Chicken cecal microbiota reduces abdominal fat deposition by regulating fat metabolism

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Research Article

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Abstract

Background:

Rapidly developed broilers are often accompanied by excessive fat deposition and markedly decreased feed utilization, which is a severe problem in the poultry industry. Recent studies established that gut microbiota significantly alters chicken fat deposition. However, what kind of cecal bacteria can regulate fat deposition and how they control fat deposition remains unclear.

Results:

The abdominal fat weight and index, and average diameter of adipocytes were markedly higher in high than in low abdominal fat deposition chickens at different time points (1, 4, and 12 months). The higher expression levels of fat synthesis related genes (*ACSL1*, *FADS1*, *CYP2C45, ACC,* and *FAS*) and a lower expression of fat catabolism genes (*CPT-1,* and *PPARα*) in the liver and abdominal fat of high abdominal fat deposition chickens, indicated that an unbalanced fat metabolism leads to excessive abdominal fat deposition. *Parabacteroides*, *Parasutterella*, *Oscillibacter,* and *Anaerofustis* were found higher in high abdominal fat deposition chickens, while *Bacteroides* and *Sphaerochaeta* were higher in low abdominal fat deposition chickens at different time points. Further, Spearman correlation analysis indicated that the relative abundance of *Parabacteroides*, *Parasutterella*, *Oscillibacter,* and *Anaerofustis* was positively correlated with abdominal fat weight and index, and the expression level of fat anabolism genes, yet the relative abundance of *Bacteroides* and *Sphaerochaeta* was negatively correlated with these factors. Furthermore, transferring fecal microbiota from adult chickens with low abdominal fat deposition into one-day-old chicks significantly decreased *Parabacteroides,* abdominal fat weight and index, and fat anabolism genes, while markedly increased *Bacteroides* and *Sphaerochaeta* and fat catabolism genes.

Conclusion:

Our findings highlighted that *Bacteroides* and *Sphaerochaeta* might reduce abdominal fat deposition, yet *Parabacteroides Parasutterella*, *Oscillibacter,* and *Anaerofustis* could promote abdominal fat deposition by regulating fat metabolism.

Introduction

In the poultry industry, genetic breeding technology and nutrition regulation unprecedentedly enhanced the growth rate and feed conversion of broilers [1]. However, rapidly developed broilers are often accompanied by excessive abdominal fat deposition [2], which is an unfavorable trait both for consumers and producers, and physiologically more than 85% of abdominal fat is useless for body functions [3]. A recent report indicated that broilers produced ≈ 3 million tons of abdominal fat around the world annually, which is causing > $2.7 billion economic loss in the poultry industry [4], a key hindrance to profitable farming [5]. Abdominal fat deposition markedly decreases feed utilization, reduces the reproduction performance of laying hens, negatively affects the slaughtering process, and significantly causes environmental pollution [2, 6, 7]. It also increases fat contents in chicken meat, which could be risky for human health by causing cardiovascular diseases [8]. Researchers have found that biologically, the abdominal adipocytes are more active cells exhibiting a higher

(0.82) heritability rate than bodyweight, breast, and leg muscles [5], resulting in fat accumulation. It is also reported that abdominal fat weight and body weight had a strong positive correlation, hindering genetic selection against fatness traits in chickens [4]. Therefore, excessive fat deposition has become a puzzle and also an emerging concern in the recent decade, and a strategy at the same time for keeping the body weight and reducing chicken abdominal fat deposition is urgently required.

Host gut harbors around 80% of the symbiont microorganisms, of which 99% are bacteria, called gut microbiota [9, 10, 11]. It has been established that gut microbiota could play a significant regulatory role in fat deposition and obesity [4, 12]. Evidence revealed that colonization of the obese microbiota promoted fat deposition in mice [13]. For example, a higher abundance of *Methanobrevibacter* and *Faecalibacterium*, while a lower abundance of *Akkermansia* increases fat deposition [4, 14]. Further studies indicated that gut microbiota influences and modulates fat metabolism, and importantly contributes to nutrient utilization, generating additional harvestable energy and resulting in abdominal fat deposition [14, 15]. For instance, *Enterococcus faecium* increases fatty acid synthase (*FAS*) and acetyl-CoA carboxylase (*ACC*) secretion in chicken liver [16], and elevated *FAS* and ACC increases fatty acid production, which incorporates into triglyceride and increases fat deposition [17]. *Klebsiella* and *Escherichia-Shigella* possess lipogenesis characteristics, and their higher abundance increases total cholesterol, low-density lipoprotein, and triglyceride concentrations in serum, which facilitate fat accumulation [18]. On the other hand, some microbiota such as *Mucispirillum schaedleri* decreases fat deposition in chickens [4], and *Sphaerochaeta* is found enriched in lean chickens [12]. *Lactobacillus johnsonii BS15* decreases fat deposition through lipoprotein lipase (*LPL*) activity and improves fat catabolism in broilers [19]. Abundant *Microbacterium* and *Sphingomonas* in chicken were positively related to fat catabolism genes in muscles and liver, which potentially reduce fat storage [20]. Previous studies indicated that gut microbiota not only can increase fat deposition but also can decrease fat deposition. In the complex network of gut microbial communities, dynamically the highest bacterial diversity is observed in the cecum [21]. Therefore, what kind of cecal bacteria could reduce abdominal fat deposition, and how they decrease fat deposition has become an interesting question.

To address this concern, chickens at three different ages (1 month, 4 months, and 12 months) with significantly different abdominal fat deposition were used in the present study. The fat metabolism levels, cecal microbial communities, and the abundances of different bacteria were compared between high and low abdominal fat deposition chickens. Spearman correlation analysis was used to find the relationship between cecal microbiota and abdominal fat deposition. Besides, transferring fecal microbiota from adult healthy chickens with low abdominal fat deposition into one-day-old chicks was performed to verify whether gut microbiota could regulate chicken fat deposition, and the fat metabolism levels in the liver and abdominal adipose tissues were also compared.

Results

2.1 The abdominal fat deposition is significantly different between high and low abdominal fat deposition chickens

Based on the abdominal fat index, the chickens were divided into high (H) and low (L) abdominal fat deposition chickens of different ages (1 month old, 4 months old, 12 months old). The abdominal fat volume (Fig. 1A),

abdominal fat weight (H vs L, 1 month old: 4.33 ± 0.31 g vs 1.12 ± 0.09 g; 4 months old: 9.58 ± 0.56 g vs 1.15 ± 0.08 g; 12 months old: 63.77 ± 6.19 g vs 19.46 ± 2.77 g) ($p < 0.0001$) (Fig. 1B), and abdominal fat index (1 month old: 1.63 ± 0.12% vs 0.48 ± 0.45%; 4 months old: 1.04 ± 0.07% vs 0.13 ± 0.01%; 12 months old: 3.11 ± 0.22% vs 0.94 ± 0.13%) ($p < 0.0001$) (Fig. 1C) were significantly higher in high abdominal fat deposition chickens. Hematoxylin and eosin (HE) staining results showed that the average diameter of abdominal adipocytes was significantly higher in high abdominal fat deposition chickens than that in low abdominal fat deposition chickens ($P < 0.0001$) (Fig. 1D). The above results showed that there were significant differences in fat deposition between high and low abdominal fat deposition chickens.

2.2 The fat metabolism is significantly different between high and low abdominal fat deposition chickens

It has been established that unbalanced fat metabolism is closely related to abdominal fat deposition, so the fat metabolism levels in the blood, abdominal fat, and liver were compared between high and low abdominal fat deposition chickens of different ages (1 month old, 4 months old, 12 months old). In blood, the concentrations of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) were markedly higher in high abdominal fat deposition chickens at some time points, yet the concentration of high-density lipoprotein cholesterol (HDL-C) was significantly higher in low abdominal fat deposition chickens at all time points ($p < 0.05$) (Fig. 2A). In abdominal fat, the relative mRNA expression of fat synthesis related genes, such as acetyl-CoA carboxylase (*ACC*), fatty acid synthase (*FAS*), and lipoprotein lipase (*LPL*), was markedly higher in high abdominal fat deposition chickens at all time points (Fig. 2B), yet the relative mRNA expression of fat catabolism related gene hormone-sensitive lipase (*HSL*) was significantly higher in low abdominal fat deposition chickens at the age of 4 and 12 months ($p < 0.05$) (Fig. 2C). In the liver, the number of hollow vesicular fat was more in high abdominal fat deposition chickens (Fig. 3A). The relative mRNA expression of fat synthesis related genes including acyl-CoA synthetase long chain family member 1 (*ACSL1*), fatty acid desaturase 1 (*FADS1*), and cytochrome P450 2C45 (*CYP2C45*) was significantly higher in high abdominal fat deposition chickens at all time points ($p < 0.05$) (Fig. 3B). Yet the relative mRNA expression of fat transport related gene apolipoprotein A-I (*APOAI*) (Fig. 3C) was significantly ($p < 0.05$) higher and fat catabolism related genes including peroxisome proliferator activated receptor alpha (*PPARα*), carnitine palmitoyl transferase 1 (*CPT-1*), leptin receptor (*LEPR*), Janus kinase 2 (*JAK2*), and signal transducer and activator of transcription 3 (*STAT3*) was higher in low abdominal fat deposition chickens at different time points (Fig. 3D). Besides, the protein expression levels of p-JAK2 and p-STAT3 were significantly higher in low abdominal fat deposition chickens at all time points ($p < 0.05$) (Fig. 4).

2.3 The cecal microbiota is significantly different between high and low abdominal fat deposition chickens

16S rRNA gene sequencing was used to compare the cecal microbiota composition between high and low abdominal fat deposition chickens at different time points. Alpha diversity analysis showed that the microbial diversity (Fig. 5A) and community abundance (Fig. 5B) in high abdominal fat deposition were higher than low abdominal fat deposition chickens. Beta diversity showed distinct separation between high and low abdominal fat deposition chickens at different time points ($p < 0.05$) (Fig. 5C). At the phylum level, Firmicutes were more

abundant in high abdominal fat deposition chickens, while Bacteroidetes were more abundant in low abdominal fat deposition chickens at all time points (Fig. 6A). The ratio of Firmicutes to Bacteroidetes was significantly higher in high abdominal fat deposition chickens at 4 months ($p < 0.01$) and 12 months ($p < 0.001$) (Fig. 6B). At the genus level, the relative abundance of *Parabacteroides*, *Parasutterella*, *Oscillibacter*, and *Anaerofustis* was higher in high abdominal fat deposition chickens (Fig. 6C), while the relative abundance of *Bacteroides* and *Sphaerochaeta* was higher in low abdominal fat deposition chickens (Fig. 6D) (Fig. S2).

2.4 Metagenomic analysis revealed distinct functional differences of the cecal microbiota between high and low abdominal fat deposition chickens

The association between cecal microbiota and carbohydrate active enzymes (CAZymes) including glycoside hydrolases (GHs), glycosyltransferases (GTs), carbohydrate esterases (CEs), auxiliary activities (AAs), carbohydrate-binding modules (CBMs), and polysaccharide lyases (PLs) was analyzed. Firmicutes and Bacteroidetes encoded more than 85% of the main CAZymes. Compared with the high abdominal fat deposition chickens, the Firmicutes encoded fewer CAZymes, yet the Bacteroidetes encoded more CAZymes in low abdominal fat deposition chickens (Fig. 7A). Further analysis indicated that 25 CAZymes were upregulated in high abdominal fat deposition chickens, and 19 of them are GHs. Other 25 CAZymes were upregulated in low abdominal fat deposition chickens, 12 of them are GHs, and 10 of them are GTs (Fig. 7B). KEGG analysis showed that the differentially expressed genes were annotated to 58 different pathways. Carbohydrate metabolism pathways including starch and sucrose metabolism, pyruvate metabolism, pentose and glucuronate interconversion, C5 branched chain dibasic acid metabolism, and propanoate metabolism were upregulated in high abdominal fat deposition chickens. Lipid metabolism pathways including fatty acid biosynthesis and fatty acid degradation were upregulated in low abdominal fat deposition chickens (Fig. 7C).

