

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

Xiaoyan Cui ^{*,†}, Zhongyong Gou,[†] Zongyong Jiang,[†] Long Li,[†] Xiajing Lin,[†] Qiuli Fan,[†] Yibing Wang,[†] and Shouqun Jiang^{†,1}

^{*}College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, P.R. China; and [†]Institute of Animal Science, Guangdong Academy of Agricultural Sciences, State Key Laboratory of Livestock and Poultry Breeding, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture and Rural Affairs, Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, P. R. China

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. Corn cob 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*), stearyl-CoA desatur-

ase (*SCD*), fatty acid synthase (*FAS*), and peroxisome proliferator-activated receptor γ (*PPAR γ*) 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-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, 4-methyl-2-oxovaleric acid, while decreasing ($P < 0.05$) those of oleic acid, glutaric acid, adipic acid, suberic acid, and L-fucose 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, corn cob meal, microbial community, microbial metabolites

2022 Poultry Science 101:101721

<https://doi.org/10.1016/j.psj.2022.101721>

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;

© 2022 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received October 7, 2021.

Accepted December 11, 2021.

¹Corresponding author: jsqun3100@sohu.com

Elnesr et al., 2022). 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.

Table 1. Composition and nutrient levels of experimental diets (% as fed-basis) for Huxu chickens from 90 to 135 d.

Components	CON ¹	CCM ¹
Ingredient, %		
Corn	81.0	66.0
Soybean meal	11.0	11.0
Corn gluten meal	1.45	2.70
Lard	2.00	2.00
<i>L</i> -Lysine HCl	0.40	0.42
<i>DL</i> -Methionine	0.20	0.22
<i>L</i> -Threonine	0.10	0.13
Limestone	1.05	1.05
Dicalcium phosphate	1.55	1.55
Salt	0.25	0.25
Premix ²	1.00	1.00
Corncob meal	0.00	13.68
Total	100.00	100.00
Nutrient composition ³		
ME (MJ/kg)	13.31	12.06
CP, %	13.00	13.00
Lys, %	0.83	0.83
Met+Cys, %	0.66	0.66
Ca, %	0.79	0.79
Nonphytate P, %	0.34	0.34
Crude fiber, %	2.53	7.13

¹Abbreviations: CON, control; CCM, corncob meal.

²Provided 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.

³Values 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 (AFW) and expressed as abdominal fat percentage (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

Table 2. Proximate composition of corncob meal.

Component ¹	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.

CCM were measured according to the methodologies described by AOAC (1997). Crude fiber content was quantified by the Weende method, whereas the neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), cellulose and lignin were measured according to the sequential methodology described by Van Soest et al. (1991). The hemicellulose content was obtained by the equation (NDF - ADF). The proximate composition of the CCM is shown in Table 2.

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 $2,000 \times g$ for 2 min and centrifuged at $800 \times g$ 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 Na_2SO_4 to remove moisture, clarified with 0.45- μm 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 protocol. The concentration and purity of RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). The specific primers 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, Waltham, 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 parameters. 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 (rarefied) 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 Metagenomic Sequencing Library Preparation guide. The V3 and V4 regions were amplified using forward primers containing the sequence 5'-CCTACGGGNGGCWGCAG-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 μL) and 6- μg internal standard lidocaine was added. The samples were left at 4°C for 20 min for protein precipitation. After 10 min of centrifugation, 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 chromatography-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 (Umetrics, Umeå, 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 ($\text{VIP} > 1$ 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

