Combined effect of microbially-derived caecal SCFA and host genetics on feed efficiency in broiler chickens

Zhengxiao He
Chinese Academy of Agricultural Sciences
Ranran Liu
Institute of Animal Sciences
Qiao Wang
Institute of Animal Sciences
Jumei Zheng
Institute of Animal Sciences
Jiqiang Ding
Institute of Animal Sciences
Jie Wen
Institute of Animal Sciences
Alan G Fahey
University College Dublin
Guiping Zhao (zhaoguiping@caas.cn)
Institute of Animal Sciences

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Abstract

Background

Improving feed efficiency is the most important goal for modern animal production. The regulatory mechanisms of controlling feed efficiency traits are extremely complex and include the functions related to host genetics and gut microbiota. Short-chain fatty acids (SCFAs), as significant metabolites of microbiota, could be used to refine the combined effect of host genetics and gut microbiota. However, the association of SCFAs with the gut microbiota and host genetics for regulating feed efficiency is far from understood.

Results

In this study, we examined host genome sequence, microbial data and SCFA concentration of caecal chyme in 300 broilers. The SNP-based heritability analysis found that the SCFA concentrations had moderate to high heritability (h^2 = 0.183 ~ 0.401). Genome-wide association studies (GWAS) showed that four out of seven SCFAs had significant associations with genome variants. One locus (gga4: 29414391–29417189) was significantly associated with propionate, locating near or inside the genes *MAML3, SETD7 and MGST2*, and had a modest effect on feed efficiency traits and the microbiota. The genetic effect of the top SNP explained 8.43% phenotype of propionate. Individuals with genotype AA had significantly different propionate concentrations (0.074 vs. 0.131 ug/mg), feed efficiency (FCR: 1.658 vs. 1.685), and relative abundance of 14 taxa compared to those with the GG genotype. *Christensenellaceae* and *Christensenellaceae_R-7_group* were identified being associated with feed efficiency, propionate concentration and top SNP genotypes, and they were found to be lipid metabolism-related. Individuals with a higher caecal abundance of these taxa showed better feed efficiency and lower concentrations of caecal SCFAs.

Conclusion

Our study concluded that host genetic variation could affect the regulation of caecal microbially-derived SCFAs, which plays a role in host feed efficiency traits. The caecal taxa *Christensenellaceae* and *Christensenellaceae_R-7_group* were identified as representative taxa contributing to the combined effect of host genetics and SCFAs on chicken feed efficiency. These findings proved strong evidence of the combined effect of host genetics and gut microbial SCFAs in regulating feed efficiency traits.

Introduction

Feed is one of the most expensive components of the farm animal industry costs, accounting for up to 70% of production costs. Strategies to improve production without additional feed supplies is vital to ensuring the profitability and sustainability of the industry. Feed efficiency (FE) depends on the relation

between the feed intake (FI) and the growth (or bodyweight gain) of an animal and is described by several traits. Feed efficiency was influenced by several factors, including the breed of the birds and their sex, age, diet, and management [1]. Thus, feed efficiency could be regulated by energy metabolism and feeding behaviours such as appetite [2, 3]. The feed conversion ratio (FCR) and residual feed intake (RFI) are two indicators commonly used to evaluate the feed efficiency of livestock [4, 5]. RFI is preferred over FCR since it reflects the variation in the efficiency of feed utilize by broilers, which is independent of growth traits [6]. The heritability of RFI was reported to be between 0.23 and 0.49, and many genome-wide association studies (GWAS) have indicated that RFI is associated with host genome variation [7–12]. Over the last 50 years, the feed efficiency of commercial breeds improved by 50% due to quantitative genetic selection [13]. Additionally, the gut microbiota can markedly affect animal feed efficiency, as symbionts influence host metabolism [14].

The chicken gastrointestinal tract (GIT) includes compartments with varied physiological roles and environments that drive the spatial distribution of microbial populations [15]. Lower species richness in the intestine of chickens is accompanied by greater feeding efficiency, but this difference is not reflected in faeces samples [16, 17]. However, several studies found that bacterial diversity within the intestinal tract is higher in birds with lower FCR or higher feed efficiency [18–22]. Because the caecum is the primary place for food fermentation in monogastric animal, many caecal microbiota studies have been conducted over a wide range of microbiota diversity. Several studies have attempted to identify the intestinal microbes associated with RFI in broiler and layer chickens [12, 19, 23–25]. Nevertheless, findings to date have been inconsistent and sometimes contradictory. The low repeatability of microbial trial might be due to the susceptibility of intestinal microbiota communities to differences in diet, environment, management, age, and breed [26]. Many studies have been done on the heritability of microbiota, showing a low average of 0.068 [27]. Furthermore, there is a broad range of microbial taxa in the environment [28], which increases the complexity microbial studies.

A previous study indicated that short-chain fatty acids (SCFAs) present in the caecum were of microbial origin in a germ-free study [29]. SCFAs are well known as energy sources [30]. Hence, identifying a more energy-efficient microbiota is necessary to develop effective strategies to improve feed utilisation. There is no previous study on the association between the host genome and SCFA production. As a previous study reported, SCFAs are produced by gut microbiota, and the interactions between the host genome and microbiota were reported [12]. SCFAs can work as signalling molecules with the help of G protein-coupled receptors (GPCRs), which are called free fatty acid receptors (FFARs) [30]. GPR43/FFAR2 and GPR41/FFAR3 can interact with the major SCFAs (acetate, propionate, and butyrate), which regulate energy expenditure, preadipocyte differentiation, and appetite control [31, 32]. Feed efficiency traits have been widely investigated in cattle and are affected by feeding behaviour energy metabolism, which could be related to SCFA metabolism due to their effects on appetite and energy homeostasis [2, 33, 34]. Previous RNA-seq results in divergent RFI groups found that differentially expressed genes, such as *CAMP, LPL, PCK1*, and *CCKAR* interact with GPCRs in lipid and energy metabolism[35–38]. Hence, there is some evidence that the gut microbiota could produce SCFAs and possibly regulating host feed efficiency through energy- and appetite-related pathways.

The caecum is the primary fermentation site in poultry and the major site of microbial SCFA production. And changes in the microbiota and SCFA production can affect feed efficiency. Therefore, assessing the relationship between host genetics and the gut microbiota, caecal SCFAs and feed efficiency will help improve our understanding of the potential biological variations in feed efficiency and design sustainable approaches to improve feed efficiency in chickens. To achieve this goal, data of host genetic, microbial taxa and SCFAs in cecum segments were used to clarify the relationship between the microbiota, caecal SCFAs and host genetic variation. The overall workflow of the present analyses is showed in Fig. 1.

Materials And Methods

Animals

All chickens were obtained from the fast-growing white-feathered pure line, produced by Xinguang Agricultural and Animal Industrials Co., Ltd. (Mile, China). This line was selected for eight generations for high body weight and feed efficiency traits. RFI testing was conducted on a total of 464 broilers. They were housed in identical individual cages (length × width × height, 30 × 25 × 45 cm) and fed ad libitum. Each day, the amount of fresh feed provided was recorded individually, and residual feed was recorded daily and removed for an intervening period during 28–40 days of age. During this period, the animals were fed a corn-soybean meal diet, and detailed information about the diet is described in Additional file 1: Table S1. The bodyweight of each chicken at 28 and 40 days of age was measured using an electronic scale. The RFI calculation method was described by Li et al. [10]. The descriptive statistics of these phenotypes are summarised in Additional file 2: Table S2. The correlation coefficient between RFI and ADFI was 0.61, and significant correlations were found in coefficients between RFI and FCR (0.79), the ratio of the breast (-0.23), and abdominal fat ratio (0.37) (Additional file 3: Figure S1).

At the age of 41 days, whole blood was collected from each bird from the wing vein using a vacuum blood tube. Furthermore, broilers were sacrificed after 2 hours of the last feed to allow time for the feed to have been digested in the GI-tract. Each bird was then euthanized by cervical dislocation. The abdominal fat tissue, whole breast muscle and thigh on the right side were carefully dissected and weighed promptly with an electronic balance (0.1 g precision). Moreover, the caecal contents (including chyme and mucosa) were collected immediately. All the samples were snap-frozen in liquid nitrogen, transported to the laboratory and stored at − 80°C for subsequent studies.

Genotyping and Quality Control

Genomic DNA was extracted from blood samples with the phenol-chloroform method. In total, 300 broilers were resequenced with 150 bp paired-end reads on an Illumina NovaSeq 6000 platform with an average depth of approximately 10×1 L coverage conducted by Beijing Compass Biotechnology Co., Ltd. (Beijing, China). Variant calling was performed according to a standardised bioinformatics pipeline for all samples [39, 40]. Specifically, clean sequencing data were aligned to the chicken reference genome (GRCg6a/galGal6: https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/-000/002/315/GCF_000002315.6_GRCg6a/) with the

Burrows-Wheeler Aligner (BWA)-MEM algorithm [41]. Then, PCR duplicates were removed, and local indel realignment and base quality score recalibration were performed with the Genome Analysis Toolkit (GATK version 3.5) [42]. Variant calling was performed via HaplotypeCaller in GVCF mode with joint genotyping on all samples. Finally, SNPs were filtered with the GATK VariantFiltration protocol. The filtering settings were as follows: variant confidence score (QUAL) < 30.0, QualByDepth (QD) < 2.0, ReadPosRankSum < −8.0, total depth of coverage (DP) < 4.0, and FisherStrand (FS) > 60.0. In addition, quality control of the reference panel was conducted with the criteria of MAF ≥ 0.05, only bi-allelic sites, genotyping missing < 0.2, mean depth value is between 3 and 30, and site quality value is higher than 30. After filtering, 9,540,946 autosome variants remained for the 486 sequenced birds, LD decay was conducted by PopLDdecay [43], and the average LD level in a 5-kb interval was 0.17 (Additional file 4: Figure S2).

