Effects of cage versus floor rearing system on goose intestinal histomorphology and cecal microbial composition

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ABSTRACT Due to the demand for modern goose production and the pressure of environmental protection, the rearing systems of geese are changing from traditional waterside rearing to intensive rearing systems such as floor rearing (**FR**) and cage rearing (**CR**) systems. However, little is known about the effects of different rearing systems on goose intestinal functions and cecal microbial composition. Therefore, this study aimed to compare intestinal histomorphology and cecal microbial composition differences in geese reared under CR and FR at 270 d of age. Histomorphological analysis showed that the ileal villus height (**VH**) to crypt depth (**CD**) ratio was significantly greater in CR than in FR ($P < 0.001$). Taxonomic analysis showed that the dominant bacteria of cecal microorganisms in both rearing systems were roughly similar, with Bacteroidota, Firmicutes, Fusobacteriota, and Proteobacteria being the dominant phyla while *Bacteroides, Fusobacterium*, and uncultured_bacterium_o_Bacteroidales being the dominant genera. Differentially abundant taxa between CR and FR were also identified using Linear Discriminant Analysis Effect Size (**LEfSe**) analysis ($P < 0.05$, LDA score > 3.5). *Megamonas* and *Anaerobiospirillum* were significantly enriched in the CR group at the genus level, while uncultured_bacterium_f_Rikenellaceae and *Sutterella* were significantly enriched in the FR group. Notably, we found that the relative abundance of uncultured_bacterium_f_Rikenellaceae was significantly negatively correlated with the ileal VH and VH/CD ($P < 0.05$). The relative abundance of *Megamonas* and *Anaerobiospirillum* were significantly negatively correlated with abdominal fat weight and relative abdominal fat weight ($P < 0.01$), whereas that of *Sutterella* was significantly positively correlated with abdominal fat weight and relative abdominal fat weight ($P < 0.01$). Furthermore, PICRUSt2 analysis indicated that the lipid metabolism pathways of cecal microorganisms were lower enriched in CR than in FR. In conclusion, compared with FR, the CR significantly changed goose ileal histomorphological characteristics and cecal microbial composition, thereby affecting goose physiological functions and production performance.

Key words: goose, rearing system, intestinal histomorphology, cecal microorganisms

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INTRODUCTION

In recent years, the market demand for geese in China has gradually expanded, and the consumption share of geese in the meat market is on the rise. The number of commercial geese increased from 180 million in 1990 to 700 million in 2020, and the world proportion also increased from about 85% to more than 90% (FAO, 2022). Driven by market demand and environmental protection pressure, the goose rearing system is changing from traditional farmers' waterside rearing to modern intensive rearing. The floor rearing (**FR**) system has become the primary system of large-scale goose rearing in China because of its low construction costs and easy management (Liu et al., 2011; Liao et al., 2021). However, compared to other commercial poultry breeds (broilers, layers and ducks) in China, geese have lower reproductive efficiency (Tóth-Baranyi, 1957). Not only are geese low in egg production, but their reproductive mode is still natural mating with low reproductive efficiency. As we all know, artificial insemination technology is an essential means to improve the reproductive efficiency of poultry. The cage rearing (**CR**) system is

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usually used as an auxiliary rearing system for artificial insemination of poultry, so it is widely used in poultry breeding (Holleman and Biellier, 1976; Ansah et al., 1980; Shaheen et al., 2021). However, CR is a new rearing system for geese, and we do not know what changes will occur to geese's production performance and physiological functions in CR.

The intestine is a vital organ responsible for nutrient absorption and waste excretion and an important site of host immunity (Chin et al., 2017). Healthy intestines in poultry are essential to perform these functions effectively, while a damaged intestine may not be able to perform one or more of these functions (Adedokun and Olojede, 2019). Some studies have shown that the intestinal mucosal barrier in chickens was the first line of defense against harmful substances that may destroy the lumen environment (Azzam et al., 2017), and balanced gastrointestinal bacteria benefit the host by maintaining normal digestion and absorption, improving intestinal barrier integrity, promoting another symbiosis, and eliminating pathogens (Burkholder et al., 2008; Song et al., 2013). It is widely recognized that different rearing systems would cause remarkable changes in poultry intestinal histomorphology and microbial composition. For example, compared with floor rearing, cage rearing changed the abundance of intestinal microbiota and increased the meat production and meat quality of broilers (Wang et al., 2021). Compared with the traditional rearing system, the dryland rearing on netting floors (**DRNF**) system changed intestinal microbial abundance and enhanced the immune ability of Shaoxing ducks (Zhao et al., 2019). Compared with floor rearing, cage rearing changed the cecal microbial abundance of ducks and affected cecal mucosa gene expression (Zhu et al., 2020). Therefore, we hypothesized that the rearing systems might also alter geese's physiological functions and production performance.