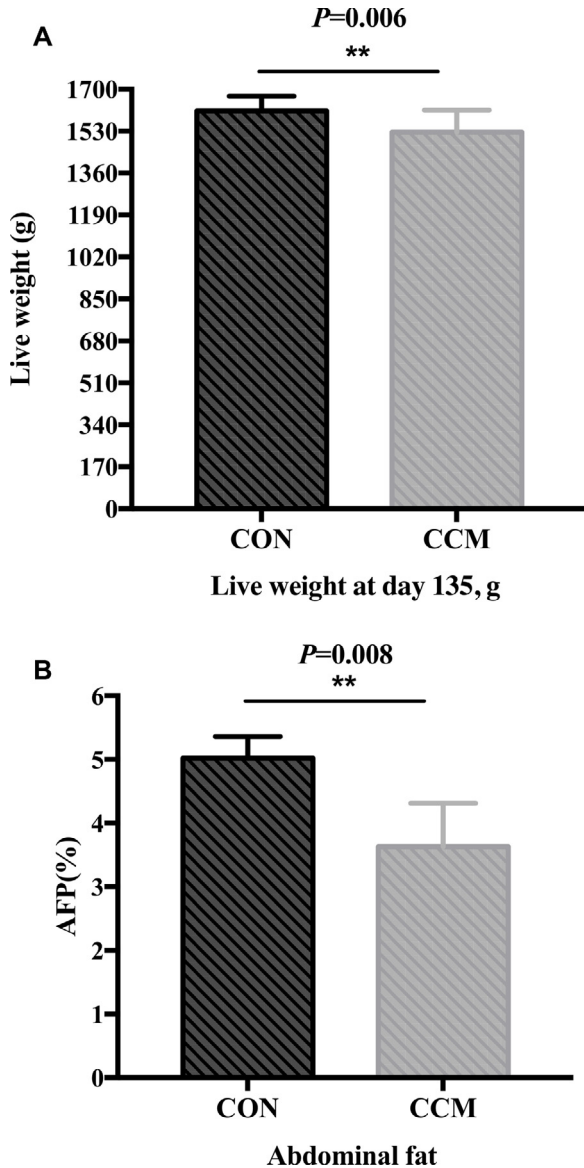


Figure 1. Effects of corn-soybean basic (CON) diet or corn-cob meal (CCM) diet on live weight and abdominal fat percentage (AFP) in chickens ($n = 10$). ** indicate significance at $P < 0.01$.

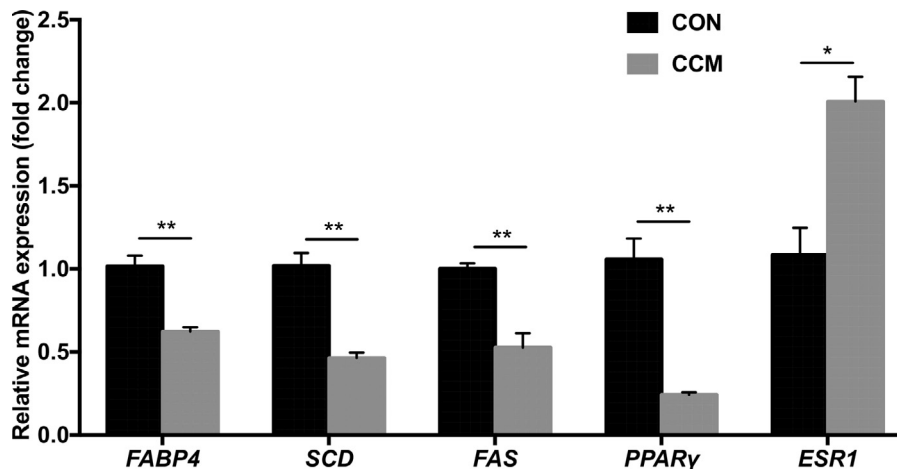


Figure 2. Effects of corn-soybean basic (CON) diet or corn-cob meal (CCM) diet on expression of lipid-related genes assessed by qRT-PCR in abdominal fat of chickens ($n = 10$). * indicate significance at $P < 0.05$, ** indicate significance at $P < 0.01$. Abbreviations: ESR1, estrogen receptor alpha; FABP4, fatty acid binding protein 4; FAS, fatty acid synthase; PPAR γ , peroxisome proliferator-activated receptor γ ; SCD, stearoyl-CoA desaturase.

using SPSS Version 19.0 (SPSS Inc., Chicago, IL). Data are shown as means \pm 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 abundances ($P < 0.01$) of fatty acid binding protein 4 (*FABP4*), stearoyl-CoA desaturase (*SCD*), fatty acid synthase (*FAS*), and peroxisome proliferator-activated receptor γ (*PPAR γ*), 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,

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

Index ¹	CON	CCM	SEM ²	P-value
Shannon	6.19 ^b	7.56 ^a	0.077	0.031
Simpson	0.092	0.095	0.014	0.279
Chao1	2,166 ^b	2,405 ^a	43.25	0.047
ACE	2,328	2,467	47.85	0.156

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

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

²SEM = standard error of mean, $n = 6$.

