The cecal ecosystem is a great contributor to intramuscular fat deposition in broilers

Chaoliang Wen ,[*,†,1] Qinli Gou,[*,†,1] Shuang Gu,[*,†] Qiang Huang,[*,†] Congjiao Sun,[*,†] Jiangxia Zheng,[*,†] and Ning Yang [*,†,2]

[*]*Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China; and* [†]*National Engineering Laboratory for Animal Breeding and Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture and Rural Affairs, China Agricultural University, Beijing 100193, China*

ABSTRACT Intramuscular fat (**IMF**) content is a meat quality trait of major economic importance in animal production. Emerging evidence has demonstrated that meat quality can be improved by regulating the gut microbiota. However, the organization and ecological properties of the gut microbiota and its relationship with the IMF content remain unclear in chickens. Here, we investigated the microbial communities of 206 cecal samples from broilers with excellent meat quality. We noted that the cecal microbial ecosystem obtained from hosts reared under the same management and dietary conditions showed clear compositional stratification. Two enterotypes, in which the ecological properties, including diversity and interaction strengths, were significantly different, described the microbial composition pattern. Compared with enterotype 2, enterotype 1, distinguished by the *Clostridia_vadinBB60_group*, had a higher fat deposition, although no discrepancy was found in growth performance and meat yield. A moderate correlation was observed in the IMF content between 2 muscle tissues, despite the IMF content of thigh muscle was 42.76% greater than that of breast muscle. Additionally, the lower abundance of cecal *vadinBE97* was related to higher IMF levels in both muscle tissues. Although *vadinBE97* accounted for 0.40% of the total abundance of genera in the cecum, it exhibited significant and positive correlations with other genera (accounting for 25.3% of the tested genera). Our results highlight important insights into the cecal microbial ecosystem and its association with meat quality. Microbial interactions should be carefully considered when developing approaches to improve the IMF content by regulating the gut microbiota in broilers.

Key words: cecum, microbial properties, enterotypes, intramuscular fat content, meat quality

2023 Poultry Science 102:102568
https://doi.org/10.1016/j.psj.2023.102568

INTRODUCTION

With the improvement in people's living standards, the demand for both meat quantity and quality increases. The appearance, flavor, and texture of meat are essential measures of sensory properties that influence the purchasing decisions of consumers. Intramuscular fat (**IMF**) is the fat that accumulates among muscle fibers or within muscle cells. Numerous studies have suggested that IMF content is a vital factor influencing meat quality, including tenderness, juiciness, flavor, and color (Hocquette et al., 2010; Sarsenbek et al., 2013; Listrat et al., 2016). Increasing IMF levels can enhance the perceived texture, aromatic notes, and most of the characteristic flavors responsible for the development of meat flavor. Thus, improvement of the IMF content of livestock and poultry meat has become one of the priorities in recent years.

The demand for chicken, the most consumed meat across the globe, is increasing faster than that for any other meat due to its advantages in price and nutritional quality and few cultural or religious barriers (Mottet and Tempio, 2017). Previous studies have investigated the genetic basis for IMF content in chickens (Cai et al., 2022; Cui et al., 2022). However, the IMF content has low to moderate heritability, ranging from 0.11 to 0.16 (Chen et al., 2008; Jiang et al., 2017; Liu et al., 2019), indicating that alternative mechanisms exist for this trait in chickens. The gut microbiome, often referred to as the second genome of animals, has coevolved with the

Received December 15, 2022.
Accepted February 2, 2023.

[1]These authors contributed equally to this work.

[2]Corresponding author: nyang@cau.edu.cn

host over thousands of years to form an intricate and mutually beneficial relationship. The gut microbiota makes an important contribution to host metabolism by contributing enzymes that are not encoded by the host genome (Nicholson et al., 2012), such as enzymes for breaking down polysaccharides. Emerging studies have demonstrated that the gut microbiota is a great contributor to fat deposition and meat quality in chickens. For instance, the regulation of *Clostridium butyricum* increased the fatty acid content of breast muscle (Yang et al., 2010), and the ingestion of probiotics significantly improved meat color (Zheng et al., 2014) and flavor characteristics (Wang et al., 2017). Moreover, the propensity for adipogenesis and the properties of muscle can be transferred from donors to recipients through fecal microbiota transplantation (Lei et al., 2022).

A postulated mechanism underlying the interactions between the gut microbiota and host fat metabolism involves the production of short-chain fatty acids (**SCFAs**), which are the main metabolites produced by microbial fermentation of undigestible carbohydrates (Frampton et al., 2020; Chen et al., 2022; Konieczka et al., 2022). Most of these metabolites are absorbed and utilized by the host and elicit effects on lipid metabolism and adipose tissue at several levels (Morrison and Preston, 2016). The cecum of chickens is a major site for microbial fermentation of dietary components. We have recently revealed that the cecal microbiota plays an important role in fat deposition (Wen et al., 2019). Additionally, the cecal microbiota has been proven to be associated with the breast muscle metabolic profile (Feng et al., 2022). Yang et al. (2022) further confirmed that supplementation of diets with prebiotics can alter the global metabolome of the cecum through microbial metabolites, including SCFAs, which significantly affect the flavor of chicken meat. These findings suggested that the cecal microbiota could be regulated to improve fat deposition in muscle and thus meat quality.

The cecum is a complex ecosystem in which commensals mostly evolve competitive or synergistic interactions with each other (Wen et al., 2021). There is certainly agreement that there are distinct microbial compositions across individuals wherein the respective gut communities show biological differences (Costea et al., 2018). The concept of enterotypes can help capture such differences. The enterotype was suggested as a predicted cluster of the gut microbiota, which describes the organization and ecological properties of the microbial ecosystem (Arumugam et al., 2011). Here, we compared the ecological diversity of the cecal microbiota between different enterotypes and evaluated the association of enterotypes with host phenotypes. The main objective of the present study was to explore the composition of cecal microbial communities and identify the special taxa associated with the IMF content in chickens. Our study uncovered the composition patterns of the cecal microbiota and improved our understanding of the relationship between the cecal microbiota and fat deposition in muscle, thus providing important insights into improving meat quality.

MATERIALS AND METHODS

Experimental Animals

A sire line from Guangdong Wen's Nanfang Poultry Breeding, Co., Ltd. (Xinxing, China) was used as the experimental animals. This strain belonged to the yellow feather broiler breed with slow growth and excellent meat quality. This experiment included 206 male chickens from one hatch. All chickens were raised in the same pen on the floor under standardized conditions of a 20:4 h light:dark cycle. Water and feed were provided ad libitum. Additional detailed information about the rearing conditions and management can be found in our previous study (Wen et al., 2018). The details of the ingredients of the diet have been previously described (Wen et al., 2021). Bird handling and study protocols were carried out in accordance with the Guide for the Animal Care and Use Committee of China Agricultural University (SYXK2018-0038).

