Dietary fiber modulates abdominal fat deposition associated with cecal microbiota and metabolites in yellow chickens

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ABSTRACT Excessive deposition of abdominal fat is a public concern in the yellow chicken industry related to human nutrition. The common practice of nutritionists is to increase the fiber content in feed to control abdominal fat deposition of chickens. Corncob meal (CCM) is the cheapest ingredient widely used in animal diets. The possible effects of CCM on chicken abdominal fat deposition and the possible mechanism involving cecal microbiota remain unknown. The objectives of this study were to investigate the effects of CCM in modulating abdominal fat deposition and the role of the cecal microbiota and their metabolites. A total of 200 ninety-day-old Huxu female chickens were divided into 2 dietary treatments, each with 10 replicates of 10 birds, and were fed two finisher diets, from 90 to 135 d. The diets were a typical corn-soybean control diet (CON) and that diet with CCM partially replacing corn and corn gluten meal. Results showed that the CCM diet markedly decreased live weight and abdominal fat percentage (P < 0.05); chickens fed the CCM diet exhibited lower (P < 0.01) expression in abdominal fat of fatty acid binding protein 4 (FABP4), stearoyl-CoA desaturase (SCD), fatty acid synthase (FAS), and peroxisome proliferator-activated receptor γ (PPARγ) but higher (P < 0.05) expression of estrogen receptor alpha (ESR1). The CCM increased the abundance of Akkermansia (P < 0.05) and markedly reduced the relative cecal abundance of Phascolarctobacterium (P < 0.01), Rikenellaceae (P < 0.05), and Faecalibacterium (P < 0.01). The metabolomic and biochemical analyses demonstrated that the CCM diet increased (P < 0.05) the concentrations of butyrate in cecal contents. The majority of the metabolites in cecal digesta with differences in abundance were organic acids. The CCM diet increased (P < 0.05) contents of (R)-5-diphosphomevalolate, pantothenic acid, 2-epi-5-epi-valiolone 7-phosphate, D-ribose 5-diphosphate, arbutin 6-phosphate, D-ribitol 5-phosphate, undecanoic acid, nicotinic acid, 4-methyl-2-oxovaleric acid, while decreasing (P < 0.05) those of oleic acid, glutaric acid, adipic acid, suberic acid, and L-fuculose 1-phosphate. In conclusion, these findings demonstrated that the dietary CCM treatment significantly decreased abdominal fat and altered the cecal microbiota and metabolite profiles of the yellow chickens.

Key words: Huxu chickens, abdominal fat, corncob meal, microbial community, microbial metabolites

INTRODUCTION

Approximately half of Chinese production of meat chickens is made up by traditional endogenous breeds, also called yellow chickens, which have appealed to consumers because of several sensory traits, including meat flavor (Jiang et al., 2000). Yellow chickens play a critical role in the meat industry because they are considered to be luxury goods for consumption due to their flavor. The Huxu chickens are a high economic value local breed in South China due to their capacity for nutrient digestibility and high tolerance of low quality feed ingredients. When Huxu chickens are fed on the basis of nutrient requirements of Chinese yellow chickens, there is excessive abdominal fat deposition which negatively affects feed efficiency and consumer acceptance. This limitation can be most effectively resolved by nutritional manipulation.

The overall trend of the poultry sector is to deliver safety for birds in the feed (Abd El-Hack et al., 2020;
Dietary fibers are usually recognized as being antinutrient factors and they negatively affect nutrient digestibility and energy utilization. There has been increased recent interest in fiber fermentation in the colon (Rose et al., 2007). Fermentation by colonic bacteria converts otherwise indigestible dietary components into end-products supplying substrates and energy for animals and potentially contributing to gut health (Rinttilä et al., 2013). Higher contents of fiber are inversely correlated with abdominal fat deposition in broiler chickens (Khempaka et al., 2009). In China, the annual production of corncob exceeds 30 million tons. Corncob contains 35% cellulose and 40% hemicellulose (Brar et al., 2016). Theoretically, corncob meal (CCM) is a suitable fiber source for all kinds of domestic animals and is far cheaper than alternatives currently in use. Using corncob as a fiber ingredient to decrease abdominal fat is legitimate and potentially serves as a means of creating huge economic value.

