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Host genomic regulation of ileal *Bradyrhizobium* abundance modulates eating speed and fat metabolism in birds: a causal mechanism study

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Abstract

Background Eating speed is a key eating behavior trait that influences energy intake and fat deposition, yet its regulation by host genetics and gut microbiota remains poorly understood in birds.

Results We systematically investigated the interplay among host genetics, gut microbiota, eating speed, and fat deposition in chickens. Phenotypic analyses revealed a positive association between eating speed and abdominal fat, and Mendelian randomization (MR) analysis identified a bidirectional feedback loop in which fat deposition promotes faster eating, which in turn exacerbates fat accumulation. Microbiome and MR analyses highlighted the ileal genus *Bradyrhizobium* as a causal regulator of both eating speed and fat deposition, with higher abundance reducing abdominal fat, triglyceride levels, and inflammatory markers. Microbiome genome-wide association studies (mGWAS) further identified host genetic variants and candidate genes, including convergent signals at *RECK*, influencing *Bradyrhizobium* abundance. Mediation analyses indicated that *Bradyrhizobium* modulates eating speed partially through its effects on abdominal fat, emphasizing a host-microbe-behavior feedback axis.

Conclusions Our findings reveal a complex interplay among host genetics, gut microbes, and eating behavior, providing mechanistic insights and potential targets for precision interventions to optimize growth and metabolic health in poultry.

Keywords Abdominal fat, *Bradyrhizobium*, Eating speed, Host genetics, Mendelian randomization

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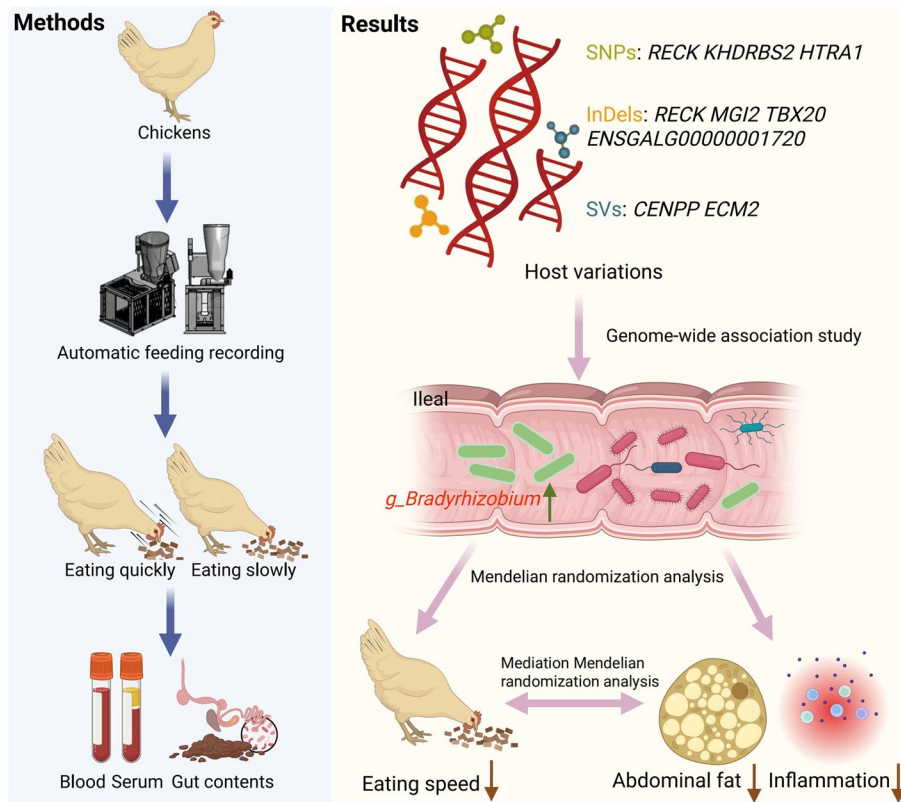
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Graphical Abstract



Background

Eating is a crucial behavioral pattern through which organisms acquire nutrients, and the alternating dominance of hunger and satiety serves as the intrinsic mechanism regulating eating behavior [1]. Eating behavior encompasses not only the total amount and frequency of food intake, but also the eating speed—a key trait that affects energy intake efficiency and metabolic status [2]. The regulation of various eating behaviors, including eating speed, is influenced by two main mechanisms. The first is associated with the host's energy homeostasis. Specifically, metabolic hormones such as insulin, leptin, and glucocorticoids act as signaling molecules that convey the body's energy status to the central nervous system, thereby modulating eating behavior [3]. The second mode involves communication between the gut microbiota and the host. Gut microbes not only regulate their own growth and maintain population levels in the intestine by modulating the host's energy balance [4], but they can also profoundly influence eating behavior through the “brain-gut axis” [5]. Mechanisms involved include

the degradation of dietary fibers and other indigestible substances, leading to the production of short-chain fatty acids (SCFAs) and other metabolites [6, 7]. Furthermore, gut microbes can release neuroactive substances or immunomodulatory factors that act directly or indirectly on intestinal endocrine cells and the vagus nerve to transmit satiety or hunger signals to the central nervous system, thus modulating the host's eating behavior [8, 9]. However, despite growing recognition of the roles of both host physiology and gut microbiota in regulating eating behavior, the precise mechanisms underlying their interaction—particularly how gut microbes and host jointly influence specific eating traits such as eating speed—remain largely unclear.

Intrinsic variability factors, such as the host's genetic background, dietary habits, environmental exposures, can profoundly shape the structure and function of the gut microbiota [10–12]. Specific host genetic polymorphisms can influence the colonization capacity and abundance of microbial taxa [13]. Currently, microbial genome-wide association studies (mGWAS) have emerged as a transformative approach for investigating genetic variations

underlying diverse bacterial phenotypes at the population genomic level [14]. Researchers have identified host genetic variants associated with the abundance of specific gut microbes. For example, polymorphisms in host genes such as *Irak3*, *Lyz1*, and *Lyz2* affect the relative abundance of *Lactococcus* and *Coriobacteriaceae* by altering the host's immune responsiveness to peptidoglycan [15]; variants in the ABO blood group gene influence the abundance of *Erysipelotrichaceae* bacteria by modulating N-acetylgalactosamine (GalNAc) concentrations in the gut [16]; and genetic variants in *MTHFD1L* and *LARGE1* are associated with differences in the abundance of *Megasphaera* and *Parabacteroides* [17]. Collectively, these findings underscore the role of host genetics in modulating gut microbial communities and highlight how host genetic variation can influence host-microbe interactions, potentially shaping the overall composition of the microbiota. However, in birds, the impact of host genetic variation on gut microbiota composition remains largely unclear.

The gut microbiota plays a pivotal role in regulating host energy homeostasis, with a particularly significant impact on lipid metabolism. Specific microbial community features—such as the ratio of Bacteroidetes to Firmicutes [18], the abundance of butyrate-producing taxa [19], and the balance of SCFAs generated from dietary fiber fermentation [20]—have been shown to modulate lipid storage and adiposity. SCFAs, particularly butyrate and propionate, act as signaling molecules to regulate the expression of genes related to lipid synthesis and oxidation in the gut and liver, thereby influencing adipocyte differentiation and lipid storage [21]. Conversely, dysbiosis can induce low-grade intestinal inflammation, increase intestinal permeability, and allow endotoxins to enter the bloodstream, triggering systemic chronic low-grade inflammation. This inflammatory state is a key driver of insulin resistance and visceral fat accumulation [22, 23]. Research concerning the influence of gut microbiota on eating behavior, particularly eating speed, has not yet been fully elucidated.

Eating behavior—including eating speed—is governed by a complex interplay between host physiological mechanisms, gut microbiota, and intrinsic variability factors such as host genetics and environmental exposures. Accumulating evidence highlights the bidirectional relationship between host genetic variation and the composition and function of the gut microbiota, which together can shape key metabolic outcomes, particularly lipid metabolism. Despite significant advances in mammals, much remains unknown about these interactions in birds. Therefore, this study aims to elucidate how host genetic factors shape microbial community composition, thereby influencing fat metabolism and ultimately

modulating the host's eating behavior, ultimately offering novel insights into the interplay between host genetics, gut microbiota, and metabolic regulation in birds.

Methods

Animals, phenotypic data and sample collection

Animal housing and management

A total of 205 slow-growing yellow-feathered male broilers, provided by Guangdong Wens Southern Poultry Farming Co., Ltd., were used in this study. The study design flow is shown in Fig. 1. All birds originated from the same batch, were hatched on the same day, and were reared under uniform conditions in floor pens bedded with fresh wood shavings to provide a comfortable and suitable environment. Birds were offered a corn-soybean meal-based diet, and no antibiotics were administered during the entire experimental period. The poultry house was equipped with 30 automated feed-intake measurement units and multiple drinking devices, and the birds had ad libitum access to feed and water. To ensure flock uniformity, a rigorous selection and culling process was conducted at 56 days of age. The flock was subjected to visual assessment, and birds exhibiting leg weakness, skeletal abnormalities, or lethargy were culled. Only healthy broilers from the same hatch batch were retained. The selected cohort ($n=205$) presented a mean initial body weight (BW56) of 1.57 ± 0.12 kg with a coefficient of variation (CV) of 7.68%.