16S rRNA gene sequencing and analysis

Three hundred caecal samples were used to conduct 16S rRNA amplicon sequencing. The total DNA of caecal content was extracted by a QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). Eight caecal samples were excluded because of DNA extraction failure. Finally, 292 microbial DNA samples were used for 16S rRNA sequencing. Seventy-nine top-ranked and seventy-nine bottom-ranked RFI samples were assigned as high and low groups. The V4 region of the 16S rRNA gene was amplified using the primer pair 515F/806R (5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-GGACTACHVGGGTWTCTAAT-3'), and the amplicons were purified and quantified using Agencourt AMPure Beads and the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA), respectively. After quantification, the barcoded V4 amplicons were pooled and subsequently sequenced using an Illumina MiSeq platform (Illumina, San Diego, CA) to generate 300 bp paired-end reads at Shenzhen BGI Technology Services Co., Ltd. For each sample, there were approximately 50000 clean reads. Amplicon sequencing bioinformatics was performed with EasyAmplicon v1.0 [44]. Paired-end sequence data were merged, quality filtered, and dereplication using VSEARCH v2.15 subcommand –fastq_mergepairs, –fastx_filter, and –derep_fulllength, respectively [45]. Then, the non-redundancy sequences were denoising into amplicon sequence variants (ASVs) with USEARCH v10.0 [46] (via -cluster_otus or unoise3). Chimaera was removed by VSEARCH –uchime_ref against the SILVA database [47]. Feature tables were created by vsearch –usearch_global. The taxonomy of the features was classified by the USEARCH sintax algorithm in SILVA v123. Diversity analysis was carried out using the vegan v2.5-6 package (https://cran.r-project.org/web/packages/vegan/), and visualised by using the ggplot2 v3.3.2 (https://cran.r-project.org/web/packages/qqman/) package in R v4.0.2. LEfSe was conducted with the online platform ImageGP (http://www.ehbio.com/ImageGP-/index.php/Home/Index/LEFSe.html)[48]. Functional profile prediction of microbial communities was conducted by PICRUSt [49], with the green genes as the reference database.

SCFA concentration determination

Three hundred caecal samples, the same as the those used for amplicon sequencing population, were used for SCFA concentration determination. Briefly, samples were thawed on ice, approximately 50 g of

sample was added to 400 μl of saturated sodium chloride solution, and 50 μl 3 mmol of saturated sodium chloride solution of hydrochloric acid was added. Ultrasonic oscillation at low temperature was conducted for 20 min. Then, 500 μl ether was added, oscillated sufficiently, and extracted for 10 mins. Next, the supernatant was centrifuged for 10 mins at 12000 r/min and 4℃. Then, 50 mg anhydrous sodium sulfate was added into the supernatant and oscillated for 3 mins. Finally, the mixture was centrifuged at 4500 r/min and 4℃ for 5 mins, and the supernatant was used for analysis. A total of 2 μl of solution was analysed by a TRACE1300-TSQ9000 gas chromatography-mass spectrometry (GC-MS) instrument (Thermo Fisher Scientific, Waltham, MA, USA) at Shenzhen BGI Technology Services Co., Ltd. To determine the absolute SCFA concentration, SCFA standards were prepared at different dilutions with ultrapure water. Then, the protocol described above was conducted to generate standard curves for the seven SCFAs.

Evaluating the effects of host genetics on SCFAs

The GWAS for SCFA concentrations was performed directly using the univariate linear mixed model (LMM) implemented in GEMMA version 0.98.1 software (https://github.com/genetics-statistics/GEMMA/releases) [50]. The concentration of SCFAs was log-2 transformed to make them follow a normal distribution (Additional file 5 and 6: Figure S3 and S4). A previous study described the detailed GWAS model in detail [10]. SNP-based heritability is implemented in GCTA (ver 1.93.3) [51].

The genome-wide significance was assessed using the GEC method [52] to infer effective independent tests. A total of 9,540,946 independent tests over all chromosomal SNPs were obtained, and 8,562,703 SNPs were retained. Then, genome-wide significant and suggestive thresholds were set to 5.84e-9 (0.05/8,562,703) and 1.17e-7(1/8,562,703), respectively. Manhattan and Q-Q plots were constructed for each trait by the qqman package (https://cran.r-project.org/web/packages/qqman/) in R (version 4.1.0). SNP positions were updated according to the GRCg6a genome version from NCBI. Identifying the closest genes to genome-wide significant and suggestive variants was performed using NCBI annotation of the GRCg6a genome version (https://www.ncbi.nlm.nih.gov/data-hub/gene/table/taxon/9031/). The variance in SCFAs explained by SNPs from GWAS results was calculated by the formula described by Shim et al [53].

Identification of the specific microbiota association

The associations between qualified taxa, feed efficiency and SCFA traits were analysed using a two-part model described by Fu et al [54]. This model accounts for both binary (present and absent) and quantitative features and is described as follows:

$$y = \begin{cases} \beta_1 b + e \\ \beta_2 q + e \end{cases} \quad (1)$$

where y is the RFI value or SCFA concentration, b is a binary feature of a specific microorganism and coded as 0 for absent or 1 for present for each sample, and q is the log10-transformed abundance of a

specific microorganism. β_1 and β_2 are the regression coefficients for the binary and quantitative models, respectively, and e is the intercept. The second part of the quantitative analysis was only for the samples in which the specific microorganism was present. The details of the two-part model are illustrated in Additional file 7: Figure S5. P values were obtained from the two-part model association analysis and adjusted by the BH method. If the adjusted P value from the binary model was less than 0.05, the presence or absence of microorganisms was considered to influence feed efficiency. If the adjusted P value from the quantitative model was less than 0.05, feed efficiency was considered to be associated with the relative abundances of the microorganisms. If the combined P value was less than 0.05, feed efficiency was considered to be associated with both the relative abundances of the microorganisms and the presence or absence of microorganisms.

A Spearman correlation analysis between microbiotas and RFI and FCR was conducted to detect specific microorganisms that significantly influenced feed efficiency. A microorganism was considered to have a significant effect if the adjusted P values from the two-part model association analysis and Spearman correlation were less than 0.05.

Statistics, plotting and others

Welch's t-test detected the difference in the pathways/taxa relative abundance with FDR correction in STAMP v2.1.3 [55]. Some scripts about data format are from Microbiome helper [56]. All the pipeline, training materials and related scripts were deposited in the EasyAmplicon project in GitHub (https://github.com/YongxinLiu/EasyAmplicon2019). The plots were generated by the ggplot2 package (https://cran.r-project.org/web/packages/ggplot2/) in the R program (version 4.1.0).

Result

Divergent RFI groups had different microbial communities

The means RFIs of the high (HRFI) and low (LRFI) RFI groups were approximately 8 g/d and − 8 g/d, respectively (Additional file 8: Figure S6. A). The richness of the high and low RFI groups was almost higher than 700 (Fig. 2. A), which presents a sufficient detection rate (Additional file 8: Figure S6. B). The PCoA analysis was conducted by Bray_Curtis distance, and the plot shows that the β-diversities of the high and low groups were significantly different (Fig. 2. B). Seven genera are the major composition of chicken in the divergent RFI groups (Fig. 2. C). The detected genus accounted for approximately half of the percentage due to the lower detected ratio, and unassigned taxonomies were allocated to the other part. Twenty-three genera were determined to be significant in terms of the relative abundance based on the Wilcoxon rank-sum test with an FDR adjusted P-value less than 0.05 (Fig. 2. D).

Significant correlations were found between SCFA, microbiota and feed efficiency traits

Comparisons of different SCFAs between the high and low RFI groups were conducted (Fig. 3. A). The propionate concentration in the HRFI group (0.090) was significantly higher than that in the LRFI group (0.081). The similar trend was also found for butyrate (0.177 vs. 0.153) between HRFI and LRFI groups.

Spearman correlations between SCFAs and growth traits were conducted among individuals (Fig. 3. B). Propionate and butyrate were positively correlated with RFI but negatively correlated with BRW and RBR. Propionate was positively correlated with ADFI. The correlations between SCFAs and family were calculated. *Christensenellaceae* was negatively correlated with propionate, and *Christensenellaceae* and *Bacillaceae* were negatively correlated with butyrate (Fig. 3. C). And only relative abundance of *Christensenellaceae* significantly differed in divergent RFI groups in above comparisons analysis. Moreover, the heatmap of Spearman correlations between SCFAs and genera can be found in Additional file 9: FigureS7. Obvious significant correlations between microbiota and SCFAs and growth traits were found.

Microbial KEGG pathways were predicted through the Greengenes database through PICRUSt. For divergent RFI groups (Fig. 4. A), the enriched differential metabolic pathways included amino acid metabolism, lipid metabolism, nucleotide metabolism, glycan biosynthesis and metabolism, and informative pathways included transcription, genetic information process and cellular processes and signalling. Groups with divergent propionate concentrations were used to conduct the KEGG prediction as propionate had the highest correlation with RFI. The top 45 propionate individuals were divided into high group (HPA, mean: 0.127), and the bottom 52 propionate individuals were divided into low group (LPA, mean: 0.046) (Additional file 10: Figure S8. A). Four types of pathways were significantly differentially enriched between the divergent PA groups (Fig. 4. B), including transcription, neurodegenerative diseases, amino acid metabolism and membrane transport. The common enriched pathways in these two groups were found to be transcription and amino acid metabolism. The microbiota between the RFI groups could cause the difference in SCFA concentration.

SCFA-related genetic variations and their effects on gut microbiota

The results showed that SCFAs had significant correlations with growth traits and gut microbiota. Thus, the association between the host genome and SCFA was examined through GWAS. Only butyrate, propionate, valerate and isovalerate were significantly associated with host variation. The Manhattan and QQ plots of propionate illustrate the correlation with feed efficiency and the significant signals found on the host genome (Fig. 5. A). Furthermore, the Manhattan and QQ plots of the other three SCFAs can be found in Additional file 11: Figure S9. The SNP-based heritability of four SCFAs ranged from 0.183 to 0.401, and the annotation of GWAS for SCFAs is shown in Table 1. One locus (gga4: 29,414,391 – 29,417,189) of propionate showed significant signals. *MAML3, MGST2*, and *SETD7* were found in a 100 kb upstream and downstream region of the Top SNP (Fig. 5. B). *MAML3* was found involved Notch signaling pathway, *MGST2* participated in glutathione metabolism, metabolism of xenobioticsm drug metabolism and metabolic pathways, *SETD7* plays roles in lysine degradation, metabolic pathways and FoxO signalling pathway. Moreover, the Top SNP found by GWAS of propionate explained approximately 8.43% of the phenotypic variance, and all of the suggestive significant SNPs were located in the intro regions of the genes (Additional file 12: TableS3). The variation in gga4: 29,417,189 resulted from a base transversion (G/A). Birds with the major genotypes had lower propionate concentration than those with the other two genotypes. The average propionate concentrations for the GG, AG and AA genotypes were

0.074, 0.096, and 0.131 ug/mg, respectively (Fig. 5. C). Meanwhile, chickens with the GG had a better feed efficiency, with low RFI (-0.630 vs. 1.467) and FCR (1.658 vs. 1.685) compared to those of the AG genotype. However, the feed efficiency of the AA genotype was not significantly different from that of the other two genotypes (Fig. 5. D&E). To further investigate the combined effects of the genotypes on phenotypes, microbiota and SCFAs, the differences were analysed among the different genotypes using a Wilcoxon rank-sum test. In addition to propionate, acetate, butyrate, and valerate also showed differential concentrations among the different genotypes. The relative abundance of fourteen taxa, including one phylum, one class, one order, three families and seven genera, differed significantly among different genotypes (Fig. 5. F).