As one of the herbivore species, the goose has a strong ability to utilize crude fiber in the grass in its gastrointestinal tract. However, the gastrointestinal tract of geese does not secrete cellulolytic and hemicellulolytic enzymes, which are mainly secreted by microorganisms in the gastrointestinal tract of geese (Guo et al., 2019). The digestibility of hemicellulose and cellulose in geese were 41.5 and 17.4%, respectively, under the action of cecal microorganisms (Lou et al., 2010; Yan et al., 2019). Therefore, the changes in geese' cecal microorganisms may affect geese's digestion and absorption capacity. Multiple studies in chickens have demonstrated that the jejunum and ileum of poultry were the major sites for absorbing most nutrients (Imondi and Bird, 1965; Renner, 1965; Hurwitz and Bar, 1970; Rodriguez-Sanchez et al., 2019), and the jejunum and ileal morphological changes may affect the digestion and absorption of nutrients in geese. Thus, the purpose of this study was to compare geese' jejunal and ileal histomorphological characteristics and cecal microbial composition between CR and FR, and to determine the relationship between differentially enriched bacteria, intestinal histomorphological characteristics, and goose slaughter traits. These results are expected to deepen our understanding of how cage and floor feeding systems affect the growth of geese and provide references for the selection of rearing systems in the process of transformation and upgrading of the goose industry.

MATERIALS AND METHODS

Ethics Statement

All experimental procedures involving animal manipulation were approved by the Institutional Animal Care and Use Committee (**IACUC**) of Sichuan Agricultural University (Chengdu Campus, Sichuan, China) under Approval No. 20160067.

Experimental Animals and Sample Collection

In this study, 60 male goslings with similar body weight were selected, which were Sichuan white goose, a native Chinese breed. All geese were hatched with the same clutch at the same time and brooded under the same condition of natural light and temperature at the waterfowl breeding experimental farm at Sichuan Agricultural University (Ya' an, Sichuan, China). After 28 d of hatching, these geese were reared in the same pen (half dormitory and swimming pool) until 120 d of age. At 120 d of age, these geese were randomly assigned to 2 rearing systems: CR and FR. All geese were reared with the same diet in Table 1 (Sanwang Agriculture and Animal Husbandry Co., Ltd, Chengdu, China). All experimental geese had free access to food and water and received the same routine immunization procedure. At 270 d of age, 15 geese were randomly selected from FR and CR, respectively, for weighing and slaughter. These

Table 1. Ingredients and nutrients composition of basal diets.

Items	Stage (28−270 d)
Ingredients	
Corn (%)	57.70
Soybean meal (%)	27.50
Wheat middling (%)	7.50
Wheat bran (%)	2.00
Calcium hydrogen phosphate (%)	1.62
Soybean oil (%)	1.40
Limestone powder (%)	0.93
NaCl (%)	0.35
Vitamin and mineral premix (%)	1.00
Total (%)	100
Nutrients	
Metabolizable energy (Mcal/kg)	2900
Dry matter (%)	87.12
Crude protein (%)	17.50
Crude fat (%)	4.13
Crude fiber (%)	3.00
Calcium (%)	0.85
Total phosphorus (%)	0.65
Available phosphorus (%)	0.40
Lysine (%)	0.85
Methionine (%)	0.40
Methionine + Cystine (%)	0.70
Threonine (%)	0.60
Tryptophan (%)	0.19

geese were first stunned with CO^2 and then euthanized by cervical dislocation. After dissection, the left breast muscle, left thigh muscle, liver, and abdominal fat were immediately separated and weighed. At the same time, 6 geese were randomly selected from CR and FR and quickly cut out about 2 cm of the mid-jejunum (15 cm from Merkel's diverticulum) and the mid-ileum (10 cm from the ileocecal junction). These intestinal segments were fixed with 4% paraformaldehyde solution for histomorphological study. Finally, gently squeeze the outer wall of the cecum with elbow forceps, and collect the cecal contents with 5 mL EP tubes. The cecal contents were stored at −80°C (Thermo, Waltham, MA) in the laboratory. All geese handling procedures were approved by the Sichuan Agricultural University Animal Welfare Committee (Ya'an, China).

In the CR system, one goose is raised per cage (length (L) × width (W) × height (H): 0.55 × 0.37 × 0.7 m, the bottom of the cage is 1.5 m from the ground. In the FR system, all geese were reared in an indoor area (L × W: 6 × 13m), which consisted of a 60 m^2 cement playground and an 18 m^2 fermentation bed. In the FR system, the stocking density could be maintained at 2.6 birds/m^2 throughout the experiment. The lighting schedule for both systems is 16 h on and 8 h off, with lights on at 08:00 AM.