2.5 Cecal microbiota was differentially related to abdominal fat deposition in chickens

Spearman correlation analysis was used to analyze the correlation between cecal microbiota and abdominal fat weight, abdominal fat index, and fat metabolism levels. The results indicated that the abundance of *Parabacteroides*, *Parasutterella*, *Oscillibacter*, and *Anaerofustis* was positively correlated with abdominal fat weight, abdominal fat index, and the expression of fat synthesis related genes in the liver and abdominal fat, while negatively correlated with the expression of fat transport and catabolism related genes in the liver and abdominal fat. On the other hand, the abundance of *Bacteroides* and *Sphaerochaeta* was positively correlated with the expression of fat transport and catabolism related genes in the liver and abdominal fat and negatively correlated with abdominal fat weight, and abdominal fat index (Fig. 8).

2.6 Fecal microbiota transplantation from low abdominal fat deposition chicken significantly decreased the abdominal fat deposition of the recipients

In order to verify the effects of gut microbiota on chicken abdominal fat deposition, fecal microbiota transplantation (FMT) from adult chickens with low abdominal fat deposition into one-day-old chicks was performed. Interestingly, an increasing trend of body weight, breast/leg muscle weight, and breast/leg muscle index was observed in the FMT group compared with the control group (Fig. 9A, B, C). The results also indicated that four weeks of FMT significantly decreased the abdominal fat volume (Fig. 9D), abdominal fat

weight (FMT: 15.18 ± 1.05 g vs Con: 18.19 ± 0.79 g) ($p < 0.05$) (Fig. 9E), and abdominal fat index (FMT: 1.02 ± 0.06% vs Con: 1.23 ± 0.04%) ($p < 0.01$) (Fig. 9F). HE staining results indicated that the average diameter of abdominal adipocytes was significantly lower in FMT group than that of the control group ($P < 0.0001$) (Fig. 9G, H). Besides, in abdominal fat, FMT significantly down-regulated the relative mRNA expression of fat synthesis related genes (*FAS* and *LPL*), and up-regulated the relative mRNA expression of *HSL* ($P < 0.05$) (Fig. 9I, J).

2.7 Fecal microbiota transplantation from low abdominal fat deposition chicken reshaped the cecal microbiota of the recipients

16S rRNA gene sequencing results showed that FMT significantly increased cecal microbial community abundance ($P < 0.0001$) (Fig. 10A), changed cecal microbiota composition ($P < 0.001$) (Fig. 10B), increased the relative abundance of Bacteroidetes, decreased the relative abundance of Firmicutes (Fig. 10C), and reduced the ratio of Firmicutes to Bacteroidetes ($P < 0.05$) (Fig. 10D). Further, FMT increased the relative abundances of *Bacteroides* and *Sphaerochaeta*, and decreased the relative abundance of *Parabacteroides* in the FMT group compared with the control group (Fig. 10E) (Fig. S3).

2.8 Reshaped cecal microbiota modulated the fat metabolism levels of the recipients

In order to verify the effects of the reshaped cecal microbiota on fat metabolism of the recipients, the fat metabolism levels in the liver and abdominal fat were investigated. HE staining results indicated the number of hollow vesicular fat was markedly less in FMT group (Fig. 11A). Q-PCR results showed that FMT significantly down-regulated the relative mRNA expression of fat synthesis related genes (*FAS*, *ACC*), and up-regulated the expression of *APOAI* and fat catabolism related genes (*PPARα*, *CPT-1*, *LEPR*, *JAK2*, and *STAT3*) ($P < 0.05$) (Fig. 11B). IHC staining results indicated that FMT significantly up-regulated the protein expression of p-JAK2 and p-STAT3 ($P < 0.05$) (Fig. 11C).

Discussion

Stable fat metabolism liberates energy to increase growth, yet unstable fat metabolism often results in an unnecessary fat deposition [22]. Fat metabolism is a complex biochemical mechanism in which fat digestion, assimilation, and transportation occur through several anabolic and catabolic reactions [20]. The produced fatty acids and glycerol are absorbed in the intestinal epithelium and transported through blood circulation to the liver, adipose tissues, and other organs [20]. Fat synthesis in the organs is regulated by fat synthesis-related genes, including *FAS, ACSL1, FADS1, CYP2C45*, and *LPL* [23]. In the present study, a significantly higher relative mRNA expression of *FAS, ACSL1, FADS1, CYP2C45*, and *LPL* was found in the liver and abdominal fat of high abdominal fat deposition chickens, suggesting more fat synthesis. Elevated *ACSL1, ACC*, and *FAS* are associated with fat deposition through increasing serum TG, TC, and LDL-C levels [24, 25]. In the present study, the increased serum TG, TC, and LDL-C levels and decreased serum HDL-C levels in high abdominal fat deposition chickens are consistent with the findings in mice [26], and in chickens [27, 28]. Simultaneously, *CPT-1* and *PPARα* are the catabolic genes that stimulate fatty acid's oxidation, resulting in energy production for chicken growth [29]. In the present study, downregulated hepatic mRNA expression of *CPT-1* and *PPARα* and a lower JAK2 expression via STAT3 activation in high abdominal fat deposition chickens increased abdominal

fat deposition in broilers [30, 31]. Notably, a significantly higher expression of *APOAI* and *HSL* and higher serum HDLC levels in low abdominal fat deposition chickens at all time points in our study facilitate fat excretion because *HSL* acts as a cleaner and, along with *APOAI*, transports cholesterol/fatty acids from fat depots to the liver for lipolysis, thus are essential to reduce fat accumulation in broilers [32, 33, 34]. Additionally, the upregulation of genes related to the differentiation of adipocytes most probably increases their proliferation and contributes to abdominal fat deposition in chickens [5, 20], and a significantly higher average diameter of abdominal adipocytes in high abdominal fat deposition chickens in our study, indicating the crucial role of adipocytes differentiation in fat deposition [35]. Therefore, more fat synthesis and less fat catabolism result in excessive fat deposition and vice versa.

It has been established that gut microbiota could control abdominal fat deposition by regulating fat metabolism [14]. It is also well-known that an obese host harbors more Firmicutes than Bacteroidetes and a higher Firmicutes/Bacteroidetes ratio [12, 36]. In the present study, we found more Firmicutes than Bacteroidetes along with a significantly higher Firmicutes/Bacteroidetes ratio in high abdominal fat deposition chickens compared with low abdominal fat deposition chickens at 4 and 12 months, which are in accordance with the above findings. *Parabacteroides* are related to several gut disorders in humans and chickens [37], and are directly linked with the obese individuals [38]. *Parasutterella* causes irritable bowel syndrome and immunosuppression in chickens [39], and increases abdominal fat percentage and deposition [40]. *Oscillibacter* is abundant in fat line chickens [41], and is associated with obesity and metabolic syndrome [42]. Likewise, an increased abundance of *Anaerofustis* in the cecum of high fat diet mice [43], and of broilers during *Clostridium perfringens*-induced infection [44], is linked with fat metabolism. Typically, these bacteria have a significant contribution in fat accumulation and are consistent with our findings of high abdominal fat deposition chickens. Interestingly, *Bacteroides* could also reduce diet-induced obesity in the obese host [45], and are associated with lipid metabolism [20]. *Bacteroides* abundance was found higher in low abdominal fat deposition chickens in our study and is positively associated with leanness traits in porcine [46], significantly higher in lean rats [47], and along with *Bacteroides, Sphaerochaeta* was also found enriched in lean chickens [12]. In the present study, higher abundances of *Parabacteroides*, *Parasutterella*, *Oscillibacter*, and *Anaerofustis* are predicted to an increasing fat deposition trend in high abdominal fat deposition chickens through fat anabolism at all time points, whereas higher abundances of *Bacteroides* and *Sphaerochaeta* are expected to the decreasing fat deposition trend in low abdominal fat deposition chickens through fat catabolism at all time points.

Firmicutes and Bacteroidetes could encode carbohydrate active enzymes (CAZymes) to degrade resistant starch and dietary fibers through hydrolysis [12, 48]. Present study found that both of these bacteria encode 85% of these enzymes, indicating their significance in nutrient metabolism. It is observed that Firmicutes are more efficient in calories absorption and thus associated with obesity [49]. In the obese microbiome, Firmicutes are enriched in genes related to synthesis of butyrate, acetate and succinate through galactosidases activity and produce nutrient transporters to facilitate their transportation [50]. Evidence revealed that Firmicutes contain phosphotransferase system (PTS) for the phosphorylation of monosaccharides/disaccharides or sugar derivatives, while Bacteroidetes are deficient in PTS, inferring that Firmicutes have additional capabilities to transport more (metabolized) carbohydrates because obesity-associated enzymes belong to PTS [51]. Both *Anaerofustis* and *Oscillibacter* are Firmicutes and in the present study, both of them are found enriched in high abdominal fat deposition chickens, suggesting that these bacteria extracted extra energy and transported it to

the adipose tissues. Other studies also found a positive association of *Anaerofustis* and *Oscillibacter* with fiber digestibility and obesity [52, 53]. On the other hand, Bacteroidetes use type VI secretion system in competition with their competitors to harvest energy from the shared resources. They possess polysaccharide utilization loci and accomplish this process through activating GHs, PLs, GTs, and CEs [54], exhibiting a distinct behavior from Firmicutes. In the present study, *Bacteroides* through GHs, GTs, and CEs activities efficiently utilized harvested energy for chicken growth instead of storing in fat depots of the low abdominal fat deposition chickens. Typically, carbohydrate metabolism is interlinked with fat metabolism because high carbohydrates of higher calories might also cause *de novo* lipogenesis and obesity [42], following a huge conversion of glucose into pyruvate (glycolysis) or into TG, indicating how both metabolic pathways together contribute to fat deposition [22, 55]. Thus, Firmicutes-mediated CAZymes activities induced fat deposition, yet Bacteroidetes-mediated CAZymes activities reduced fat deposition.

Increasing evidences indicated that reshaping gut microbiota by fecal microbiota transplantation (FMT) could reduce abdominal fat deposition [12, 56]. It was reported that *Bacteroides* and *Sphaerochaeta* enriched in lean chickens, and *Parabacteroides* enriched in obese individuals [12, 38]. Another study reported that FMT could increase *Bacteroides*, which maintain gut homeostasis [57]. Interestingly, a higher abundance of *Sphaerochaeta* and *Bacteroides*, and a lower abundance of *Parabacteroides* were observed in the FMT group of the present study, which are consistent with our results in low abdominal fat deposition chickens. Other studies also described that FMT could significantly attenuate fat deposition in high fat diet mice [58] because lower LPL level decreases adipogenesis by reducing triglyceride hydrolysis in the adipose tissues [59]. In the present study, the decreased anabolism (*FAS*, *LPL* in abdominal fat and *ACC*, *FAS* in liver), and increased catabolism (hepatic *PPARα, CPT-1, LEPR, JAK2*, and *STAT3*) gene's expressions were found in the FMT group compared with the control. Besides, a significantly higher expression of *HSL* in abdominal fat, and *APOAI*, *p-JAK2*, and *p-STAT3* in the liver of FMT group rather than the control group was also observed. Thus, in our study, the findings of FMT and control groups are consistent with the results of low and high abdominal fat deposition chickens respectively, indicating that FMT might be applied to reshape gut microbiota, which in turn might reduce fat deposition by modulating fat metabolism [60].

Conclusion

Taken together, the current findings indicated that the unbalanced fat metabolism leads to excessive abdominal fat deposition. Early colonization of *Parabacteroides*, *Parasutterella*, *Oscillibacter*, and *Anaerofus* upregulates the expression of fat anabolism genes, which increases abdominal fat deposition. However, early colonization of *Bacteroides* and *Sphaerochaeta* upregulates the expression of fat catabolism genes, which reduces abdominal fat deposition and benefits the muscle growth of the chickens. Moreover, FMT significantly decreased *Parabacteroides*, increased *Bacteroides* and *Sphaerochaeta*, and upregulated the expression of fat catabolism genes. FMT might be applied as a strategy in reducing abdominal fat deposition and at the same time promoting the growth of muscles.