Table 1
Annotation of the GWAS results on SCFAs

Traits	SNP-h^2	GGA	Locus	Top SNP	MAF	Gene	Pathway
Propionate	0.183	4	29,414,391 - 29,417,189	29,417,189	0.191	*MAML3*	Notch signaling pathway
						MGST2 (50k upstream)	Glutathione metabolism Metabolism of xenobiotics Drug metabolism Metabolic pathways
						SETD7 (70k upstream)	Lysine degradation Metabolic pathways FoxO signalling pathway
Butyrate	0.242	2	-	11,072,248	0.106	NA	
		7	-	1,661,848	0.253	lnc_RNA	
Valerate	0.389	6	28,682,597 - 28,802,237	28,682,597	0.261	*TDRD1*	
		6		28,792,315	0.362	*ABLIM1*	
		6		28,721,051	0.375	*AFAP1L2*	
Isovalerate	0.401	6	28,682,597 - 28,802,237	28,682,597	0.261	*TDRD1*	
		6		28,721,051	0.374	*AFAP1L2*	

The column " SNP-h^2" is the SNP-based heritability; the column "GGA" is the Gallus gallus chromosome; the column "Locus" is the region of SNPs above the suggestive line; the column "Top SNP" is highest the P-value SNPs in each gene; the column "MAF" is the allele frequency of the first listed marker.

Christensenellaceae_R-7_group **was identified as the biomarker related to the host genome, feed efficiency and SCFAs**

As described above, 14 taxa were associated with the top SNP associated with propionate. Two-part association and Spearman correlation analyses were used to identify the microbial taxa related to feed

efficiency and propionate. The results of the two-part association model are presented in Additional file 13: Table S4. RFI and FCR were used to identify the representative taxa for feed efficiency traits, and 21 taxa were found (Additional file 14: Figure S10. A). Eight taxa were identified as associated with propionate using the same method (Additional file 14: Figure S10. B). The biomarkers were selected based on the intersection of the SNP-affected taxa and the taxa related to feed efficiency and propionate concentration. Two taxa, *Christensenellaceae* and *Christensenellaceae_R-7_group*, were identified as biomarkers related to host genome, feed efficiency and propionate concentration (Fig. 6. A). These taxa were also detected in the microbial composition detection among divergent RFI and PA groups (Fig. 2 & Additional file 1: Figure S8. D). Significant negative correlations were found with RFI, FCR and propionate concentration. Slight negative correlations were found with BW40 and ADFI (Fig. 6. B). By comparing the relative abundance of these two taxa, it was found that *Christensenellaceae_R-7_group* was the only genus found in *Christensenellaceae* family, because of the mean proportions of these two taxa were the same. The relative abundance of *Christensenellaceae_R-7_group* was approximate 1 ~ 2%, and higher relative abundance was found in LRFI (Fig. 6. C) and LPA group (Fig. 6. D).

Discussion

The effect of RFI-related traits on the microbial community

Feed efficiency is a complex trait influenced by feed-intake and body weight. Early feed efficiency studies reported correlations between the gut microbiota and gut microbial community [21, 25]. The lack of difference in α-diversity between the HRFI and LRFI groups here agrees with the results of previous studies in chickens [23], and similar results were also found in pigs [57]. Different groups from one population might show a similar α-diversity, and divergent RFI selection of pig lines indicated different α-diversity [58]. A difference in β-diversity was discovered in the divergent RFI groups, indicating that the specific microbiota affects RFI. However, different breeds and diets can influence the microbial composition, leading to different RFI related microbiotas with different traits [12, 23, 59, 60]. There was a faecal microbiota transplant (FMT) trial that identified that the faecal microbiota from chickens with high feeding efficiency could improve the feed efficiency in other chickens, and three microbial taxa (*Lactobacillus, Dorea, and Ruminococcus*) changed in abundance after chickens received this treatment [24]. Our results agree with Metzler's result [24], one member of Ruminococcus (*Ruminococcaceae_UCG_014*) played an essential role in the low-RFI group, two members of Ruminococcus (*Ruminococcaceae_UCG_008* and *Ruminococcaceae_UCG_009*) and Lactobacillus were enriched in high-RFI chickens. It was reported that a positive association between improved feed efficiency and the relative abundance of *Butyricicoccus* and *Faecalibacterium* is considered to be beneficial for the health of the animals [61]. However, our results did not agree with some previous studies, even in a white broiler population. In recent reports, *Oscillibacter* and *Butyricicoccus* were more abundant in low-RFI chickens, and *Subdoligranulum variabile* and two *Peptostreptoccaceae* members were negatively correlated with feed efficiency [23]. In our study, *Butyricicoccus* presented a slightly positive correlation with RFI and nearly no correlation with SCFAs. Meanwhile, *Subdoligranulum* showed a negative correlation with RFI, and a slightly negative correlation with butyrate, propionate and acetate

concentrations. These microbial taxa showed correlations with SCFAs, which could be a primary explanation for why propionate and butyrate concentrations were significantly correlated with RFI.

SCFAs are representative metabolites of gut microbiota function in feed efficiency

Our study found that propionate and butyrate concentration were significantly different between the HRFI and LRFI groups. Propionic acid is beneficial to the human body as it may play a role in satiety and energy homeostasis via specific mechanisms, including activation of free fatty acid receptors, reducing lipogenesis levels and glucose homeostasis [2]. Butyrate, the anionic part of dissociated butyric acid and its salts, has been implicated in various host physiological functions, including energy homeostasis, obesity, immune system regulation, cancer, and even brain function [62, 63]. Butyrate was reported to induce the relative mRNA expression of Mucin 2 and its secretion in goblet-like cells [64], as well as promote the assembly of occludin through the AMPK pathway [65].

Christensenellaceae and *Christensenellaceae_R-7_group* were the two taxa found to be biomarkers that showed correlations with propionate concentration, feed efficiency and locus genotype effects. *Christensenellaceae* has been widely investigated in the human gut, which suggests that it is highly heritable, regulated by host genetics and inversely related to host body mass index (BMI) [66, 67]. High feed efficiency traits are usually accompanied by leanness and health performance, and it was reported that *Christensenellaceae* was more enriched in the leaner individuals [68]. Faecal propionate concentration was negatively correlated with feed efficiency [69]. A similar result was reported by Wang et al [70], who showed that probiotics improved the feed efficiency, with decreased propionate concentration in the rumen. In a previous broiler study, butyrate supplementation improved feed efficiency without affecting the growth rate and decreasing abdominal fat deposition [71]. In this study, butyrate and propionate concentration showed positive correlations with RFI, which indicated negative correlations with feed efficiency. One hypothesis is that a larger proportion of butyrate was transported into the blood. Some literature reported that blood butyrate concentrations could be associated with feed efficiency [72, 73]. An in vivo/vitro assay reported that approximately 90% of SCFA would be absorbed in the hindgut [74]. However, in our study, blood metabolites were not measured. The dynamic balance between the lumen concentration and blood concentration of butyrate could be a focus of future studies.

The association between SCFA and host genome variants

Our study proved that seven caecal SCFAs were moderately to highly heritable and was the first to conduct the GWAS for caecal SCFAs. SNPs associated with propionate concentration were located near *MAML3, SETD7* and *MGST2. MAML3*, one protein of the Mastermind-like proteins family, is a transcriptional coactivator for Notch signalling, and Notch signalling plays a pivotal role in development and homeostasis [75, 76]. *MAML3* was also associated with metastatic and WNT signalling activation [77]. Notch and WNT signalling pathways are critical components of the intestinal stem cell signalling network [78]. The weighted SNPs were annotated with *SETD7*, which is involved in lysine degradation in pigs with low feed efficiency [79]. Lysine intake plays an essential role in intestinal lysine transport and promotes feed intake associated with the piglet gut microbiome [80]. No reports have reported the

correlation between feed efficiency and *MGST2*, but *MGST2* was not enriched in a range of metabolic pathways, indicating a potential effect on feed efficiency [81]. Similar regions and genes were found to be associated with valerate and isovalerate. *TDRD* is associated with spermatogenesis [82]. *AFAP1L2* is an adaptor activator of the PI3K-AKT pathway [83], and *ABLIM1* is involved in the PI3K/Akt/Rac1 pathway [84]. These results agreed with those of previous studies showing that SCFAs function as signalling molecules in several pathways [85]. In this study, the propionate associated genomic variants showed a correlation with feed efficiency traits, indicating that the function of *MAML3, SETD7* and *MGST2*, needs further investigation.

The regulation mechanism of feed efficiency with SCFA involved

SCFAs are the major end products from the fermentation of gut microbiota. Propionate can be biosynthesized from the succinate, acrylate and propanediol pathways by using succinate, lactate and deoxyhexose sugar as substrates, respectively [86, 87]. Butyrate can be transformed from butyryl-CoA by phosphotransbutyrylase and butyrate kinase in a direct pathway and by the butyryl-CoA:acetate CoA-transferase route [88, 89]. In our study, the divergent RFI and PA groups had significant differential pathways in transcription and amino acid metabolism. Genes encoding SCFA producing enzymes were found to be active in a range of microbial strains [90, 91]. Thus, enzyme activity could be a part of explaining the differential transcription levels observed in the present study. Pyruvate is involved in many energy metabolism pathways and is correlated with butyryl-CoA and acetate CoA metabolism, which are substrates for SCFA production. Moreover, pyruvate was reported to be a product of amino acid metabolism [92–94]. Thus, the difference in amino acid metabolism found in the RFI and PA divergent groups could be explained by the fact that pyruvate was produced by amino acid metabolism. This resulted in different SCFA levels, and these varied SCFA concentrations could influence RFI.