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.

Compared with CON, relative abundance of *Kiritima-tiellaeota* 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

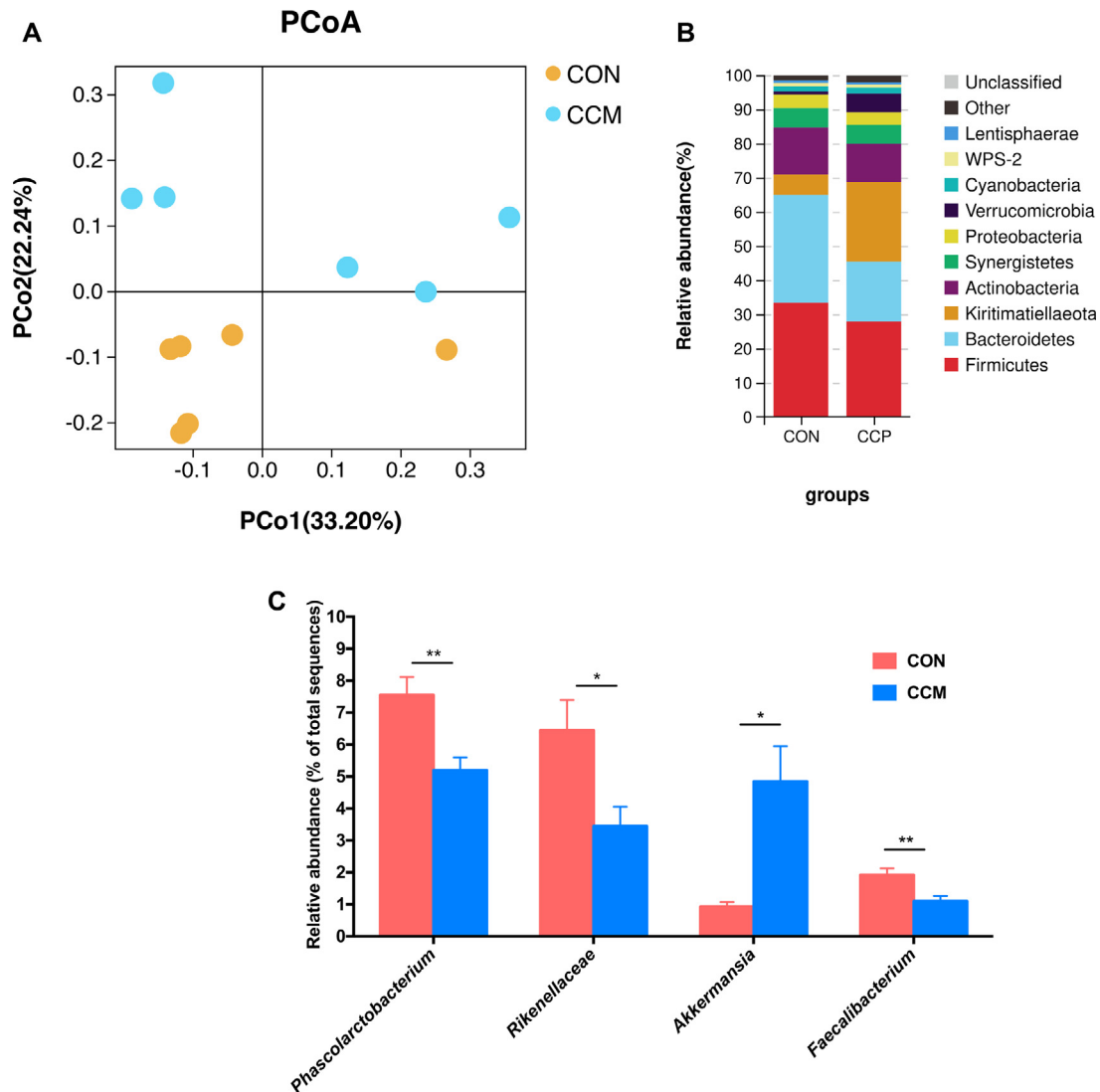


Figure 3. (A) Principal coordinates analysis (PCoA) of bacterial communities in the cecal digesta of chickens (based on the Bray distance). The percentage of variation explained by PCo1 and PCo2 are indicated in the axes. (B) Phylum-level relative abundance of 16S rRNA gene sequences from the cecal digesta of chickens ($n = 6$). (C) Significantly changed bacteria genera by corncob 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, corncob meal; CON, control.