Histological Observation

The tissue samples of jejunum and ileum fixed with 4% paraformaldehyde were decalcified with decalcification solution, dehydrated with ethanol, transparent with xylene, and embedded in paraffin. Each tissue was cut into 3 sections with well-oriented parts using a Leica rm2235 microtome, then dewaxed with xylene and stained with H&E. Microscope images were taken at 40 × (Nikon, Tokyo, Japan). The villus height (**VH**), crypt depth (**CD**), and intestinal wall thickness (**IWT**) of jejunum and ileum were measured and recorded by Image-Pro Plus 6.0 software, and the VH/CD was calculated. Each section selected 5 complete and straight villi and crypts for measurement. A total of 15 complete and straight villi and crypts were recorded in each tissue, and then the total average was calculated.

DNA Extraction and 16S rRNA Amplicons Sequencing

Microbial DNA was extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Norcross, GA) according to the manufacturer's instructions. The ratios of 260 nm/280 nm and 260 nm/230 nm were used as indicators of DNA quality and quantity. Three samples were excluded due to poor DNA quality. Finally, 27 samples (14 for FR and 13 for CR) were used for subsequent sequencing.

The V3−V4 hypervariable regions of the bacterial 16S rRNA genes were amplified with the primers 338-F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806-R (5′-GGACTACNNGGGTATCTAAT-3′) using a thermocycler PCR system (Bio-Rad T100, Germany). The PCR reactions were conducted with a high-fidelity polymerase in the following program: 60 s of denaturation at 98°C, 30 cycles of 10 s at 98°C, and 30s for annealing at 50°C, and 30 s for elongation at 72°C, and a final extension at 72°C for 5 min. Purified amplicons were pooled in equimolar amounts and sequenced on Illumina NovaSeq 6,000 platform (2 × 250 paired ends).

Bioinformatics Analysis

The paired-end reads of the samples were assembled with FLASH (v.1.2.11) (Magoč and Salzberg, 2011); only sequences that overlapped for more than 10 bp were assembled according to their overlapping sequence. Then, the low-quality reads that met the following criteria were discarded: 1) reads containing ambiguous characters and 2) read lengths shorter than 400 bp. These assembled reads were processed and taxonomy assigned using QIIME2 (v.2021.2) (Bolyen et al., 2019). Using the denoise-paired method, amplicon sequence variants (**ASV**) were determined with DADA2. The SILVA138 release was used as the reference database for the taxonomic assignment (Quast et al., 2013), and the taxonomic classification of phyla, classes, orders, families, genera, and species was obtained.

Alpha and Beta diversity were calculated using QIIME2 (v.2021.2) software. Unweighted UniFrac distance metrics were obtained to generate principal coordinate analysis (**PCoA**). Random subsampling of reads was performed to fit the sample with the lowest number of reads in the entire dataset (28,535 sequences). The community structure between the 2 rearing systems was compared by measuring Chao1 species richness, Shannon diversity, Faith_pd, evenness, and Observed_features. Statistical differences between 2 rearing systems at different taxonomic assignments were calculated using Linear Discriminant Analysis Effect Size (**LEfSe**) (Segata et al., 2011) using criteria, $P < 0.05$, LDA score >
3.5. PICRUSt2 (v.2.4.1) (Langille et al., 2013) was used to predict the metabolic pathways of cecal microorganisms, and the group differences were compared using STAMP (v.2.1.3) software (Parks and Beiko, 2010). Welch's *t*-tests (two−sided) were used for two−group comparisons. Welch's inverted confidence interval (**CI**) method was used for CI calculation. All *P*-values were adjusted with the Benjamini−Hochberg procedure and had a false discovery rate of 0.05. A *P*-value of ≤0.05 was considered significant, and a *P*-value of ≤0.10 was considered a trend.

Statistical Analysis

Differences in slaughter traits and intestinal histomorphological characteristics between CR and FR were analyzed using either ANOVA or nonparametric test according to the homogeneity of variance test results.

Table 2. Comparison of some slaughter traits of geese under the cage and floor rearing system.