Under the anaerobic conditions in the colon, the microflora produce an array of metabolic end-products such as volatile short-chain fatty acids, gases (e.g., H2 and CH4), and favors microflora species considered to be healthy for animals (Awad et al., 2016). In clinical studies, dietary fiber increases short-chain fatty acids, particularly butyrate, and lowers pH to reduce intestinal disease (Noakes et al., 1996; Jenkins et al., 1998). Corncob was chosen here as the fiber source because of its low cost and widespread availability. It was intended that the corncob would serve as the primary dietary component microbially fermented in the hindgut enabling examining how dietary levels affect lipogenesis in the yellow chickens. The Huxu local breed and feeds with and without CCM were used to examine the cecal microbiota and metabolites related to abdominal fat deposition. The present study determines to reveal possible way and mechanism of CCM modulating chicken’s abdominal fat deposition associate with cecal microbiota and metabolites. Further, to provide a scientific basis and technical support for commercial diet. In order to achieve the demand for reducing abdominal fat in local yellow-feathered chickens.

**MATERIALS AND METHODS**

**Animals and Experimental Design**

Animal care and procedures followed The Chinese Guidelines for Animal Welfare, and the present study was approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences (Guangzhou, PRC).

A total of 200 one-day-old Huxu female chicks of the same genetic background, were obtained from Guangdong Wiz Agricultural Science & Technology Co. Ltd. (Guangzhou, PRC). All birds were fed a 2-phase starter and grower diet under the same feeding management from 1 to 90 d. The experimental diet formula, based on the Chinese Feeding Standard of Chicken (2004) and the Feed Database in China (2018), is shown in Table 1.

| Component                  | CON (%) | CCM (%) |
|----------------------------|---------|---------|
| Ingredient, %              |         |         |
| Corn                       | 81.0    | 66.0    |
| Soybean meal               | 11.0    | 11.0    |
| Corn gluten meal           | 1.45    | 2.70    |
| Lard                       | 2.00    | 2.00    |
| L-Lysine HCl               | 0.40    | 0.42    |
| D,L-Methionine             | 0.20    | 0.22    |
| L-Threonine                | 0.10    | 0.13    |
| L-Methionine               | 1.05    | 1.05    |
| Dicalcium phosphate        | 1.55    | 1.55    |
| Salt                       | 0.25    | 0.25    |
| Premix 1                   | 1.00    | 1.00    |
| Corncob meal               | 0.00    | 13.68   |
| Total                      | 100.00  | 100.00  |

1. Abbreviations: CON, control; CCM, corncob meal.
2. Provided the following per kilogram of diet: Vitamin A, 6,000 IU; Vitamin D3, 500 IU; Vitamin E, 20 IU; Vitamin K3, 0.50 mg; Vitamin B1, 2.1 mg; Vitamin B2, 3.0 mg; Vitamin B6, 3.5 mg; Vitamin B12, 0.01 mg; pantothenic acid, 10 mg; niacin, 15 mg; biotin, 0.15 mg; folic acid, 0.45 mg; choline chloride, 500 mg; Fe, 80 mg; Cu, 7 mg; Mn, 60 mg; Zn, 65 mg; I, 0.35 mg; Se, 0.23 mg.
3. Values were calculated from data provided by Feed Database in China (2018).

Birds were weighed at 90 d (body weight, BW 1.08 ± 0.00 kg) and randomly divided into 2 dietary treatments, each with 10 replicates of 10 birds. The control chickens were fed a corn-soybean basic (CON) diet and the treatment chickens were fed this diet with 13.68% corncob meal (CCM) substituting for reduced content of corn and corn gluten meal. The birds were raised for the 45-d experiment during the finisher phase (from 90 to 135 d) in an environmentally controlled room in three-story step cages. No chickens died during the experiment.

**Sample Collection**

At the end of the experiment, on d 135, one bird with typical BW of each replicate was anesthetized and then killed by experienced technicians following a 12-h overnight fast. The abdominal fat was dissected in the same area for all chickens. Abdominal fat samples were weighed, snap-frozen in liquid nitrogen, and stored at −80°C for RNA extraction. The remaining abdominal fat tissues were removed and weighed (AFP, %) as a percentage of BW. Cecal contents were collected and snap-frozen and then stored at −80°C for measuring volatile compounds, 16 s sequencing and metabolomics analyses.