Conclusion

Our study provides strong evidence of a combined effect of host genetics and gut microbial metabolites, specifically SCFAs, in regulating feed efficiency traits. Our study concluded that host genetic variation could regulate the caecal microbially derived SCFAs, which play a role in host feed efficiency related traits. The SNP-based heritability results suggest that the SCFAs had moderate to high heritabilities (h^2 = 0.183 ~ 0.401). The GWAS showed that four out of seven SCFAs have significant associations with genome variants. SCAFs concentrations, microbiota and feed efficiencies were significantly different among different genotypes for the top SNP. Caecal *Christensenellaceae* and *Christensenellaceae_R-7_group* were identified as biomarkers contributing to the combined effect of host genetics and SCFAs on chicken feed efficiency.

Abbreviations

SCFA: Short-chain fatty acid; FE: Feed efficiency; FI: Feed intake; FCR: Feed conversion ratio; RFI: Residual feed intake; GWAS: Genome-wide association study; GIT: Gastrointestinal tract; MAF: Minor Allele

Frequency; LD: Linkage disequilibrium; ASV: Amplicon sequence variants; GC-MS: Gas chromatography-mass spectrometry; LMM: Linear mixed model; PA: Propionate; FMT: Fecal microbiota transplant; BMI: Body mass index.

Declarations

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Authors' contributions

ZH contributed to performing the study, the analysis of data and the writing of the manuscript. RL contributed to the analysis, interpretation of data, and reviewing of the manuscript. QW & JW contributed to the analysis of data and reviewing of the manuscript. JZ & JD contributed to the sample and data collection. AF & GZ contributed to the design of the study, interpretation of data, and reviewing of the manuscript.

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Availability of data and materials

The sequencing data generate in this study are available in the Genome Sequence Archive (GSA: https://ngdc.cncb.ac.cn/gsa/). The accession number of 16S RNA gene sequencing data is CRA005940. The accession number of resequencing data is CRA006625.

Ethics approval and consent to participate

All experimental procedures with broilers were performed according to the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). Ethical approval on animal survival was given by the animal welfare and ethics committee of the Institute of Animal Sciences (IAS), Chinese Academy of Agricultural Sciences (CAAS, Beijing, China) with the following reference number: IAS2020–83.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sell-Kubiak E, Wimmers K, Reyer H, Szwaczkowski T: Genetic aspects of feed efficiency and reduction of environmental footprint in broilers: a review. *J Appl Genet* 2017, 58(4):487-498.http://doi.org/10.1007/s13353-017-0392-7
2. Byrne CS, Chambers ES, Morrison DJ, Frost G: The role of short chain fatty acids in appetite regulation and energy homeostasis. *International journal of obesity (2005)* 2015, 39(9):1331-1338.http://doi.org/10.1038/ijo.2015.84
3. So PW, Yu WS, Kuo YT, Wasserfall C, Goldstone AP, Bell JD, Frost G: Impact of resistant starch on body fat patterning and central appetite regulation. *PLoS One* 2007, 2(12):e1309.http://doi.org/10.1371/journal.pone.0001309
4. Atchley WR, Gaskins CT, Anderson D: Statistical Properties of Ratios. I. Empirical Results. *Systematic Biology* 1976, 25(2):137-148.http://doi.org/10.2307/2412740 %J Systematic Biology
5. Archer JA, Arthur PF, Herd RM, Parnell PF, Pitchford WS: Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. *J Anim Sci* 1997, 75(8):2024-2032.http://doi.org/10.2527/1997.7582024x
6. Kennedy BW, van der Werf JH, Meuwissen TH: Genetic and statistical properties of residual feed intake. *J Anim Sci* 1993, 71(12):3239-3250.http://doi.org/10.2527/1993.71123239x
7. Pakdel A, van Arendonk JA, Vereijken AL, Bovenhuis H: Genetic parameters of ascites-related traits in broilers: correlations with feed efficiency and carcase traits. *British poultry science* 2005, 46(1):43-53.http://doi.org/10.1080/00071660400023805
8. N'Dri AL, Mignon-Grasteau S, Sellier N, Tixier-Boichard M, Beaumont C: Genetic relationships between feed conversion ratio, growth curve and body composition in slow-growing chickens. *British poultry science* 2006, 47(3):273-280.http://doi.org/10.1080/00071660600753664
9. Aggrey SE, Karnuah AB, Sebastian B, Anthony NB: Genetic properties of feed efficiency parameters in meat-type chickens. *Genetics Selection Evolution* 2010, 42(1):25.http://doi.org/10.1186/1297-9686-42-25
10. Li W, Liu R, Zheng M *et al*: New insights into the associations among feed efficiency, metabolizable efficiency traits and related QTL regions in broiler chickens. *J Anim Sci Biotechnol* 2020, 11:65.http://doi.org/10.1186/s40104-020-00469-8
11. Li W, Zheng M, Zhao G *et al*: Identification of QTL regions and candidate genes for growth and feed efficiency in broilers. *Genetics Selection Evolution* 2021, 53(1):13.http://doi.org/10.1186/s12711-021-00608-3
12. Wen C, Yan W, Mai C, Duan Z, Zheng J, Sun C, Yang N: Joint contributions of the gut microbiota and host genetics to feed efficiency in chickens. *Microbiome* 2021, 9(1):126.http://doi.org/10.1186/s40168-021-01040-x

13. Zuidhof MJ, Schneider BL, Carney VL, Korver DR, Robinson FE: Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poultry Science* 2014, 93(12):2970-2982.http://doi.org/https://doi.org/10.3382/ps.2014-04291
14. Grice EA, Segre JA: The Human Microbiome: Our Second Genome. *Annual Review of Genomics and Human Genetics* 2012, 13(1):151-170.http://doi.org/10.1146/annurev-genom-090711-163814
15. Maki JJ, Klima CL, Sylte MJ, Looft T: The Microbial Pecking Order: Utilization of Intestinal Microbiota for Poultry Health. *Microorganisms* 2019, 7(10).http://doi.org/10.3390/microorganisms7100376
16. Siegerstetter SC, Schmitz-Esser S, Magowan E *et al*: Intestinal microbiota profiles associated with low and high residual feed intake in chickens across two geographical locations. *PLoS One* 2017, 12(11):e0187766.http://doi.org/10.1371/journal.pone.0187766
17. Bae Y, Koo B, Lee S, Mo J, Oh K, Mo IP: Bacterial diversity and its relationship to growth performance of broilers. *Korean J Vet Res* 2017, 57(3):159-167.http://doi.org/10.14405/kjvr.2017.57.3.159
18. Stanley D, Geier MS, Denman SE, Haring VR, Crowley TM, Hughes RJ, Moore RJ: Identification of chicken intestinal microbiota correlated with the efficiency of energy extraction from feed. *Veterinary microbiology* 2013, 164(1-2):85-92.http://doi.org/10.1016/j.vetmic.2013.01.030
19. Yan W, Sun C, Yuan J, Yang N: Gut metagenomic analysis reveals prominent roles of Lactobacillus and cecal microbiota in chicken feed efficiency. *Scientific Reports* 2017, 7(1):45308.http://doi.org/10.1038/srep45308
20. Torok VA, Hughes RJ, Mikkelsen LL *et al*: Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. *Appl Environ Microbiol* 2011, 77(17):5868-5878.http://doi.org/10.1128/aem.00165-11
21. Stanley D, Denman SE, Hughes RJ *et al*: Intestinal microbiota associated with differential feed conversion efficiency in chickens. *Applied Microbiology and Biotechnology* 2012, 96(5):1361-1369.http://doi.org/10.1007/s00253-011-3847-5
22. Singh KM, Shah T, Deshpande S, Jakhesara SJ, Koringa PG, Rank DN, Joshi CG: High through put 16S rRNA gene-based pyrosequencing analysis of the fecal microbiota of high FCR and low FCR broiler growers. *Molecular biology reports* 2012, 39(12):10595-10602.http://doi.org/10.1007/s11033-012-1947-7
23. Liu J, Stewart SN, Robinson K, Yang Q, Lyu W, Whitmore MA, Zhang G: Linkage between the intestinal microbiota and residual feed intake in broiler chickens. *Journal of animal science and biotechnology* 2021, 12(1):22-22.http://doi.org/10.1186/s40104-020-00542-2
24. Metzler-Zebeli BU, Siegerstetter SC, Magowan E, Lawlor PG, NE OC, Zebeli Q: Fecal Microbiota Transplant From Highly Feed Efficient Donors Affects Cecal Physiology and Microbiota in Low- and High-Feed Efficient Chickens. *Front Microbiol* 2019, 10:1576.http://doi.org/10.3389/fmicb.2019.01576
25. Siegerstetter SC, Petri RM, Magowan E, Lawlor PG, Zebeli Q, O'Connell NE, Metzler-Zebeli BU: Feed Restriction Modulates the Fecal Microbiota Composition, Nutrient Retention, and Feed Efficiency in

Chickens Divergent in Residual Feed Intake. *Front Microbiol* 2018, 9:2698.http://doi.org/10.3389/fmicb.2018.02698