Phenotypic measurements

The eating behavior of eating speed trait was recorded and calculated using an automated feed-intake recording system. This system, based on radio-frequency identification (RFID) technology, enables precise feeding management and continuous monitoring of individual eating behaviors in poultry. Specifically, each bird was fitted with an RFID leg band, and antennas installed inside the feeders automatically recorded individual identification, entry and exit times, and changes in feeder weight. All data were transmitted in real time and stored on a central computer system. To improve measurement accuracy, the recording period was set from 56 to 76 days of age (21 consecutive days), as early-stage chicks tend to feed in groups, and their small body size increases the likelihood of misrecording due to accidental entry into feeder gaps, which may interfere with data quality. The formula for calculating eating speed is as follows:

$$\text{Eating Speed} = \frac{\text{FIV}}{\text{FDV}}$$

where FIV is feeding intake per visit, FDV is feeding duration per visit.

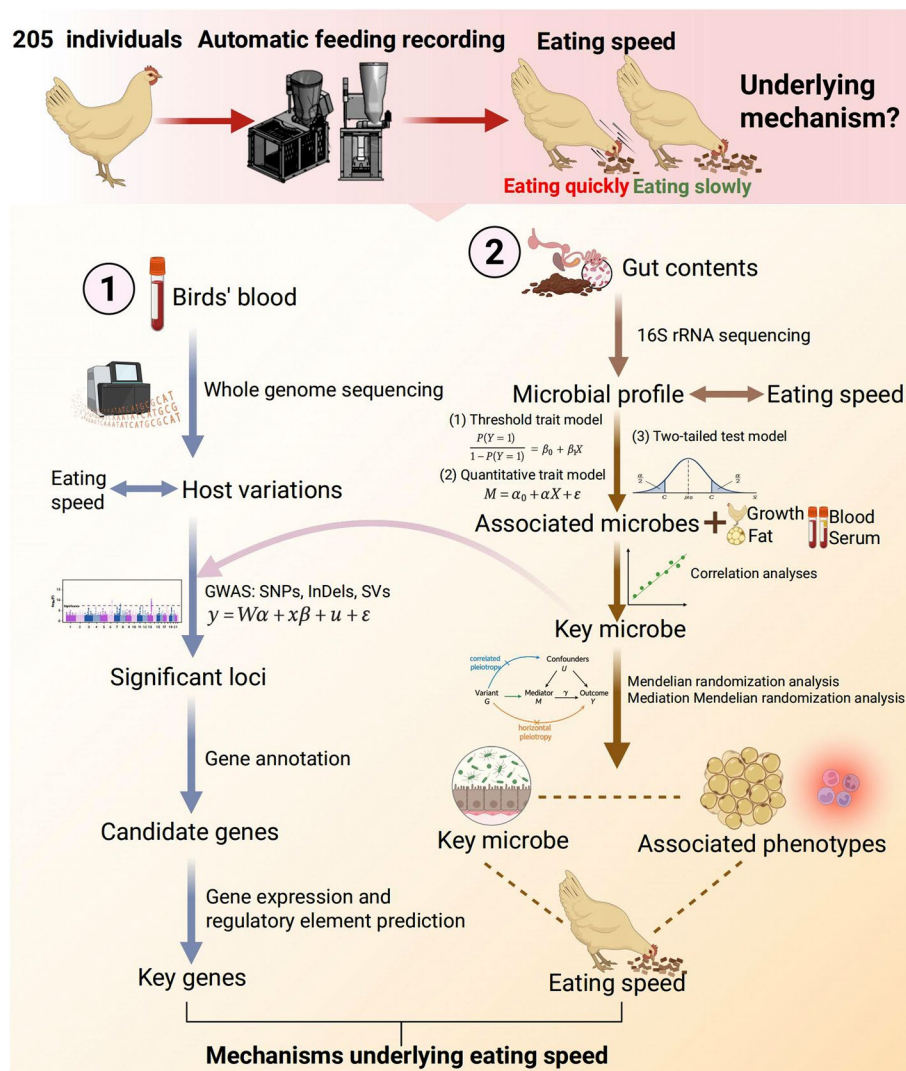


Fig. 1 Study design and workflow. To investigate the underlying mechanisms of eating speed in chickens, we analyzed eating speed records from 205 individuals and integrated host whole-genome sequencing data with gut microbiota profiles. Using genome-wide association analyses, correlation analyses, and Mendelian randomization plus mediation approaches, we delineated a feedback axis linking host genetics, a key microbe (*Bradyrhizobium*), fat deposition, metabolic and inflammatory traits, and eating speed

At 78 days of age, all birds were humanely euthanized via cervical dislocation and dissected. Abdominal fat weight (AFW) was measured using an electronic balance with an accuracy of 0.1 g, and the abdominal fat weight percentage (AFWP) was calculated as follows:

$$AFWP = \frac{AFW}{AFW + W} \times 100$$

where W represents the eviscerated carcass weight.

Sample collection

Prior to slaughter, approximately 5 mL of blood was collected from the brachial vein of each of the 205

experimental male broilers using procoagulant vacuum blood collection tubes. To ensure complete coagulation and separation, the tubes were placed at a 45° incline at room temperature for 30 min, followed by low-speed centrifugation at 3,000×g for 15 min. The resulting supernatant was transferred into 2 mL centrifuge tubes for subsequent biochemical analyses. Concentrations of total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-CH), high-density lipoprotein cholesterol (HDL-CH), triglycerides (TG) were determined using an automated biochemical analyzer (Hitachi 7020, Japan) [24]. The following hematological parameters were measured using an automated hematology analyzer: white

blood cell count (WBC), neutrophil count (NE), lymphocyte count (LY), monocyte count (MO), eosinophil count (EO), neutrophil percentage (NE%), lymphocyte percentage (LY%), monocyte percentage (MO%), eosinophil percentage (EO%), red blood cell count (RBC), hemoglobin (HGB), hematocrit value (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW). Additionally, approximately 1 mL of blood was collected from each bird via the brachial vein into anticoagulant-containing centrifuge tubes, thoroughly mixed, and stored at -20°C for subsequent extraction of host genomic DNA. Fecal samples were obtained by gently pressing the abdomen to collect excreta directly from the cloaca. Immediately after dissection, intestinal contents, including both chyme and mucosa were collected from the duodenum, jejunum, ileum, and cecum. All samples were placed in 2 mL cryovials, snap-frozen in liquid nitrogen, and subsequently stored at -80°C for long-term preservation.

Whole-genome resequencing and data processing

Host genomic DNA was extracted from blood samples using TIANamp Genomic DNA Kit (Cat#DP318, TIAN-GEN, Beijing, China) and assessed for quality. Qualified DNA was fragmented into short segments using an ultrasonic disruptor, followed by end repair, poly(A) tailing, adapter ligation, PCR amplification, purification, and quantification to construct sequencing libraries. Libraries passing quality control were sequenced on the Illumina HiSeq 2500 platform with 150 bp paired-end reads at an average depth of $10\times$ to ensure data stability and accuracy.

16S rRNA gene sequencing and analysis

Microbial DNA was extracted from the duodenum, jejunum, ileum, and cecal contents, as well as fecal samples, of 205 experimental birds using the QIAamp DNA Stool Mini Kit (Cat#51504, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The V4 region of the 16S rRNA gene was amplified using primers 520F and 802R, with a unique 7 bp barcode sequence incorporated at the 5' end of the forward primer for sample identification. PCR products were purified, quantified prior to library preparation. Libraries passing quality control were sequenced on the Illumina MiSeq platform, generating 300 bp paired-end reads.

Raw sequencing reads were first assigned to samples based on their 5' barcode sequences, and high-quality reads were retained by filtering out sequences shorter than 150 bp, with a Phred score below 20, containing

ambiguous bases, or with homopolymer runs exceeding 8 bp. Paired-end reads were merged using FLASH, and chimeric sequences were removed within the QIIME2 (v.2021.4) framework. Denoising was performed using the DADA2 plugin in QIIME2 to infer exact amplicon sequence variants (ASVs) without operational taxonomic unit (OTU) clustering, thereby improving sequence accuracy by removing sequencing errors [25–27]. ASVs were further filtered to retain those with a relative abundance greater than 10^{-6} and detected in at least one sample. Taxonomic classification of ASVs was performed using the classify-sklearn algorithm in QIIME2 against the SILVA reference database, generating microbial community profiles at multiple taxonomic ranks. All processed ASV data underwent stringent quality control to ensure compliance with established quality standards.