Indicator	CR (n = 13)	FR (n = 14)	*P*-value
Body weight (kg)	4.42 ± 0.27	4.56 ± 0.35	0.275
Liver weight (g)	65.45 ± 12.16	60.56 ± 5.58	0.187
Breast muscle weight (g)	478.55 ± 65.60	455.46 ± 54.79	0.338
Thigh muscle weight (g)	449.06 ± 46.74	432.93 ± 40.63	0.347
Abdominal fat weight (g)	115.68 ± 47.67	145.93 ± 31.82	0.062
Relative liver weight (%)	1.48 ± 0.27	1.33 ± 0.12	0.076
Relative breast muscle weight (%)	10.81 ± 1.45	10.01 ± 0.98	0.106
Relative thigh muscle weight (%)	10.17 ± 0.97	9.50 ± 0.48	0.032
Relative abdominal fat weight (%)	2.58 ± 0.95	3.19 ± 0.62	0.058

Abbreviations: CR, cage rearing system; FR, floor rearing system.
All results are presented as the mean ± standard deviation (S.D.).

Using CR and FR data sets as the total data set, Spearman's correlation coefficients of cecal microorganisms with slaughter traits and intestinal histomorphological parameters were determined by IBM SPSS statistics (version 20). The differences were considered to be significant at $P < 0.05$. The data are presented as the mean ± standard deviation (S.D.).

RESULTS

Effects of Cage vs. Floor System on Goose Slaughter Traits

As shown in Table 2, the rearing systems did not significantly affect the body weight, liver weight, breast muscle weight, relative breast muscle weight, and thigh muscle weight. However, CR relative thigh muscle weight was significantly higher than FR ($P < 0.05$). Moreover, the relative liver weight ($P = 0.076$), abdominal fat weight ($P = 0.062$), and relative abdominal fat weight ($P = 0.058$) tended to be lower in CR than in FR (Table 2).

Effects of Cage vs. Floor System on Goose Intestinal Histomorphology

In jejunal histomorphology, the VH, CD, and VH/CD of jejunum in CR were similar to those in FR. At the same time, the IWT of CR was slightly higher than in FR, although these differences were not significant. In ileal histomorphology, the VH of the ileum in CR is slightly higher than in FR, and the CD of the ileum is slightly lower than in CR. Thus, the VH/CD of the ileum was greater in CR than in FR ($P = 0.032$, Table 3).

Effects of Cage vs. Floor System on Goose Cecal Microbial Composition

After quality control and filtering, 886,995 high-quality reads were generated from 27 samples, with an average of 32,851 reads per sample. These reads were assigned using the DADA2 analysis pipeline in QIIME2, and 2,953 ASVs were identified. These ASVs were subsequently classified into 27 phyla, 44 classes, 95 orders, 141 families, 286 genera, and 556 species.

The cecal microbial complexity was estimated based on the alpha-diversity indices (Chao1, Faith_pd, Shannon, Evenness, and Observed_features). As shown in Table 4, the Observed_features, Evenness, Faith_pd, Chao1, and Shannon indices of CR were slightly lower than those of FR; however, these differences were not significant between the two systems. In unweighted UniFrac PCoA, the first principal coordinate (**PCo1**) explained 14.58% of variations among samples and PCo2 explained 9.114% of variations (Figure 1A). The sample dots from the 2 rearing systems showed distinct distances, and the PCoA plots showed an apparent clustering of the microbial communities based on the rearing systems. The ANOSIM test (Figure 1B) also revealed significant differences in the cecal microbial communities between CR and FR (R = 0.198, $P = 0.002$).

As for cecal microorganisms, 27 phyla and 286 genera were identified from samples under 2 rearing systems. Taxonomic analysis showed that the dominant bacteria of cecal microorganisms in two rearing systems were roughly similar, with Bacteroidota, Firmicutes, Fusobacteriota, and Proteobacteria being the dominant phyla (Figure 1D) while *Bacteroides, Fusobacterium*, uncultured_bacterium_o_Bacteroidales being the dominant genera (Figure 1C). Moreover, the Bacteroidota/Firmicutes ratio of the cecum was slightly greater

Table 3. Comparison of intestinal histomorphological parameters of geese under the cage and floor rearing system.

Intestinal segment	Parameters	CR	FR	*P*-value
Jejunum	Number	n = 5	n = 6	
	Villus height (μm)	1105.92 ± 380.41	1193.64 ± 374.03	0.710
	Crypt depth (μm)	202.60 ± 52.14	232.85 ± 129.63	0.638
	Villus height/Crypt depth (μm/μm)	5.97 ± 3.19	6.10 ± 2.93	0.980
	Intestine wall thickness (μm)	463.60 ± 142.45	383.51 ± 77.83	0.266
Ileum	Number	n = 6	n = 6	
	Villus height (μm)	1038.17 ± 208.30	943.98 ± 265.56	0.510
	Crypt depth (μm)	137.61 ± 24.40	150.21 ± 28.43	0.429
	Villus height/Crypt depth (μm/μm)	7.57 ± 1.04	6.24 ± 0.79	0.032
	Intestine wall thickness (μm)	421.41 ± 76.09	417 ± 46.99	0.917

Abbreviations: CR, cage rearing system; FR, floor rearing system.
All results are presented as the mean ± standard deviation (S.D.).

