiency is an important economic trait. With the extension of the laying cycle, feed utilization tends to decline (Yuan et al., 2015a). Therefore, improving feed efficiency in the late laying period of chicken is a key problem that breeders need to solve; further continued improvement will be aided by better understanding of the factors influencing feed efficiency.

Genetic and breeding strategies are effective to enhance feed efficiency in laying hens, feed utilization has been improved through artificial selection of feed efficiency traits (Thiruvenkadan et al., 2010). Understanding the genetic background of feed efficiency is essential and would contribute to chicken breeding and further genomic studies.

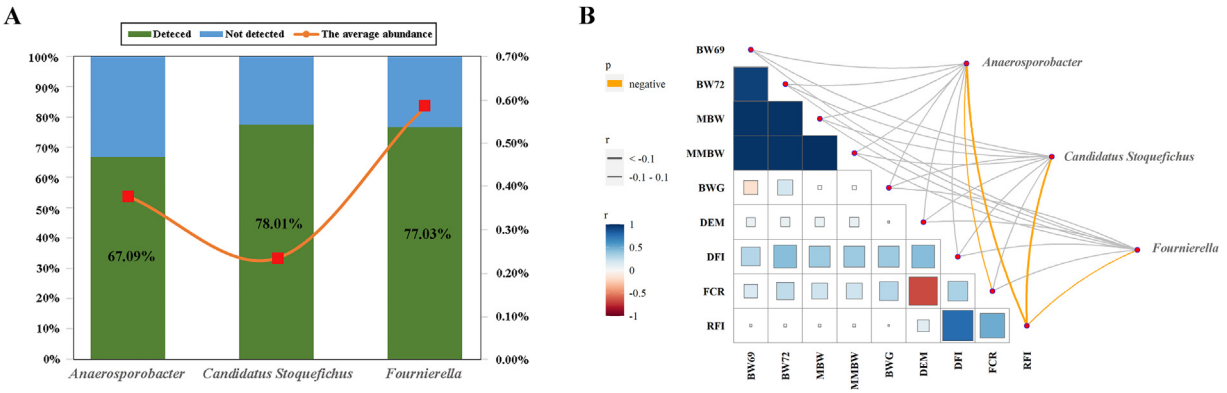

Figure 6. Details of feed efficiency-associated microorganisms. (A) Relative abundance and detection rate of RFI-associated genera. (B) Pearson correlation between RFI-associated microorganisms and host phenotypes. Yellow lines represent significant correlations and grey lines represent insignificant correlations.

The heritability of a16 to 42 wk of age finnish Leghorn population was reflected by RFI values of 0.46 (Schulman et al., 1994). Another study in a brown egg layer line found that the heritability estimate of the RFI was moderate (0.47) (Wolc et al., 2013). As for FCR, the heritability estimates ranged from 0.208 to 0.452 for FCR from day-1 to 16th wk of age in a selected line of Rhode Island Red chicken (Das et al., 2015). A recent study in heat-challenged commercial white egg-laying hens found that the heritability of FCR was 0.23 from 24 wk of age to 28 wk of age (Rowland et al., 2019). Moreover, in 2 laying periods of chickens from a White Leghorn and Dongxiang reciprocal cross, estimates for heritability of RFI and FCR were 0.21 and 0.19, repectively, from 37 and 40 wk, and 0.29 and 0.13,respectively, from 57 and 60 wk (Yuan et al., 2015a). In general, different breeds and ages of chickens showed different heritabilities of the FCR and RFI, and the moderately estimated heritability in our study was consistent with these previous studies. The moderate estimated heritability indicated the presence of sufficient genetic variability for the traits in the population. The selection for either of these 2 indices (FCR and RFI) can undoubtedly increase the feed efficiency.

FCR was negatively correlated with DEM but positively correlated with DFI phenotypically and genetically, suggesting the selection for FCR may lead to similar ratios but different DFI and outputs (Schulman et al., 1994; Difford et al., 2016; Camarinha-Silva et al., 2017). The positive and high correlation between RFI and FI in our study agreed with a previous study on two laying periods of chickens (Yuan et al., 2015a). The genetic and phenotypic correlation between RFI and DEM is weak (Schulman et al., 1994), suggesting that selection for RFI can improve the feed efficiency by less FI and supplying the same amount of egg mass, and RFI is a more desirable trait for characterizing feed efficiency. These results are important for the continued development of strategies to improve feed efficiency in chicken breeding and production.

In addition to host genetics, the gut microbiota is also important for feed efficiency (Yan et al., 2017). Difford et al. (2016) first proposed the proportion of microbial variance to phenotypic variance as "microbiability" in dairy cattle. In pigs, Camarinha-Silva et al. (2017) and Weishaar et al. (2020) reported the presence of a medium to high microbiability of daily gain, feed intake, and feed conversion rate. Our previous study demonstrated that the cecal microbiota accounted for 28% of the RFI variance in broiler chickens (Wen et al., 2021). Medium-to-high microbiabilities for feed-related traits have also been identified in Japanese quails (Vollmar et al., 2020). The microbiability estimate for RFI was medium in our population, confirming that the effect of the gut microbiome on feed efficiency in pigs and poultry was medium to high (Khanal et al., 2021).