Genetic variant detection

Genetic variant detection included the identification of single-nucleotide polymorphisms (SNPs), insertions and deletions (InDels), and structural variants (SVs). Based on processed BAM files, SNPs and InDels were called using the GATK v.4.2.0.0 toolkit. Variants were first called from each sample using the HaplotypeCaller module to generate gVCF files, which were then merged and jointly genotyped with the GenotypeGVCFs module. SNPs and InDels were subsequently filtered according to predefined quality control (QC) thresholds. For SNPs, the criteria were root mean square mapping quality (MQ) > 10.0 , fisher strand (FS) < 60.0 , quality by depth (QD) > 10.0 , mapping quality rank sum test (MQRankSum) > -12.5 , and read position rank sum test (ReadPosRankSum) > -8.0 . For InDels, the criteria were QD > 2.0 , FS < 200.0 , ReadPosRankSum > -20.0 , and QUAL > 30.0 . After QC, the dataset was further filtered using PLINK (v.1.9) [28] to ensure a sample call rate $> 90\%$ and SNP call rate $> 95\%$. Genotype imputation was performed using Beagle (v.4.0) [29], followed by a secondary QC step to improve reliability.

Structural variants were detected using Manta (v1.6.0) [30] and DELLY (v1.3.3) [31], employing both read-pair and split-read approaches to identify deletions, inversions, duplications, and translocations. SV call sets from both tools were merged using SURVIVOR (v.10.7) [32], and the combined dataset was subjected to QC and functional annotation to ensure high-confidence SV calls. Functional annotation of significant SNPs, InDels, and SVs was conducted using SnpEff [33], which predicts the impact of variants on transcript structure, exonic regions, and protein function. Among multiple annotation levels, the highest-priority category was selected as

the definitive functional label for each variant to ensure standardization and accuracy in downstream analyses.

Genome-wide association analysis

To identify candidate genes regulating microbial abundance and eating speed, a univariate linear mixed model (ULMM) implemented in GEMMA (v.0.98.5) [34] was applied to perform a genome-wide association study (GWAS), assessing the associations between genetic variants (SNPs, InDels, SVs) and gut microbial abundance and eating speed. The model was defined as:

$$y = W\alpha + x\beta + u + \varepsilon$$

where y is the vector of microbial abundance phenotypes, W is the covariate matrix, x is the genotype, β denotes the effect size of the genetic variant, u represents the random effect, and ε is the residual error. To account for population structure, genomic relationship matrices (GRMs) were constructed separately for each type of genetic variant using standardized genotype matrices (X) by calculating the mean and variance of each column. Correlation analysis revealed substantial heterogeneity between the SV-based GRM and those derived from other variant types; thus, SV-based GWAS analyses were calibrated using the SNP-based GRM. The top four principal components of the host genome were included as covariates to further control for population stratification [35].

Multiple testing thresholds were determined using the Bonferroni correction method [36]. The effective number of independent tests for SNPs was estimated as 1,535,191 using the simpleM method [17], resulting in genome-wide significance thresholds of $-\log_{10}(0.05/1,535,191) = 7.488$ for SNPs, 7.426 for InDels, and 5.425 for SVs. Suggestive significance thresholds were set at 6.186, 6.125, and 4.184 for SNPs, InDels, and SVs, respectively. Manhattan and quantile-quantile (Q-Q) plots were generated in R to visualize GWAS results. Significant and suggestively significant loci were annotated against the chicken reference genome using SnpEff to identify candidate genes.

Identification of key microbes related to feed behavior

To identify microbial taxa significantly associated with eating behavior, we employed a two-part association model for analysis [37]. Specifically, on one hand, we converted the microbial detection status into a binary variable (threshold trait): when the relative abundance of a given taxon was greater than 0, it was assigned a value of 1, and when undetected, a value of 0. Logistic regression analysis was then conducted between the threshold trait data and eating speed. The model was defined as:

$$\frac{P(Y = 1)}{1 - P(Y = 1)} = \beta_0 + \beta_1 X$$

where $P(Y = 1)$ is the probability that the microbe is detected, Y is whether a certain microbe is detected, β_0 is intercept term, β_1 is regression coefficient, X is eating speed phenotypic value. On the other hand, for samples where the taxon was detected (relative abundance > 0), its relative abundance was treated as a continuous variable, and linear regression analysis was performed using a quantitative trait model in combination with eating speed. The model was defined as:

$$M = \alpha_0 + \alpha_1 X + \varepsilon$$

where M is microbial relative abundance, α_0 is intercept term, α_1 is regression coefficient, X is eating speed phenotypic value, ε is error term. After the analyses were completed, we extracted results from both models: when the threshold trait (binary variable) model yielded $P < 0.05$, it indicated a significant association between the presence/absence of the microbe and eating behavior trait; when the quantitative trait (continuous variable) model yielded $P < 0.05$, it suggested that the relative abundance level of the microbe was significantly correlated with eating behavior trait. Microbes identified as associated with eating behavior trait through these analyses were considered potential associated taxa.

Finally, to further validate taxa significantly associated with eating speed, we performed a two-tailed phenotypic comparison restricted to taxa with a detection rate greater than 30% based on relative abundance. Specifically, the eating speed phenotype was ranked by value, and the top 20% and bottom 20% of individuals were selected to form high and low phenotype groups, respectively. Using the STAMP (v2.1.3) [38] software platform, we compared the differences in microbial relative abundance between the two groups using the Wilcoxon rank-sum test. When the test result was $P < 0.05$, it indicated an association between eating speed and microbial abundance. These findings were further integrated with the results of the two-part association model for comprehensive analysis.

Mendelian randomization analysis

A bidirectional two-sample Mendelian randomization (MR) analysis was conducted using the TwoSampleMR package (v.0.5.6) [39] in R. GWAS summary statistics for gut microbial abundance, abdominal fat, metabolic traits, blood parameters, and eating speed were used as both exposure and outcome datasets in the analysis. For each MR direction, single-nucleotide polymorphisms (SNPs) with $P < 1 \times 10^{-5}$ in the exposure GWAS were selected as instrumental variables.

The primary MR analysis was conducted using the inverse variance weighted (IVW) method, with MR-Egger regression and the weighted median method applied as complementary approaches. To further evaluate the validity of the instrumental variables, heterogeneity testing and horizontal pleiotropy testing were performed. Sensitivity analyses included single-SNP analysis and leave-one-out analysis, along with corresponding visualizations to assess the influence and robustness of individual instruments.

Mediation Mendelian randomization analysis

A two-step mediation MR analysis was performed in this study. First, the selected significant microbial taxon was treated as the exposure, AFW and AFWP were treated as mediators, and the causal effect of the exposure on the mediator (β_1) was estimated using the IVW method in a two-sample MR framework. Subsequently, AFW or AFWP was treated as the exposure and eating speed as the outcome, and the causal effect of the mediator on the outcome (β_2) was estimated. In the second stage, eating speed was used as the exposure, AFW and AFWP as mediators, and β_1 was estimated using the IVW method; then AFW or AFWP was used as the exposure and the significant microbial taxon as the outcome to estimate β_2 . The mediation effect was quantified using the product method ($\beta_1 \times \beta_2$), and its statistical significance was assessed using a z-test. The total effect (β) of the microbial taxon or eating behavior on the outcome variable was obtained via the IVW method, and the direct effect was calculated by subtracting the mediation effect from the total effect.

Results

Eating speed profile of the experimental population and its relationships with growth, fat deposition, and physiological traits

This study involved 205 birds, with eating events recorded using automatic recording feeders in a common pen. Host genetic information was obtained through whole-genome sequencing, while gut microbiome composition—covering duodenum, jejunum, ileum, cecum, and feces—was profiled by 16S rRNA gene sequencing. Fat deposition, serum biochemical indices, and blood inflammatory traits were measured to assess the impact of eating speed (Fig. 1).

Statistical analysis of eating speed behavior in the flock revealed a unimodal distribution with moderate dispersion. The mean eating speed was 1.69 g/min, ranging from 0.43 to 3.43 g/min, with a coefficient of variation of 33.63% (Fig. 2A), indicating substantial variability in this trait. Normality was evaluated using both a formal normality test and Q-Q plot (Fig. 2B), the results

confirmed that eating speed followed a normal distribution ($P=0.38$). The two extreme groups of birds differed markedly: the fastest group (top 20%) averaged 2.50 g/min, nearly three times higher than the slowest group (bottom 20%; 0.91 g/min; $P<0.01$) (Fig. 2C).