The CCM was purchased from the local market. After complete drying in an oven, the materials were ground. Total dietary fiber (TDF), ash, protein, and fat of the
suitable amount of anhydrous Na₂SO₄ to remove mois-
ture, clarified with 0.45-um syringe filters and analyzed by
gas chromatography-mass spectrometry (GC-MS).

**Volatile Compounds**

For the analyses of volatile compounds according to
Rideout et al. (2004), the digesta samples (2 g) were
extracted with 100% methanol (6 mL), homogenized at
2,000 × g for 2 min and centrifuged at 800 × g for
20 min. The clear supernatant (1 mL) was transferred
into an ampule and 0.2 mL metaphosphoric acid solution
was added. The supernatant was treated with a
suitable amount of anhydrous Na₂SO₄ to remove mois-
ture, clarified with 0.45-um syringe filters and analyzed by
gas chromatography-mass spectrometry (GC-MS).

**Quantitative Real-Time PCR**

Total RNA was isolated from 100 mg of frozen
abdominal fat using the Trizol reagent (Invitrogen,
Carlsbad, CA), according to the manufacturer’s proto-
col. The concentration and purity of RNA were deter-
mined using a NanoDrop 2000 spectrophotometer
(Thermo Scientific, Wilmington, DE). The specific prim-
ers of examined genes (Table S1) were designed using
Primer Premier 6.0 software. RNA isolation and real-
time PCR procedures were completed as previously
described (Cui et al., 2016).

**DNA Extraction**

DNA was extracted from samples of cecal digesta from
6 chickens in each treatment. The method was slightly
modified from the instructions in TIANamp Stool DNA
Kits from Tiangen Biotech (Beijing, PR China), as
described below. The sample vortexing step was
replaced with a bead-beating homogenization using 1.4-
mm Ceramic Bead Tubes in a PowerLyzer-24
homogenizer (MP Biomedicals, Santa Ana, CA 92707)
to enhance the cell lysis. The DNA concentrations of the extracts were measured fluorometrically with the Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Wal-
tham, MA), after which the DNAs were stored at −80°C
until 16S rDNA library preparation.

**16S rRNA Gene Amplification and Microbiota Community Analysis**

The microbial 16S rRNA profiles of the DNA extracts
were analyzed with Pandaseq v2.8 with default param-
ters. Chimeras were identified and removed using
USEARCH 6.1 within QIIME. The QIIME script “add qiime labels.py” was used to combine the non-chimeric sequence in the GreenGenes 13.08 database. Sequence reads were filtered with a quality-score acceptance rate of
20 or better, and the generated operational taxonomic unit (OTU) table was filtered by dropping out OTU representing 0.05% of the total sequence count. Then, to minimize the effect of intrasample variation in the sequencing efficiency, samples were subsampled (rare-
fied) by random sampling without replacement to the
lowest common sequencing depth. The V3–V4 library
preparation and sequencing were performed at FISABIO
(Va­len­cia, ESP). The V3–V4 region of the bacterial 16S
rRNA gene was amplified by following the 16S Metage-
nomic Sequencing Library Preparation guide. The V3
and V4 regions were amplified using forward primers
containing the sequence 5′-CCTACGGGNGGCWGC-
CAG-3' and reverse primers containing the sequence 5′-
GGACTACHVGGGTATCTAAT-3'.

**Liquid Chromatography-Mass Spectrometry Metabolomics Analyses**

The cecal digesta 50-mg frozen samples were diluted
with methanol water (500 uL) and 6-ug internal stan-
dard lidocaine was added. The samples were left at 4°C
for 20 min for protein precipitation. After 10 min of cen-
trifugation, the supernatant was transferred to vials with
micro-inserts. Metabolomics analysis was performed by
Gene Denovo Biotechnology (Guangzhou, PRC) using
in-house methodology based on a Waters liquid chroma-
tography-mass spectrometry (LC-MS) system. The
processed data were analyzed by principal component
analysis (PCA) and partial least squares discriminant
analysis (PLS-DA) using SIMCA-P14.0 software (Ume-
metrics, Umea, SE). Differentially abundant metabolites
between CON and CCM treatments were identified from
variable importance in projection (VIP) from PLS-DA
and Student’s t tests (VIP > 1 and P < 0.05).