Phenotype Measurements

The feed intake (**FI**) of each broiler was quantified with an automatic feeder during the fast-growing period from 56 to 76 d of age. The body weight (**BW**) of each broiler at 56 and 76 d of age was measured with an electronic scale. The body weight gain (**BWG**) and feed conversion ratio from 56 to 76 d of age were calculated. To obtain morphological traits, the breast width (**BrW**), fossil bone length (**FBL**), shank length (**SL**), and shank circumference (**SC**) of all birds were measured. At the end of 11 wk of age, chickens were euthanized by exsanguination. The serum was separated from whole blood by centrifugation. The concentrations of triglyceride (**TG**) and low-density lipoprotein cholesterol (**LDL-CH**) in the serum were evaluated. Carcass traits, including subcutaneous fat thickness (**SFT**), abdominal fat weight, left breast weight, left leg weight, liver weight (**LiW**), bile weight (**BiW**), and cecal length (**CL**) were assessed. The percentages of abdominal fat (**AFP**), single breast muscle (**BMP**), and single leg (**LP**) were calculated. The descriptive statistics of phenotypic observations were described in our previous study (Wen et al., 2018).

IMF Content Determination

After slaughter, samples of breast muscle and thigh muscle were separately collected and stored at −20°C for subsequent determination of the IMF content. Prior to lipid extraction, the muscle samples were minced with a meat grinder and dried in an oven at 65°C for 12 h and 105°C for 12 h. The dried samples were then ground using a sample mill to generate a homogenous sample. The IMF of the breast (**IMFb**) and thigh muscle (**IMFt**) were extracted with anhydrous ether in a Soxhlet extractor (Luo et al., 2022). This Soxhlet extraction protocol was as follows: approximately 2 g samples were placed in a porous thimble and extracted in anhydrous

ether for 48 h within individual extraction tins. The residue solvent was allowed to evaporate for an additional 20 min before being placed in an oven for 8 h at 105°C to further remove any residual solvent. The variation in dry sample weight before and after extraction was used to calculate the IMF content. The formula of IMF calculation is expressed as:

$$\text{IMFcontent} = \frac{\text{The variation in dry sample weight before and after extraction}}{\text{Dry weight of the muscle sample before extraction}}$$

16S rRNA Sequence Processing

The cecal contents were scraped with a small steel spoon. Samples were immediately snap frozen in liquid nitrogen and then placed at −80°C until DNA extraction. Microbial DNA was extracted from all cecal samples using a QIAamp DNA Stool Mini Kit following the manufacturer's protocol (QIAGEN, Hilden, Germany). The V4 region of the 16S rRNA gene was amplified with the unique barcoded PCR primers 520F (5'-AYTGGGYDTAAAGNG-3') and 802R (5'-TACNVGGGTATCTAATCC-3') and sequenced (2 × 300 bp) on an Illumina MiSeq (Illumina, Inc., San Diego, CA) to a target depth of 60,000 reads per sample. The obtained sequences were processed with QIIME2 (Bolyen et al., 2019), and amplicon sequencing variants (**ASVs**) were assigned with DADA2. A phylogenetic tree was constructed with MAFFT and FastTree2, while taxonomic classification was conducted using a naive Bayesian classifier pretrained using the 520F/802R primers on the SILVA 138 database (Quast et al., 2012). Singletons were removed for this study. Alpha diversity was measured as Shannon's diversity and observed ASVs. The ASV tables and taxonomic classification were then exported for subsequent analysis.

Enterotype Classification

Enterotype analysis of cecal samples was performed according to the partitioning around medoid-based clustering protocols using Jensen–Shannon divergence of the normalized genus counts (Wu et al., 2011). The genera that were present in more than 50% of the cecal samples were selected for enterotype clustering. Enterotype identification was performed using the R program with the cluster packages. The maximum number of clusters evaluated was set at 20. The optimal number of enterotypes was identified by selecting the number that gave the highest Calinski−Harabasz index in the clustering model. Beta diversity at the genus level was estimated using the Jensen–Shannon divergence to calculate distances between the samples and visualized using principal coordinate analysis (**PCoA**).

Differential Analysis Between Enterotypes

The differences in alpha diversity (including Shannon index and observed ASVs) and phenotypes between the enterotypes were conducted by one-way analysis of variance (**ANOVA**) in the R program. The Wilcoxon rank-sum test was used to determine the changes in the taxa characteristics between enterotypes. The difference was considered statistically significant if the adjusted P value was less than 0.05. To determine the differences in microbial communities among each enterotype, Spearman and Pearson correlations for microbial genera that were detected in greater than 30% of individuals were quantified using the psych package in the R program, and P values were adjusted for FDRs using the BH method. Before the correlation was calculated, the relative abundance of each genus was $\log_{10}$-transformed. The correlation patterns were further filtered to select only adjusted P values less than 0.05 in both the Spearman and Pearson correlation analyses. Correlation networks of specific enterotypes were then visualized by using Cytoscape (Shannon et al., 2003).

Uncovering the IMF Content-Related Microbiota

Pairwise phenotypic correlations among fatness traits were evaluated using the corr.test function in R. To identify specific taxa that were significantly associated with the IMF level, a total of 84 genera detected in more than 30% of cecal samples were used for the following statistical analysis. A random-forest model (Liaw and Wiener, 2002) was carried out based on these genera to identify biomarkers of the IMF-related microbiota using the randomForest Package in R. Additionally, one-way ANOVA was used to test the difference in IMF traits between chickens with the highest 20% (N = 41) and lowest 20% (N = 41) abundances of specific genera. Additionally, the Wilcoxon rank-sum test was performed to determine the difference in the relative abundance of each genus between the highest 20% (N = 41) and lowest 20% (N = 41) trait-ranked chickens. A genus was considered significant if the P values from the ANOVA and Wilcoxon rank-sum test were all less than 0.05.

RESULTS

Microbial Characteristics of Cecal Enterotypes

16S rRNA gene sequencing produced 11,686 features from 206 chickens. After quality control, a total of 4,943 features were obtained and annotated to represent 154 genera by taxonomy classification. The enterotype clustering and PCoA analyses showed that the microbial communities were distinguished as 2 clusters, which we designated ET1 and ET2 (Figure 1A). The number of broilers associated with ET1 and ET2 was 110 and 96, respectively. The Shannon index of ET2 was significantly higher than that of ET1 ($P < 0.01$, Figure 1B). Additionally, the average observed ASV was 247 in ET1