Given the wide variability in eating speed, we next investigated its potential influence on growth, metabolism, and inflammation through correlation analyses. Eating speed showed moderate to strong correlations with growth traits ($r=0.15$ to 0.23 , $P<0.05$; Fig. 2D), including mean body weight and body weight gain, suggesting that faster eaters tend to achieve superior growth performance. Moreover, eating speed was positively associated with fat deposition; specifically, it correlated with AFW ($r=0.21$, $P<0.05$; Fig. 2D) and AFWP ($r=0.18$, $P<0.05$). This finding implies that increased energy intake in fast eaters may be preferentially directed toward fat storage, which could partly explain the observed positive correlations between eating speed and body weight traits. In contrast, eating speed exhibited few direct associations with metabolic or inflammatory indicators (Fig. 2D), implying that its effects on growth and adiposity are likely mediated by mechanisms beyond direct systemic metabolic or immune regulation. To further elucidate these mechanisms, we next explored the roles of host genetics and gut microbiota in shaping eating speed and its consequences.

Investigation of genetic variants and gut microbes associated with eating speed

We performed genome-wide association analyses using SNPs, InDels, and SVs data to examine host genetic regulation of the eating speed phenotype. No loci reached genome-wide or suggestive significance thresholds (Fig. S1). Therefore, we subsequently focused on the potential influence of the gut microbiota on eating speed.

The next question we addressed was whether gut microbes are associated with eating speed. To this end, we applied two-part association models across six taxonomic ranks (phylum, class, order, family, genus, and species) in the duodenum, jejunum, ileum, cecum, and feces. The quantitative-trait model (Fig. 3A) and threshold-liability model (Fig. 3B) identified 233 and 231 taxa with statistically significant associations ($P<0.05$), respectively (Table S1). Their overlap yielded 139 taxa, comprising 2 phyla, 6 classes, 16 orders, 34 families, 61 genera, and 20 species. To reduce false positives, we further performed an extreme-tail differential-abundance test by comparing the top and bottom 20% of birds ranked by eating speed ($n=42$ per group; Fig. 3C). Five taxa remained significant across all three analyses: in the duodenum, the phylum Planctomycetota, the order Phycisphaerales, and

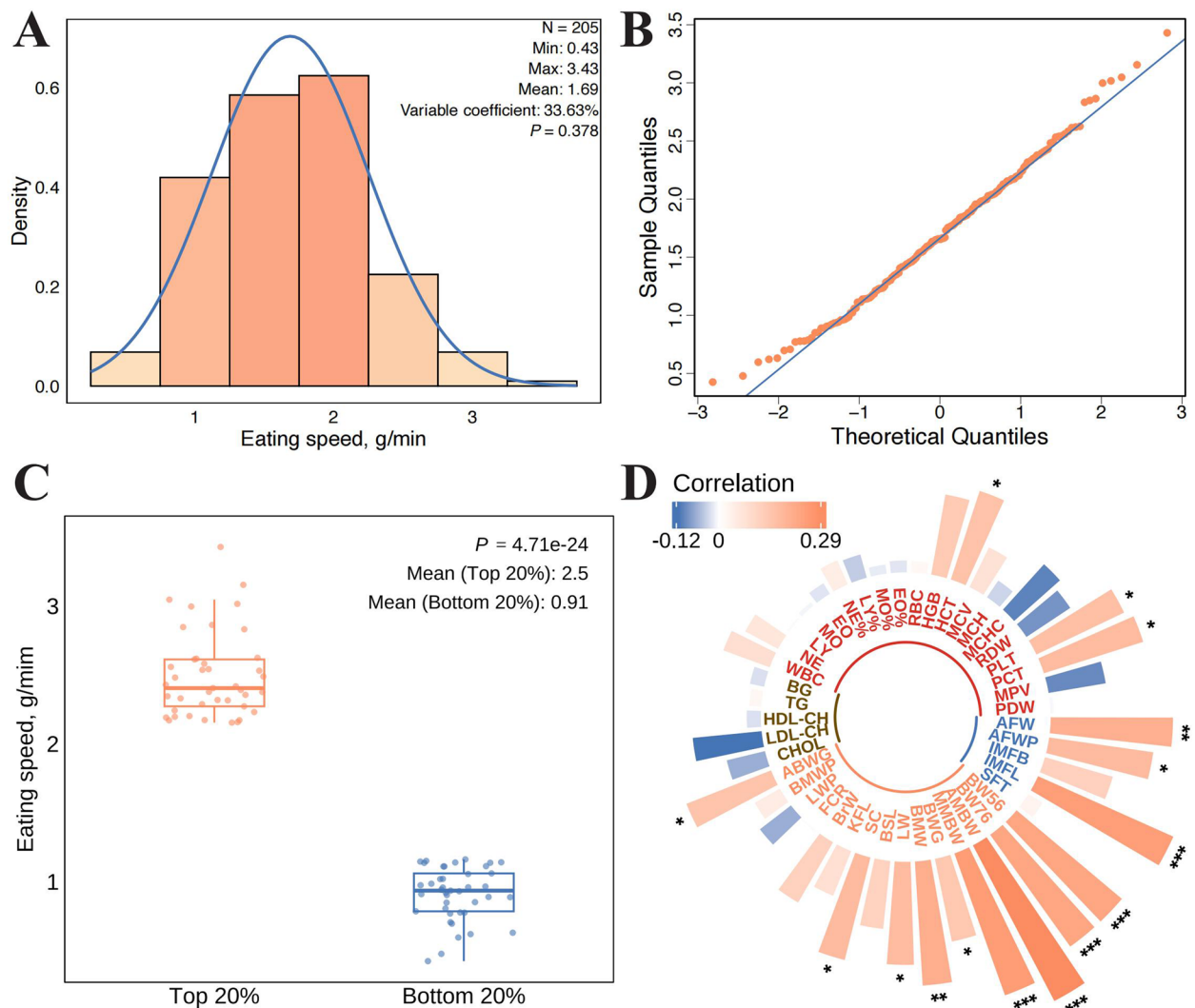


Fig. 2 Phenotypic analyses profile the eating speed trait and reveal its effects on growth and adiposity. **A** The distribution of eating speed in birds includes number of individuals, the mean, range, and coefficient of variation, normality test *P*-value. **B** Quantile-quantile (Q-Q) plot of the normality test. **C** Comparison of eating speed between the top 20% and bottom 20% of birds. **D** Correlation analysis between eating speed and growth, fat deposition, metabolic, and inflammatory traits. Traits are color-coded in the inner circle: orange = growth traits, blue = fat traits, brown = metabolic traits, red = inflammatory traits

the family Phycisphaeraceae; in feces, the family Beijerinckiaceae; and in the ileum, the genus *Bradyrhizobium* (Fig. 3D).

Having identified microbes associated with eating speed, we next examined whether these taxa are linked to metabolic and inflammatory indicators and could thereby mediate the effects of eating speed on growth and fat deposition. Correlation analyses revealed that in the duodenum, Planctomycetota was significantly associated with the inflammatory indicator LY ($r=0.16$, $P<0.05$). In the ileum, *Bradyrhizobium* showed significant correlations with both metabolic (TG; $r=-0.16$, $P<0.05$) and inflammatory indicators (WBC, LY;

$r=-0.16$ and -0.19 , respectively, $P<0.05$). Notably, ileal *Bradyrhizobium* was negatively associated with body weight traits (BW76, MMBW, and BWG, $r=-0.17$, -0.17 and -0.15 , respectively, $P<0.05$) as well as fat deposition traits (AFW and AFWP, $r=-0.22$ and -0.19 , respectively, $P<0.05$), suggesting a potential protective role against excessive body weight gain, particularly through limiting fat accumulation. By contrast, Phycisphaerales, Phycisphaeraceae, and Beijerinckiaceae were associated only with a limited number of metabolic, fat deposition and inflammatory indicators, such as MO, HGB, PDW, and IMFL (Fig. 3E).