26. Diaz Carrasco JM, Casanova NA, Fernandez Miyakawa ME: Microbiota, Gut Health and Chicken Productivity: What Is the Connection? *Microorganisms* 2019, 7(10).http://doi.org/10.3390/microorganisms7100374
27. Grieneisen L, Dasari M, Gould TJ *et al*: Gut microbiome heritability is nearly universal but environmentally contingent. *Science* 2021, 373(6551):181-186.http://doi.org/10.1126/science.aba5483
28. Kurilshikov A, Medina-Gomez C, Bacigalupe R *et al*: Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet* 2021, 53(2):156-165.http://doi.org/10.1038/s41588-020-00763-1
29. Annison EF, Hill KJ, Kenworthy R: Volatile fatty acids in the digestive tract of the fowl. *The British journal of nutrition* 1968, 22(2):207-216.http://doi.org/10.1079/bjn19680026
30. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F: From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* 2016, 165(6):1332-1345.http://doi.org/10.1016/j.cell.2016.05.041
31. Nøhr MK, Pedersen MH, Gille A *et al*: GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* 2013, 154(10):3552-3564.http://doi.org/10.1210/en.2013-1142
32. Nøhr MK, Egerod KL, Christiansen SH, Gille A, Offermanns S, Schwartz TW, Møller M: Expression of the short chain fatty acid receptor GPR41/FFAR3 in autonomic and somatic sensory ganglia. *Neuroscience* 2015, 290:126-137.http://doi.org/10.1016/j.neuroscience.2015.01.040
33. Dorji J, MacLeod IM, Chamberlain AJ *et al*: Mitochondrial protein gene expression and the oxidative phosphorylation pathway associated with feed efficiency and energy balance in dairy cattle. *J Dairy Sci* 2021, 104(1):575-587.http://doi.org/10.3168/jds.2020-18503
34. Menezes ACB, Valadares Filho SC, Benedeti PDB, Zanetti D, Paulino MF, Silva FF, Caton JS: Feeding behavior, water intake, and energy and protein requirements of young Nellore bulls with different residual feed intakes. *J Anim Sci* 2020, 98(9).http://doi.org/10.1093/jas/skaa279
35. Xu Z, Ji C, Zhang Y *et al*: Combination analysis of genome-wide association and transcriptome sequencing of residual feed intake in quality chickens. *BMC genomics* 2016, 17:594-594.http://doi.org/10.1186/s12864-016-2861-5
36. Liu R, Liu J, Zhao G *et al*: Relevance of the intestinal health-related pathways to broiler residual feed intake revealed by duodenal transcriptome profiling. *Poult Sci* 2019, 98(3):1102-1110.http://doi.org/10.3382/ps/pey506
37. Xiao C, Deng J, Zeng L, Sun T, Yang Z, Yang X: Transcriptome Analysis Identifies Candidate Genes and Signaling Pathways Associated With Feed Efficiency in Xiayan Chicken. *Frontiers in Genetics* 2021, 12.http://doi.org/10.3389/fgene.2021.607719

38. Tan Z, Wang Y, Yang T *et al*: Differentially expressed genes in the caecal and colonic mucosa of Landrace finishing pigs with high and low food conversion ratios. *Sci Rep* 2017, 7(1):14886.http://doi.org/10.1038/s41598-017-14568-6
39. DePristo MA, Banks E, Poplin R *et al*: A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011, 43(5):491-498.http://doi.org/10.1038/ng.806
40. Van der Auwera GA, Carneiro MO, Hartl C *et al*: From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Current protocols in bioinformatics* 2013, 43(1110):11.10.11-11.10.33.http://doi.org/10.1002/0471250953.bi1110s43
41. Li H: Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv e-prints* 2013:arXiv:1303.3997
42. McKenna A, Hanna M, Banks E *et al*: The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010, 20(9):1297-1303.http://doi.org/10.1101/gr.107524.110
43. Zhang C, Dong S-S, Xu J-Y, He W-M, Yang T-L: PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics (Oxford, England)* 2018, 35(10):1786-1788.http://doi.org/10.1093/bioinformatics/bty875
44. Liu YX, Qin Y, Chen T, Lu M, Qian X, Guo X, Bai Y: A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein & cell* 2021, 12(5):315-330.http://doi.org/10.1007/s13238-020-00724-8
45. Rognes T, Flouri T, Nichols B, Quince C, Mahé F: VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 2016, 4:e2584.http://doi.org/10.7717/peerj.2584
46. Edgar RC: Search and clustering orders of magnitude faster than BLAST. *Bioinformatics (Oxford, England)* 2010, 26(19):2460-2461.http://doi.org/10.1093/bioinformatics/btq461
47. Quast C, Pruesse E, Yilmaz P *et al*: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research* 2013, 41(Database issue):D590-596.http://doi.org/10.1093/nar/gks1219
48. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C: Metagenomic biomarker discovery and explanation. *Genome Biol* 2011, 12(6):R60.http://doi.org/10.1186/gb-2011-12-6-r60
49. Langille MG, Zaneveld J, Caporaso JG *et al*: Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 2013, 31(9):814-821.http://doi.org/10.1038/nbt.2676
50. Zhou X, Stephens M: Genome-wide efficient mixed-model analysis for association studies. *Nat Genet* 2012, 44(7):821-824.http://doi.org/10.1038/ng.2310
51. Yang J, Lee SH, Goddard ME, Visscher PM: GCTA: A Tool for Genome-wide Complex Trait Analysis. *The American Journal of Human Genetics* 2011, 88(1):76-82.http://doi.org/https://doi.org/10.1016/j.ajhg.2010.11.011

52. Li MX, Yeung JM, Cherny SS, Sham PC: Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Human genetics* 2012, 131(5):747-756.http://doi.org/10.1007/s00439-011-1118-2
53. Shim H, Chasman DI, Smith JD *et al*: A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. *PloS one* 2015, 10(4):e0120758-e0120758.http://doi.org/10.1371/journal.pone.0120758
54. Fu J, Bonder MJ, Cenit MC *et al*: The Gut Microbiome Contributes to a Substantial Proportion of the Variation in Blood Lipids. *Circulation Research* 2015, 117(9):817-824.http://doi.org/doi:10.1161/CIRCRESAHA.115.306807
55. Parks DH, Tyson GW, Hugenholtz P, Beiko RG: STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics (Oxford, England)* 2014, 30(21):3123-3124.http://doi.org/10.1093/bioinformatics/btu494
56. Comeau AM, Douglas GM, Langille MGI, Eisen J: Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. *mSystems* 2017, 2(1):e00127-00116.http://doi.org/doi:10.1128/mSystems.00127-16
57. Si J, Feng L, Gao J *et al*: Evaluating the association between feed efficiency and the fecal microbiota of early-life Duroc pigs using 16S rRNA sequencing. *AMB Express* 2020, 10(1):115.http://doi.org/10.1186/s13568-020-01050-2
58. Aliakbari A, Zemb O, Billon Y, Barilly C, Ahn I, Riquet J, Gilbert H: Genetic relationships between feed efficiency and gut microbiome in pig lines selected for residual feed intake. *Journal of animal breeding and genetics* 2021, 138(4):491-507.http://doi.org/10.1111/jbg.12539
59. Wen C, Yan W, Sun C *et al*: The gut microbiota is largely independent of host genetics in regulating fat deposition in chickens. *ISME J* 2019, 13(6):1422-1436.http://doi.org/10.1038/s41396-019-0367-2
60. Chen C, Fang S, Wei H *et al*: Prevotella copri increases fat accumulation in pigs fed with formula diets. *Microbiome* 2021, 9(1):175-175.http://doi.org/10.1186/s40168-021-01110-0
61. Lingbeek MM, Borewicz K, Febery E, Han Y, Doelman J, van Kuijk SJA: Short-chain fatty acid administration via water acidifier improves feed efficiency and modulates fecal microbiota in weaned piglets. *J Anim Sci* 2021, 99(11).http://doi.org/10.1093/jas/skab307
62. Bourassa MW, Alim I, Bultman SJ, Ratan RR: Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neuroscience letters* 2016, 625:56-63.http://doi.org/10.1016/j.neulet.2016.02.009
63. Di Sabatino A, Morera R, Ciccocioppo R *et al*: Oral butyrate for mildly to moderately active Crohn's disease. *Aliment Pharmacol Ther* 2005, 22(9):789-794.http://doi.org/10.1111/j.1365-2036.2005.02639.x
64. Gao X, Chen Y, Chen Z *et al*: Identification and antimicrobial activity evaluation of three peptides from laba garlic and the related mechanism. *Food Funct* 2019, 10(8):4486-4496.http://doi.org/10.1039/c9fo00236g

65. Peng L, Li ZR, Green RS, Holzman IR, Lin J: Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *The Journal of nutrition* 2009, 139(9):1619-1625.http://doi.org/10.3945/jn.109.104638
66. Goodrich JK, Waters JL, Poole AC *et al*: Human genetics shape the gut microbiome. *Cell* 2014, 159(4):789-799.http://doi.org/10.1016/j.cell.2014.09.053
67. Waters JL, Ley RE: The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. *BMC biology* 2019, 17(1):83-83.http://doi.org/10.1186/s12915-019-0699-4
68. McCormack UM, Curião T, Buzoianu SG *et al*: Exploring a Possible Link between the Intestinal Microbiota and Feed Efficiency in Pigs. *Appl Environ Microbiol* 2017, 83(15).http://doi.org/10.1128/aem.00380-17
69. Jiang H, Fang S, Yang H, Chen C: Identification of the relationship between the gut microbiome and feed efficiency in a commercial pig cohort. *J Anim Sci* 2021, 99(3).http://doi.org/10.1093/jas/skab045
70. Wang H, Yu Z, Gao Z *et al*: Effects of compound probiotics on growth performance, rumen fermentation, blood parameters, and health status of neonatal Holstein calves. *J Dairy Sci* 2021.http://doi.org/10.3168/jds.2021-20721
71. Yang Q, Chen B, Robinson K *et al*: Butyrate in combination with forskolin alleviates necrotic enteritis, increases feed efficiency, and improves carcass composition of broilers. *J Anim Sci Biotechnol* 2022, 13(1):3.http://doi.org/10.1186/s40104-021-00663-2
72. Dechow CD, Baumrucker CR, Bruckmaier RM, Blum JW: Blood plasma traits associated with genetic merit for feed utilization in Holstein cows. *J Dairy Sci* 2017, 100(10):8232-8238.http://doi.org/10.3168/jds.2016-12502
73. Leão JM, Coelho SG, Lage CFA *et al*: How Divergence for Feed Efficiency Traits Affects Body Measurements and Metabolites in Blood and Ruminal Parameters on Pre-Weaning Dairy Heifers. *Animals (Basel)* 2021, 11(12).http://doi.org/10.3390/ani11123436
74. Bai Y, Zhou X, Zhao J *et al*: Sources of Dietary Fiber Affect the SCFA Production and Absorption in the Hindgut of Growing Pigs. *Front Nutr* 2022, 8:719935-719935.http://doi.org/10.3389/fnut.2021.719935
75. Saint Just Ribeiro M, Wallberg AE: Transcriptional mechanisms by the coregulator MAML1. *Current protein & peptide science* 2009, 10(6):570-576.http://doi.org/10.2174/138920309789630543
76. Kitagawa M: Notch signalling in the nucleus: roles of Mastermind-like (MAML) transcriptional coactivators. *Journal of biochemistry* 2016, 159(3):287-294.http://doi.org/10.1093/jb/mvv123
77. Alzofon N, Koc K, Panwell K *et al*: Mastermind Like Transcriptional Coactivator 3 (MAML3) Drives Neuroendocrine Tumor Progression. *Molecular cancer research : MCR* 2021, 19(9):1476-1485.http://doi.org/10.1158/1541-7786.Mcr-20-0992
78. Katoh M, Katoh M: WNT antagonist, DKK2, is a Notch signaling target in intestinal stem cells: augmentation of a negative regulation system for canonical WNT signaling pathway by the Notch-DKK2 signaling loop in primates. *International journal of molecular medicine* 2007, 19(1):197-201