**Statistical Analyses**

Replicate served as the experimental unit. The effects of
dietary supplementation with CCM on live weight,
abdominal fat percentage, gene transcripts, and contents
of volatile compounds were assessed by Student’s t test

| Component¹ | Content   |
|------------|-----------|
| Protein (g/100 g DM) | 2.98 |
| Fat (g/100 g DM) | 0.54 |
| Ash (g/100 g DM) | 1.67 |
| TDF (g/100 g DM) | 91.23 |
| ADF (g/100 g DM) | 1.23 |
| NDF (g/100 g DM) | 86.89 |
| DH (Hemicellulose; g/100 g DM) | 44.54 |
| ADL (Lignin; g/100 g DM) | 3.78 |
| ADC (Cellulose; g/100 g DM) | 38.56 |

¹Abbreviations: ADL, acid detergent lignin; ADC, acid detergent cellulose; ADF, acid detergent fiber; DH, detergent hemicellulose; DM, dry matter; NDF, neutral detergent fiber; TDF, total dietary fiber.
using SPSS Version 19.0 (SPSS Inc., Chicago, IL). Data are shown as means ± SEM. Differences were considered to be statistically significant at $P < 0.05$ or $P < 0.01$.

**RESULTS**

**Live Weight and Abdominal Fat Percentage**

The live weight and AFP in chickens are presented in Figure 1. Compared to chickens fed the CON diet, live weight and AFP of chickens fed the CCM diet were significantly decreased by 5.33% ($P = 0.006$) and 27.69% ($P = 0.008$), respectively.

**Expression in Abdominal Fat of Genes Related to Lipid Metabolism**

As shown in Figure 2, chickens fed the CCM diet exhibited lower transcript abundances ($P < 0.01$) of fatty acid binding protein 4 (FABP4), stearoyl-CoA desaturase (SCD), fatty acid synthase (FAS), and peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$), together with higher expression ($P < 0.05$) of estrogen receptor alpha (ESR1) compared with birds on the CON diet.

**Microbiota Composition of the Cecal Digesta**

Sequences of 16S rRNA gene amplicons from cecal contents were examined to investigate the effects of CCM supplementation on the gastrointestinal microbiota. Totals of 29 phyla, and more than 200 genera were identified in the present study. Taxonomy results of the major bacteria are shown in Table S2. Alpha diversity was applied in analyzing complexity of species diversity through Shannon, Simpson, Chao1, and ACE indices and all results are displayed in Table 3. The CCM treatment increased ($P < 0.05$) the Shannon and Chao 1 indices compared to those in the CON group.
whereas there was no significant difference in the Simpson and ACE indices, indicating that CCM treatment changed diversity and richness of the microflora. The PCoA with Bray distance results showed that the CCM and CON dietary treatments were well separated (Figure 3A). Phylum distributions of the gut microbiome for the CCM treatment are shown in Figure 3B.

Table 3. Effects of corn cob meal on α- diversity of cecal bacterial communities.

| Index     | CON  | CCM  | SEM  | P-value |
|-----------|------|------|------|---------|
| Shannon   | 6.19b| 7.56a| 0.077| 0.031   |
| Simpson   | 0.092| 0.095| 0.014| 0.279   |
| Chao1     | 2.166b| 2.405a| 43.25| 0.047   |
| ACE       | 2.328| 2.467| 47.85| 0.156   |

abMeans within a row with different superscripts are different at \( P < 0.05 \).

1Abbreviations: ACE, abundance-based coverage estimator; CON, control; CCM, corn cob meal.

2SEM = standard error of mean, \( n = 6 \).

Compared with CON, relative abundance of *Kiritimatiellaeota* in chickens supplemented with CCM increased \( (P < 0.05) \), whereas that of *Bacteroidetes* decreased \( (P < 0.01) \). Concerning genus distributions (Figure 3C), treatment with CCM reduced the relative abundance of *Phascolarctobacterium* \( (P < 0.01) \), *Rikenellaceae* \( (P < 0.05) \) and *Faecalibacterium* \( (P < 0.01) \), while *Akkermansia* \( (P < 0.05) \) were markedly increased compared with those in the CON diet.