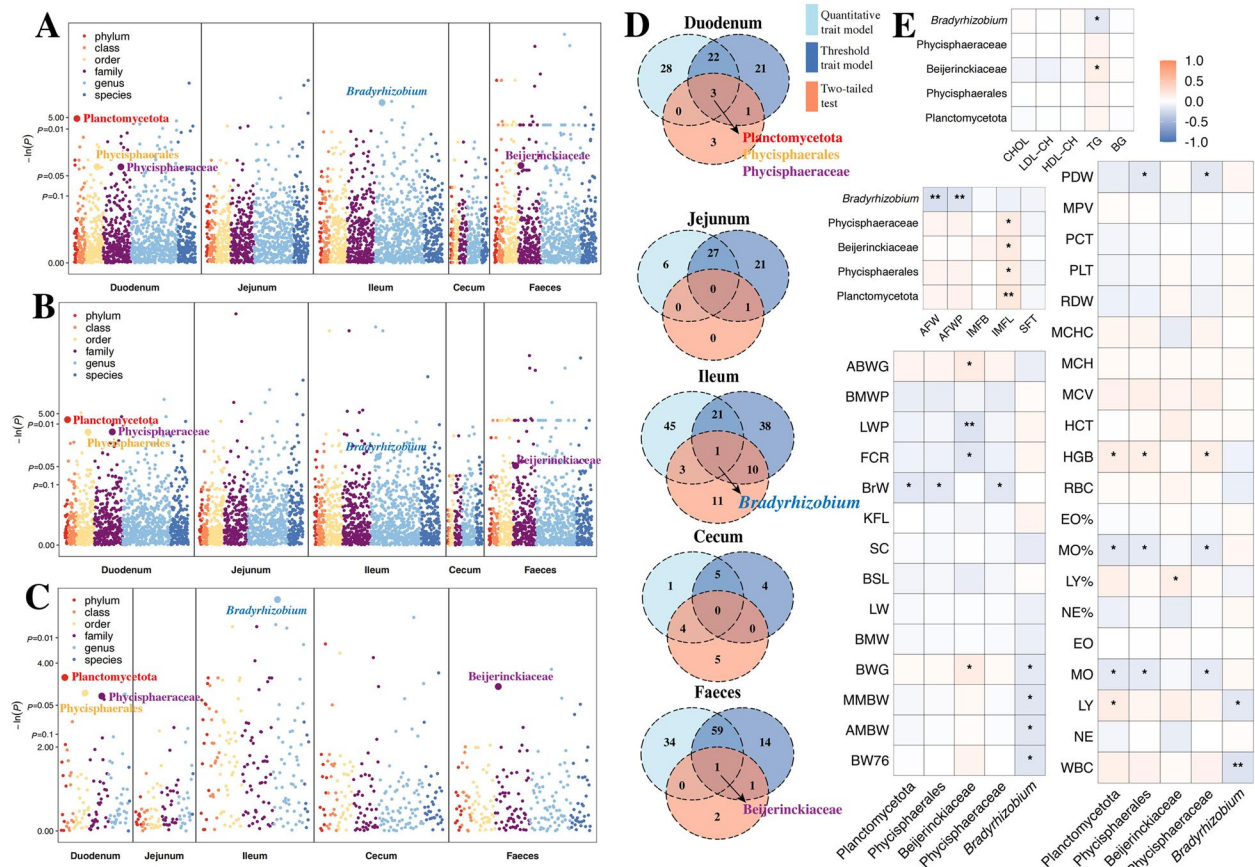


Fig. 3 Microbiome analyses reveal the key microbes having influence on eating speed. **A** The relationships of eating speed with microbial taxa through quantitative trait model tests. The x-axis indicates microbes from different gut segments and taxonomic ranks. **B** The relationships of eating speed with microbial taxa through threshold model tests. **C** The relationships of eating speed with microbial taxa through two-tailed tests. **D** Overlaps of significant microbial taxa among gut regions and models as shown by Venn diagrams. The five highlighted microbes were identified as overlapping significant microbes ($P < 0.05$) by all three models within the same gut segment. **E** Correlation analysis of the five significant microbes with growth, fat deposition, metabolic, and inflammatory traits. ($*P < 0.05$; $**P < 0.01$). CHOL=cholesterol; LDL-CH=low-density lipoprotein cholesterol; HDL-CH=high-density lipoprotein cholesterol; TG=triglycerides; BG=blood glucose; AFW=abdominal fat weight; AFWP=abdominal fat weight percentage; IMFB=intramuscular fat of breast muscle; IMFL=intramuscular fat of leg muscle; SFT=subcutaneous fat thickness; BW76=body weight at 76 days; AMBW=average body weight; MMBW=metabolic mid-body weight; BWG=body weight gain; BMW=breast muscle weight; LW=leg weight; BSL=body slant length; SC=shin circumference; KFL=keel length; BrW=breast weight; FCR=feed conversion ratio; LWP=leg weight percentage; BMWP=breast muscle weight percentage; ABWG=average body weight gain; WBC=white blood cell count; NE=neutrophil count; LY=lymphocyte count; MO=monocyte count; EO=eosinophil count; NE%=neutrophil percentage; LY%=lymphocyte percentage; MO%=monocyte percentage; EO%=eosinophil percentage; RBC=red blood cell count; HGB=hemoglobin; HCT=hematocrit; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; RDW=red cell distribution width; PLT=platelet count; PCT=plateletcrit; MPV=mean platelet volume; PDW=platelet distribution width

Collectively, these results suggest that specific gut microbes, particularly ileal *Bradyrhizobium*, may partially mediate the effects of eating speed on growth performance and adiposity, highlighting a potential microbial contribution to host phenotypic variation.

Causal relationships among eating speed, ileal *Bradyrhizobium*, fat deposition, and metabolic and inflammatory traits

Although we observed significant associations among eating speed, ileal *Bradyrhizobium*, body weight, fat

deposition, and metabolic and inflammatory indicators, whether these links reflect causal relationships remained unclear. To address this, we carried out bidirectional MR analysis. The results revealed that increased abundance of the ileal *Bradyrhizobium* exerts pleiotropic regulatory effects on host metabolism, inflammation and eating speed (Fig. 4A). Specifically, MR analysis suggested a causal effect of higher *Bradyrhizobium* abundance on reduced eating speed ($\beta = -8.30$, 95% CI: -14.74 to -1.87 , $P = 0.01$), decreased AFW ($\beta = -395.54$ g, 95% CI: -711.88 to -79.21 , $P = 0.014$), decreased AFWP