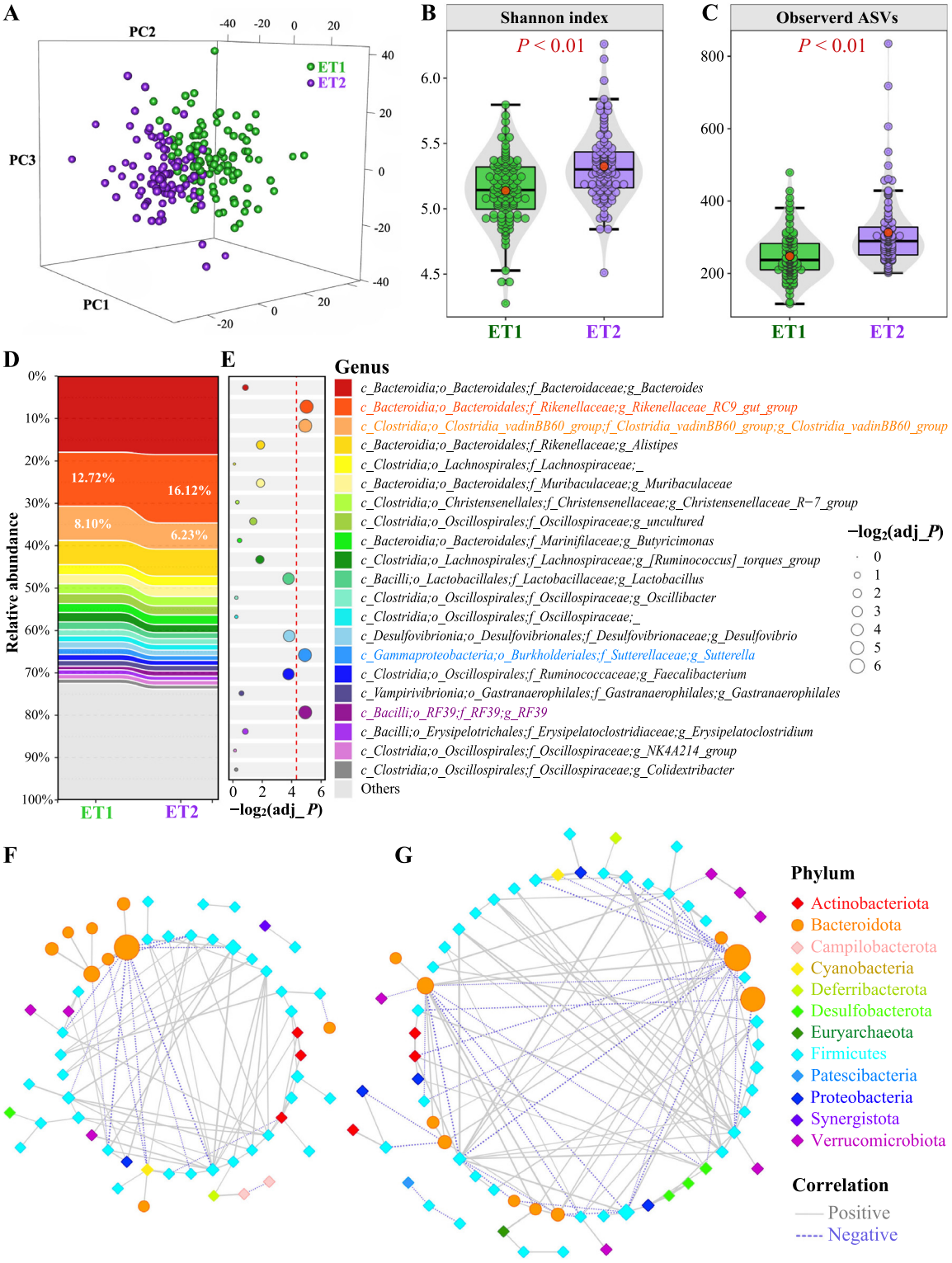

Figure 1. Microbial characteristics of the cecum in broilers. (A) Enterotype identification in 206 chickens using principal coordinate analysis. The boxplots show the difference in the Shannon index (B) and observed ASVs (C) between the two cecal enterotypes. Each point represents an individual. The center red point indicates the mean value in the corresponding enterotypes. (D) Proportions of genera characteristic of each enterotype. Only genera with an abundance of >1.0% are presented. (E) Comparison of the relative abundance of genera between the enterotypes. (F and G) Bacterial cooccurrence network in ET1 and ET2, respectively. The size of the nodes was proportional to the relative abundance of the genus. The color of the nodes represents the phylum. The gray lines and blue dashed lines represent positive and negative correlations, respectively. Abbreviation: ASVs, amplicon sequencing variants.

samples, which was significantly less than the 313 in ET2 ($P < 0.01$, Figure 1C).

Proportions of the genera of each enterotype are presented in Figure 1D. The two enterotypes had similar dominant genera. *Bacteroides*, *Rikenellaceae_RC9_gut_group*, and *Clostridia_vadinBB60_group* were the 3 most abundant genera in both enterotypes. At an FDR of 5%, however, 4 genera with abundance greater

than 1% differed between the 2 enterotypes (Figure 1E). Among these, the relatively high levels of the genera *Rikenellaceae_RC9_gut_group* (phylum Bacteroidota) and *Clostridia_vadinBB60_group* (phylum Firmicutes) distinguished the 2 enterotypes. The relative abundances of *Rikenellaceae_RC9_gut_group* and *Clostridia_vadinBB60_group* in ET1 were 12.72% and 8.10%, whereas the 2 genera in ET2 accounted for 16.12% and 6.23% of the total abundance, respectively.

A correlation analysis of core genera was then constructed to explore the microbial interactions between enterotypes. As shown in Figures 1F and 1G, the core genera strongly correlated (that is, they co-occurred or avoided each other) with those of other genera, suggesting that the enterotypes are in fact driven by groups of species that together contribute to the preferred community compositions. However, the interactions in ET1 were relatively simple compared with those in ET2.

Differences in Phenotypes Between the Cecal Enterotypes

As described by the above results, the community properties differed between ET1 and ET2. We further performed ANOVA on varied phenotypes between broilers belonging to different cecal enterotypes. As shown in Figure 2A, the IMFt and SFT exhibited significant differences between the 2 enterotypes ($P < 0.05$), although no discrepancy was found in growth performance (including BW, BWG, FI, and FCR), yield of edible carcass meat (including BMP and LP), body size (including BrW, FBL, SC, and SL), and organ features (including LiW, BiW, and CL). Additionally, the difference in LDL-CH between ET1 and ET2 was close to the significance level ($P = 0.06$).

We subsequently focused on fat-related traits, including IMFt, IMFb, SFT, AFP, LDL-CH, and TC, and found that fat deposition content (Figures 2B–2E) and blood biochemical parameters (Figures 2F and 2G) were both higher in the ET1 group than in the ET2 group. In particular, the IMFt content of ET1 broilers was 33.32 ± 9.95 mg/g, which was much greater than that of chickens classified as ET2, with a value of 29.50 ± 7.98 mg/g ($P < 0.01$, Figure 2B). These findings suggest an intimate link between the cecal microbiota and the amount of fat deposition in the chicken muscle and adipose tissue.

Relationships Among Fat-Related Traits

Meat from chicken is generally considered white. However, one of the important differences between chicken breasts and thighs is their fat content. In the present study, the IMF content of thigh muscle was 31.55 ± 9.26 mg/g, which was 42.76% greater than that in breast muscle at 22.10 ± 5.24 mg/g ($P < 0.01$). The phenotypic correlations among fat-related traits are presented in Figure 3. A moderate and positive correlation (r = 0.37, $P < 0.01$) was found in the IMF content between breast muscle and thigh muscle, indicating that improvement in the IMF content and probably meat quality in breast and thigh could be simultaneously targeted in broilers. The relationships between SFT and AFP, AFP and LDL-CH, and LDL-CH and TG were 0.23, −0.21, and 0.28, respectively. The IMF content of both muscle tissues was not significantly associated with 2 adipose tissues (AFP and SFT) or blood biochemical parameters (LDL-CH and TG), suggesting that IMF deposition was not associated with the amount of adipose tissue.

IMF-Related Microbiota and Their Characteristics

To identify potential IMF-related cecal taxa across both muscle tissues, we first chose random forest models to identify the genera that were the best predictors of IMFt and IMFb. As indicated in Figure 4A, *vadinBE97*, *Escherichia-Shigella*, *[Ruminococcus]_torques_group*, and *Lactobacillus* were found to be important genera with the greatest effect on predicting the IMF content of the thigh. The top 4 IMFb-related signature genera were *vadinBE97*, *[Ruminococcus]_torques_group*, *Mucispirillum*, and *Subdoligranulum* (Figure 4B). Notably, the genera *vadinBE97* and *[Ruminococcus]_torques_group* were observed in both random forest analyses.