Table 4. Responses in the cecal concentrations (mg/g DM¹ cecal digesta) of major volatile compounds in the chickens fed the experimental diets

Item	CON ²	CCM ²	SEM ³	<i>P</i> -value
Acetic acid	25.42	35.73	13.71	0.098
Butyric acid	5.12 ^b	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 $P < 0.05$.

¹DM, dry matter.

²CON, control; CCM, corncob meal.

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

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

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-diphosphomevalote, 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.

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.,

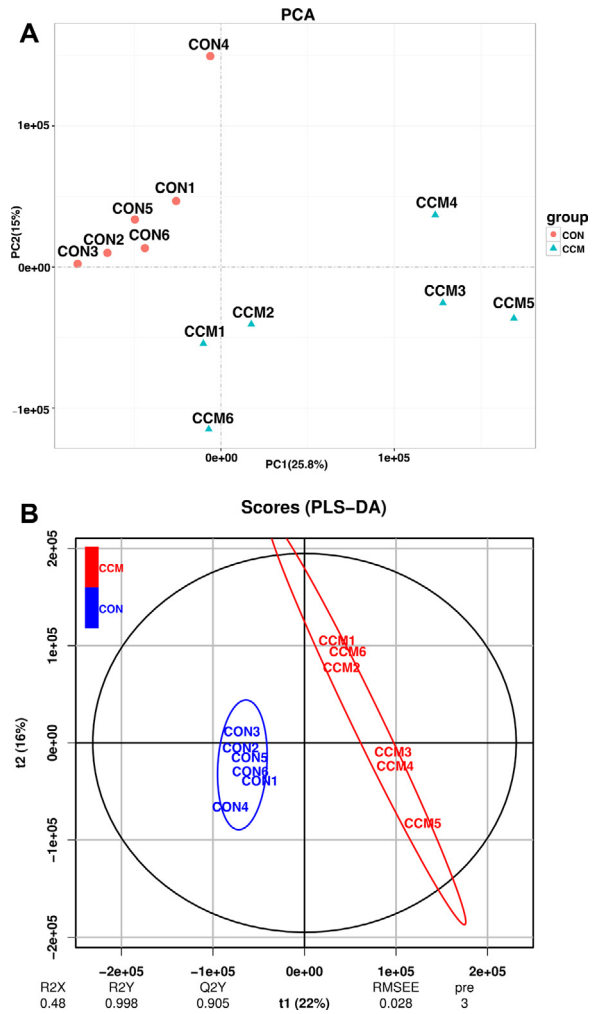


Figure 4. A. Principal components analysis (PCA) of cecal metabolites from chickens ($n = 6$) fed corn-soybean basic (CON) diet or corncob meal (CCM) diet. Figure 4B. Partial least squares discriminant analysis (PLS-DA) of the cecal metabolites from chickens ($n = 6$) of CON and CCM treatment. PCA and PLS-DA models demonstrating a separation between CON and CCM treatment.

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 Huxu 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 (Ruehe, 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*, *PPAR γ* , *FAS*) 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

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

Metabolites	RT	m/z	log ₂ FC(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-diphosphate	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
Nicotinic acid	638.3	394	1.07	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-Fucose 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, corncob meal; RT, retention time; m/z, mass to charge ratio; FC, fold change; VIP, variable importance in projection.

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 *PPAR γ* 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* expression was lower in fat chickens; maybe *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 *PPAR γ* , *FAS*, and *RXR γ* . The *PPAR γ* plays an important role in the regulation of abdominal fat deposition, and is regulator of abdominal adipocyte development (Hocquette, 2010). Previous work showed that *PPAR γ* 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 adiporegulatory genes regulating 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 fermented 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.

Corn cob 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).

Previous pig research indicated that fiber, especially celluloses and hemicelluloses, is hydrolyzed in the large intestine, increasing effective overall fiber fermentation

(Varel and Yen., 1997; Wang et al., 2012). Soluble fiber from sugar beet was fermented in human cecum and supplied energy to epithelial cells (Castiglia-Delavaud et al., 1998). Rideout et al. (2008) showed that soluble guar gum fiber significantly enhanced production of volatile short-chain fatty acids in the large intestine. The present study, with chickens, presumably would have primarily involved the cecum.