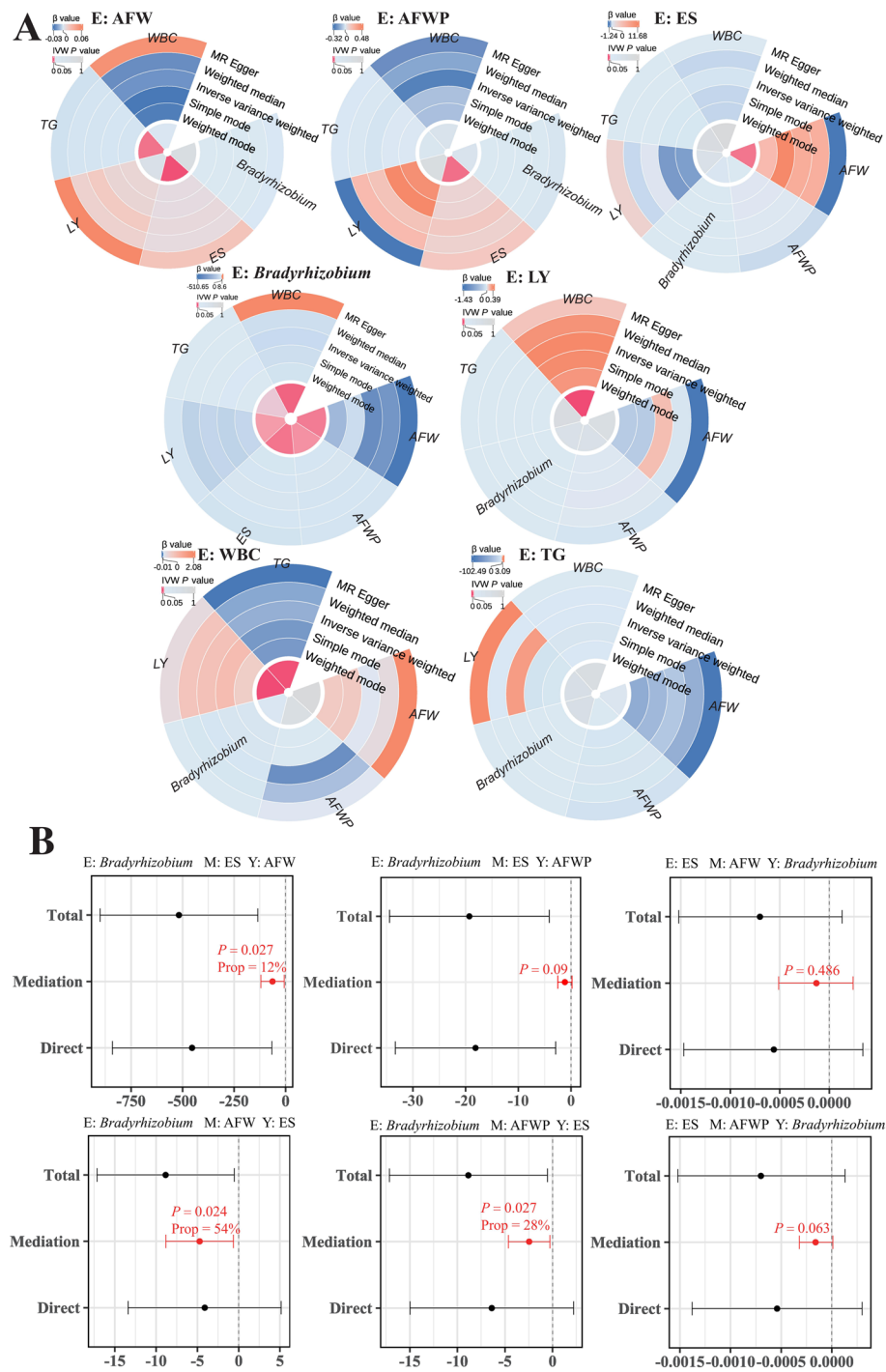


Fig. 4 Mendelian randomization and mediation Mendelian randomization analyses confirm the causal effects of ileal *Bradyrhizobium* on eating speed and fat deposition, and reveal bidirectional positive feedback loop between eating speed and fat deposition. **A** Mendelian randomization analysis of *Bradyrhizobium*, eating speed, metabolic, fat deposition and inflammatory traits. Each circular plot displays results from multiple statistical analysis methods; from inner to outer rings, the methods are: Weighted Median, Simple Mode, Inverse Variance Weighted, Weighted Median, and MR Egger. Colored segments represent effect sizes (β values). The central circle shows the P values from the Inverse Variance Weighted method, with colors indicating different p values. **B** Mediation Mendelian randomization analysis of the causal relationships among ileal *Bradyrhizobium*, eating speed and abdominal fat. Prop is the proportion of the mediation effect. E=exposure; M=mediator; Y=outcome. AFW=abdominal fat weight; AFWP=abdominal fat weight percentage; CHOL=cholesterol; LDL-CH=low-density lipoprotein cholesterol; TG=triglycerides; HDL-CH=high-density lipoprotein cholesterol; BG=blood glucose; WBC=white blood cell count; NE=neutrocyte; LY=lymphocyte count; ES=eating speed. Total=the total effect, Mediation=the mediation effect, Direct=the direct effect, and Prop is the proportion of the mediation effect

($\beta = -14.55$, 95% CI: -27.01 to -2.10 , $P=0.022$), and lower TG levels ($\beta = -0.96$, 95% CI: -1.898 to -0.014 , $P=0.047$). Conversely, increased AFW or AFWP was causally associated with elevated eating speed ($\beta = 0.01$, 95% CI: 0.004 to 0.015 , $P=0.001$; $\beta = 0.15$, 95% CI: 0.04 to 0.26 , $P=0.009$), while increased eating speed further promoted AFW ($\beta = 6.92$, 95% CI: 1.51 to 12.33 , $P=0.012$), thereby revealing a bidirectional positive feedback loop between eating speed and fat deposition. In addition, MR analysis indicated that greater *Bradyrhizobium* abundance causally reduced inflammation parameters, including WBC ($\beta = -53.21$, 95% CI: -91.52 to -14.89 , $P=0.006$) and LY ($\beta = -73.60$, 95% CI: -138.08 to -9.11 , $P=0.025$). Together, these findings identify ileal *Bradyrhizobium* as a potential microbial regulator of eating behavior, fat deposition, and immune homeostasis, highlighting its importance as a candidate target for improving growth efficiency and metabolic health.

To confirm the robustness of the causal associations, we performed sensitivity analyses using the MR-Egger regression, weighted median method, single-SNP analysis, and leave-one-out analysis. All approaches yielded effect estimates consistent with those from the IVW method, reinforcing the robustness of our findings (Table S2). The MR-Egger intercept test provided no evidence of horizontal pleiotropy ($P>0.05$; Table S3). Moreover, Cochran's Q test indicated no significant heterogeneity among the instrumental variables ($P>0.05$; Table S4), further supporting the validity of the MR assumptions.

Having established that ileal *Bradyrhizobium* exerts causal effects on both eating speed and fat deposition traits (AFW/AFWP), and that eating speed and AFW mutually influence one another, we next asked whether *Bradyrhizobium* affects eating speed indirectly through fat deposition, or conversely, affects fat deposition indirectly through eating speed. To address this, we performed mediation Mendelian randomization analysis. The results indicated that *Bradyrhizobium* indirectly inhibited eating speed partly by reducing AFW or AFWP (Fig. 4B). Specifically, AFW mediated 54% of the total effect of *Bradyrhizobium* on eating speed (mediation effect $\beta = -4.73$, 95% CI: -8.83 to -0.64 , $P=0.024$; total effect $\beta = -8.85$, $P=0.037$), while AFWP mediated 28% of the effect ($\beta = -2.46$, 95% CI: -4.64 to -0.27 , $P=0.027$). At the same time, due to the bidirectional positive feedback loop between eating speed and fat deposition, *Bradyrhizobium* was found to indirectly suppress AFW/AFWP by reducing eating speed. In this pathway, eating speed mediated 12.29% and 6.06% of the total effect of *Bradyrhizobium* on AFW and AFWP with $P=0.027$ and 0.09 , respectively. By contrast, the reverse mediation pathway—where eating speed affects *Bradyrhizobium*

abundance via abdominal fat—was not significant. In the reverse pathway, the mediation effects of eating speed on *Bradyrhizobium* via abdominal fat were not significant.

Identification of host genetic variants and genes in regulating ileal *Bradyrhizobium*

The important role of ileal *Bradyrhizobium* in eating speed and fat deposition prompted us to investigate factors governing its abundance. Because all birds were raised under the same environment conditions, we hypothesized that host genetics may contribute to the inter-individual variation in ileal *Bradyrhizobium* abundance. To test this, we performed mGWAS using SNP, InDel, and SV data. We identified 8 SNPs, 4 InDels, and 5 SVs that surpassed the significance threshold (Fig. 5A). The Q-Q plot showed that the distribution of observed P -values closely matched the expected values under the null hypothesis, with slight deviation at the tail, indicating potential genetic associations (Fig. 5B).

Using a linear regression main-effect model, loci such as SNP4 (Chr3:86,220,914 G>A), InDel1 (Chr1:12,509,836 CAT>C), SV5 (Chr33:6,229,923 dup:tandem), and SNP5 (Chr6:32,249,009 C>T) exhibited positive significant effects on *Bradyrhizobium* ($P<0.05$; Fig. 5C). Furthermore, based on a model incorporating all second-order interaction terms, we identified the top 10 genotype interactions most significantly associated with *Bradyrhizobium*. Among these, SNP3 (Chr2:55,985,285 A>G) and SV2 (Chr12:3,769,275 del99 insT) displayed a significant synergistic effect ($P<0.05$), whereas InDel3 (Chr2:55,992,464 TC>T) and SV2 (Chr12:3,769,275 del99 insT) exhibited a significant antagonistic effect ($P<0.05$; Fig. 5D).

To further evaluate the effects of the genetic variants on ileal *Bradyrhizobium* abundance, we conducted genotype-based analyses. The results revealed that the abundance of *Bradyrhizobium* significantly differed across 13 loci, with individuals carrying the mutated alleles exhibiting higher microbial abundance. No significant differences were observed at the remaining four loci (Fig. 5E).

Further annotation of the 17 identified variants indicated that these loci were mapped to eight genes: *KHDRBS2*, *HTRA1*, *MAGI2*, *TBX20*, *RECK*, *CENPP*, *ECM2*, and *ENSGALG00000001720*. Notably, both the SNP3 (Chr2:55,985,285 A>G), identified in the SNP analysis, and InDel3 (Chr2:55,992,464 TC>T), identified in the InDel analysis, mapped to the same gene, *RECK*, providing convergent evidence that prioritizes *RECK* as a host gene potentially modulating ileal *Bradyrhizobium* abundance.