Material And Methods

4.1 Animals

The Institutional Animal Care and Use Committee of Huazhong Agricultural University (HZAUCH-2018-008), Wuhan, China) approved all the animal procedures, and all methods were performed in accordance with the relevant guidelines and regulations.

Newly hatched chickens (Turpan cockfighting × White Leghorn) were reared under similar husbandry conditions on the chicken farm of Huazhong Agricultural University. At the age of 1, 4, and 12 months, 120 chickens were randomly selected for each time point. Based on the abdominal fat index, the chickens at each time point were categorized into two groups, namely the high abdominal fat deposition group (H) and the low abdominal fat deposition group (L) (n = 10, 5 males and 5 females). For the fecal microbiota transplantation (FMT) experiment, the chickens with high body weight and low abdominal fat deposition were selected as FMT donors. 60 one-day-old white feather broilers were selected as recipients.

4.2 Selection of FMT donors

Two adult female white Leghorn chicken × Turpan fighting chicken possibly having high or low abdominal fat deposition were scanned with computed tomography (CT) instrument (Aquilion PRIME Tsx-303A, Canon Medical, Japan). Pari software was used to mark the abdominal fat in different frame images of each chicken (Fig. S1), and then Python language was used to write programs to analyze the images and calculate the volume of the body and abdominal fat of each chicken. The volume of the body was 2.22 dm^3 and 2.50 dm^3, and the volume of abdominal fat was 0.06 dm^3 and 0.15 dm^3, respectively. Similarly, the volume percentage of abdominal fat was 2.66% and 5.92%. The chicken with less abdominal fat volume percentage was selected as FMT donor. After FMT experiment, the two chickens were dissected to get the abdominal fat weight and index. The abdominal fat weight was 74.3 g and 161.2 g, and the abdominal fat index was 3.12% and 5.78%, respectively, which are consistent with the CT results and indicated that the FMT donor has low abdominal fat deposition.

4.3 Preparation of fecal suspension

Every morning, once the donor chickens defecated, the white part of the fecal materials was removed as it contains uric acid. Then 10 g of feces were collected in the sterile tube (50 mL) and gently mixed with 60 mL of 0.75% normal saline. The mixture was kept on the ice for settling down the precipitates. The supernatant was obtained and filtered with the sterile gauze to get fecal suspension.

4.4 Animal treatment

60 one-day-old white feather broilers were selected as recipients and randomly divided into FMT group and control group (n = 30). Broilers in the FMT group were orally administrated with 1 mL fecal microbiota suspension, while 1 mL 0.75% saline was used as a substitute in the control group for 28 days. At the age of 42 days, they were sacrificed and samples were collected.

4.5 Sample collection

After fasting for 12 hours, the chickens were weighed and sacrificed, then blood, liver, abdominal adipose tissue, and left cecum were collected. The abdominal adipose tissue was measured as well. For gut microbiota analysis, the cecal content (1 to 1.5 g per bird) was collected into two sterilized centrifuge tubes (1.5 mL) and snap-frozen in liquid nitrogen, then stored at -80°C for sequencing. For analysis of lipometabolic parameter,

blood samples (3 mL per bird) were centrifuged at 3,000× *g* at 4°C for 15 min to get the serum, and then it was stored at − 80 ℃ for subsequent blood biochemical index analysis. For histo-morphological analysis, freshly harvested liver and abdominal adipose tissues were fixed in 4% paraformaldehyde solution. For gene expression analysis, parts of freshly harvested liver and abdominal adipose tissues were snap-frozen in liquid nitrogen and then stored at -80°C.

4.6 Muscle or abdominal fat index calculation

The muscle or abdominal fat index was calculated using the following formula: muscle index = muscle weight (g)/ body weight (g) × 100%, abdominal fat index = abdominal fat weight (g)/ body weight (g) × 100%.

4.7 16S rRNA and Metagenomic genes sequencing

Microbial genomic DNA was extracted from the chicken's cecal content using Fast DNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA), according to manufacturer's instructions. The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR amplification of the 16S rRNA gene was performed as follows: an initial denaturation (3 min) at 95 ℃ following 27 cycles of denaturing (30 s) at 95 ℃, annealing (30 s) at 55 ℃, extension (45 s) at 72 ℃, and single extension (10 min) at 72 ℃, and ended at 4 ℃. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Illumina MiSeq PE300 platform (Illumina, San Diego, USA) was used for 16S rRNA gene sequencing. For 20 chickens at the age of 4 months with metagenomic sequencing, the same DNA extract was fragmented to an average size of about 400 bp using Covaris M220 (Gene Company Limited, China) for paired-end library construction, which was constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Illumina NovaSeq platform (Illumina, San Diego, CA, USA) was used for metagenomic sequencing.

4.8 16S rRNA gene sequencing data processing

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0, and merged by FLASH version 1.2.7. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA database (Silva 132) using a confidence threshold of 0.7. For α and β diversity measurements, the sequencing depth was minimized by subsampling the readings of each sample. The lowest valid reads of cecal microbiota of high and low abdominal fat deposition chickens at the age of 1 month were 25,339, the lowest effective reading of cecal microbiota of high and low abdominal fat deposition chickens at the age of 4 months was 30,671, and the lowest effective reading of cecal microbiota of high and low abdominal fat deposition chickens at the age of 12 months was 45,053. Similarly, the lowest valid reads of cecal microbiota in the control and FMT chickens were 14,960. The α-diversity was described using the Shannon index and Chao index. Principal coordinates analysis (PCoA) based on Bray-Curtis was used to estimate the dissimilarity in the community structure. The community composition at the phylum level and the change of abundance at the genus level were visualized by bar chart and histogram. Linear discriminant analysis effect size (LEfSe) was performed to detect differentially abundant taxa across groups using the default parameters linear discriminant analysis (LDA > 2).

4.9 Metagenomic sequencing data processing

The low-quality reads (length < 50 bp or with a quality value < 20 or having N bases) were removed by fastp (https://github.com/OpenGene/fastp, version 0.20.0). Reads were aligned to the chicken genome by burrows-wheeler alignment (BWA) tool (http://bio-bwa.sourceforge.net, version 0.7.9a), and any hit associated with the reads and their mated reads were removed. The optimized sequence was spliced and assembled, and contigs ≥ 300 bp were selected as the final assembly result, and then the contigs were used for further gene prediction and annotation. Open reading frames (ORFs) from each assembled contig were predicted using MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/). The predicted ORFs with length ≥ 100 bp were retrieved and translated into amino acid sequences. A non-redundant gene catalog was constructed using CD-HIT (http://www.bioinformatics.org/cd-hit/, version 4.6.1) with 90% sequence identity and 90% coverage. Reads after quality control were mapped to the non-redundant gene catalog with 95% identity using SOAPaligner (http://soap.genomics.org.cn/, version 2.21), and gene abundance in each sample was evaluated. Public data used for taxonomic analysis and gene functional classification included the integrated NCBI-NR database, KEGG database, and CAZy database. Amino acid sequence of non-redundant gene was aligned to NR database and KEGG database respectively with an e-value cutoff of $1e^{-5}$ using Diamond (http://www.diamondsearch.org/index.php, version 0.8.35), and obtained the species annotation and KEGG function corresponding to the gene. Carbohydrate-active enzymes annotation was conducted using hmmscan (http://hmmer.janelia.org/search/hmmscan) against CAZy database (http://www.cazy.org/) with an e-value cutoff of $1e^{-5}$.

4.10 Blood parameters analysis

For the analysis of different blood parameters, the serum concentrations of triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were determined using a Rayto Chemistry Analyzer (Chemray 800, China) according to the manufacturer's instructions with the commercial diagnostic kits (Shenzhen Rayto Life Science Co., Ltd). Briefly, the serum samples were thoroughly mixed with the reaction solution in the recommended proportion and maintained at 37 ° C for 10 minutes. Finally, the absorbance for each sample was measured, and the total concentrations were calculated according to the following formula. Total concentrations = Absorbance of sample / Absorbance of calibration solution × Calibration concentrations (mmol per liter).

4.11 Hematoxylin and eosin (HE) staining

For morphological observation, liver and abdominal fat tissue samples were embedded in paraffin, and the sections were prepared. Liver tissues were cut into 3 μm thick sections, and abdominal fat tissues were cut into 7 μm thick sections with a rotary slicer (LEICA 819, Leica, Germany). HE staining was performed according to the routine protocol, and the stained tissue sections were examined with the light microscope (BH-2, Olympus, Japan) using a digital camera (DP72, Olympus, Japan).

4.12 Real-time quantitative polymerase chain reaction (qPCR)

In order to detect the expression of fat metabolism related genes on mRNA level, total RNA was extracted from abdominal adipose and liver tissues using Trizol reagent (Takara, Japan) following the instructions of the

manufacturer. RNA (1μg) from each sample was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan). The qPCR reaction mixture (10 μL) consisted of 5 μL of SYBR (Takara, Japan), 0.4 μL of forward and reverse primer, 3.2 μL of ddH_2O, and 1 μL of template cDNA. The qPCR reaction is carried out on Bio-Rad CFX Connect real-time qPCR detection system (Bio-Rad, Hercules, CA, USA). The steps are as follows: 5 min pre-denaturation at 95°C, following 30 s denaturation at 95°C (40 cycles), 30 s annealing at 60°C, and 15 s elongation at 72°C. The sequences of primers were listed in Table 1 with reference gene (β-actin). Gene expression levels were quantified using the $2^{-\Delta\Delta CT}$ method.

Table 1
Primers used for real-time qPCR

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Gene Bank No.
β-actin	TTGTTGACAATGGCTCCGGT	TCTGGGCTTCATCACCAACG	NM_205518.2
ACC	TCCAGCAGAACCGCATTGACAC	GTATGAGCAGGCAGGACTTGGC	NM_205505.2
FAS	GCTCTGCGTCTGCTTCAGTCTAC	GGTACAGGACTCTGCCATCAATGC	NM_205155.4
LPL	TGGACATTGGTGACCTGCTTATGC	TCGCCTGACTTCACTCTGACTCTC	NM_205282.2
ACSL1	GACTAATGGTCACAGGAGCAGCAC	CCAGGCATTGACAGTGAGCATCC	NM_001012578.2
FADS1	CCGTGCCACTGTGGAGAAGATG	GCCTAGAAGCAACGCAGAGAAGAG	XM_040673219.1
CYP2C45	AACAAGCACCACCACACGATACG	GGTCAGCCACGCAAGGTCTTC	NM_001001752.3
APOAI	GTGACCCTCGCTGTGCTCTT	CACTCAGCGTGTCCAGGTTGT	NM_205525.5
PPARα	TGCTGTGGAGATCGTCCTGGTC	CTGTGACAAGTTGCCGGAGGTC	XM_040699549.1
CPT-1	ACAGCGAATGAAAGCAGGGT	GCCATGGCTAAGGTTTTCGT	NM_001012898.1
LEPR	CACTCGCTGGGAACACTTGA	TTCAGCAGCCCATCGTTTCT	NM_204323.2
JAK2	GAGCGTGAGAATGCCACTGAC	TGGAGGACAGCACTTGATGAAC	NM_001030538.3
STAT3	GCCGAATCACAACTACAGACTC	CTGACTTTGGTGGTGAACTGC	NM_001030931.3
HSL	GAGGCACAGCGTCTTCTTTAGG	GGCACGAACTGGAACCCGAG	XM_040695201.1

4.13 Immunohistochemistry (IHC)

Following the steps described in earlier studies, immunohistochemical staining was performed to observe the protein distribution and expression in the liver. Briefly, the sections were dewaxed twice in xylene and rehydrated in graded series ethanol. The antigen was retrieved in sodium citrate buffer (pH 6.0) using a microwave oven (MYA-2270M, Haier, Qingdao, China) for 18 min, i.e., three min at 700 W and fifteen min at 116 W, and then cooled for 2 to 3 h at room temperature. Endogenous peroxidase was inactivated with 3% hydrogen peroxide (H_2O_2), and tissue sections were incubated with 5% bovine serum albumin (BSA) (boster, China) at 37 ℃ for 30

minutes to block nonspecific binding sites. Then, the sections were incubated with primary antibodies of rabbit anti-JAK2 (1:100) (A11497, ABclonal Technology, Wuhan, China), rabbit anti-p-JAK2 (1:100) (AP0531, ABclonal Technology, Wuhan, China), rabbit anti-STAT-3 (1:100) (A1192, ABclonal Technology, Wuhan, China) and rabbit anti-p-STAT3 (1:100) (AP0474, ABclonal Technology, Wuhan, China). Subsequently, the horseradish peroxidase (HRP)-conjugated secondary antibody (Proteintech, China) was used to incubate the tissue sections for 30 min at 37°C. After diaminobenzidine (DAB) (Proteintech, China) staining, the sections were counterstained with hematoxylin, cleaned and dehydrated until they became transparent, and finally sealed with neutral gum and coverslips. Finally, we used a light microscope (BH-2, Olympus, Japan) with a digital camera (DP72, Olympus, Japan) to examine the sections.