79. Banerjee P, Carmelo VAO, Kadarmideen HN: Genome-Wide Epistatic Interaction Networks Affecting Feed Efficiency in Duroc and Landrace Pigs. *Front Genet* 2020, 11:121.http://doi.org/10.3389/fgene.2020.00121
80. Yin J, Han H, Li Y *et al*: Lysine Restriction Affects Feed Intake and Amino Acid Metabolism via Gut Microbiome in Piglets. *Cellular Physiology and Biochemistry* 2017, 44(5):1749-1761.http://doi.org/10.1159/000485782
81. Thulasingam M, Orellana L, Nji E, Ahmad S, Rinaldo-Matthis A, Haeggström JZ: Crystal structures of human MGST2 reveal synchronized conformational changes regulating catalysis. *Nat Commun* 2021, 12(1):1728.http://doi.org/10.1038/s41467-021-21924-8
82. Honda S, Miki Y, Miyamoto Y, Kawahara Y, Tsukamoto S, Imai H, Minami N: Oocyte-specific gene Oog1 suppresses the expression of spermatogenesis-specific genes in oocytes. *The Journal of reproduction and development* 2018, 64(4):297-301.http://doi.org/10.1262/jrd.2018-024
83. Tanga N, Kuboyama K, Kishimoto A *et al*: The PTN-PTPRZ signal activates the AFAP1L2-dependent PI3K-AKT pathway for oligodendrocyte differentiation: Targeted inactivation of PTPRZ activity in mice. *Glia* 2019, 67(5):967-984.http://doi.org/10.1002/glia.23583
84. Jin SH, Kim H, Gu DR, Park KH, Lee YR, Choi Y, Lee SH: Actin-binding LIM protein 1 regulates receptor activator of NF-κB ligand-mediated osteoclast differentiation and motility. *BMB reports* 2018, 51(7):356-361.http://doi.org/10.5483/bmbrep.2018.51.7.106
85. Gonzalez-Santana A, Diaz Heijtz R: Bacterial Peptidoglycans from Microbiota in Neurodevelopment and Behavior. *Trends in molecular medicine* 2020, 26(8):729-743.http://doi.org/10.1016/j.molmed.2020.05.003
86. Scott KP, Martin JC, Campbell G, Mayer CD, Flint HJ: Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "Roseburia inulinivorans". *Journal of bacteriology* 2006, 188(12):4340-4349.http://doi.org/10.1128/jb.00137-06
87. Hetzel M, Brock M, Selmer T, Pierik AJ, Golding BT, Buckel W: Acryloyl-CoA reductase from Clostridium propionicum. An enzyme complex of propionyl-CoA dehydrogenase and electron-transferring flavoprotein. *European journal of biochemistry* 2003, 270(5):902-910.http://doi.org/10.1046/j.1432-1033.2003.03450.x
88. Duncan SH, Barcenilla A, Stewart CS, Pryde SE, Flint HJ: Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl Environ Microbiol* 2002, 68(10):5186-5190.http://doi.org/10.1128/aem.68.10.5186-5190.2002
89. Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ: Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *Journal of bacteriology* 2004, 186(7):2099-2106.http://doi.org/10.1128/jb.186.7.2099-2106.2004
90. Louis P, Young P, Holtrop G, Flint HJ: Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol* 2010, 12(2):304-314.http://doi.org/10.1111/j.1462-2920.2009.02066.x

91. Sheridan PO, Louis P, Tsompanidou E *et al*: Distribution, organization and expression of genes concerned with anaerobic lactate utilization in human intestinal bacteria. *Microbial genomics* 2022, 8(1).http://doi.org/10.1099/mgen.0.000739

92. Liu K, Yu H, Sun G *et al*: Semi-rational design of L-amino acid deaminase for production of pyruvate and D-alanine by Escherichia coli whole-cell biocatalyst. *Amino acids* 2021, 53(9):1361-1371.http://doi.org/10.1007/s00726-021-03067-8

93. Weghoff MC, Bertsch J, Müller V: A novel mode of lactate metabolism in strictly anaerobic bacteria. *Environ Microbiol* 2015, 17(3):670-677.http://doi.org/10.1111/1462-2920.12493

94. Huynh TYL, Oscilowska I, Sáiz J, Nizioł M, Baszanowska W, Barbas C, Palka J: Metformin Treatment or PRODH/POX-Knock out Similarly Induces Apoptosis by Reprograming of Amino Acid Metabolism, TCA, Urea Cycle and Pentose Phosphate Pathway in MCF-7 Breast Cancer Cells. *Biomolecules* 2021, 11(12).http://doi.org/10.3390/biom11121888

Figures

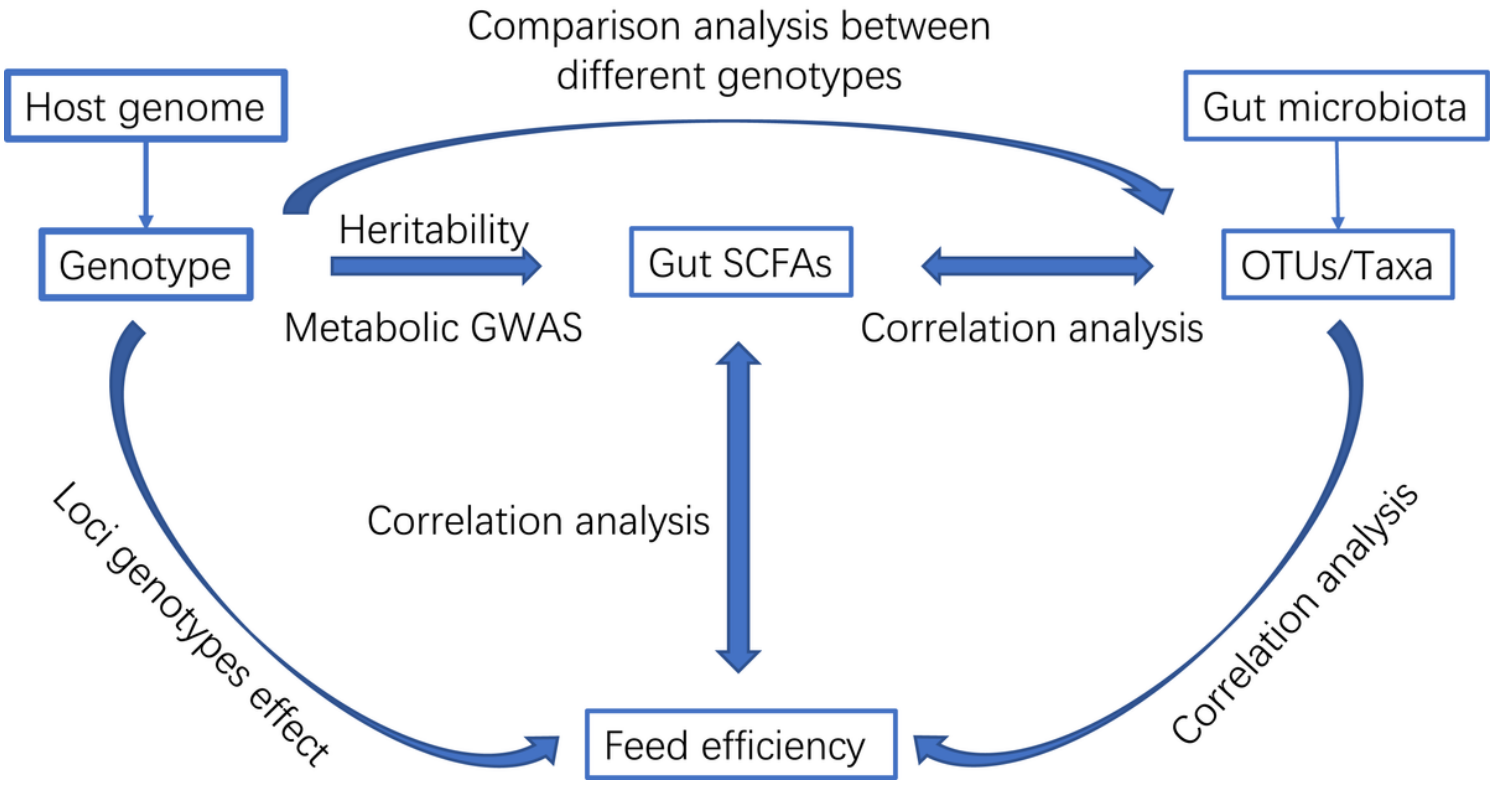

Figure 1

The overall workflow.