Table 4. Comparison of cecal microorganisms alpha-diversity of geese under cage and floor rearing system.

Indices	CR (n = 13)	FR (n = 14)	*P*-value
Observed_features	398.62 ± 71.92	414.29 ± 90.62	0.625
Faith_pd	27.31 ± 4.00	27.92 ± 4.18	0.702
Evenness	0.79 ± 0.48	0.80 ± 0.06	0.619
Shannon	6.85 ± 0.59	6.98 ± 0.77	0.609
Chao1	399.08 ± 71.90	415.21 ± 90.40	0.614

Abbreviations: CR, cage rearing system; FR, floor rearing system.
All results are presented as the mean ± standard deviation (S.D.).

in CR (2.01) than in FR (1.95), and the relative abundance of Fusobacteriota and Proteobacteria were lower in CR than in FR (Supplementary Table 1). Differentially abundant taxa were identified using LEfSe analysis ($P < 0.05$, LDA score > 3.5). The results showed that the abundance of *Sutterella* (genus), Burkholderiales (order), Sutterellaceae (family), and uncultured_bacterium_f_Rikenellaceae (genus) were lower in CR than in FR, whereas the abundance of *Megamonas* (genus), Veillonellales-Selenomonadales (order), Negativicutes (class), Selenomonadaceae (family), *Anaerobiospirillum* (genus), Succinivibrionaceae (family), and Aeromonadales (order) were higher in CR than in FR ($P < 0.05$, Figures 2B and 2C). Furthermore, PICRUSt2 analysis indicated that the lipid metabolism pathways of cecal microorganisms were lower enriched in CR than in FR. ($P < 0.01$, Figure 2A).

Correlation of Goose Slaughter Traits and Intestinal Histomorphological Parameters With Differentially Enriched Bacteria

Spearman correlation coefficient was used to evaluate the relationships between slaughter traits, intestinal histomorphological parameters, and relative abundance of differentially enriched bacteria in geese. The results showed that the relative abundance of uncultured_bacterium_f_Rikenellaceae was significantly negatively correlated with the ileal VH and VH/CD ($P < 0.05$). The relative abundance of *Anaerobiospirillum* was significantly positively correlated with thigh muscle weight and relative thigh muscle weight ($P < 0.05$). The relative abundance of *Megamonas* was significantly positively correlated with relative breast muscle weight ($P < 0.05$). Moreover, the relative abundance of *Megamonas* and *Anaerobiospirillum* were significantly negatively correlated with abdominal fat weight and relative abdominal