4.14 Statistical analysis

Under 10 x 20 microscope, 10 abdominal fat HE stained sections were selected from each group, and 5 visual fields were randomly selected for the image acquisition. The average diameter of abdominal fat adipocytes was measured with image pro plus 6.0 (Media Cybernetics, USA). Under a 10 x 40 microscope, 10 liver immunohistochemical sections were selected from each group and five positive visual fields were randomly selected from each section for the image acquisition. Image Pro Plus 6.0 was used to calculate the integral optical density of positive signals. GraphPad Prism 6.0 (Media Cybernetics, USA) was used to analyze the test data. The measurement data were expressed as mean ± standard error of the mean (mean ± SEM). The statistical significance of the mean values in the comparisons of two groups was determined using Student's t-test. Value of $p < 0.05$ was considered statistically significant.

Declarations

Ethics approval and consent to participate

The current scientific investigation was conducted following the rules and regulations of the ethics committee for the use of animals, Huazhong Agricultural University (HZAUCH-2018-008), Wuhan, China.

Consent for publication

Not applicable.

Availability of data and materials

The raw 16S rRNA gene and metagenomic sequencing data are available at the NCBI Sequence Read Archive (SRA), under BioProject PRJNA837471.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

YC, MA, ZYM, TWH, QYL, HP, XLZ, AAN, ARA, E-SMA-K, DSS, and HZL all contributed to the conceptual design for this project and the experiments within the manuscript. YC, MA, ZYM, and TWH performed the experiments and data analysis, contributed to animal handling, samples, and clinical data collection. MA, DSS, XLZ, AAN, ARA, E-SMA-K, and HZL edited and critically revised the manuscript. YC, MA, DSS, and HZL wrote the manuscript. DSS and HZL supervised the writing, experimentation, analysis, reviewing of the manuscript, funding acquisition, and project administration. All authors read and approved the final version of the manuscript.

References

1. Tallentire CW, Leinonen I, Kyriazakis I. Breeding for efficiency in the broiler chicken: A review. Agron Sustain Dev. 2016;36(4):66.
2. Moreira GCM, Boschiero C, Cesar ASM, Reecy JM, Godoy TF, Pértille F, et al. Integration of genome wide association studies and whole genome sequencing provides novel insights into fat deposition in chicken. Sci Rep. 2018;8(1):16222.
3. Fouad AM, El-Senousey HK. Nutritional Factors Affecting Abdominal Fat Deposition in Poultry: A Review. Asian-Australas J Anim Sci. 2014;27(7):1057–68.
4. Wen C, Yan W, Sun C, Ji C, Zhou Q, Zhang D, et al. The gut microbiota is largely independent of host genetics in regulating fat deposition in chickens. ISME j. 2019;13(6):1422–36.
5. Abdalla BA, Chen J, Nie Q, Zhang X. Genomic Insights Into the Multiple Factors Controlling Abdominal Fat Deposition in a Chicken Model. Front Genet. 2018;9:262.
6. Zhang XY, Wu MQ, Wang SZ, Zhang H, Du ZQ, Li YM, et al. Genetic selection on abdominal fat content alters the reproductive performance of broilers. Animal. 2018;12(6):1232–41.
7. Li D, Zhang K, Pan Z, Yu M, Lu Y, Wang G, et al. Antibiotics promote abdominal fat accumulation in broilers. Anim Sci J. 2020;91(1):e13326.
8. Milićević D, Vranić D, Mašić Z, Parunović N, Trbović D, Nedeljković-Trailović J, et al. The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. Lipids Health Dis. 2014;13:42.
9. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99(4):1877–2013.
10. Ballal SA, Gallini CA, Segata N, Huttenhower C, Garrett WS. Host and gut microbiota symbiotic factors: lessons from inflammatory bowel disease and successful symbionts. Cell Microbiol. 2011;13(4):508–17.
11. Akhtar M, Chen Y, Ma Z, Zhang X, Shi D, Khan JA, et al. Gut microbiota-derived short chain fatty acids are potential mediators in gut inflammation. Anim Nutr. 2022;8:350–60.

12. Xiang H, Gan J, Zeng D, Li J, Yu H, Zhao H, et al. Specific Microbial Taxa and Functional Capacity Contribute to Chicken Abdominal Fat Deposition. Front Microbiol. 2021;12:643025.
13. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.
14. Cui X, Gou Z, Jiang Z, Li L, Lin X, Fan Q, et al. Dietary fiber modulates abdominal fat deposition associated with cecal microbiota and metabolites in yellow chickens. Poult Sci. 2022;101(4):101721.
15. Polansky O, Sekelova Z, Faldynova M, Sebkova A, Sisak F, Rychlik I. Important Metabolic Pathways and Biological Processes Expressed by Chicken Cecal Microbiota. Appl Environ Microbiol. 2015;82(5):1569–76.
16. Zhao X, Guo Y, Guo S, Tan J. Effects of Clostridium butyricum and Enterococcus faecium on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. Appl Microbiol Biotechnol. 2013;97(14):6477–88.
17. Wang W-W, Wang J, Zhang H-J, Wu S-G, Qi G-H. Supplemental Clostridium butyricum Modulates Lipid Metabolism Through Shaping Gut Microbiota and Bile Acid Profile of Aged Laying Hens. Front Microbiol. 2020;11:600-.
18. Zhang T, Ding H, Chen L, Lin Y, Gong Y, Pan Z, et al. Antibiotic-Induced Dysbiosis of Microbiota Promotes Chicken Lipogenesis by Altering Metabolomics in the Cecum. Metabolites. 2021;11(8):487.
19. Wang H, Ni X, Qing X, Zeng D, Luo M, Liu L, et al. Live Probiotic Lactobacillus johnsonii BS15 Promotes Growth Performance and Lowers Fat Deposition by Improving Lipid Metabolism, Intestinal Development, and Gut Microflora in Broilers. Front Microbiol. 2017;8:1073.
20. Zhang X, Hu Y, Ansari AR, Akhtar M, Chen Y, Cheng R, et al. Caecal microbiota could effectively increase chicken growth performance by regulating fat metabolism. Microb Biotechnol. 2021;15(3):844–61.
21. Saxena S, Saxena VK, Tomar S, Sapcota D, Gonmei G. Characterisation of caecum and crop microbiota of Indian indigenous chicken targeting multiple hypervariable regions within 16S rRNA gene. Br Poult Sci. 2016;57(3):381–9.
22. Suzuki S, Kobayashi M, Murai A, Tsudzuki M, Ishikawa A. Characterization of Growth, Fat Deposition, and Lipid Metabolism-Related Gene Expression in Lean and Obese Meat-Type Chickens. J Poult Sci. 2019;56(2):101–11.
23. Resnyk CW, Chen C, Huang H, Wu CH, Simon J, Le Bihan-Duval E, et al. RNA-Seq Analysis of Abdominal Fat in Genetically Fat and Lean Chickens Highlights a Divergence in Expression of Genes Controlling Adiposity, Hemostasis, and Lipid Metabolism. PLoS One. 2015;10(10):e0139549.
24. Li T, Li X, Meng H, Chen L, Meng F. ACSL1 affects Triglyceride Levels through the PPARγ Pathway. Int J Med Sci. 2020;17(6):720–7.
25. Wan X, Yang Z, Ji H, Li N, Yang Z, Xu L, et al. Effects of lycopene on abdominal fat deposition, serum lipids levels and hepatic lipid metabolism-related enzymes in broiler chickens. Animal bioscience. 2021;34(3):385–92.
26. Chen Y, Lu W, Jin Z, Yu J, Shi B. Carbenoxolone ameliorates hepatic lipid metabolism and inflammation in obese mice induced by high fat diet via regulating the JAK2/STAT3 signaling pathway. Int Immunopharmacol. 2019;74:105498.
27. Rahnama M, Bouyeh M, Kadim I, Seidavi A, Elghandour MMMY, Reddy PRK, et al. Effect of dietary inclusion of lecithin with choline on physiological stress of serum cholesterol fractions and enzymes,

abdominal fat, growth performance, and mortality parameters of broiler chickens. Anim Biotechnol. 2020;31(6):483–90.

28. Zhou Y, Chen Z, Lin Q, Yang Y, Hang Y, Zhou X, et al. Nuciferine reduced fat deposition by controlling triglyceride and cholesterol concentration in broiler chickens. Poult Sci. 2020;99(12):7101–8.
29. Kim D-H, Jeong D, Kang I-B, Kim H, Song K-Y, Seo K-H. Dual function of Lactobacillus kefiri DH5 in preventing high-fat-diet-induced obesity: direct reduction of cholesterol and upregulation of PPAR-α in adipose tissue. Mol Nutr Food Res. 2017;61(11):1700252.
30. Xie Z, Shen G, Wang Y, Wu C. Curcumin supplementation regulates lipid metabolism in broiler chickens. Poult Sci. 2019;98(1):422–9.
31. Wu L, Chen G, Liu W, Yang X, Gao J, Huang L, et al. Intramuscular injection of exogenous leptin induces adiposity, glucose intolerance and fatty liver by repressing the JAK2-STAT3/PI3K pathway in a rat model. Gen Comp Endocrinol. 2017;252:88–96.
32. Pirany N, Bakrani Balani A, Hassanpour H, Mehraban H. Differential expression of genes implicated in liver lipid metabolism in broiler chickens differing in weight. Br Poult Sci. 2020;61(1):10–6.
33. Zheng A, Luo J, Meng K, Li J, Bryden WL, Chang W, et al. Probiotic (Enterococcus faecium) induced responses of the hepatic proteome improves metabolic efficiency of broiler chickens (Gallus gallus). BMC Genomics. 2016;17(1):89.
34. Niu J-L, Zhang J, Wei L-Q, Zhang W-J, Nie C-X. Effect of Fermented Cottonseed Meal on the Lipid-Related Indices and Serum Metabolic Profiles in Broiler Chickens. Animals. 2019;9(11):930.
35. Guo L, Sun B, Shang Z, Leng L, Wang Y, Wang N, et al. Comparison of adipose tissue cellularity in chicken lines divergently selected for fatness. Poult Sci. 2011;90(9):2024–34.
36. Stojanov S, Berlec A, Štrukelj B. The Influence of Probiotics on the Firmicutes/Bacteroidetes Ratio in the Treatment of Obesity and Inflammatory Bowel disease. Microorganisms. 2020;8(11):1715.
37. Geng S, Huang S, Ma Q, Li F, Gao Y, Zhao L, et al. Alterations and Correlations of the Gut Microbiome, Performance, Egg Quality, and Serum Biochemical Indexes in Laying Hens with Low-Protein Amino Acid-Deficient Diets. ACS Omega. 2021;6(20):13094–104.
38. Pu S, Khazanehei H, Jones PJ, Khafipour E. Interactions between Obesity Status and Dietary Intake of Monounsaturated and Polyunsaturated Oils on Human Gut Microbiome Profiles in the Canola Oil Multicenter Intervention Trial (COMIT). Front Microbiol. 2016;7:1612.
39. Lee SH, Bang S, Jang HH, Lee EB, Kim BS, Kim SH, et al. Effects of Allium hookeri on gut microbiome related to growth performance in young broiler chickens. PLoS One. 2020;15(1):e0226833.
40. Guo B, Li D, Zhou B, Jiang Y, Bai H, Zhang Y, et al. Comparative characterization of bacterial communities in geese consuming of different proportions of ryegrass. PloS one. 2019;14(10):e0223445.
41. Hou Q, Kwok L-Y, Zheng Y, Wang L, Guo Z, Zhang J, et al. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. Sci Rep. 2016;6(1):37376.
42. Ijaz MU, Ahmad MI, Hussain M, Khan IA, Zhao D, Li C. Meat Protein in High-Fat Diet Induces Adipogensis and Dyslipidemia by Altering Gut Microbiota and Endocannabinoid Dysregulation in the Adipose Tissue of Mice. J Agric Food Chem. 2020;68(13):3933–46.