To further confirm the above results, all the broilers were successively ranked by the value of IMF traits or the relative abundance of prevalent genera, and the highest and lowest 20% of chickens for each ranking were selected for subsequent statistical tests. As shown in Figure 4C, nine and four genera had significantly different abundances between the highest and lowest IMFt or IMFb groups by the Wilcoxon rank-sum test, respectively. We then performed ANOVA to detect the difference in IMF phenotypes between the chickens with the highest and lowest abundances of each genus. Three and 3 signature genera were found to be associated with the IMFt and IMFb contents, respectively. Among these, the genus *vadinBE97* was observed in all 4 significance tests (Figure 4C and Supplemental Table S1).

The genus *vadinBE97* belongs to the phylum Verrucomicrobiota and accounted for 0.40% of the total abundance of genera in the cecum. Compared with the chickens with the highest abundance of cecal *vadinBE97*, the IMF level in both muscle tissues was significantly higher in the chickens with the lowest abundance ($P < 0.01$, Figures 4D and 4E). Notably, there were no significant differences in AFP, SFT, LDL-CH and TG between the 2 groups ($P > 0.05$). We subsequently focused on the relationship between *vadinBE97* and other genera. Of the 83 prevalent genera, we found that 21 genera exhibited significant and positive correlations with *vadinBE97* (Figure 5 and Supplemental Table S2). In particular, 5 and 12 genera belonged to the phyla Verrucomicrobiota and Firmicutes, which accounted for 100% (5/5) and 25% (12/48) of the tested genera, respectively. These findings indicated that *vadinBE97* engages in a considerable number of interactions with other genera.

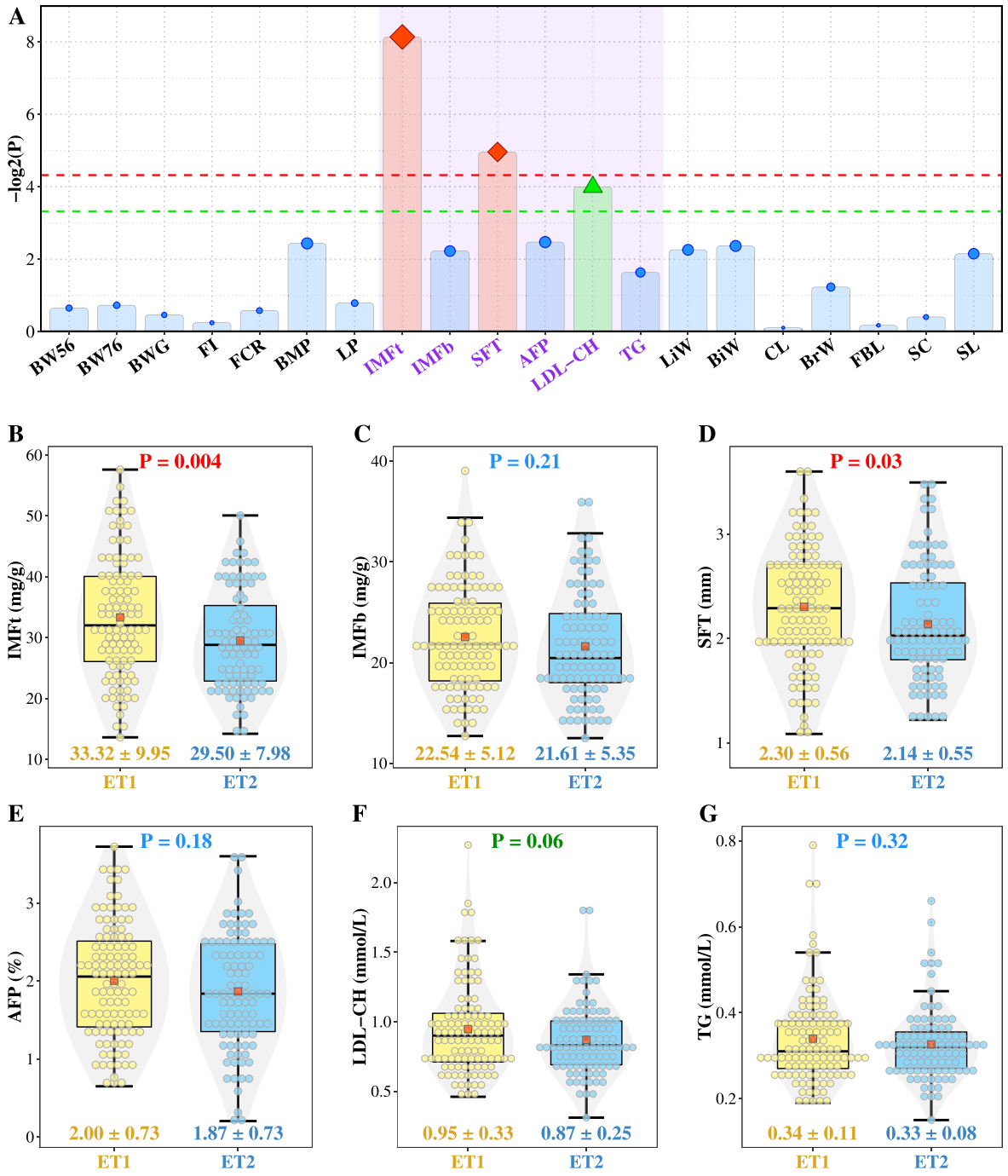

Figure 2. Comparison of phenotypes between cecal enterotypes. (A) *P* values obtained from ANOVA for each trait between the two cecal enterotypes. The horizontal red and green lines indicate significance ($P = 0.05$) and suggestive significance thresholds ($P = 0.10$). Abbreviations: BW56 and BW76, body weight at 56 and 76 d of age, respectively; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; BMP, LP and AFP, the percentage of single breast muscle, single leg and abdominal fat, respectively; IMFb and IMFt, the intramuscular fat content of the breast and thigh muscle, respectively; SFT, subcutaneous fat thickness; LDL-CH and TG, the concentrations of low-density lipoprotein cholesterol and triglyceride in the serum, respectively; LiW and BiW, liver weight and bile weight, respectively; CL, cecal length. BrW, breast width; FBL, fossil bone length; SL, shank length; SC, shank circumference. (B–G) The boxplots show the differences in fat-related traits and blood biochemical parameters. Each point represents an individual. The center red point indicates the mean value in the corresponding enterotypes, and the data are expressed as the means ± SDs.