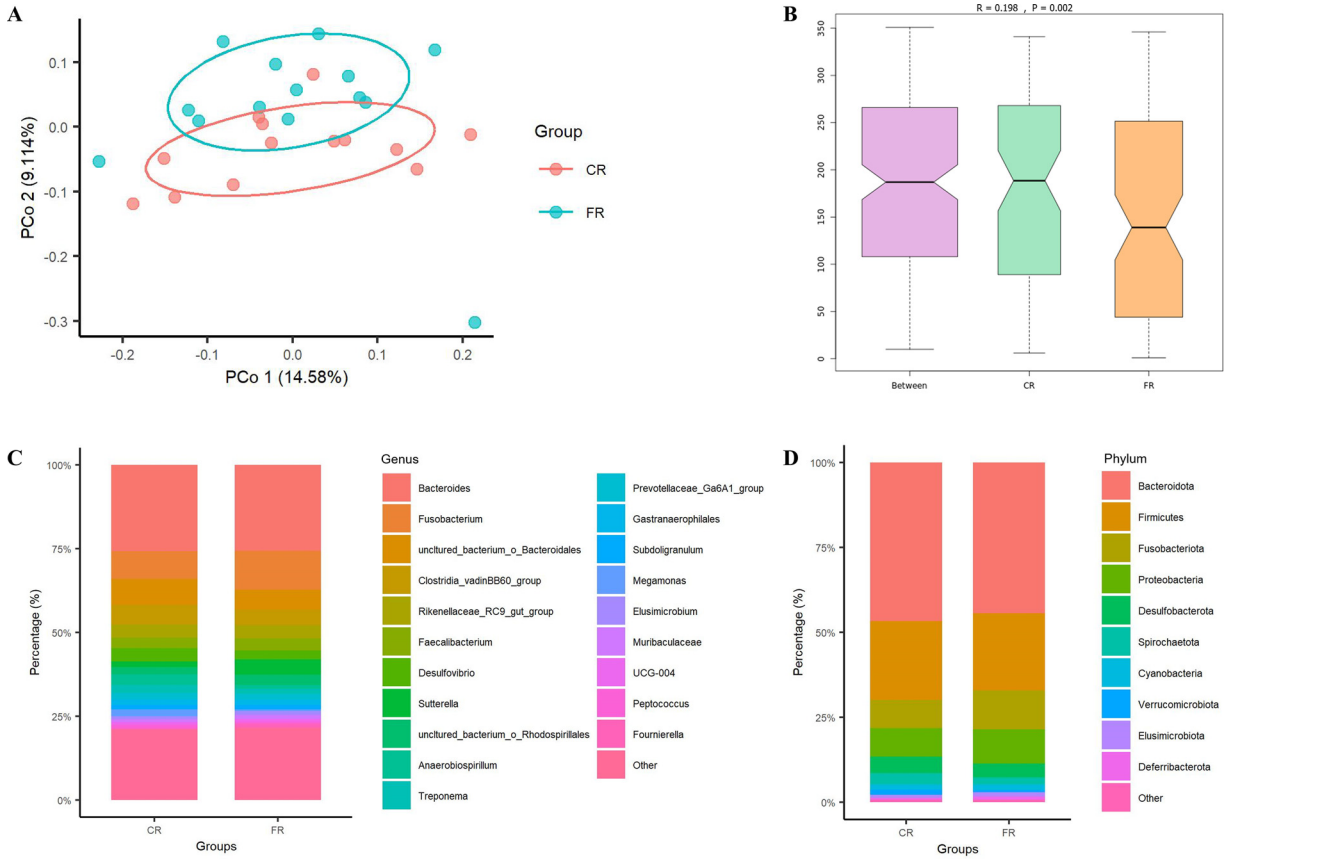

Figure 1. Beta diversity and species composition of cecal microorganisms. (A) PCoA (based on unweighted UniFrac distance) of goose cecal microorganisms in CR and FR. (B) Box plot of intergroup and intragroup beta distance (ANOSIM analysis) of CR and FR. "Between" indicates the difference between groups, and "CR" and "FR" respectively indicate the difference within groups. R > 0, the difference between groups is greater than the difference within the group, indicating that the experimental grouping is effective. (C) Bacterial community composition at the genus level of CR and FR. (D) Bacterial community composition at the phyla level of different rearing systems. PCo1 and PCo2 on the x-and y-axis represent two principle discrepancy components among groups, the percentage in brackets indicates the contribution to the discrepancy component. Dots represent samples. Abbreviations: CR, cage rearing system; FR, floor rearing system.

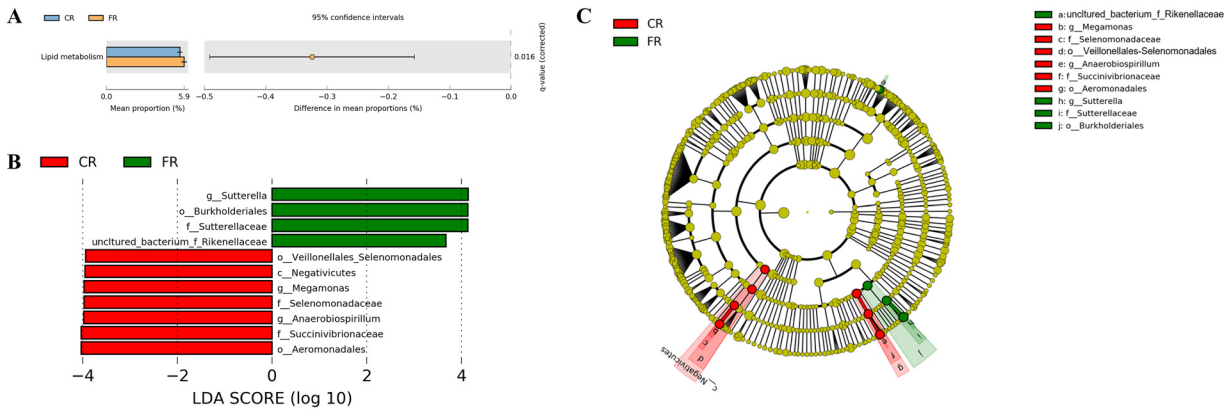

Figure 2. LEfSe analyses and KEGG analyses of goose cecal microorganisms. (A)Differential functional pathways of cecal microorganisms. (B) Linear discriminant analysis (LDA) score distribution of cecal microorganisms between CR and FR. (C) Cladogram indicating statistical differences of the cecal microbial populations between CR and FR. Abbreviations: CR, cage rearing system; FR, floor rearing system.

fat weight ($P < 0.01$), but the relative abundance of *Sutterella* was significantly positively correlated with abdominal fat weight and relative abdominal fat weight ($P < 0.01$, Figure 3).