43. Patrone V, Minuti A, Lizier M, Miragoli F, Lucchini F, Trevisi E, et al. Differential effects of coconut versus soy oil on gut microbiota composition and predicted metabolic function in adult mice. BMC Genomics. 2018;19(1):808.
44. Lu M, Li RW, Zhao H, Yan X, Lillehoj HS, Sun Z, et al. Effects of Eimeria maxima and Clostridium perfringens infections on cecal microbial composition and the possible correlation with body weight gain in broiler chickens. Res Vet Sci. 2020;132:142–9.
45. Wu J, Wang K, Wang X, Pang Y, Jiang C. The role of the gut microbiome and its metabolites in metabolic diseases. Protein Cell. 2021;12(5):360–73.
46. He M, Fang S, Huang X, Zhao Y, Ke S, Yang H, et al. Evaluating the Contribution of Gut Microbiota to the Variation of Porcine Fatness with the Cecum and Fecal Samples. Front Microbiol. 2016;7:2108.
47. Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. The British journal of nutrition. 2012;107(4):601–13.
48. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017;8(2):172–84.
49. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, et al. The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? Nutrients. 2020;12(5):1474.
50. Krajmalnik-Brown R, Ilhan Z-E, Kang D-W, DiBaise JK. Effects of Gut Microbes on Nutrient Absorption and Energy Regulation. Nutr Clin Pract. 2012;27(2):201–14.
51. Ibrahim M, Anishetty S. A meta-metabolome network of carbohydrate metabolism: Interactions between gut microbiota and host. Biochem Biophys Res Commun. 2012;428(2):278–84.
52. Niu Q, Li P, Hao S, Kim SW, Du T, Hua J, et al. Characteristics of Gut Microbiota in Sows and Their Relationship with Apparent Nutrient Digestibility. Int J Mol Sci. 2019;20(4):870.
53. Li T-T, Huang Z-R, Jia R-B, Lv X-C, Zhao C, Liu B. Spirulina platensis polysaccharides attenuate lipid and carbohydrate metabolism disorder in high-sucrose and high-fat diet-fed rats in association with intestinal microbiota. Food Res Int. 2021;147:110530.
54. Wexler AG, Goodman AL. An insider's perspective: Bacteroides as a window into the microbiome. Nat Microbiol. 2017;2:17026.
55. Granchi C. ATP citrate lyase (ACLY) inhibitors: An anti-cancer strategy at the crossroads of glucose and lipid metabolism. Eur J Med Chem. 2018;157:1276–91.
56. Lai Z-L, Tseng C-H, Ho HJ, Cheung CKY, Lin J-Y, Chen Y-J, et al. Fecal microbiota transplantation confers beneficial metabolic effects of diet and exercise on diet-induced obese mice. Sci Rep. 2018;8(1):15625.
57. Li X, Kang Y, Huang Y, Xiao Y, Song L, Lu S, et al. A strain of Bacteroides thetaiotaomicron attenuates colonization of Clostridioides difficile and affects intestinal microbiota and bile acids profile in a mouse model. Biomed Pharmacother. 2021;137:111290.
58. Zhao R, Ji Y, Chen X, Hu Q, Zhao L. Polysaccharide from Flammulina velutipes attenuates markers of metabolic syndrome by modulating the gut microbiota and lipid metabolism in high fat diet-fed mice. Food Funct. 2021;12(15):6964–80.
59. Napolitano M, Covasa M. Microbiota Transplant in the Treatment of Obesity and Diabetes: Current and Future Perspectives. Front Microbiol. 2020;11:590370-.

60. Niederwerder MC. Fecal microbiota transplantation as a tool to treat and reduce susceptibility to disease in animals. Vet Immunol Immunopathol. 2018;206:65–72.

Figures

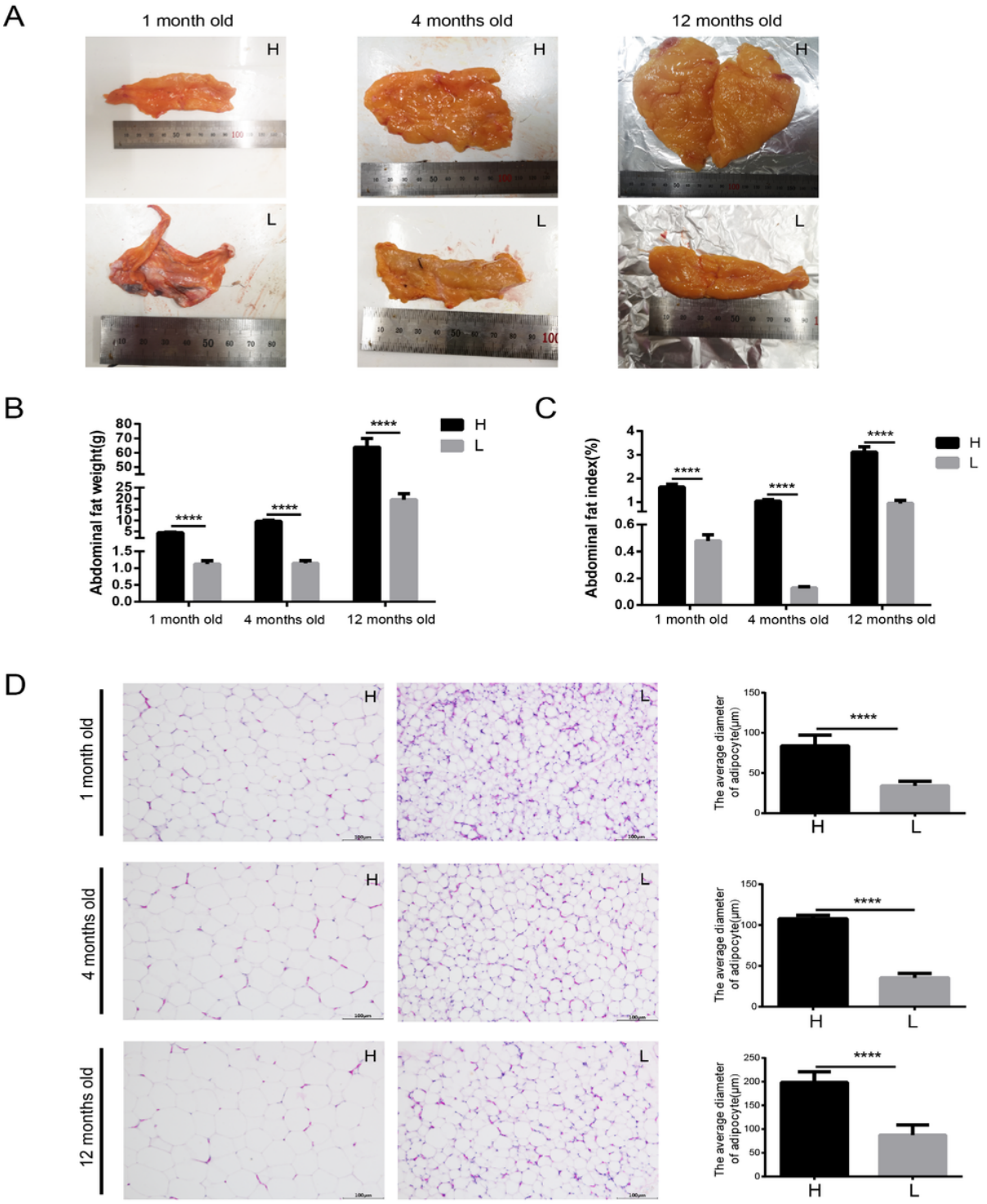

Figure 1

Analysis of the differences of abdominal fat deposition between high and low abdominal fat deposition chickens at different months

(A) The comparison of abdominal fat volume between high and low abdominal fat deposition chickens at different months. (B) The comparison of abdominal fat weight between high and low abdominal fat deposition chickens at different months. (C) The comparison of abdominal fat index between high and low abdominal fat deposition chickens at different months. (D) HE staining sections of fat abdominal adipose tissues and the comparison of an average diameter of adipocytes in high and low abdominal fat deposition chickens at different months. Scale bars = 100 μm. H represents high abdominal fat chickens, and L represents low abdominal fat chickens. All data were presented as mean ± SEM. ****p < 0.0001.

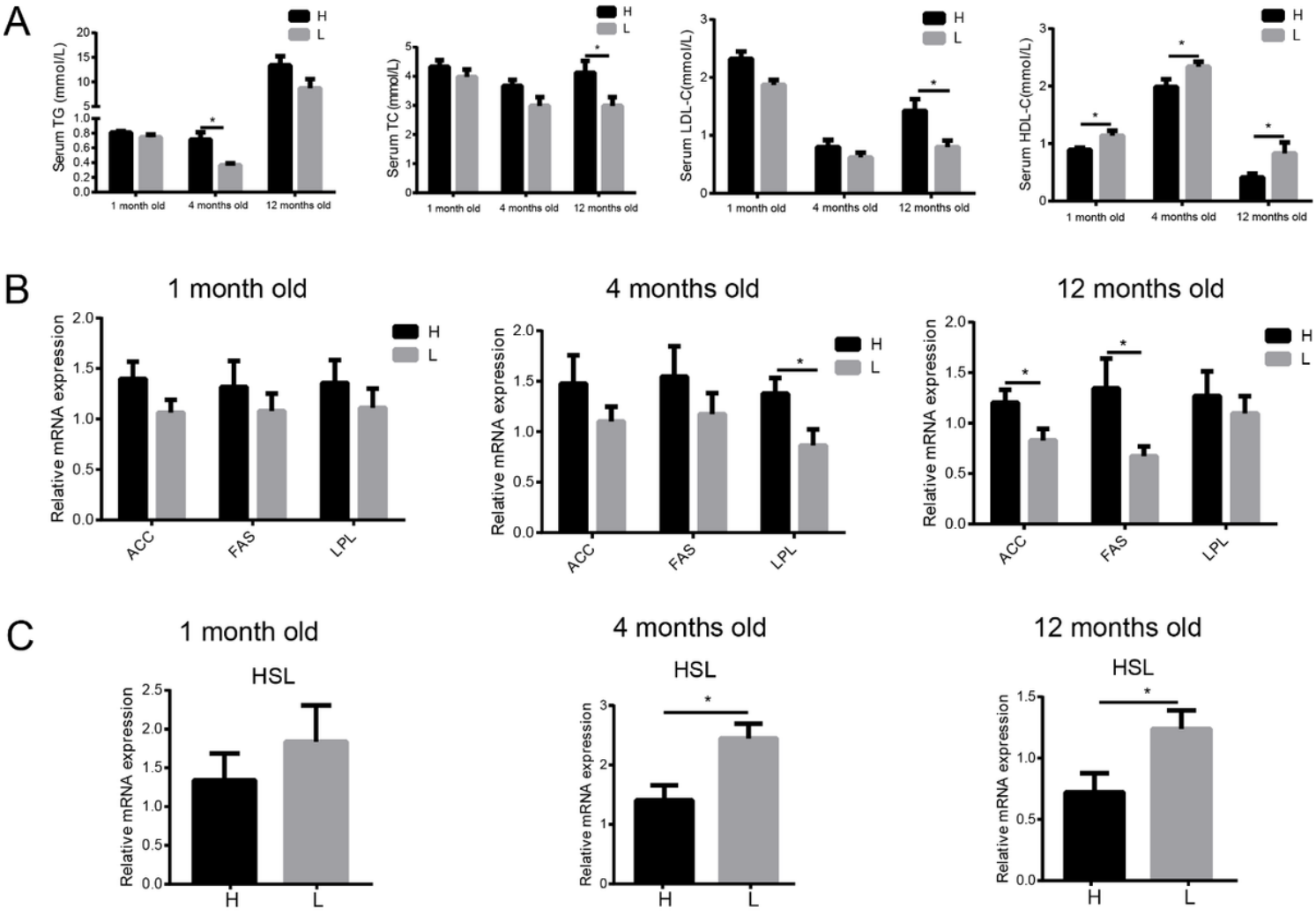

Figure 2

Analysis of fat metabolism differences in blood and abdominal fat between high and low abdominal fat deposition chickens at different months