Moreover, the lower estimate of microbiability compared with heritability in our study and a previous study (Wen et al., 2021) implied that host genetics is a more important determinant for the feed efficiency in chicken. Similarly, a lower estimate of microbiability than that of heritability for RFI, FCR, and DFI was found in pigs (Khanal et al., 2021). In addition to feed efficiency-related traits, the lower estimate of microbiability compared with heritability was also found in fat deposition traits in chicken (Wen et al., 2019) and pigs (Tang et al., 2020). However, higher estimates of microbiability for feed efficiency than their corresponding heritabilities were also found in pigs (Camarinha-Silva et al., 2017). Interestingly, a study in swine found that that the proportion of variance captured by the microbiome varied over time (Khanal et al., 2021). The differences of the comparison between microbiability and heritability may account for host age, population structure, genetic relatedness, different traits and environmental factors.

Multiple factors, including diet (Fouad and El-Senousey, 2014), medication (Weersma et al., 2020), and genetics (Bonder et al., 2016b) influence the gut microbiota composition. The role of host genetics in shaping intestinal flora has been investigated in humans (Zoetendal et al., 2001) and livestock (Xiao et al., 2015; Pandit et al., 2018; Bergamaschi et al., 2020). In our study, no significant difference was observed between genetics kinships, which is consistent with the finding that microbiome composition is not significantly associated with genetic ancestry in humans (Rothschild et al., 2018). Similar to previous studies, genera with high heritability identified in this study belonged to Firmicutes (Xiao et al., 2015) and Proteobacteria (Bergamaschi et al., 2020), and accounted for a low proportion of the tested fecal microbiota (Difford et al., 2018). Our previous study also showed that no significant relationship between host genetic kinship and gut community have been found at five different gut microbial sites in broilers (Wen et al., 2021). However, several studies in different species have indicated that host genetics influence gut microbial composition (Org et al., 2015; Goodrich et al., 2016; Bonder et al., 2016a; Li et al., 2019; Aliakbari et al., 2021; Grieneisen et al., 2021). Whether host genetic variation plays a role in determining microbial composition is debatable and could be attributed to different populations and sampling sites. A disadvantage of this study is that it only collected fecal samples. Although fecal sampling is non-invasive and convenient, it does not fully represent the entire intestine (Yan et al., 2019), and we should take multiple intestinal segments into account in the following research. Overall, our observations implied that host genetics plays a minor role in determining microbiome composition, and most of the variation in the gut microbial community is due to factors other than host genetics.

Considering the difference in finding significant taxa by using corncob and DESeq2 (Nearing et al., 2022), two-tailed tests including corncob, LEfSe, and DESeq2 were used for intersection of significant differential taxa. Moreover, a two-part model of all birds was also used to in our study for pinpointing more reliably microorganisms. We identified that *Anaerosporobacter, Candidatus Stoquefichus*, and *Fournierella* were more

abundant in feed-efficient birds. *Candidatus Stoquefichus* belonging to the family Erysipelotrichaceae, has been reported to be negatively correlated with serum inflammatory cytokines in mice (Yang et al., 2021). Moreover, strong evidence supports an association between Erysipelotrichaceae and host lipid metabolism and inflammation (Kaakoush., 2015). Therefore, we speculate that *Candidatus Stoquefichus* facilitates the establishment of the intestinal barrier by inhibiting the production of inflammatory cytokines, which is beneficial for host energy absorption.

Fournierella and *Anaerosporobacter* have been reported to be significantly associated with intrahepatic fat accumulation (Yaskolka Meir et al., 2021). *Fournierella* might be involved in bile secretion and tryptophan metabolism in rabbits with diarrhea (Wang et al., 2022). Anaerosporobacter may cause vascular damage and worsen renal function in murine models (Li et al., 2020) and was reported to be associated with an increase in trimethylamine oxide (TMAO) content in vivo (Wang et al., 2016). In addition, non-alcoholic fatty liver disease patients have a lower fecal abundance of *Anaerosporobacter* (Wong et al., 2013). However, there have been no studies on the relationship between fecal *Fournierella* or *Anaerosporobacter* and feed efficiency. The precise mechanism by which these two bacterial taxa affect feed efficiency warrants further study. Interestingly, the heritability estimates of *Anaerosporobacter* and *Candidatus Stoquefichus* were relatively high, indicating that genetic selection can be used to regulate the abundance of flora and improve the host feed efficiency for specific microbiota.

In conclusion, our study described feed efficiency and its relevant traits in the late laying period of a Rhode Island Red pure line chickens. The moderate heritability estimates for both RFI and FCR suggested that feed efficiency can be directly improved by proper selection programs. We found that host genetics play a more important role in shaping feed efficiency than fecal microbiota and the effect of host relatedness kinship on fecal microbial distance was weak. Several genera, including *Anaerosporobacter, Candidatus Stoquefichus*, and *Fournierella* were significantly associated with residual feed intake. Our findings provide a promising strategy to improve feed efficiency from the perspective of the host genetic background and microorganisms.

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DISCLOSURES

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102393.

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