DISCUSSION

Our results show that the rearing systems may affect the slaughter traits, intestinal structure, and cecal microbial composition of geese, but most indicators are not significant. The relative breast muscle weight and relative thigh muscle weight in CR are higher than in FR, but the relative abdominal fat weight is lower than in FR. This means that the meat production performance of the goose in CR is higher than in FR. This contradicts other studies, they found that ducks in FR had better meat production performance than CR (Zhu et al., 2020). The differences may be caused by the change in feed intake of geese. Some studies have found that the changes in the rearing systems will affect the feed intake of poultry (Starčević et al., 2021; Yan et al., 2021).

Intestinal morphology is used as an indicator of intestinal health as values are often indicative of digestive and absorptive capacity. Intestinal VH affects the actual surface area for nutrient digestion and absorption (Mayhew and Middleton, 1985), while CD and IWT reflect the rate of intestinal cell proliferation and the intensity of energy metabolism (Blackmore et al., 2017; Greenwood-Van Meerveld et al., 2017). In this study, the VH, CD, and IWT of the jejunum and ileum of the geese in 2 systems were similar, which may indicate that the intestinal absorption capacity and the proliferation rate of intestinal wall cells in CR were not significantly different from those in FR. Besides, the ratio of villus height to crypt depth is often used as a single measure of intestinal health. The VH/CD values in CR geese were significantly higher than in FR, indicating that the ileum of geese under CR may be more resistant to disease or toxin challenges.

The intestinal microbiota is a dynamic entity influenced by environmental and nutritional factors (Paoli et al., 2019). Previous studies have demonstrated that different rearing systems can affect the intestinal microbial composition of chickens (Wang et al., 2021;

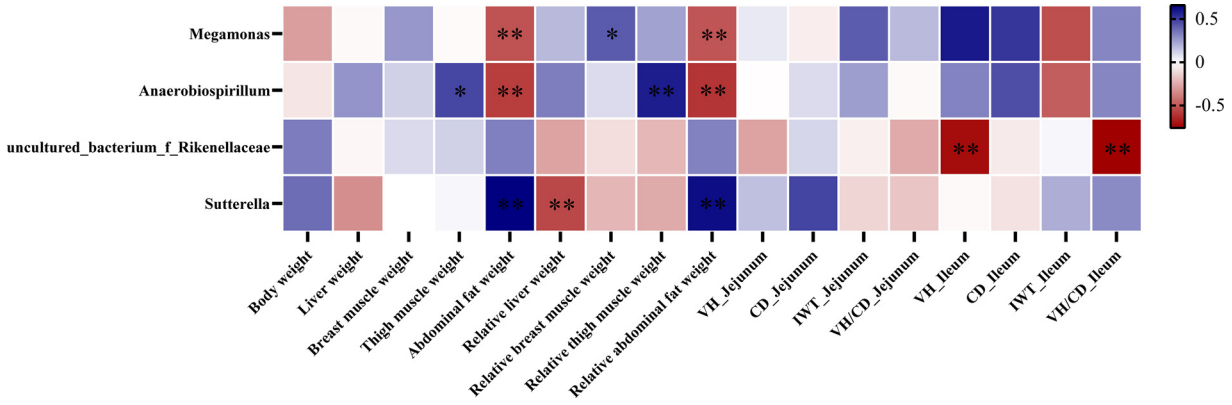

Figure 3. Correlation of slaughter traits and intestinal histomorphological parameters with differentially detected bacteria. * indicates significant correlation at 0.05 level; ** indicates significant correlation at 0.01 level. Abbreviations: CD_Jejunum, crypt depth of jejunum; CD_Ileum, crypt depth of ileum; IWT_Jejunum, intestine wall thickness of jejunum; IWT_Ileum, intestine wall thickness of ileum; VH/CD_Jejunum, the ratio of villus height to crypt depth of jejunum; VH/CD_Ileum, the ratio of villus height to crypt depth of ileum; VH_Jejunum, villus height of jejunum; VH_Ileum, villus height of ileum.