(A) The comparison of serum triglycerides (TG) concentrations (mmol/L), serum total cholesterol (TC) concentrations (mmol/L), serum LDL-C concentrations (mmol/L), and serum HDL-C concentrations (mmol/L) between high and low abdominal fat deposition chickens at 1, 4, and 12 months. (B) The comparison of relative mRNA expression of fat synthesis related genes between high and low abdominal fat deposition chickens at 1, 4, and 12 months (q-PCR) (C) The comparison of relative mRNA expression of fat catabolism

related genes between high and low abdominal fat deposition chickens at 1, 4, and 12 months (q-PCR). H represents high abdominal fat chickens, and L represents low abdominal fat chickens. All data were presented as mean ± SEM. *p < 0.05.

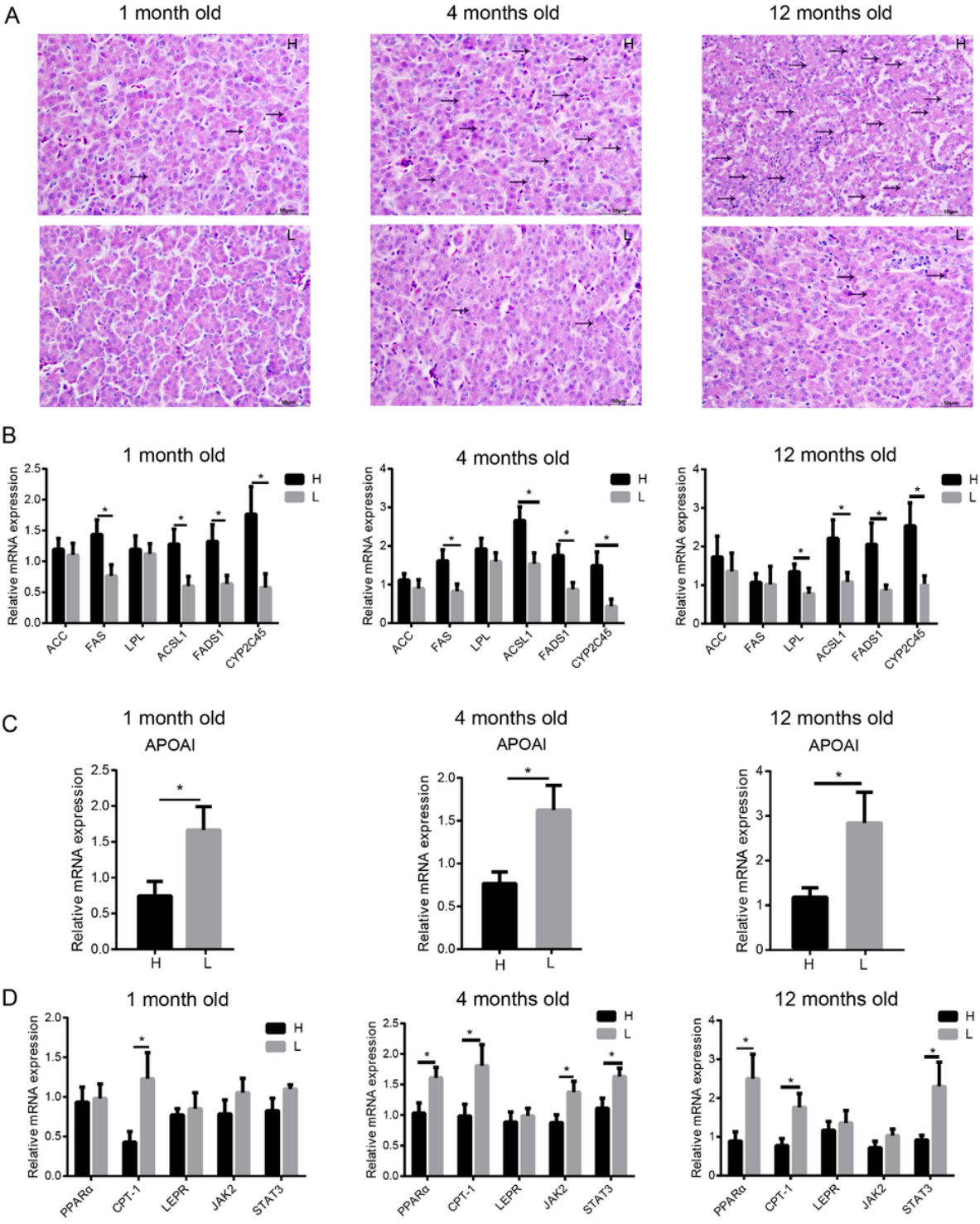

Figure 3

Analysis of fat metabolism differences in liver between high and low abdominal fat deposition chickens at different months

(A) HE staining sections of fat content in hepatocytes of the chickens at 1, 4, and 12 months. The fat droplets (white) are indicated with the arrows in the figures. (B) The comparison of relative mRNA expression of fat synthesis related genes between the high and low abdominal fat deposition chickens at 1, 4, and 12 months (q-PCR). (C) The comparison of relative mRNA expression of fat transport related genes between the high and low abdominal fat deposition chickens at 1, 4, and 12 months (q-PCR). (D) The comparison of relative mRNA expression of fat catabolism related genes between the high and low abdominal fat deposition chickens at 1, 4, and 12 months (q-PCR). Scale bars = 50 μm. H represents high abdominal fat chickens, and L represents low abdominal fat chickens. All data were presented as mean ± SEM. *p < 0.05.

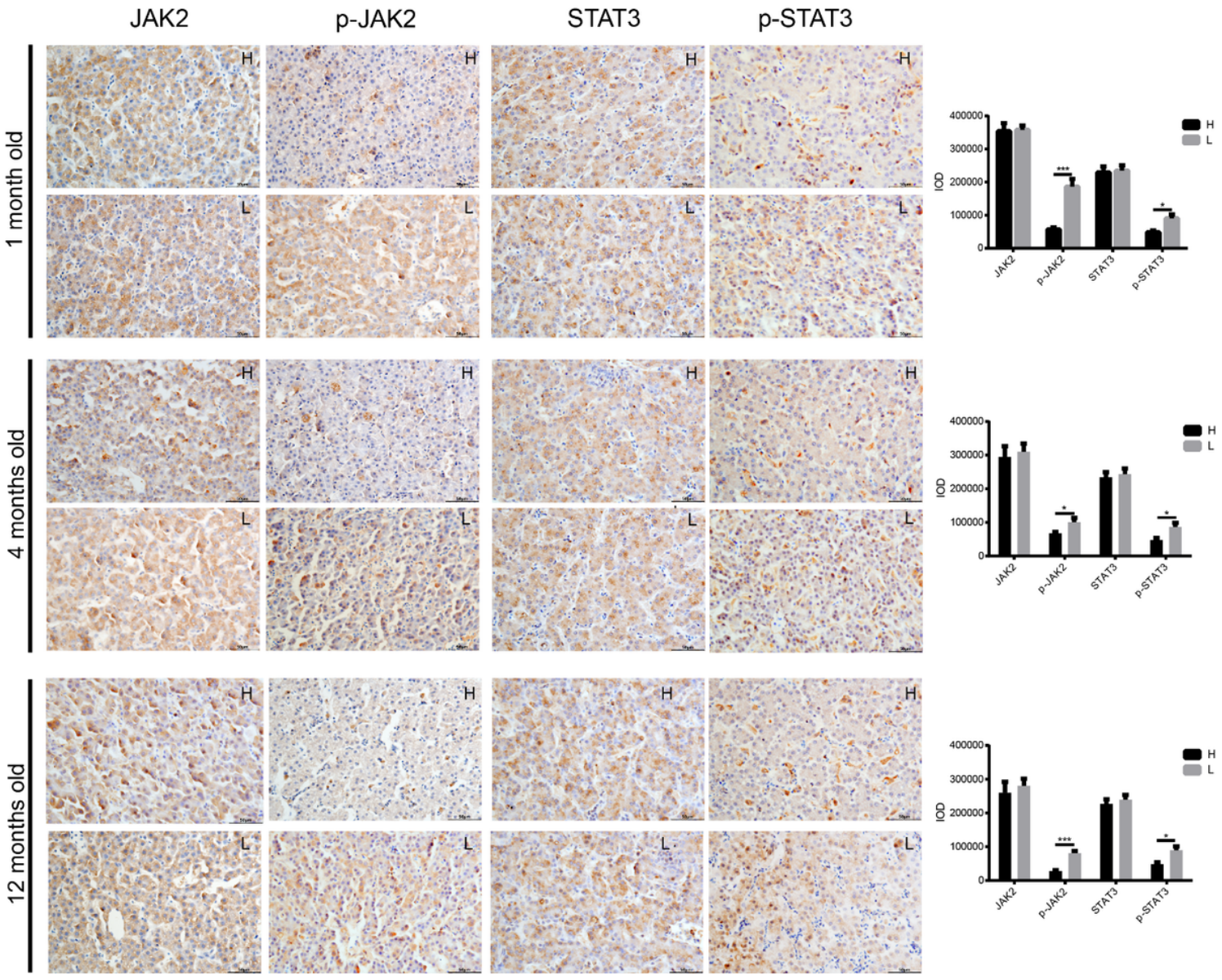

Figure 4

Comparing the expression of fat metabolism related proteins in liver between high and low abdominal fat deposition chickens at different months

The protein distribution and expression levels of JAK2, p-JAK2, STAT3, p-STAT3 in high and low abdominal fat deposition chickens at 1, 4, and 12 months old, respectively (IHC). Scale bars = 50 μm. H represents high abdominal fat chickens, and L represents low abdominal fat chickens. All data were presented as mean ± SEM. *p < 0.05, ***p < 0.001.