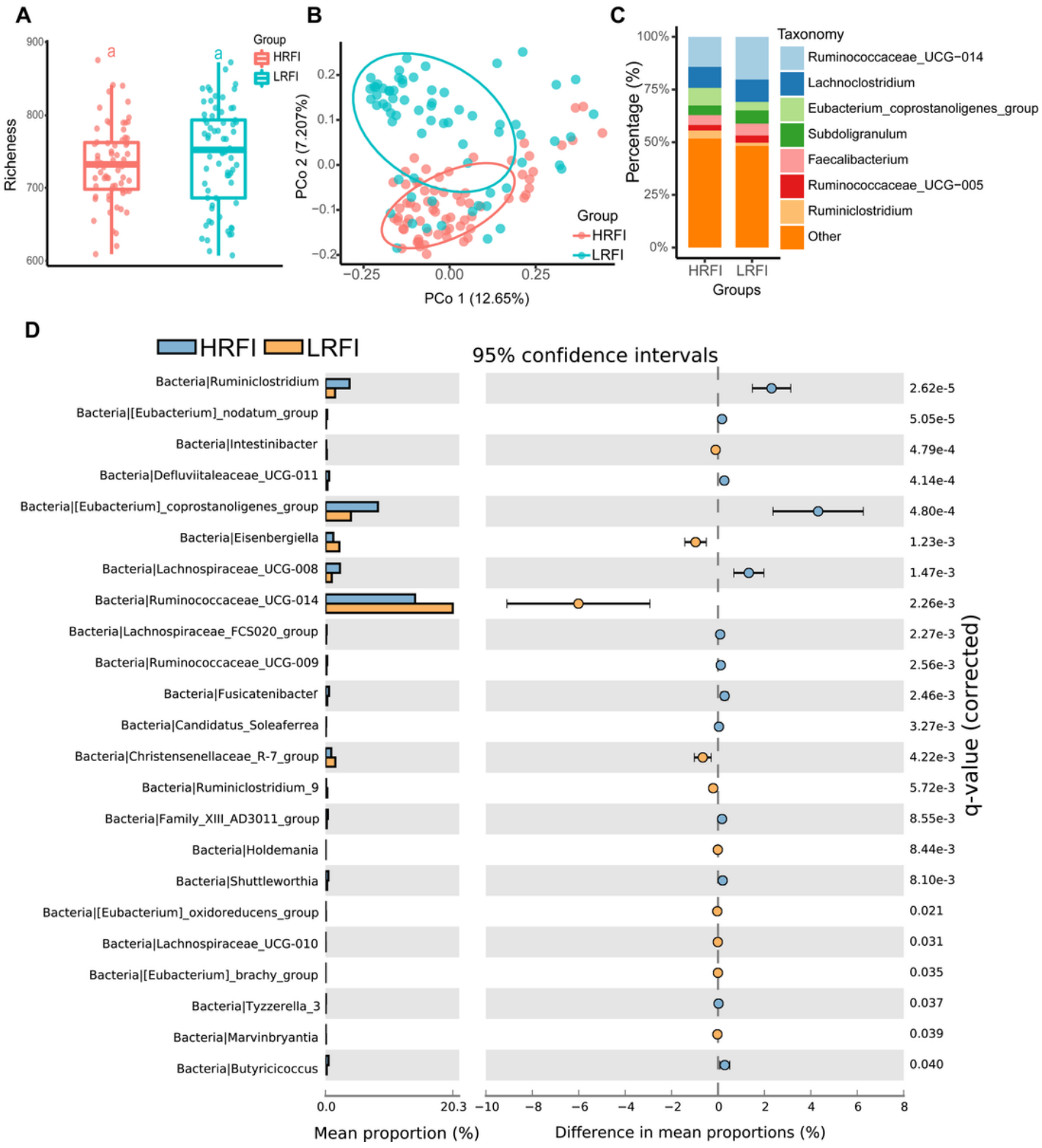

Figure 2

The microbiota composition between the high RFI and low RFI groups. A. The α-diversity index richness compared between the two groups. B. The Principal Co-ordinates Analysis on the ASV level. C. The cecum microbiota composition of two groups on the genus level. D. Comparisons of genus components between the two groups.

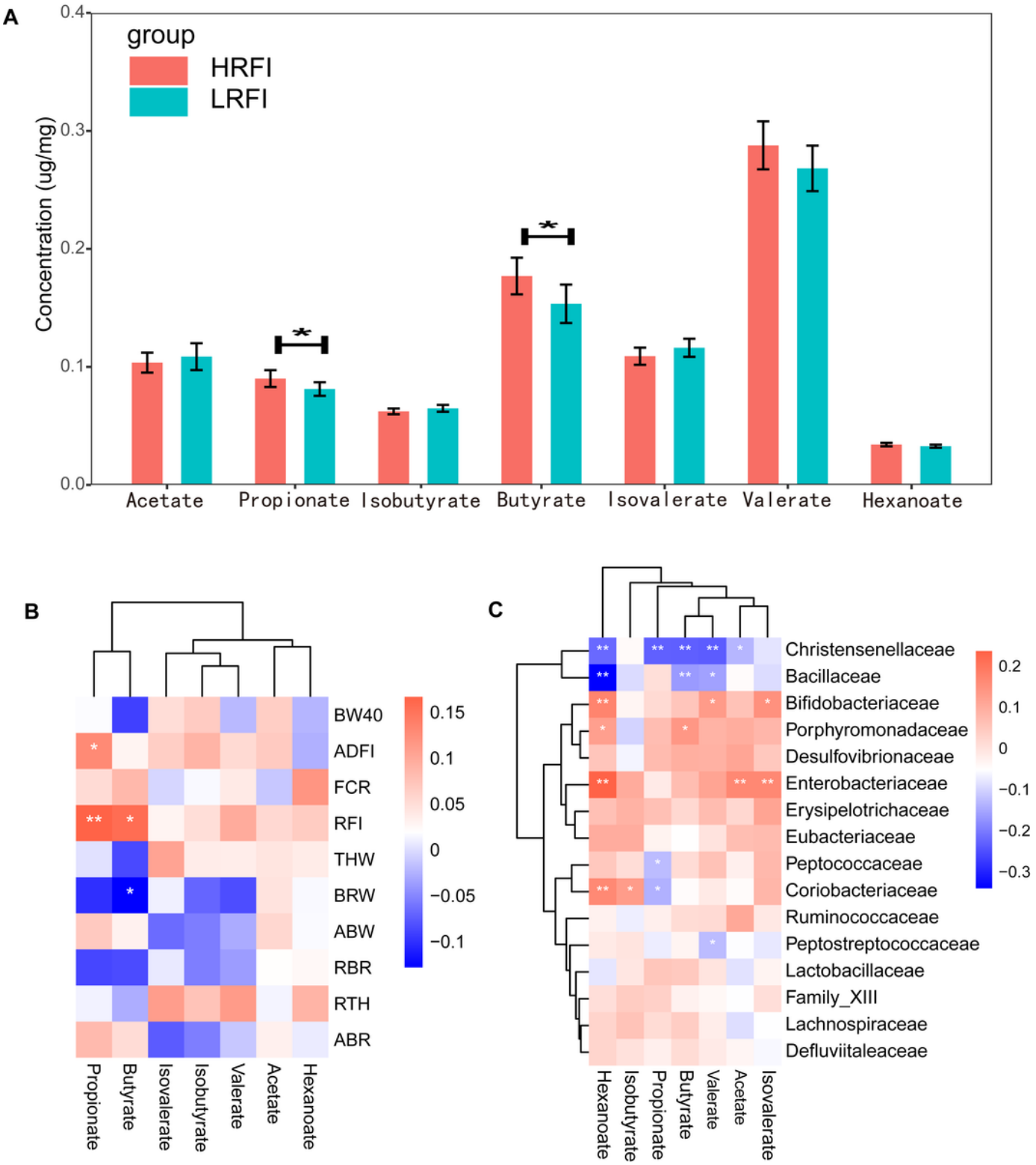

Figure 3

Correlation between SCFAs and feed efficiency and microbial biomarkers of feed efficiency. A. Bar plots of the concentration of SCFAs among high and low RFI groups. B. Spearman correlation between SCFAs and growth performance. C. Spearman correlation between SCFAs and taxa at the family level.

BW40: body weight at 40 days of age; ADFI: average daily feed intake; THW: thigh weight; BRW: breast weight; ABW: abdominal fat weight; RTH: ratio of thigh weight; RBR: ratio of breast weight; ABR: ratio of abdominal fat.