Wiersema et al., 2021) and ducks (Wang et al., 2018a; Zhu et al., 2020). To our knowledge, this study was the first to compare the effects of CR and FR on the cecal microbial composition in geese. We revealed significant changes in goose cecal microbial composition and diversity between CR and FR. Our results showed that the dominant phyla under both rearing systems were Bacteroides and Firmicutes, consistent with the results of several previously published studies on geese (Li et al., 2017; Yan et al., 2019). Moreover, we found that the cecal Bacteroidetes/Firmicutes were greater in CR than in FR, and the abdominal fat weight tended to be lower in CR than in FR. This is consistent with the results of a previous study (Ley et al., 2005), which found that lean animals had higher levels of Bacteroidetes but lower levels of Firmicutes among their intestinal microbes. Furthermore, differentially enriched bacterial species were also observed in geese' cecal contents between the 2 rearing systems. In this study, results from the LEfSe analysis showed that *Megamonas* and *Anaerobiospirillum* were significantly enriched in CR while uncultured_bacterium_f_Rikenellaceae and *Sutterella* were significantly enriched in FR. *Megamonas* and *Anaerobiospirillum* may be involved in fat deposition and energy metabolism. A study found that the abundance of *Megamonas* in male chickens was significantly positively correlated with glycogen phosphorylase L (**PYGL**) expression in the cecum (Cui et al., 2021), and the PYGL gene was involved in glycan metabolism. Compared to fatty-type (**FT**) Pekin ducks, *Anaerobiospirillum* has significantly enriched in the cecum of lean-type (**LT**) ducks (Yang et al., 2022). In this study, we also found that the relative abundance of *Megamonas* ($R = -0.52$ and $R = -0.51$) and *Anaerobiospirillum* ($R = -0.58$ and $R = -0.60$) were significantly negatively correlated with abdominal fat weight and relative abdominal fat weight ($P < 0.01$). Besides, our results showed that *Sutterella* ($R = 0.66$ and $R = 0.62$) was positively correlated with abdominal fat weight and relative abdominal fat weight ($P < 0.01$). *Sutterella* may promote fat deposition in geese of FR. Previous studies have shown that *Sutterella* has been implicated in obesity-related metabolic disorders (Miller et al., 2015), and *Sutterella* abundance is positively correlated with the obesity phenotype in rats (Wang et al., 2018b). In this study, the PICRUSt2 analysis results further showed that geese cecal microorganisms' lipid metabolism capability significantly decreases in CR than in FR. Several studies have demonstrated that lipid metabolism pathways are associated with fat deposition and are typically significantly enriched in the intestinal microbiota of obese individuals (Chávez-Carbajal et al., 2019; Wang et al., 2020). Therefore, compared with FR, CR changed the composition and functions of cecal microorganisms of geese, which may lead to less fat deposition in geese in CR.

The intestinal microbiota plays a crucial role in host health and metabolism, and pathogenic bacteria may cause deleterious effects (Jha and Berrocoso, 2015). Our results showed that the relative abundance of Fusobacteriota and Proteobacteria were lower in CR than in FR. Fusobacteriota is generally considered an opportunistic pathogen (Brennan and Garrett, 2019), and is closely associated with diseases such as periodontitis (Krisanaprakornkit et al., 2000; Bhattacharyya et al., 2016), appendicitis (Swidsinski et al., 2011), cancer (Guo et al., 2018), and tumors (Kostic et al., 2013). Proteobacteria is composed of many pathogens and autonomous microorganisms, such as *Escherichia coli, Salmonella*, and many nitrogen-fixing bacteria, which secrete various pro-inflammatory factors (Hiippala et al., 2018). In addition, our results showed that uncultured_bacterium_f_Rikenellaceae was negatively correlated with VH and VH/CD of the ileum. Most species of the Rikenellaceae family are considered pathogenic bacteria that can increase intestinal inflammation or produce mutagenic toxins (Sun et al., 2017). Therefore, compared with FR, the CR may change the abundance of pathogenic bacteria in cecal microorganisms, possibly reducing inflammation and damage to the intestinal mucosa.

CONCLUSIONS

In conclusion, our results showed that CR and FR geese have differences in slaughter traits, intestinal histomorphological characteristics, and cecal microbial composition. CR has higher values for relative thigh muscle weight, relative liver weight, abdominal fat weight, and relative abdominal fat weight, and the ileal VH/CD ratio of geese in CR increased. Moreover, CR increased the cecal relative abundance of *Megamonas* and *Anaerobiospirillum*, and decreased the colonization of uncultured_bacterium_f_Rikenellaceae and *Sutterella* in the cecum of geese. The abdominal fat weight and relative abdominal fat weight were lower in CR than in FR, and the lipid metabolism pathways of cecal microorganisms were significantly lower enriched in CR than in FR.

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DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101931.

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