Wong et al. (2006) reported that the reduced deposition of abdominal fat associated with dietary fiber depended on the fermented substrate. In the present study with chickens, the cecal content of pantothenic acid was higher in birds fed corn meal. Pantothenic acid is a component of coenzyme A and so is part of acetyl coenzyme A, a key intermediary in fatty acid synthesis and degradation (Takahashi et al., 2015). A deficiency of pantothenic acid leads to fat accumulation in the body. In the present study, nicotinic acid was 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 *PPAR γ* transcripts in abdominal fat were reduced and *ESR1* was increased. Similarly, the majority of the metabolites in cecal digesta with differences in abundance were organic acids, including (R)-5-diphosphomevalate, D-ribose 5-diphosphate, arbutin 6-phosphate, 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.

ACKNOWLEDGMENTS

The authors sincerely thank W. Bruce Currie (Emeritus Professor, Cornell University, Ithaca, NY) for his help in presentation of this manuscript.

This work was funded by National Key R&D Project (2021YFD1300004), Key Realm R&D Program of Guangdong Province (2020B0202090004), China Agriculture Research System of MOF and MARA (CARS-41), National Natural Science Foundation of China (31802104), the Natural Science Foundation from Guangdong Province (2021A1515012412, 2021A1515010830), the Science and Technology Program of Guangdong Academy of Agricultural Sciences (202106TD, R2019PY-QF008).

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

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.psj.2022.101721](https://doi.org/10.1016/j.psj.2022.101721).

REFERENCES

- Abd El-Hack, M. E., S. S. Elnesr, M. Alagawany, A. Gado, A. E. Noreldin, and A. A. Gabr. 2020. Impact of green tea (*Camellia sinensis*) and epigallocatechin gallate on poultry. *World. Poult. Sci. J.* 76:49–63.
- Awad, W. A., F. Dublecz, C. Hess, K. Dublecz, B. Khayal, and M. Hess. 2016. *Campylobacter jejuni* colonization promotes the translocation of *Escherichia coli* to extra-intestinal organs and disturbs the short-chain fatty acids profiles in the chicken gut. *Poult. Sci.* 95:2259–2265.
- Bhuiyan, M. M., Z. Cheng, M. S. Bari, and P. A. Iji. 2021. High fibre diet reduced the energy cost of production and abdominal fat of broiler chickens. *Adv. Anim. Vet. Sci.* 9:1585–1593.
- Brar, K. K., S. Kaur, and B. S. Chadha. 2016. A novel staggered hybrid SSF approach for efficient conversion of cellulose/hemicellulosic fractions of corncob into ethanol. *Renew. Energ.* 98:16–22.
- Castiglia-Delavaud, C., E. Verdier, J. M. Besle, J. Vernet, and M. Vermorel. 1998. Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects. *Br. J. Nutr.* 80:343–352.
- Catherine, D., R. Nicolas, D. Roger, G. Bernard, T. Henryk, D. Barbara, and D. Nathalie. 2002. Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. *J. Nutr.* 132:967–973.
- Chartrin, P., M. D. Bernadet, G. Guy, J. Mourot, J. F. Hocquette, N. Rideau, M. J. Duclos, and E. Baéza. 2006. Does overfeeding enhance genotype effects on energy metabolism and lipid deposition in breast muscle of ducks? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 145:413–418.
- Chen, J., and L. Vitetta. 2020. The role of butyrate in attenuating pathobiont-induced hyperinflammation. *Immune Netw.* 20:e15.
- Cohen, P., M. S. Miyazaki, N.D. Hagge-Greenberg, A. W. S. Liedtke, A. A. Sharma, R. Hudgins, L.C., and J. M. F. Ntambi, J.M. 2002. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science.* 297:240–253.
- Cui, X. Y., Y. Y. Li, R. R. Liu, G. P. Zhao, M. Q. Zheng, Q. H. Li, and J. Wen. 2016. Follicle-stimulating hormone increases the intramuscular fat content and expression of lipid biosynthesis genes in chicken breast muscle. *J. Zhejiang Univ. Sci. B.* 17:303–310.
- Elnesr, S. S., A. R. H. Abdel-Razik, and H. A. Elwan. 2022. Impact of humate substances and *Bacillus subtilis* PB6 on thyroid activity and histomorphometry, iron profile and blood haematology of quail. *J. Anim. Physiol. Anim. Nutr.* 106:110–117.
- Fujimoto, T., H. Imaeda, K. Takahashi, E. Kasumi, and A. Andoh. 2013. Decreased abundance of *faecalibacterium prausnitzii* in the gut microbiota of Crohn's disease. *J. Gastroenterol. Hepatol.* 28:613–619.
- Hamer, H. M., D. Jonkers, K. Venema, S. Vanhoutvin, F. J. Troost, and R.-J. Brummer. 2008. Review article: the role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* 27:104–119.
- Hocquette, F. J. 2010. Endocrine and metabolic regulation of muscle growth and body composition in cattle. *Animal* 4:1797–1809.
- Hummasti, S., C. Hong, J. B. Steven, and P. Tontonoz. 2008. HRASLS3 is a PPAR γ -selective target gene that promotes adipocyte differentiation. *J. Lipid Res.* 49:2535–2544.
- Jenkins, D., V. Vuksan, C. Kendall, P. Würsch, R. Jeffcoat, S. Waring, C. C. Mehling, E. Vidgen, L. Augustin, and E. Wong. 1998. Physiological effects of resistant starches on fecal