To explore the expression profiles of these candidate genes, we queried the public expression database and summarized tissues with the highest transcript abundance (Fig. 5F). Gene expression prediction revealed

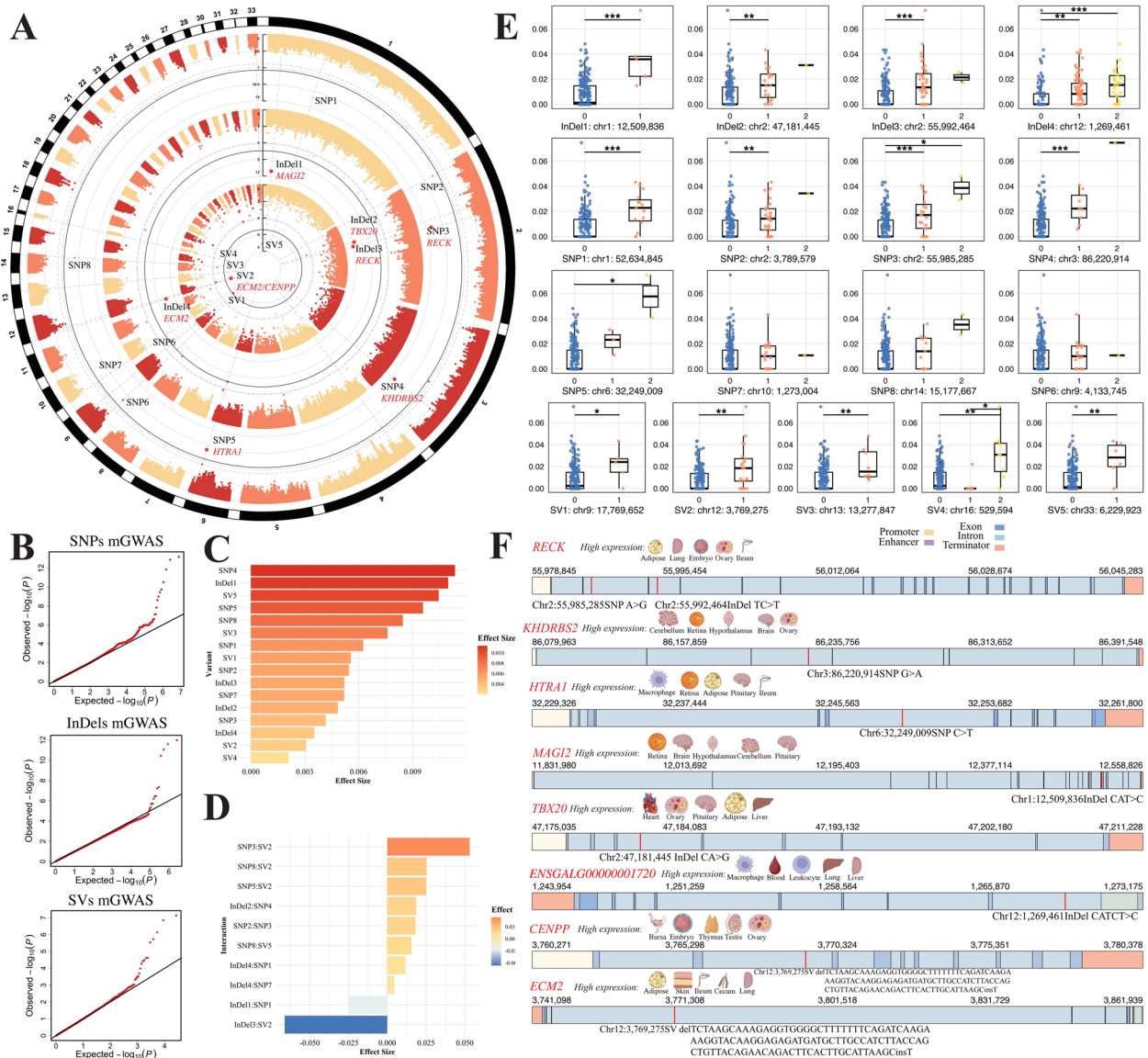


Fig. 5 mGWAS and following analyses identify the significant genetic variants and genes in regulating ileal *Bradyrhizobium*. **A** mGWAS results of *Sodoligranulum* SNPs, InDels and SVs. Black solid and gray dashed lines indicate significance and suggestive thresholds, respectively. In order from outside to inside: SNPs, InDels, SVs. SNP1=Chr1:52,634,845 A>G; SNP2=Chr2:3,789,579 C>T; SNP3=Chr2:55,985,285 A>G; SNP4=Chr3:86,220,914 G>A; SNP5=Chr6:32,249,009 C>T; SNP6=Chr9:4,133,745 C>T; SNP7=Chr10:1,273,004 A>T; SNP8=Chr14:15,177,667 G>A; InDel1=Chr1:12,509,836 CAT>C; InDel2=Chr2:47,181,445 GA>G; InDel3=Chr2:55,992,464 TC>T; InDel4=Chr12:1,269,461 CATCT>C; SV1=Chr9:17,769,652 dup:tandem; SV2=Chr12:3,769,275 del99 insT; SV3=Chr13:13,277,847 del; SV4=Chr16:529,594 del; SV5=Chr33:6,229,923 dup:tandem. **B** Q-Q plot of *Bradyrhizobium* SNPs, InDels and SVs mGWAS. **C** Main effects of variants on *Bradyrhizobium*. **D** Top 10 epistatic effects. **E** The genotyping of the potential seventeen significant SNPs, SVs and InDels loci for *Bradyrhizobium* ($P < 0.05$; $**P < 0.01$; $***P < 0.001$). The x-axis represents different genotypes at each variant site (0=homozygous reference, 1=heterozygous, 2=homozygous alternate). The y-axis indicates the relative abundance of the microbe, where 0.05 corresponds to 5%. **F** Relative expression prediction of candidate genes in various tissues and the genomic localization of the identified variants within the gene structures

that all the candidate genes are expressed across gut segments, with particularly high expression in the ileum (Fig. S2). To gain insight into how the identified variants may regulate these genes, we integrated predictive regulatory element analysis with the chicken regulatory

element atlas and validated the findings using ChickenGTEx expression data. Regulatory element prediction showed that these loci are primarily located within enhancer regions in the duodenum, ileum, cecum,

hypothalamus, cortex, and cerebellum, as well as within weak repressed polycomb regions in the liver (Fig. S3).

Together, these results suggest that host genetic variation, particularly involving the *RECK* gene, may shape ileal *Bradyrhizobium* abundance through enhancer-mediated regulation in gut tissues, providing a genetic basis for host–microbe interactions that influence eating behavior and fat deposition.

Discussion

In this study, we systematically dissected the relationships among eating speed, fat deposition, host genetics, and the gut microbiota in birds. While no host genetic variants were directly associated with eating speed, we identified the ileal *Bradyrhizobium* as a key microbial factor exerting causal effects on both eating speed and fat deposition. Mendelian randomization and mediation analyses further revealed that *Bradyrhizobium* not only directly modulates eating speed and abdominal fat deposition but also participates in a bidirectional feedback loop between these traits, underscoring its pleiotropic regulatory role. Moreover, microbiome genome-wide association analyses pinpointed host genetic variants and candidate genes, including convergent signals at *RECK* gene, that may underlie inter-individual variation in *Bradyrhizobium* abundance. Together, our findings fill a critical knowledge gap, as the role of eating speed in relation to metabolic syndrome in birds has remained largely unexplored. These results provide new insights into the host-microbe-trait axis, highlight the important role of gut microbiota in regulating animal behavior and metabolism, and emphasize the importance of the indirect host genetics mediated through gut microbiome.

Consistent with evidence from humans [40], our results in chickens show that faster eating is associated with increased abdominal fat deposition, suggesting that the metabolic consequences of rapid food intake reflect conserved or convergently evolved regulatory pathways across species. In humans, faster eating speed is linked to a higher risk of metabolic syndrome, including central obesity, hypertension, and elevated triglycerides [41–45]. Prior to this study, however, such associations had not been well-established in birds. Here, we demonstrate that eating speed in chickens is positively correlated with body weight and fat deposition, which is consistent with observations in humans [46–48]. The parallel findings across humans and chickens indicate that rapid food intake may engage conserved metabolic pathways that channel excess energy toward fat storage, thereby increasing metabolic risk. Thus, recognizing eating speed as a modifiable factor could have implications not only for reducing human

metabolic disease risk but also for improving livestock health and productivity.

Importantly, the relationship between eating speed and fat deposition is bidirectional rather than unidirectional. In this study, we identified a positive feedback loop in which AFW causally promotes eating speed ($\beta = 0.009$, $P = 0.001$), a phenomenon rarely reported previously. Mechanistic evidence from other studies suggests that excessive fat intake, via apolipoproteins, can directly affect specific brain neurons, altering protein degradation pathways and leading to abnormal hunger sensitivity and increased foraging behavior [49]. Furthermore, exposure to high-fat diets has been found to reconfigure the eating behavior system that maintains homeostasis and hedonic drive, prompting animals to seek higher-calorie food and then leading to overeating and diabetes [50]. These findings indicate that excessive abdominal fat can enhance eating behavior, which in turn further exacerbates fat accumulation ($\beta = 6.92$, $P = 0.012$), highlighting a self-reinforcing mechanism that may contribute to the development of metabolic syndrome across species.