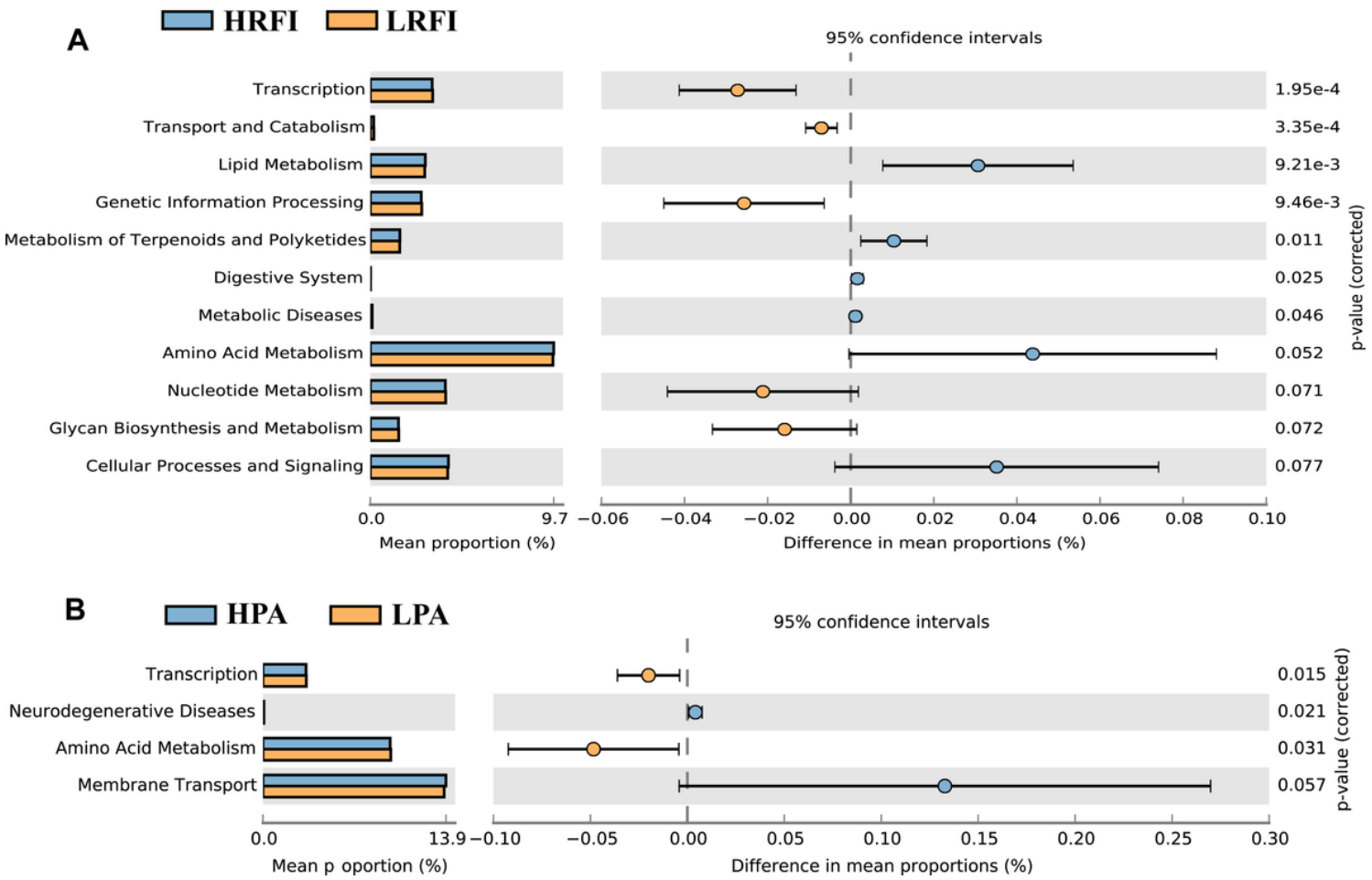

Figure 4

KEGG pathway prediction by the Greengenes database. A. Comparison between divergent RFI groups. B. Comparison between divergent Propionate group.

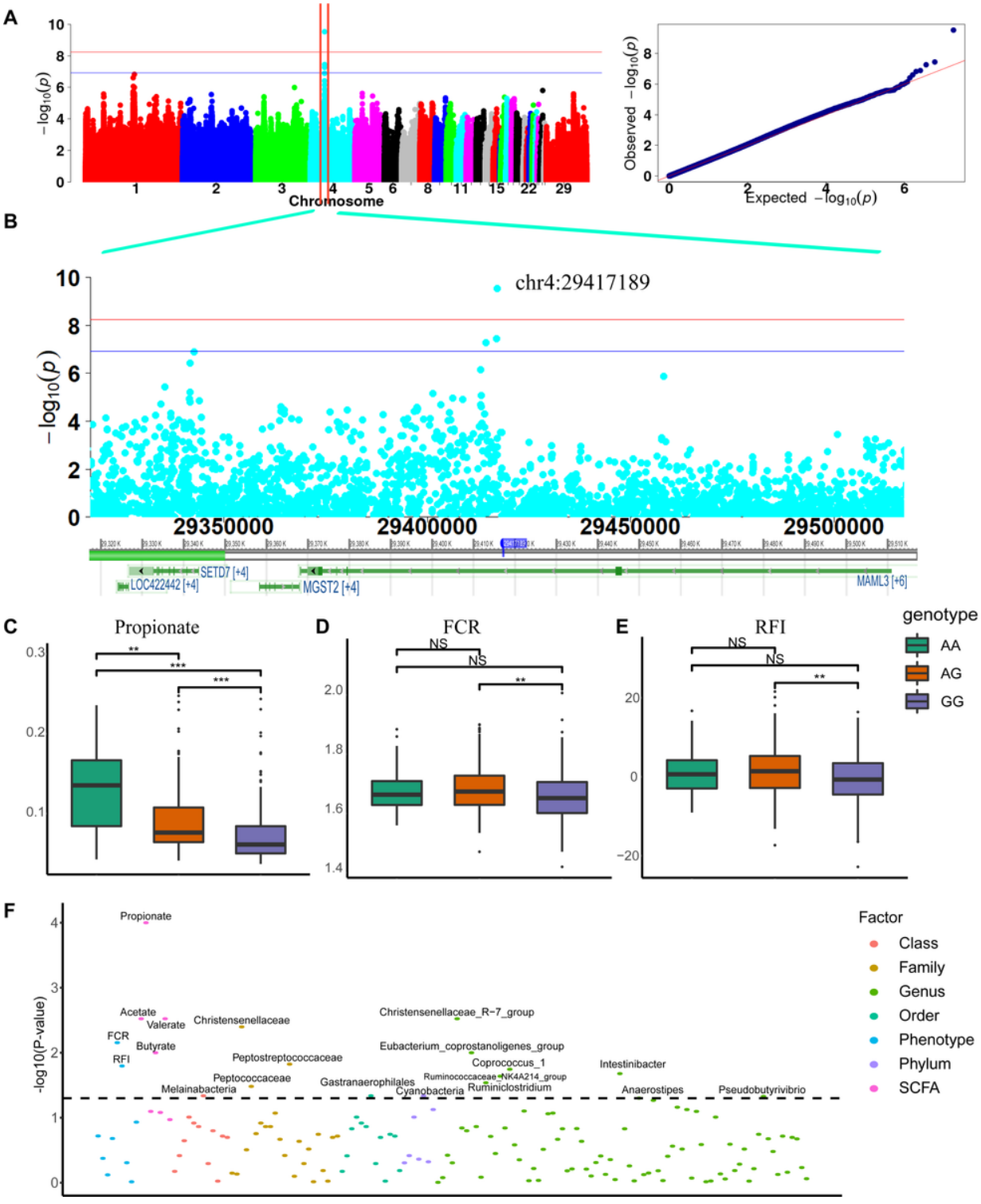

Figure 5

GWAS for propionate and associated SNP effects on RFI and microbiota. A. The Manhattan and QQ plot of propionate. B. The significant region on chromosome 4 and gene distribution. C D E. The effect of loci genotyping on propionate, FCR and RFI, ***, ** and ns represent adjusted P values < 0.001, < 0.01, and > 0.05, respectively. F. Overview of locus effect on grow performance, SCFA, and microbiota.

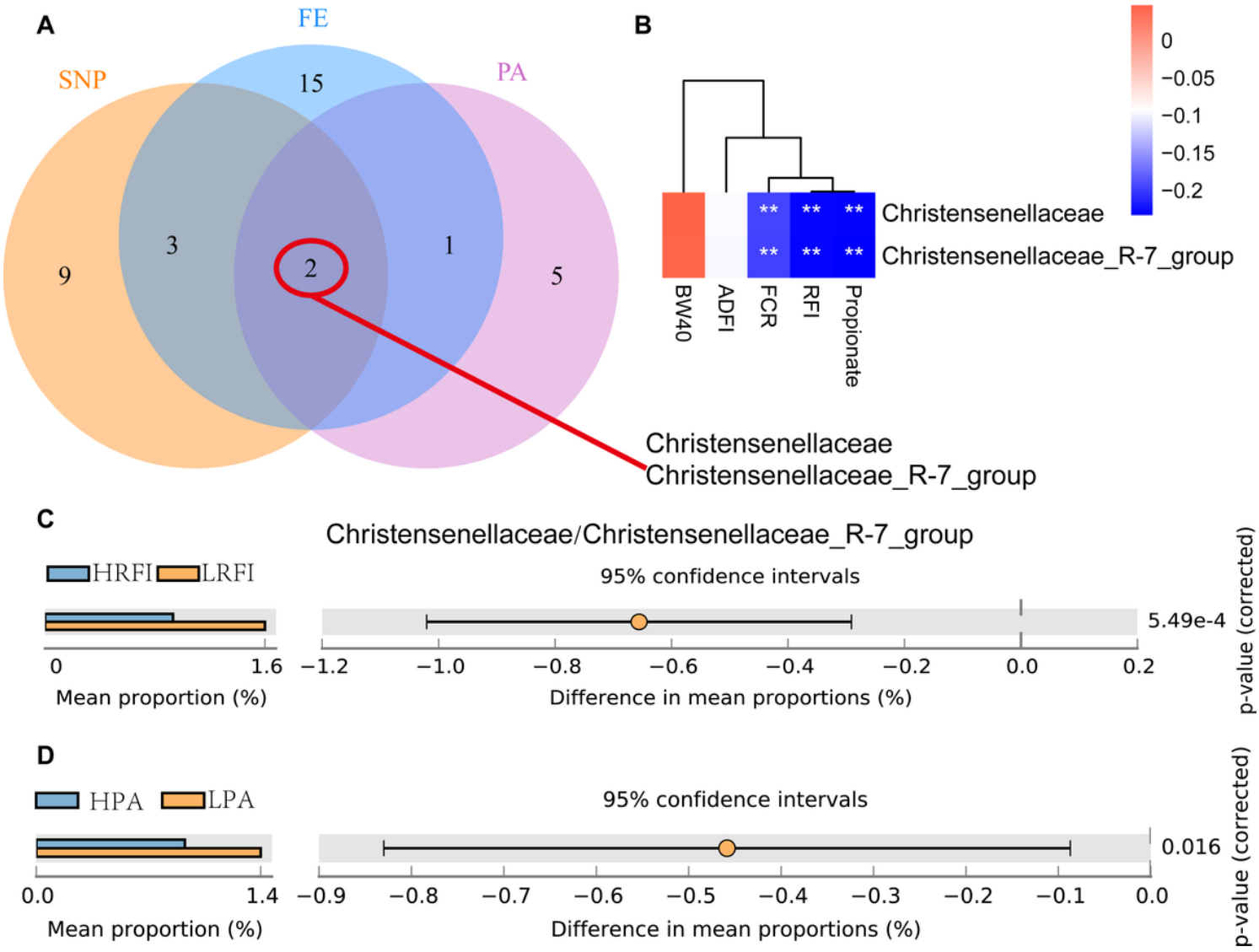

Figure 6

Biomarker determination from feed efficicency, propionate and SNP genotyping effects. A. The Venn diagram of selecting the biomarkers from SNP (locus genotypes), FE (feed efficiency) and PA (propionate) effects. B. Spearman correlation between the biomarkers and different traits. C & D. Comparisons of Christensenellaceae/Christensenellaceae_R-7_group among divergent RFI and PA groups, respectively.

Supplementary Files

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