- bulk, short chain fatty acids, blood lipids and glycemic index. *J. Am. Coll. Nutr.* 17:609–616.
- Jiang, X. S., and A. F. Groen. 2000. Chicken breeding with local breeds in china - a review. *Asian Austral. J. Anim. Sci.* 13:1482–1498.
- Khempaka, S., W. Molee, and M. Guillaume. 2009. Dried cassava pulp as an alternative feedstuff for broilers: effect on growth performance, carcass traits, digestive organs, and nutrient digestibility. *J. Appl. Poultry Res.* 18:487–493.
- Kim, H., Y. Jeong, S. Kang, H. J. You, and G. E. Ji. 2020. Co-culture with bifidobacterium catenulatum improves the growth, gut colonization, and butyrate production of faecalibacterium prausnitzii: in vitro and in vivo studies. *Microorganisms* 8:788.
- Lai, H. S. 2005. Effects of a high-fiber diet on hepatocyte apoptosis and liver regeneration after partial hepatectomy in rats with fatty liver. *JPEN J. Parenter. Enteral Nutr.* 29:401.
- Lee, K. C., D. Y. Kil, and W. J. Sul. 2017. Cecal microbiome divergence of broiler chickens by sex and body weight. *J. Microbiol.* 55:939–945.
- Liu, D., X. Wang, L. Kong, and Z. Chen. 2015. Nicotinic acid regulates glucose and lipid metabolism through lipid independent pathways. *Curr. Pharm. Biotechnol.* 16:3–10.
- Mariat, D., O. Firmesse, F. Levenez, V. Guimares, and J. P. Furet. 2009. The firmicutes/bacteroides ratio of the human microbiota changes with age. *BMC Microbiol.* 9:123.
- Nie, C. X., W. J. Zhang, W. X. Ge, Y. F. Liu, Y. Q. Wang, and J. C. Liu. 2015. Effect of cottonseed meal fermented with yeast on the lipid-related gene expression in broiler chickens. *Braz. J. Poult. Sci.* 16:57–64.
- Niu, J. L., J. Zhang, L. Q. Wei, W. J. Zhang, and C. X. Nie. 2019. Effect of fermented cottonseed meal on the lipid-related indices and serum metabolic profiles in broiler chickens. *Animals* 9:930.
- Niu, J., J. Zhang, L. Wei, X. Ma, W. Zhang, and C. Nie. 2020. Cottonseed meal fermented by candida tropical reduces the fat deposition in white-feather broilers through cecum bacteria-host metabolic cross-talk. *Appl. Microbiol. Biotechnol.* 104:4345–4357.
- Noakes, M., P. M. Clifton, P. J. Nestel, R. L. Leu, and G. H. Mcintosh. 1996. Effect of high-amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertriglyceridemia. *Am. J. Clin. Nutr.* 64:944–951.
- Ou, Z., L. Deng, Z. Lu, F. Wu, and Y. Peng. 2020. Protective effects of Akkermansia muciniphila on cognitive deficits and amyloid pathology in a mouse model of Alzheimer's disease. *Nutr. Diabetes.* 10:12–22.
- Rideout, T. C., M. Z. Fan, J. P. Cant, R. Wagner, and C. 2004 P. Stonehouse. 2004. Excretion of major odor-causing and acidifying compounds in response to dietary supplementation of chicory inulin in growing pigs. *J. Anim. Sci.* 82:1678–1684.
- Rideout, T. C., S. V. Harding, P. J. Jones, and M. Z. Fan. 2008. Guar gum and similar soluble fibers in the regulation of cholesterol metabolism: current understandings and future research priorities. *Vasc. Health. Risk Manag.* 4:1023–1033.