DISCUSSION

It is generally accepted that a higher IMF content has a positive effect on the sensory quality of meat. Growing evidence regarding the gut-muscle axis has indicated that muscle metabolism can be improved by regulating the gut microbiota (Frampton et al., 2020). The cecal microbiota is recognized as a strong determinant of host physiology and metabolism and has attracted extensive attention in chickens. However, the organization and ecological properties of the microbial ecosystem remain underinvestigated. In this study, two dominant enterotypes for the cecal microbiota were identified. To the best of our knowledge, this is the first enterotype-like

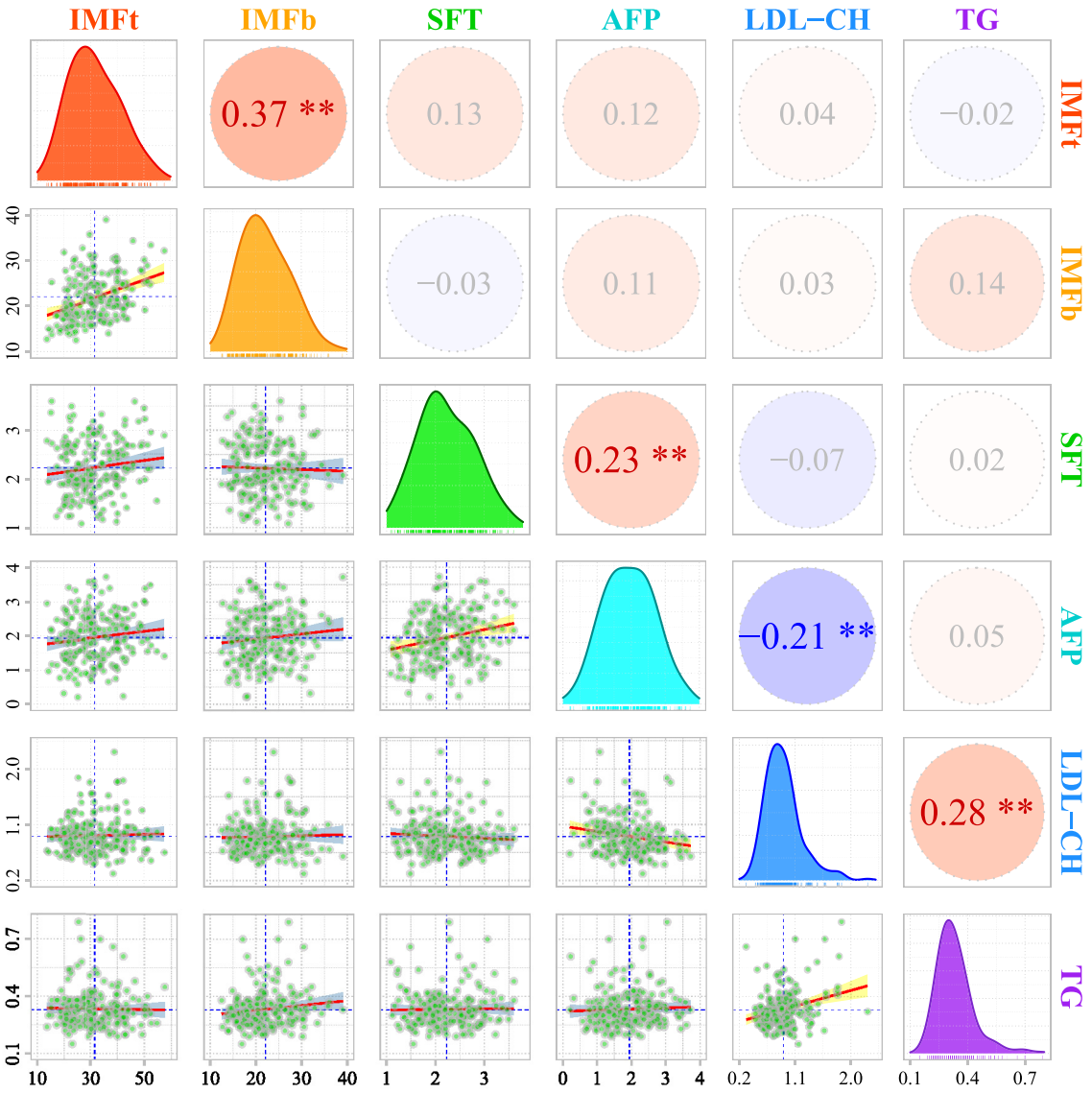

Figure 3. Relationships among fat-related traits and blood biochemical parameters. Estimates of Pearson correlations are shown above the diagonal. ** indicates $P < 0.01$. Scatter plots with trend lines and 95% confidence intervals for the phenotype value between two traits are shown below the diagonal. The diagonal shows the density plot of each trait.

clustering analysis of the cecum of chickens. The observed microbial differences between enterotypes support the notion that they have varying community properties, such as diversity and interaction strengths. Moreover, fat-related microbial compositional differences within the cecum suggest that the cecal microbiota is possibly associated with the IMF content in broilers. We further identified the genus *vadinBE97*, which was significantly correlated with the IMF content of both thigh and breast muscle in chickens. Specifically, the lower abundance of cecal *vadinBE97* was related to a higher IMF content in both muscle tissues. These results suggest the potential for the application of the gut-muscle axis in the regulation of IMF content in chickens.

The amount of IMF varies between muscle types. The breast and thigh muscles are the 2 largest proportions of edible carcass on chickens (Mai et al., 2021). The total lipid content of thigh muscle is higher than that of breast muscle (Pikul et al., 1985; Milicevic et al., 2014; Mahiza et al., 2021), although their shape can be slightly similar. In our study, the IMF content of thigh muscle was 42.76% greater than that of breast muscle, which is consistent with the results of a study on a commercial breed (Crespo and Esteve-Garcia, 2001). A moderate correlation was found in the IMF content between the 2 muscle tissues, while the relationship of the IMF content with adipose tissues was negligible. Similarly, Zerehdaran et al. (2004) found that the correlation between IMF and abdominal fat and skin weight was close to zero. A major reason for this is that the compositions of fat are different among tissues. The abdominal and subcutaneous fat had very similar fatty acid patterns and differed significantly from the composition of the fat extracted from the breast and thigh (Hrdinka et al., 1996; Crespo and Esteve-Garcia, 2001). These findings suggested that the improvement in IMF would not result in high accumulation of adipose tissues with little economic value.