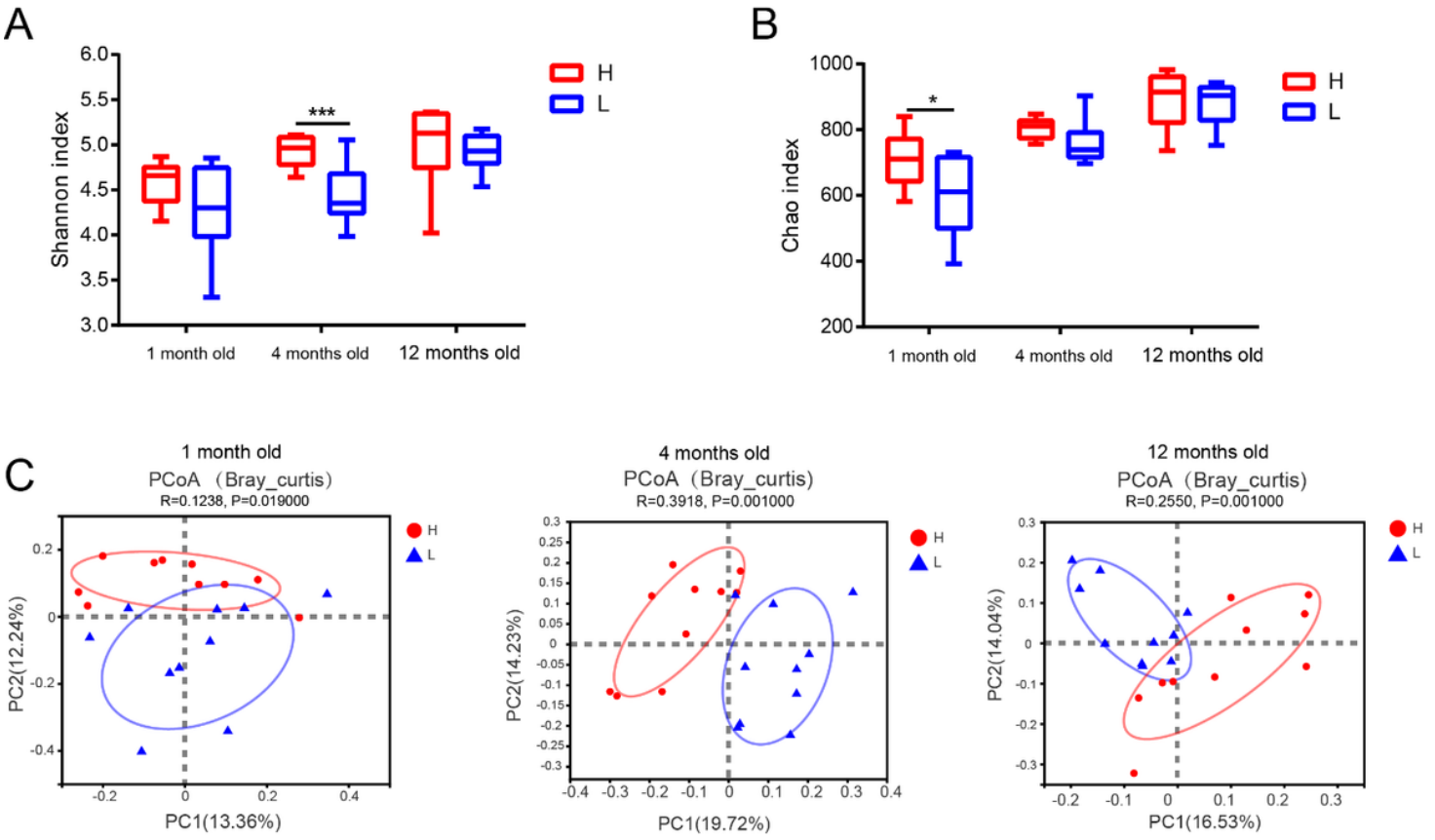

Figure 5

Comparison of microbial α and β diversity in cecum between high and low abdominal fat deposition chickens at different months

The comparison of microbial community diversity measured with the Shannon index (A) and Chao index (B) between high and low abdominal fat deposition chickens at different months. (C) The comparison of the principal co-ordinates analysis (PCoA) based on OTU between high and low abdominal fat deposition chickens at 1, 4, and 12 months. H represents high abdominal fat chickens, and L represents low abdominal fat chickens. *p < 0.05, ***p < 0.001.

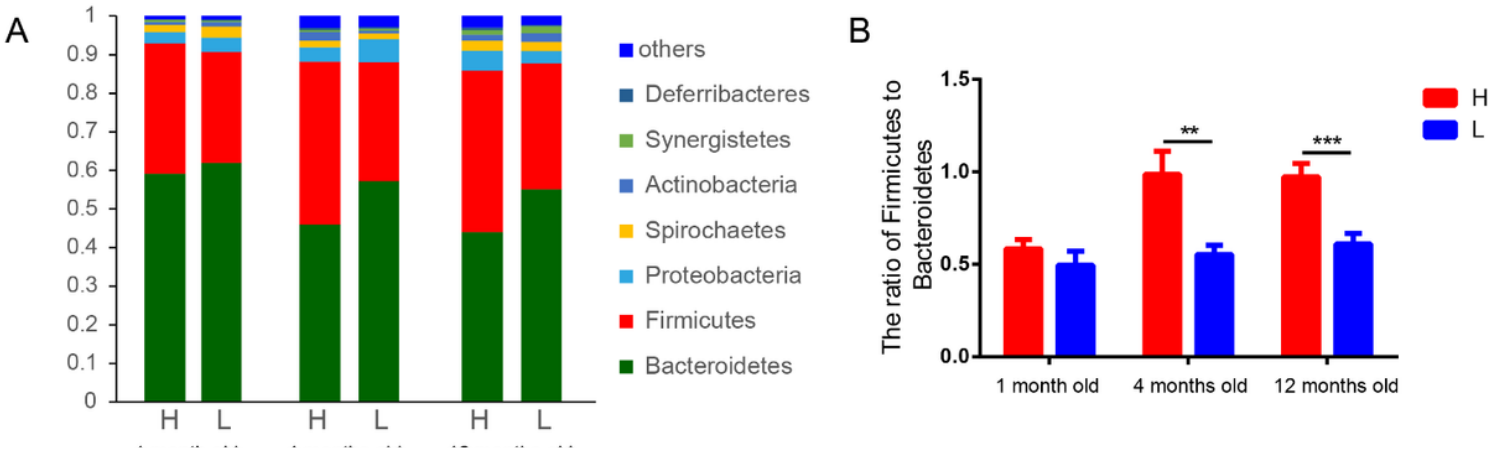

Figure 6

Analysis of microbial community composition and difference in cecum between high and low abdominal fat deposition chickens at different months

(A) Cecal microbiota community composition at the phylum level in chickens at different months. (B) The comparison of the Firmicutes/Bacteroidetes ratio between high and low abdominal fat deposition chickens at different months. (C-D) The comparison of the relative abundance of target genera (*Parabacteroides*, *Parasutterella, Oscillibacter*, *Anaerofustis, Bacteroides, Sphaerochaeta*) between high and low abdominal fat deposition chickens at different months, respectively. H represents high abdominal fat chickens, and L represents low abdominal fat chickens. All data were presented as mean ± SEM. *p < 0.05 **p < 0.01, ***p < 0.001.

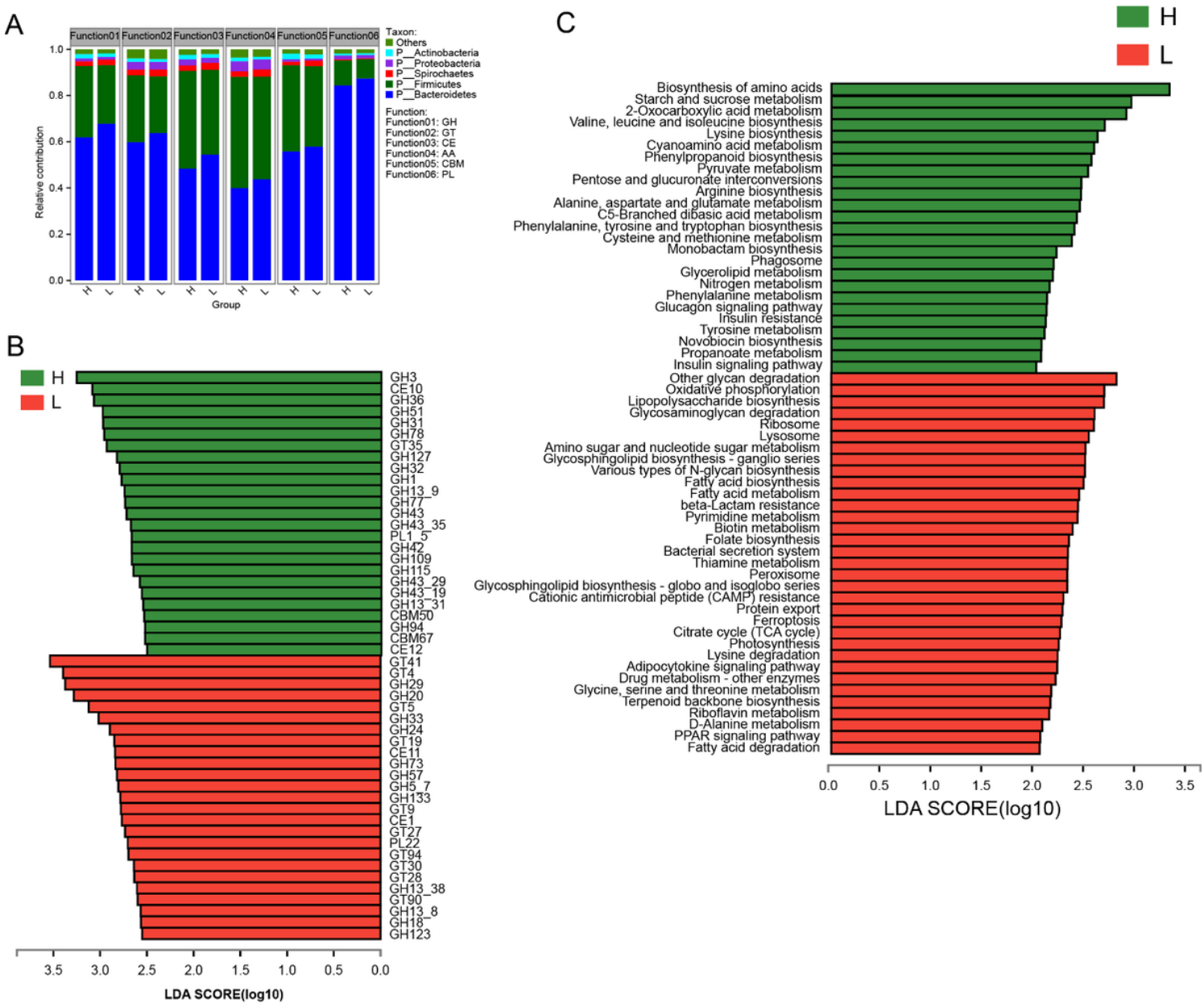

Figure 7

Analysis of functional differences of cecal microbiota between high and low abdominal fat deposition chickens at four months

(A) The comparison of the relative contribution of cecal microbiota (at phylum level) to carbohydrate active enzymes (CAZymes) between high and low abdominal fat deposition chickens. (B) The comparison of carbohydrate enzymatic activities of cecal microbiota between high and low abdominal fat deposition chickens. (C) The comparison of KEGG differential pathways of cecal microbiota between high and low abdominal fat deposition chickens. H represents high abdominal fat chickens, and L represents low abdominal fat chickens. LDA score (log10) > 2.0.