- Rinttila, T., and J. Apajalahti. 2013. Intestinal microbiota and metabolites-implications for broiler chicken health and performance. *J. Appl. Poult. Res.* 22:647–658.
- Rose, D. J., M. T. Demeo, A. Keshavarzian, and B. R. Hamaker. 2007. Influence of dietary fiber on inflammatory bowel disease and colon cancer: importance of fermentation pattern. *Nutr. Rev.* 65:51–62.
- Ruheer, R. T. 2018. Dietary fiber and its effect on obesity: a review article. *Adv. Med. Sci.* 23:23–30.
- Shih, M., Y. N. Chiu, M. C. Hu, I. C. Guo, and B. C. Chung. 2011. Regulation of steroid production: analysis of cyp11a1 promoter. *Mol. Cell Endocrinol.* 336:80–84.
- Sokol, H., B. Pigneur, L. Watterlot, O. Lakhdari, L. G. Bermudez-Humaran, J. J. Gratadoux, and P. Langella. 2018. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of crohn disease patients. *Proc. Natl. Acad. Sci. U. S. A.* 105:16731–16736.
- Sun, M., W. Wu, Z. Liu, and Y. Cong. 2017. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. *J. Gastroenterol.* 52:1–8.
- Takahashi, K., T. Fukuwatari, and K. Shibata. 2015. Exercise and a high fat diet synergistically increase the pantothenic acid requirement in rats. *J. Nutr. Sci. Vitaminol.* 61:215–221.
- Tang, R., and L. Li. 2021. Modulation of short-chain fatty acids as potential therapy method for Type 2 diabetes mellitus. *Can. J. Infect Dis. Med.* 2021:1–13.
- Tannenbaum, B. M., D. N. Brindley, G. S. Tannenbaum, M. F. Dallman, and M. J. Meaney. 1997. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. *Am J. Physiol.* 273:E1168.
- Tu, P., X. Bian, C. Liang, B. Gao, H. Ru, T. J. Knobloch, C. M. Weghorst, and K. Lu. 2018. Characterization of the functional changes in mouse gut microbiome associated with increased Akkermansia muciniphila population modulated by dietary black raspberries. *ACS Omega* 3:10927–10937.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Varel, V. H., and J. T. Yen. 1997. Microbial perspective on fiber utilization by swine. *J. Anim. Sci.* 75:2715–2722.
- Wang, W., T. Archbold, M. S. Kimber, J. Li, J. S. Lam, and M. Z. Fan. 2012. The porcine gut microbial metagenomic library for mining novel cellulases established from growing pigs fed cellulose-supplemented high-fat diets. *J. Anim. Sci.* 90:400–412.
- Wong, J., R. D. Souza, C. Kendall, A. Emam, and D. Jenkins. 2006. Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* 40:235–243.
- Yu, X., R. Liu, G. Zhao, M. Zheng, J. Chen, and J. Wen. 2014. Folate supplementation modifies CCAAT/enhancer-binding protein α methylation to mediate differentiation of preadipocytes in chickens. *Poult. Sci.* 93:2596–2603.
- Zhou, L., M. Zhang, Y. Wang, D. R. Gregory, H. Liu, T. Yu, X. Chen, D. Tang, L. Xu, and Y. Yin. 2018. Faecalibacterium prausnitzii produces butyrate to maintain Th17/Treg balance and to ameliorate colorectal colitis by inhibiting histone deacetylase. *Inflamm. Bowel Dis.* 24:S110–S111.