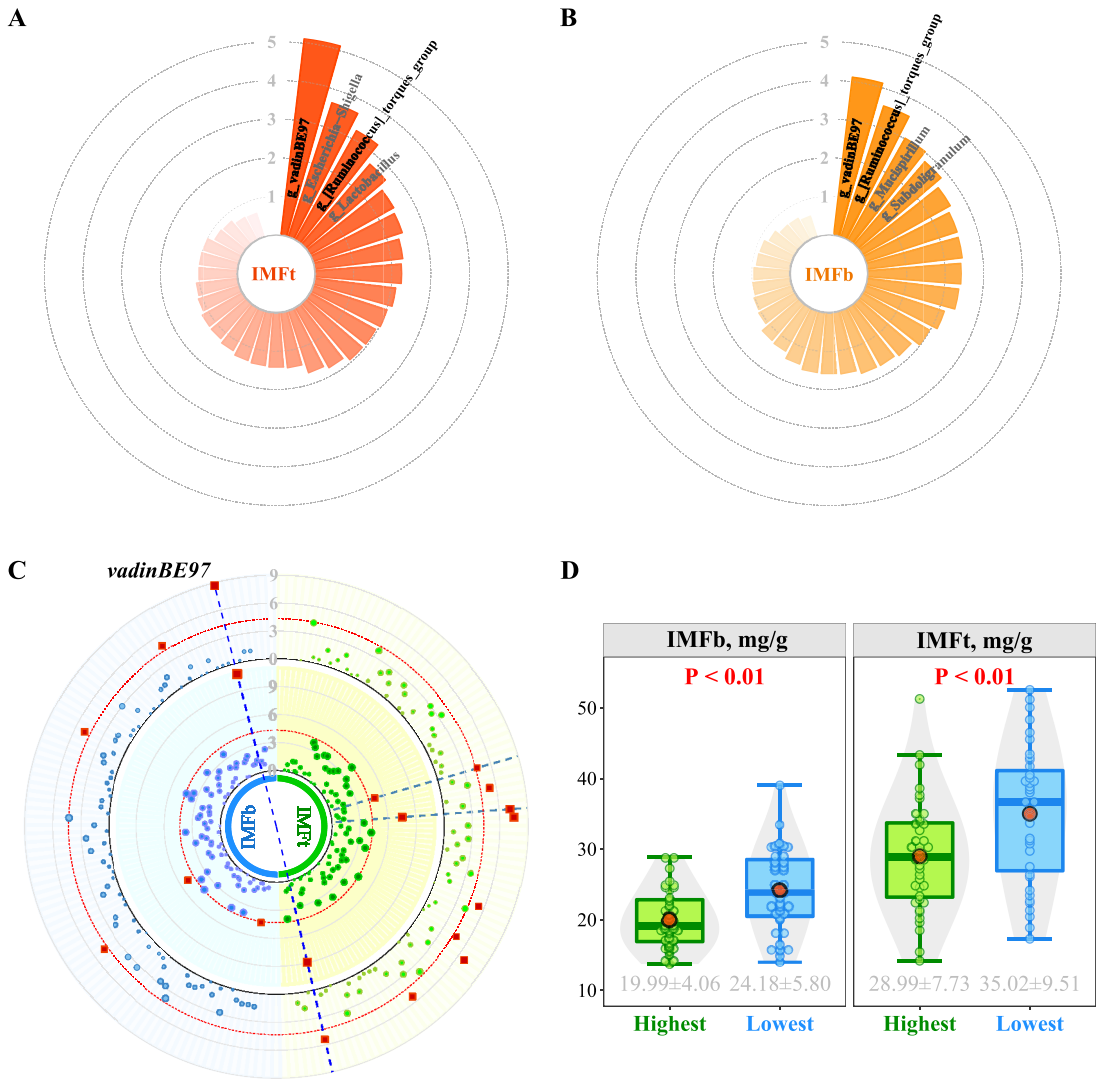

Figure 4. Identification of IMF-related bacteria. IMF-related microbiota identified by a random forest regression model. The variable importance based on the prevalent genus (prevalence > 30%) by random forest for prediction of the IMF content of thigh (A) and breast (B). (C) *P* values for the Wilcoxon rank-sum test (outer circle) and ANOVA (inner circle). The *P* values for the significance test are plotted as $-\log_2(P)$. The dashed red line shows the significance threshold ($P = 0.05$). Each point represents a genus, and the red point indicates that the *P* value passed the significance threshold. The dashed gray line indicates that the *P* values from the Wilcoxon rank-sum test and ANOVA are both less than 0.05. (D and E) Difference in the IMF content of thigh and breast between the chickens with the highest and lowest abundance of cecal *vadinBE97*, respectively. Each point represents an individual. The center red point indicates the mean value in the corresponding group, and the data are expressed as the means ± SDs.

As a complex trait, IMF is influenced by various factors, including host genetics (Chen et al., 2008; Luo et al., 2022) and diet (Ma et al., 2015). In recent years, growing evidence has indicated that skeletal muscle properties, including lipid metabolism, are closely linked to the gut microbiota (Frampton et al., 2020). Considering the importance and complexity of the gut ecosystem, there is great interest in identifying patterns of microbial composition, as they may help us assess the importance of the function and ecology of the gut microbiome. The enterotype summarizes the microbial characteristics using mathematical methods, which have been deeply investigated in humans (Arumugam et al., 2011; Wu et al., 2011; Costea et al., 2018). The analysis of public datasets spanning several nations and continents reveals that human gut communities are generally classified into 3 enterotypes, which are significantly associated with the health status of individuals (Arumugam et al., 2011). Several associations between enterotypes and animal production have also been reported, although the gut composition of these animals is distinct from that of humans. For example, the cluster classification of the pig microbiota was significantly associated with porcine growth traits (Ramayo-Caldas et al., 2016). Guo et al. (2021) found that the enterotype of yaks plays a key role in mediating nutritional homeostasis at high altitudes. In chickens, duodenal enterotypes have been found to be linked to fat deposition (Yuan et al., 2020). Thus, analysis of whole community organization is an important frontier in the life sciences.

To better understand the contribution of the cecal ecosystem to hosts, we examined the possibility of defining enterotypes and their relationship with phenotypes. Analysis of 206 cecal samples of broilers revealed that individuals could be classified into 2 enterotypes

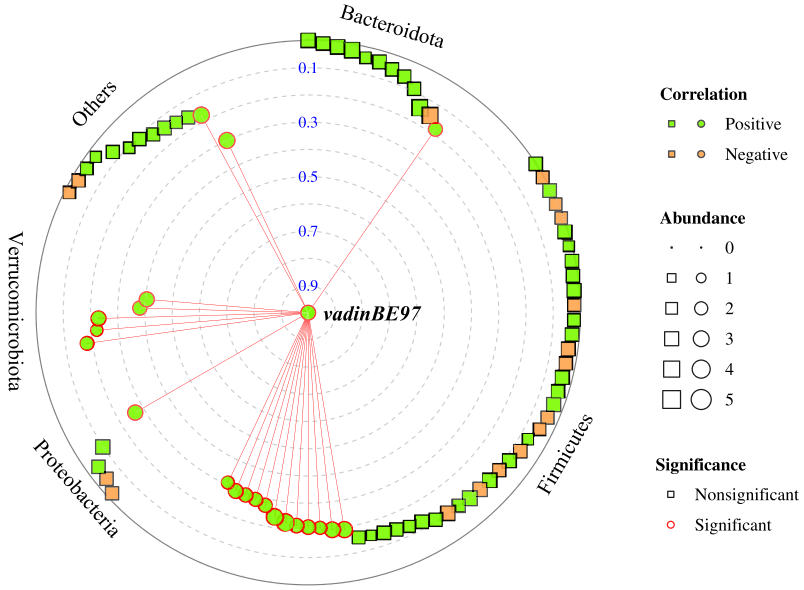

Figure 5. Spearman correlation between *vadinBE97* and other prevalent genera. The size and shape of the points represent the relative abundance and significance level, respectively. The green and orange points indicate positive and negative correlations, respectively. Gray circles indicate the scale of correlation from 0 to 0.9. The red lines indicate that the genera are significantly correlated with *vadinBE97*.

distinguished by *Clostridia_vadinBB60_group* (ET1) and *Rikenellaceae_RC9_gut_group* (ET2). *Clostridiales_vadinBB60_group* is considered an SCFA-producing bacterial group (Zhang et al., 2022) and is positively correlated with feed efficiency (Mccormack et al., 2017). Lin et al. (2016) observed that an unclassified *Clostridiales_vadinBB60_group* was increased in relative abundance in rats fed a high-fat diet for 4 wk, that is, during the preobesity state, indicating its possible role in metabolism. In our study, broilers associated with ET1 had a higher IMF content and SFT than those associated with ET2, although the alpha diversity and ecological community interactions were higher in ET2 individuals. These findings allow us to foresee that microbial ecosystems may be relevant for farm animal production.