### Cecal Volatile Compounds

The effects of CON and CCM diets on the contents in cecal digesta of major volatile compounds are shown in Table 4. Concentrations of acetic acid, propionic acid, isobutyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, hexanoic acid, p-cresol, 4-ethylphenol, indole, skatole, and total volatile fatty acids (VFA) in cecal contents did not differ \( (P > 0.05) \) between the

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**Figure 3.** (A) Principal coordinates analysis (PCoA) of bacterial communities in the cecal digesta of chickens (based on the Bray distance). The percentage of variation explained by PCo1 and PCo2 are indicated in the axes. (B) Phylum-level relative abundance of 16S rRNA gene sequences from the cecal digesta of chickens \( (n = 6) \). (C) Significantly changed bacteria genera by corn cob meal diet treatment. The values were expressed as the medians \( (n = 6) \). Statistical differences were calculated by t test, * indicate significance at \( P < 0.05 \), ** indicate significance at \( P < 0.01 \). Abbreviations: CCM, corn cob meal; CON, control.
diets. In comparison with the CON diet, the CCM diet resulted in a higher proportion ($P < 0.05$) of butyric acid in the cecal contents of chickens.

**Fermentation Metabolites in the Cecal Digesta**

Metabolomic profiles of the cecal contents of chickens that were fed CON or CCM diets were characterized using LC-MS. As shown in Figure 4, PCA and PLS-DA models revealed clear separation between birds fed CON and CCM diets. Of 538 metabolites shown to differ (VIP > 1 and $P < 0.05$) between the 2 diets, 148 were annotated and identified; the list of differentially abundant metabolites is presented in Table S3. Some typical and representative metabolites are represented in Table 5. The majority of the metabolites in cecal contents differing in abundance were organic acids. The CCM diet increased ($P < 0.05$) contents of (R)-5-diphosphomevalate, pantothenic acid, 2-epi-5-epi-valiolone 7-phosphate, D-ribose 5-diphosphate, arbutin 6-phosphate, D-ribitol 5-phosphate, undecanoic acid, nicotinic acid, and 4-methyl-2-oxovaleric acid, while decreasing ($P < 0.05$) those of oleic acid, glutaric acid, adipic acid, suberic acid, L-fuculose 1-phosphate, and phosphoenolpyruvate.

**Table 4.** Responses in the cecal concentrations (mg/g DM$^1$ cecal digesta) of major volatile compounds in the chickens fed the experimental diets

| Item                  | CON$^2$ | CCM$^2$ | SEM$^3$ | $P$-value |
|-----------------------|---------|---------|---------|-----------|
| Acetic acid           | 25.42   | 35.73   | 13.71   | 0.098     |
| Butyric acid          | 5.12$^a$| 12.19$^a$| 3.90    | 0.013     |
| Propionic acid        | 19.35   | 30.75   | 10.26   | 0.087     |
| Isobutyric acid       | 1.13    | 1.50    | 0.72    | 0.423     |
| 2-Methylbutyric acid  | 1.41    | 1.84    | 1.02    | 0.654     |
| Isovaleric acid       | 2.25    | 3.16    | 1.93    | 0.125     |
| Valeric acid          | 5.10    | 9.22    | 3.80    | 0.095     |
| Hexanoic acid         | 6.14    | 7.26    | 4.64    | 0.577     |
| Indole                | 0.0374  | 0.0433  | 0.0321  | 0.434     |
| Skatole               | 0.0898  | 0.1493  | 0.0739  | 0.773     |
| Total VFA$^4$         | 66.14   | 101.44  | 31.52   | 0.088     |

$ab$Means within a row with different superscripts are different at $P < 0.05$.

$^1$DM, dry matter.

$^2$CON, control; CCM, corncob meal.

$^3$SEM = standard error of mean, $n = 10$.

$^4$Total VFA is sum of all of the analyzed volatile compounds.