As an important component of the animal gut microbiota and a potential regulator of fat deposition, *Bradyrhizobium* plays a role far beyond its traditional function in symbiotic nitrogen fixation. It can produce beneficial metabolites and influence host nutritional metabolism, immune function, and disease processes. For example, the study in pigs fed fermented mulberry leaves showed that *Bradyrhizobium* synthesizes proteins via nitrogen fixation, potentially reducing the host's energy expenditure for essential amino acid synthesis and thereby indirectly lowering compensatory feeding requirements [51]. In broiler chickens, dietary herbal supplements significantly increased the relative abundance of *Bradyrhizobium* in the liver microbiota, which was associated with enhanced hepatic antioxidant capacity and downregulation of genes involved in cholesterol synthesis [52]. This helps explain in our study why an increase in the abundance of *Bradyrhizobium* is associated with a reduction in abdominal fat deposition. Moreover, supplementation with dietary fiber in piglets has been found to increase ileal *Bradyrhizobium* abundance and gut butyrate levels [53], a SCFA that supports intestinal epithelial proliferation, maintains gut barrier integrity, and exerts anti-inflammatory effects [54]. The result further validates the relationship observed in this study between the abundance of *Bradyrhizobium* and decreased WBC and LY in blood, suggesting a role for this microbe in alleviating systemic inflammation.

Beyond dietary regulation, the abundance of *Bradyrhizobium* is also partly shaped by host genetics. Our mGWAS analysis identified key variants and eight candidate genes—*MAGI2*, *RECK*, *HTRA1*, *KHDRBS2*, *TBX20*, *CENPP*,

ECM2, and *ENSGALG00000001720*—that are strongly associated with *Bradyrhizobium* abundance. Although these genes function in diverse pathways, they likely act synergistically to influence microbial colonization in the gut. In this study, we identified significant located InDel within *MAGI2*. *MAGI2* encodes a scaffolding protein primarily located at tight junctions. Functionally, *MAGI2* is known to interact with and stabilize PTEN, a negative regulator of the PI3K/Akt signaling pathway [55]. Given the central role of the PI3K/Akt pathway in regulating glucose metabolism and lipid synthesis, we speculate that *MAGI2* may modulate metabolic traits through insulin signaling sensitivity. Previous studies have indicated that *MAGI2* downregulation is closely related to increased barrier permeability and inflammatory bowel disease (IBD) susceptibility [56]. Furthermore, *MAGI2* variations have been linked to mental and eating disorders, which are often accompanied by gut microbiota dysbiosis [57]. These findings suggest that *MAGI2* may serve as a critical node in the gut-brain axis, mediating the bidirectional communication between the microbiota and the host's nervous system to co-regulate eating behavior and fat metabolism. *HTRA1* is an important regulatory factor in the TGF- β signaling pathway. In the gut, *HTRA1* may play a role in maintaining immune homeostasis by regulating the TGF- β pathway. Notably, convergent SNP and InDel variants mapped to *RECK* gene, which primarily functions as a potent inhibitor of multiple matrix metalloproteinases (MMPs) [58]. *RECK* inhibits MMP activity, reducing the degradation of extracellular matrix (ECM), thus maintaining vascular barrier integrity and reducing the leakage of inflammatory mediators. In the gut, inflammation is a key factor leading to intestinal barrier damage and the development of various gut diseases. The expression of *RECK* and its regulation of inflammation in the gut suggest that it may play a potential role in maintaining intestinal homeostasis. Other genes such as *TBX20* [59], *CENPP* [60], *ECM2* [61], and *KHDRBS2* [62] play important roles in regulating cardiovascular development, cell cycle, cell adhesion, blood-brain barrier permeability, and selective splicing. They may indirectly influence the integrity and cell turnover of the intestinal mucosa, thus regulating the colonization and abundance of gut microbiota.

Overall, our findings position ileal *Bradyrhizobium* as a central mediator linking host genetics, gut microbial composition, and key metabolic and behavioral traits. By shaping eating speed and abdominal fat deposition—and interacting with these traits in a bidirectional feedback loop—this microbe exemplifies how gut microbiota can influence complex host phenotypes. The dual regulation by diet and host genotype underscores the integrative nature of the host-microbe-trait axis, suggesting that targeted manipulation of specific microbial taxa could offer

novel strategies to modulate eating behavior, optimize growth efficiency, and improve metabolic health in livestock. Given that *Bradyrhizobium* is not a conventional probiotic typically used for direct supplementation, we envision practical manipulation through two complementary avenues. First, host genetic selection serves as a primary strategy; incorporating the identified significant markers, such as those within the *RECK* gene, into genomic selection programs could allow for the breeding of chicken lines naturally predisposed to enrich this beneficial taxon. Second, dietary modulation offers a parallel approach. As supported by previous studies, the abundance of *Bradyrhizobium* can be effectively enhanced through the inclusion of specific dietary fibers or herbal supplements, which act as prebiotics to create a favorable gut niche. Future studies at higher taxonomic resolution, larger sample size, and with experimental validation will be critical to translate these insights into precision interventions.

Conclusion

This study reveals interactions among host genetics, the gut microbiota, eating speed, and fat deposition in chickens. Using phenotypic analyses, Mendelian randomization, and microbiome profiling, we found that the ileal genus *Bradyrhizobium* is causally associated with eating speed and abdominal fat; increased abundance is associated with reduced abdominal fat, triglycerides, and inflammatory markers. mGWAS implicates host genes (such as *RECK*) in the regulation of this taxon. These findings point to potential strategies for modulating feeding behavior and metabolic health via microbial or genetic interventions.

Abbreviations

ABWG	Average body weight gain
AFW	Abdominal fat weight
AFWP	Abdominal fat weight percentage
AMBW	Average body weight
BG	Blood glucose
BMW	Breast muscle weight
BMWP	Breast muscle weight percentage
BrW	Breast width
BSL	Body slant length
BW76	Body weight at 76 days
BWG	Body weight gain
CENPP	Centromere protein P
CHOL	Cholesterol
ECM2	Extracellular matrix protein 2
EO%	Eosinophil percentage
EO	Eosinophil count
ES	Eating speed
FCR	Feed conversion ratio
FDV	Feeding duration per visit
FIV	Feeding intake per visit
GWAS	Genome-wide association study
HCT	Hematocrit
HDL-CH	High-density lipoprotein cholesterol
HGB	Hemoglobin
HTRA1	HtrA serine peptidase 1

IMFB	Intramuscular fat of breast muscle
IMFL	Intramuscular fat of leg muscle
InDel	Insertion-deletion
KFL	Keel length
KHDRBS2	KH RNA binding domain containing, signal transduction associated 2
LDL-CH	Low-density lipoprotein cholesterol
LW	Leg weight
LWP	Leg weight percentage
LY%	Lymphocyte percentage
LY	Lymphocyte count
MAGI2	Membrane associated guanylate kinase, WW and PDZ domain containing 2
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mGWAS	Microbiome genome-wide association studies
MMBW	Metabolic mid-body weight
MO%	Monocyte percentage
MO	Monocyte count
MPV	Mean platelet volume
MR	Mendelian randomization
NE%	Neutrophil percentage
NE	Neutrophil count
PCT	Plateletcrit
PDW	Platelet distribution width
PLT	Platelet count
RBC	Red blood cell count
RDW	Red cell distribution width
RECK	Reversion inducing cysteine rich protein with Kazal motifs
RFID	Radio-frequency identification
SC	Shin circumference
SCFA	Short-chain fatty acid
SFT	Subcutaneous fat thickness
SNP	Single nucleotide polymorphism
SV	Structural variation
TG	Triglycerides
WBC	White blood cell count

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40104-026-01369-z>.

Additional file 1: Fig. S1. Genome-wide association analyses of eating speed with SNPs, InDels, and SVs.

Additional file 2: Fig. S2. Predicted expression levels of candidate genes across tissues.

Additional file 3: Fig. S3. Predicted regulatory element localization of candidate loci across tissues.

Additional file 4: Table S1. Raw merged taxa association results from quantitative-trait model and threshold-liability model.

Additional file 5: Table S2. Single-variant MR estimates and leave-one-out sensitivity tests in Mendelian randomization.

Additional file 6: Table S3. Summary of MR-Egger intercept tests for directional horizontal pleiotropy.

Additional file 7: Table S4. Results of Cochran Q heterogeneity test.

Authors' contributions

ZY: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft and editing. ZSS: Methodology, Formal analysis, Visualization. WP: Writing – review and editing, Investigation. YWW: Writing – review & editing, Methodology. LXX: Writing – review and editing, Investigation. DYC: Writing – review and editing, Investigation. LCX: Investigation, Methodology. LF: Writing – review and editing, Investigation. YN: Resources, Conceptualization, Methodology, Writing – review and editing. YW: Resources,

Funding acquisition, Project administration, Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review and editing. All authors reviewed and approved the manuscript.

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Data availability

The raw data are available from the Sequence Read Archive with accession numbers PRJNA449436, PRJNA449437, and PRJNA449438.

Declarations

Ethics approval and consent to participate

All experimental procedures involving animals followed the ethical guidelines and were approved by the Animal Care and Use Committee of China Agricultural University (Permit number: AW08059102-1).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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