Host microbial ecosystems are vastly complex, and many different bacteria occur, each with the potential to interact with the host by modulating metabolism (Foster et al., 2017). Thus, we further investigated which bacteria play crucial roles in IMF deposition. The most notable difference in relative abundance across IMF-ranked broilers occurred for members of *vadinBE97*. The lower abundance of cecal *vadinBE97* was linked with a higher IMF content of both breast and thigh muscles, whereas no significant association was found between *vadinBE97* and adipose tissues. These findings corroborated our aforementioned results regarding approaches to improving the IMF content by regulating the gut microbiota without affecting the accumulation of adipose tissues. Although the actual roles of the genus *vadinBE97* in IMF deposition are largely unknown, a recent study in yaks found that an unclassified genus belonging to the vadinBE97 family was positively correlated with the SCFAs of rumen fluid and the IMF content (Du et al., 2021), suggesting that *vadinBE97* may regulate IMF deposition by affecting the concentration of SCFAs. The differentially abundant cecal taxa identified could potentially be exploited as biomarkers for IMF content. However, additional research is needed to investigate the reliability of the IMF-associated microbial taxa identified here, that is, across batches of broilers or rearing environments.

Additionally, *vadinBE97* is part of the ecosystem and has the potential to exert diverse effects on neighboring bacteria, despite presenting at low relative abundances. The repurposing of communities from individual microbiota to microecological systems will provide a more comprehensive view to understand and study the potential interplay between the microbiota and host. Therefore, community ecology and potential microbial interactions must be carefully considered when developing approaches to improve the IMF content by regulating the cecal microbiota.

In conclusion, this study has clearly described the ecological properties of the cecum in broilers and provided evidence on the existence of links between the cecal microbial communities and the IMF content. Broilers could be classified into 2 enterotypes, in which fat deposition was significantly different. The improvement in IMF would not result in high accumulation of adipose tissues. It should be noted that the IMF-associated compositional differences were relatively subtle, presenting interplay with neighboring bacteria. Nonetheless, the differentially abundant cecal taxa identified could potentially be exploited as biomarkers for IMF. Specifically, a lower relative abundance of the genus *vadinBE97* was found in broilers that had a higher IMF content in breast and thigh muscle tissues. Although the results provide potential possibilities for manipulating the meat quality properties of broilers by modulating the gut microbiota, intervention studies are required to confirm the insights provided to improve meat quality in the future.

ACKNOWLEDGMENTS

We thank Guangdong Wen's Nanfang Poultry Breeding Co., Ltd., for providing the experimental chickens.

This work was supported by the National Natural Science Foundation of China (32102535 and 31930105) and the National Key Research and Development Program of China (2022YFF1000204).

DISCLOSURES

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102568.

REFERENCES

Arumugam, M., J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G. R. Fernandes, J. Tap, T. Bruls, J. Batto, M. Bertalan, N. Borruel, F. Casellas, L. Fernandez, L. Gautier, T. Hansen, M. Hattori, T. Hayashi, M. Kleerebezem, K. Kurokawa, M. Leclerc, F. Levenez, C. Manichanh, H. B. Nielsen, T. Nielsen, N. Pons, J. Poulain, J. Qin, T. Sicheritz-Ponten, S. Tims, D. Torrents, E. Ugarte, E. G. Zoetendal, J. Wang, F. Guarner, O. Pedersen, W. M. de Vos, S. Brunak, J. Doré, J. Weissenbach, S. D. Ehrlich, and P. Bork. 2011. Enterotypes of the human gut microbiome. Nature 473:174–180.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodriguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. Bin Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. I. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vazquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:1091.

Cai, B., M. Ma, J. Zhang, S. Kong, Z. Zhou, Z. Li, B. A. Abdalla, H. Xu, X. Zhang, R. A. Lawal, and Q. Nie. 2022. Long noncoding RNA *ZFP36L2-AS* functions as a metabolic modulator to regulate muscle development. Cell Death. Dis. 13:389.

Chen, B., D. Li, D. Leng, H. Kui, X. Bai, and T. Wang. 2022. Gut microbiota and meat quality. Front. Microbiol. 13:951726.

Chen, J. L., G. P. Zhao, M. Q. Zheng, J. Wen, and N. Yang. 2008. Estimation of genetic parameters for contents of intramuscular fat and inosine-5′-monophosphate and carcass traits in Chinese Beijing-You chickens. Poult. Sci. 87:1098–1104.

Costea, P. I., F. Hildebrand, M. Arumugam, F. Bäckhed, M. J. Blaser, F. D. Bushman, W. M. de Vos, S. D. Ehrlich, C. M. Fraser, M. Hattori, C. Huttenhower, I. B. Jeffery, D. Knights, J. D. Lewis, R. E. Ley, H. Ochman, P. W. O Toole, C. Quince, D. A. Relman, F. Shanahan, S. Sunagawa, J. Wang, G. M. Weinstock, G. D. Wu, G. Zeller, L. Zhao, J. Raes, R. Knight, and P. Bork. 2018. Enterotypes in the landscape of gut microbial community composition. Nat. Microbiol. 3:8–16.

Crespo, N., and E. Esteve-Garcia. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. Poult. Sci. 80:71–78.

Cui, H., L. Liu, X. Liu, Y. Wang, N. Luo, X. Tan, Y. Zhu, R. Liu, G. Zhao, and J. Wen. 2022. A selected population study reveals the biochemical mechanism of intramuscular fat deposition in chicken meat. J. Anim. Sci. Biotechnol. 13:54.

Du, M., C. Yang, Z. Liang, J. Zhang, Y. Yang, A. A. Ahmad, P. Yan, and X. Ding. 2021. Dietary energy levels affect carbohydrate metabolism-related bacteria and improve meat quality in the *Longissimus Thoracis* muscle of Yak (*Bos grunniens*). Front. Vet. Sci. 8:718036.

Feng, Y., D. Liu, Y. Liu, X. Yang, M. Zhang, F. Wei, D. Li, Y. Hu, and Y. Guo. 2022. Host-genotype-dependent cecal microbes are linked to breast muscle metabolites in Chinese chickens. iScience 25:104469.

Foster, K. R., J. Schluter, K. Z. Coyte, and S. Rakoff-Nahoum. 2017. The evolution of the host microbiome as an ecosystem on a leash. Nature 548:43–51.

Frampton, J., K. G. Murphy, G. Frost, and E. S. Chambers. 2020. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. Nat. Metab. 2:840–848.

Guo, N., Q. Wu, F. Shi, J. Niu, T. Zhang, A. A. Degen, Q. Fang, L. Ding, Z. Shang, Z. Zhang, and R. Long. 2021. Seasonal dynamics of diet-gut microbiota interaction in adaptation of yaks to life at high altitude. NPJ Biofilms Microbiomes 7:38.

Hocquette, J. F., F. Gondret, E. Baéza, F. Médale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4:303–319.

Hrdinka, C., W. Zollitsch, W. Knaus, and F. Lettner. 1996. Effects of dietary fatty acid pattern on melting point and composition of adipose tissues and intramuscular fat of broiler carcasses. Poult. Sci. 75:208–215.

Jiang, M., W. L. Fan, S. Y. Xing, J. Wang, P. Li, R. R. Liu, Q. H. Li, M. Q. Zheng, H. X. Cui, J. Wen, and G. P. Zhao. 2017. Effects of balanced selection for intramuscular fat and abdominal fat percentage and estimates of genetic parameters. Poult. Sci. 96:282–287.