**DISCUSSION**

Excessive abdominal fat deposition is a concern in the yellow chicken industry because it reduces feed efficiency. Most of it is removed and discarded during evisceration so this appreciable energetic component of the carcass is considered to be waste in chicken meat production. Previous research has shown that dietary fiber influences abdominal fat. Natural cellulose was shown to decrease fat deposition in rats. Lai (2005) found that dietary fiber-reduced hepatocyte apoptosis in rats, which resulted in reduced abdominal fat deposition. Similar result was found in broilers that the dietary fiber reduced abdominal fat significantly (Bhuiyan et al., 2021). Catherine et al. (2002) also demonstrated that increasing dietary supplementation with cellulose in rats and chickens resulted in reduced abdominal fat. CCM used here in Huixu chickens, this Yellow-feathered chicken is an important local breed in southern China. The meat is very popular because it satisfies consumers’ sensory preferences. CCM significantly reduced abdominal fat of chickens. This result is consistent with some scientific evidence suggesting that higher intakes of dietary fiber may reduce fat deposition (Ruhee, 2018). It is not clear how dietary fiber mechanistically influences fat distribution. One explanation is that fiber intake plays a role in controlling insulin secretion or other lipogenic hormones (Tang et al., 2021). Another possible reason is that intake of fiber influences hypothalamic-pituitary-adrenal activity and may reduce fat deposition (Tannenbaum et al., 1997).

In the current study, the expression of several relevant genes (FABP4, SCD, PPARγ, FA4) was generally decreased in abdominal fat by supplementation with CCM, and only ESR1 was increased. In poultry, reverse changes in expression of these genes are associated with
increased abdominal fat deposition. For example, Cui et al. (2016) demonstrated that follicle-stimulating hormone increased abdominal fat deposition of chickens and also increased FABP4, FAS, and PPARγ expression in abdominal fat. The ability of FABP4 to synthesize lipids contributes partially to lipid deposition in chicken abdominal fat. Shih et al. (2011) found that FABP4 impacts fat deposition through lipolysis. Increasing evidence indicates that SCD-1 contributes to lipid metabolism and fat deposition in mammals (Cohen et al., 2002). In the current study with chickens, SCD-1 gene expression was reduced in abdominal fat in birds supplemented with CCM. The present study also showed that dietary CCM reduced abdominal fat expression of PPARγ, FAS, and RXRG. The PPARγ plays an important role in the regulation of abdominal fat deposition, and is regulator of abdominal adipocyte development (Hocquette, 2010). Previous work showed that PPARγ is a target gene in the lipogenic pathway and induces expression of lipogenic genes (Hummasti et al., 2008). Yu et al. (2014) reported that FAS is a mediator of adipose tissue lipogenesis and its expression was decreased here in birds fed CCM. The results are consistent with the capacity to synthesize lipids partially determining lipid deposition in abdominal fat (Chartrin et al., 2006; Cui et al., 2016). Additional studies are needed to demonstrate that ESR1, altered by dietary fiber, can affect lipid metabolism.

Recently, some scientists suggested that feed fermentation is a way to manipulate fat deposition in chickens. Niu et al. (2019) reported that fermented cottonseed meal altered the lipid-related metabolites and decreased fat deposition in chickens. The results presented here also found similar results, CCM reduced fat deposition significantly associate with cecal microbiota and metabolites. Nie et al. (2015) reported that feeding cottonseed meal decreased abdominal fat in broilers.

Corncob fiber altered the microbial diversity. Akkermansia, Faecalibacterium were the main microbes in the cecal microbiome in yellow chickens. The contents of Akkermansia, and Faecalibacterium were significantly altered here by corncob fiber. Niu et al. (2020) reported that the predominant microbial flora in cecum consisted Bacteroidetes (53.55%), Firmicutes (33.75%), and Proteobacteria (8.61%). Fermented cottonseed meal diet increased the relative abundance of Bacteroides but decreased obese microbial, including Faecalibacterium, Lachnospiraceae, Ruminococcaceae, and Anaerofilum. Akkermansia are considered to be “lean microbes.” On the other hand, Faecalibacterium are described to be “obese microbes” (Mariat et al., 2009). Faecalibacterium was more abundant in the fat chickens, which is in alignment with the findings of Lee et al. (2017) who also reported an enrichment of this genus in high fat male chickens. Similar results were observed in human clinical research; obese subjects had lower Akkermansia abundance and that of Faecalibacterium was accordingly higher (Tu et al., 2018). It is established that reduced abundance of Akkermansia is associated with obesity (Ou et al., 2020) which might partly explain the relationship with excessive fat deposition. Faecalibacterium is a major bacterium in the non-ruminant gut (Kim et al., 2020). It produces short-chain fatty acids that are absorbed in the cecum and colon and can be used for energy and as substrates for gluconeogenesis and lipogenesis in hepatocytes (Sun et al., 2017). Faecalibacterium produces butyrate, further, butyrate is major energy source in the cecum and colon (Zhou et al., 2018). It plays an important role by regulating gene expression and apoptosis (Hamer et al., 2008). The most studied one is Faecalibacterium which increases butyrate levels (Chen and Vitetta, 2020). Contradictory evidence also exists because butyric acid was increased in the birds given corn meal whereas Faecalibacterium abundance decreased. This finding seems to be counter-intuitive. Some research has found that Faecalibacterium abundance decreased with inflammatory disease (Sokol et al., 2008; Fujimoto et al., 2013).

Table 5. Differentially abundant metabolites in the cecal contents of chickens fed CON or CCM diet1

| Metabolites                            | RT     | m/z   | log2FC(CCM/CON) | VIP    | P-value |
|----------------------------------------|--------|-------|-----------------|--------|---------|
| (R)-5-Diphosphomevalote                | 304.8  | 307   | 2.66            | 1.18   | 0.000   |
| Pantothenic acid                       | 45.6   | 218   | 2.66            | 1.43   | 0.000   |
| 2-epi-5-epi-Valiolone 7-phosphate      | 304.8  | 271   | 2.09            | 1.66   | 0.001   |
| D-Ribose 5-phosphate                   | 169.8  | 309   | 1.94            | 1.04   | 0.012   |
| Arbutin 6-phosphate                    | 535.3  | 351   | 1.57            | 1.54   | 0.031   |
| D-Ribitol 5-phosphate                  | 161.5  | 231   | 1.39            | 1.39   | 0.001   |
| Undecanoic acid                        | 346.5  | 185   | 1.25            | 2.60   | 0.016   |
| Neotinic acid                          | 638.3  | 394   | 1.67            | 2.04   | 0.033   |
| 4-Methyl-2-oxovaleric Acid             | 68.8   | 129   | 0.52            | 1.04   | 0.022   |
| Oleic acid                             | 535.9  | 281   | -0.69           | 16.95  | 0.008   |
| Glutaric acid                          | 35.5   | 131   | -0.99           | 1.57   | 0.046   |
| Adipic acid                            | 26.8   | 145   | -1.01           | 2.61   | 0.013   |
| Suberic acid                           | 37.6   | 173   | -1.15           | 2.31   | 0.009   |
| L-Fuculose 1-phosphate                 | 152.5  | 243   | -2.23           | 1.14   | 0.015   |
| Phosphoenolpyruvate                    | 28.1   | 167   | -4.83           | 2.71   | 0.007   |

All differently-abundant metabolites listed here are those with VIP > 1 and P < 0.05, n = 6.

1Abbreviations: CON, control; CCM, corn cob meal; RT, retention time; m/z, mass to charge ratio; FC, fold change; VIP, variable importance in projection.
was increased markedly in chickens fed the CCM diet. Faecalibacterium dancos of producing bacteria were increased by CCM diet. The abundance of butyrate and number of VFA-producing bacteria were increased, but abdominal fat was significantly reduced. Higher concentrations of nicotinic acid cause lipid lowering (Liu et al., 2015), perhaps accounting for part of the effect of reducing abdominal fat.

In conclusion, fat deposition, cecal microbiotas and metabolites differed as a result of dietary addition of CCM. The live weight and AFP were significantly decreased by feeding the CCM diet. The relative abundances of FABP4, SCD, FAS, and PPARγ transcripts in abdominal fat were reduced and ESRI was increased. Similarly, the majority of the metabolites in cecal digesta with differences in abundance were organic acids, including (R)-5-diphosphomevalote, D-ribose 5-diphosphate, arbutin 6-diphosphate, D-ribitol 5-phosphate, undecanoic acid, 4-methyl-2-oxovaleric acid, glutaric acid, adipic acid, suberic acid, L-fuculose 1-phosphate, and phosphoenolpyruvate. The concentration of butyrate and number of VFA-producing bacteria were increased by CCM diet. The abundances of Phascolarctobacterium, Rikenellaceae, and Faecalibacterium were reduced and that of Akkermansia was increased markedly in chickens fed the CCM diet.

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DISCLOSURES

All authors have read and approved this version of the paper and no conflict of interest exists in the submission of this manuscript. Finally, this paper is our original unpublished work and has not been submitted to any other journal for reviews.

SUPPLEMENTARY MATERIALS

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