Konieczka, P., I. Wojtasik-Kalinowska, A. Poltorak, M. Kinsner, D. Szkopek, B. Fotschki, J. Juskiewicz, J. Banach, and M. Michalczuk. 2022. Cannabidiol affects breast meat volatile compounds in chickens subjected to different infection models. Sci. Rep. 12:18940.

Lei, J., Y. Dong, Q. Hou, Y. He, Y. Lai, C. Liao, Y. Kawamura, J. Li, and B. Zhang. 2022. Intestinal microbiota regulate certain meat quality parameters in chicken. Front. Nutr. 9:747705.

Liaw, A., and M. Wiener. 2002. Classification and Regression by randomForest. R News 2:18–22.

Lin, H., Y. An, F. Hao, Y. Wang, and H. Tang. 2016. Correlations of fecal metabonomic and microbiomic changes induced by high-fat diet in the pre-obesity state. Sci. Rep. 6:21618.

Listrat, A., B. Lebret, I. Louveau, T. Astruc, M. Bonnet, L. Lefaucheur, B. Picard, and J. Bugeon. 2016. How muscle structure and composition influence meat and flesh quality. Sci. World J. 2016:3182746.

Liu, R., M. Zheng, J. Wang, H. Cui, Q. Li, J. Liu, G. Zhao, and J. Wen. 2019. Effects of genomic selection for intramuscular fat content in breast muscle in Chinese local chickens. Anim. Genet. 50:87–91.

Luo, N., J. Shu, X. Yuan, Y. Jin, H. Cui, G. Zhao, and J. Wen. 2022. Differential regulation of intramuscular fat and abdominal fat deposition in chickens. BMC Genomics 23:308.

Ma, J. S., W. H. Chang, G. H. Liu, S. Zhang, A. J. Zheng, Y. Li, Q. Xie, Z. Y. Liu, and H. Y. Cai. 2015. Effects of flavones of sea

buckthorn fruits on growth performance, carcass quality, fat deposition and lipometabolism for broilers. Poult. Sci. 94:2641–2649.
Mahiza, M., H. I. Lokman, and E. B. Ibitoye. 2021. Fatty acid profile in the breast and thigh muscles of the slow- and fast-growing birds under the same management system. Trop. Anim. Health Prod. 53:409.
Mai, C., C. Wen, Z. Xu, G. Xu, S. Chen, J. Zheng, C. Sun, and N. Yang. 2021. Genetic basis of negative heterosis for growth traits in chickens revealed by genome-wide gene expression pattern analysis. J. Anim. Sci. Biotechnol. 12:52.
McCormack, U. M., T. Curiao, S. G. Buzoianu, M. L. Prieto, T. Ryan, P. Varley, F. Crispie, E. Magowan, B. U. Metzler-Zebeli, D. Berry, O. O'Sullivan, P. D. Cotter, G. E. Gardiner, and P. G. Lawlor. 2017. Exploring a possible pink between the intestinal microbiota and feed efficiency in pigs. Appl. Environ. Microbiol. 83:e317–e380.
Milicevic, D., D. Vranic, Z. Masic, N. Parunovic, D. Trbovic, J. Nedeljkovic-Trailovic, and Z. Petrovic. 2014. The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. Lipids Health Dis. 13:42.
Morrison, D. J., and T. Preston. 2016. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes 7:189–200.
Mottet, A., and G. Tempio. 2017. Global poultry production: current state and future outlook and challenges. Worlds Poult. Sci. J. 73:245–256.
Nicholson, J. K., E. Holmes, J. Kinross, R. Burcelin, G. Gibson, W. Jia, and S. Pettersson. 2012. Host-gut microbiota metabolic interactions. Science 336:1262–1267.
Pikul, J., D. E. Leszczynski, and F. A. Kummerow. 1985. Influence of fat content and composition on malonaldehyde concentration in chicken meat and skin. Poult. Sci. 64:311–317.
Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic. Acids. Res. 41:D590–D596.
Ramayo-Caldas, Y., N. Mach, P. Lepage, F. Levenez, C. Denis, G. Lemonnier, J. J. Leplat, Y. Billon, M. Berri, J. Dore, C. Rogel-Gaillard, and J. Estelle. 2016. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J. 10:2973–2977.
Sarsenbek, A., T. Wang, J. K. Zhao, and W. Jiang. 2013. Comparison of carcass yields and meat quality between Baicheng-You chickens and Arbor Acres broilers. Poult. Sci. 92:2776–2782.
Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13:2498–2504.
Wang, Y., J. Sun, H. Zhong, N. Li, H. Xu, Q. Zhu, and Y. Liu. 2017. Effect of probiotics on the meat flavour and gut microbiota of chicken. Sci. Rep. 7:6400.
Wen, C., W. Yan, C. Mai, Z. Duan, J. Zheng, C. Sun, and N. Yang. 2021. Joint contributions of the gut microbiota and host genetics to feed efficiency in chickens. Microbiome 9:126.
Wen, C., W. Yan, C. Sun, C. Ji, Q. Zhou, D. Zhang, J. Zheng, and N. Yang. 2019. The gut microbiota is largely independent of host genetics in regulating fat deposition in chickens. ISME J. 13:1422–1436.
Wen, C., W. Yan, J. Zheng, C. Ji, D. Zhang, C. Sun, and N. Yang. 2018. Feed efficiency measures and their relationships with production and meat quality traits in slower growing broilers. Poult. Sci. 97:2356–2364.
Wu, G. D., J. Chen, C. Hoffmann, K. Bittinger, Y. Y. Chen, S. A. Keilbaugh, M. Bewtra, D. Knights, W. A. Walters, R. Knight, R. Sinha, E. Gilroy, K. Gupta, R. Baldassano, L. Nessel, H. Li, F. D. Bushman, and J. D. Lewis. 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334:105–108.
Yang, C., M. Qiu, Z. Zhang, X. Song, L. Yang, X. Xiong, C. Hu, H. Pen, J. Chen, B. Xia, H. Du, Q. Li, X. Jiang, and C. Yu. 2022. Galacto-oligosaccharides and xylo-oligosaccharides affect meat flavor by altering the cecal microbiome, metabolome, and transcriptome of chickens. Poult. Sci. 101:102122.
Yang, X., B. Zhang, Y. Guo, P. Jiao, and F. Long. 2010. Effects of dietary lipids and Clostridium butyricum on fat deposition and meat quality of broiler chickens. Poult. Sci. 89:254–260.
Yuan, Z., W. Yan, C. Wen, J. Zheng, N. Yang, and C. Sun. 2020. Enterotype identification and its influence on regulating the duodenum metabolism in chickens. Poult. Sci. 99:1515–1527.
Zerehdaran, S., A. L. Vereijken, J. A. van Arendonk, and E. H. van der Waaijt. 2004. Estimation of genetic parameters for fat deposition and carcass traits in broilers. Poult. Sci. 83:521–525.
Zhang, J., W. Jin, Y. Jiang, F. Xie, and S. Mao. 2022. Response of milk performance, rumen and hindgut microbiome to dietary supplementation with Aspergillus oryzae fermentation extracts in dairy cows. Curr. Microbiol. 79:113.
Zheng, A., J. Luo, K. Meng, J. Li, S. Zhang, K. Li, G. Liu, H. Cai, W. L. Bryden, and B. Yao. 2014. Proteome changes underpin improved meat quality and yield of chickens (*Gallus gallus*) fed the probiotic Enterococcus faecium. BMC Genomics 15:1167.