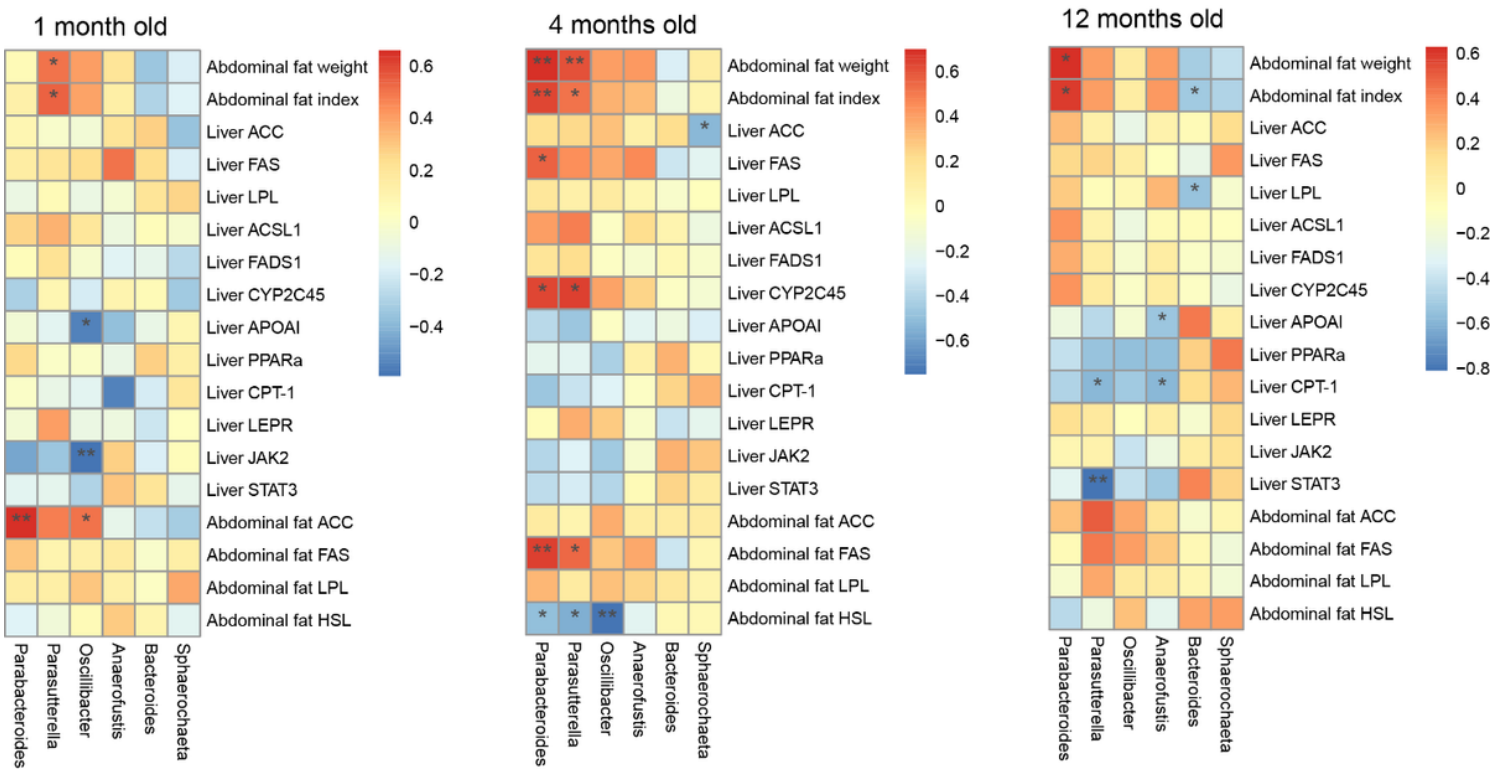

Figure 8

Correlation between cecum microbiota and abdominal fat deposition factors in high and low abdominal fat deposition chickens at different months

The association of different bacteria with the abdominal fat deposition and its related fat accumulation factors. Red color indicates positive correlation, and blue color indicates negative correlation. *p < 0.05, **p < 0.01.

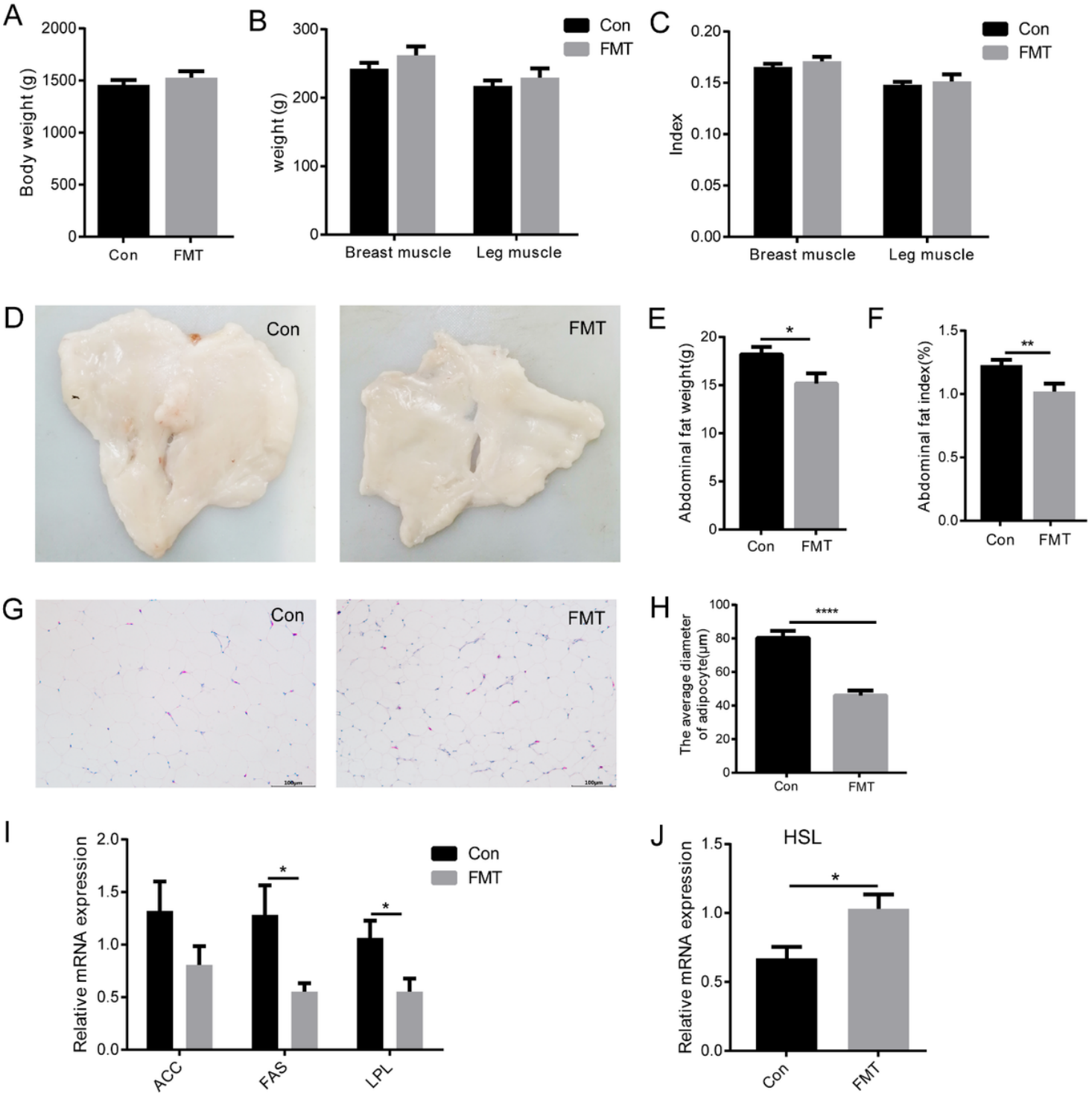

Figure 9

FMT altered the fat deposition of chickens

An increasing trend of body weight (A), breast and leg muscle weight (B), and breast and leg muscle index (C) in the FMT group compared with the control group. (D) The comparison of abdominal fat tissues of chickens between the control and FMT groups. (E) The comparison of abdominal fat weight between the control and FMT groups. (F) The comparison of abdominal fat index between the control and FMT groups. (G-H) HE staining sections of abdominal adipose tissues and the comparison of an average diameter of adipocytes

between the control and FMT groups. (I-J) The comparison of relative mRNA expression of fat synthesis related genes and fat catabolism related genes in abdominal fat between the control and FMT groups (q-PCR). Scale bars = 100 μm, Con represents the control group, and FMT represents the fecal microbiota transplantation group. All data were presented as mean ± SEM. * p < 0.05，**p < 0.01, **** p < 0.0001.

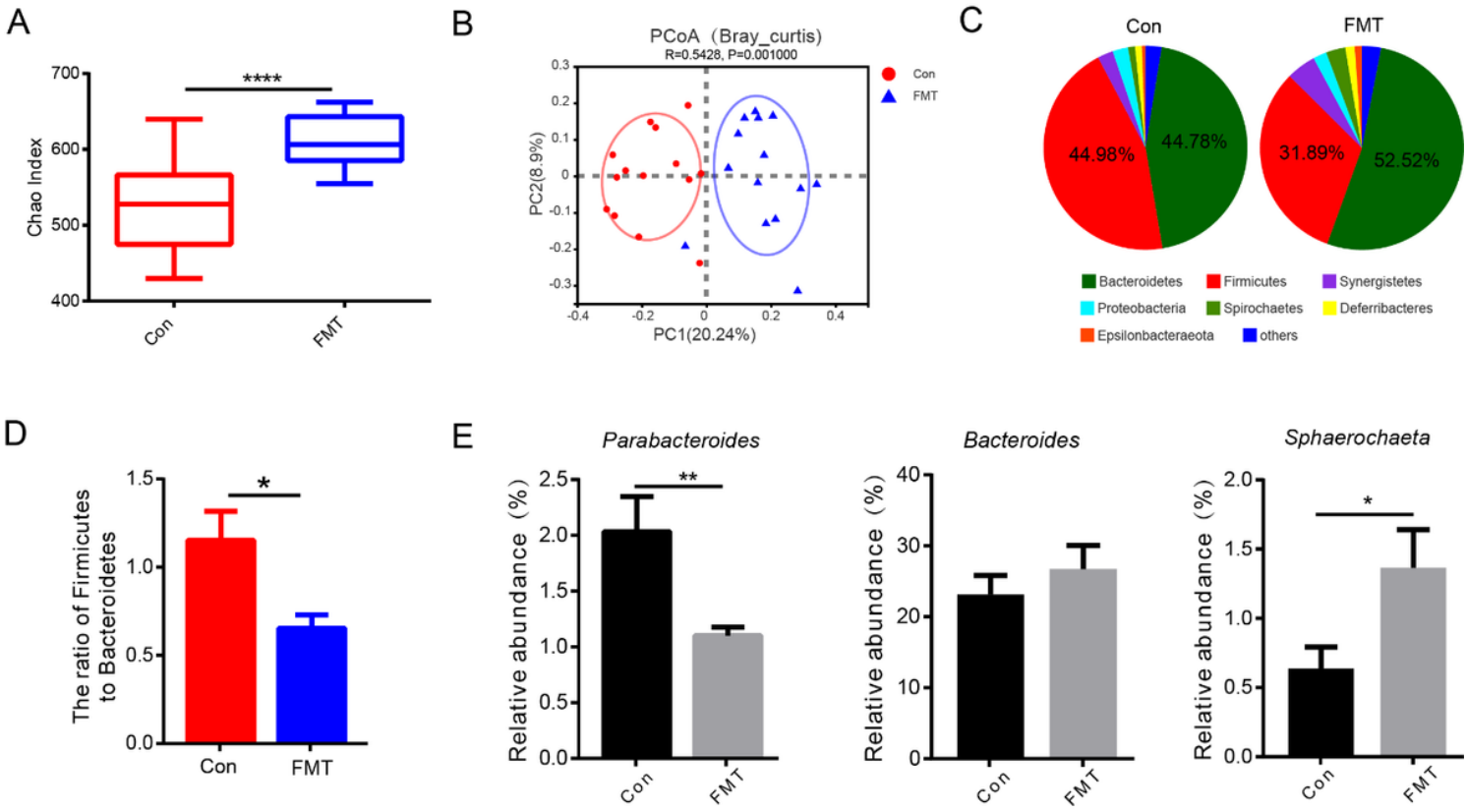

Figure 10

FMT changed the composition of chicken cecal microbiota

For 16S rRNA sequencing, 28 white feather broilers (14 in each group with equal males and females) were randomly selected for cecal microbiota analysis in the control and FMT groups. (A) The comparison of Chao index. (B) Principal co-ordinates analysis (PCoA) analysis. (C) Cecal microbiota community composition at the phylum level. (D) The ratio of Firmicutes to Bacteroidetes. (E) The relative abundance of *Parabacteroides*. *Bacteroides*, and *Sphaerochaeta*. Con represents the control group, and FMT represents the fecal microbiota transplantation group. All data were presented as mean ± SEM. * p < 0.05，**p < 0.01, **** p < 0.0001.

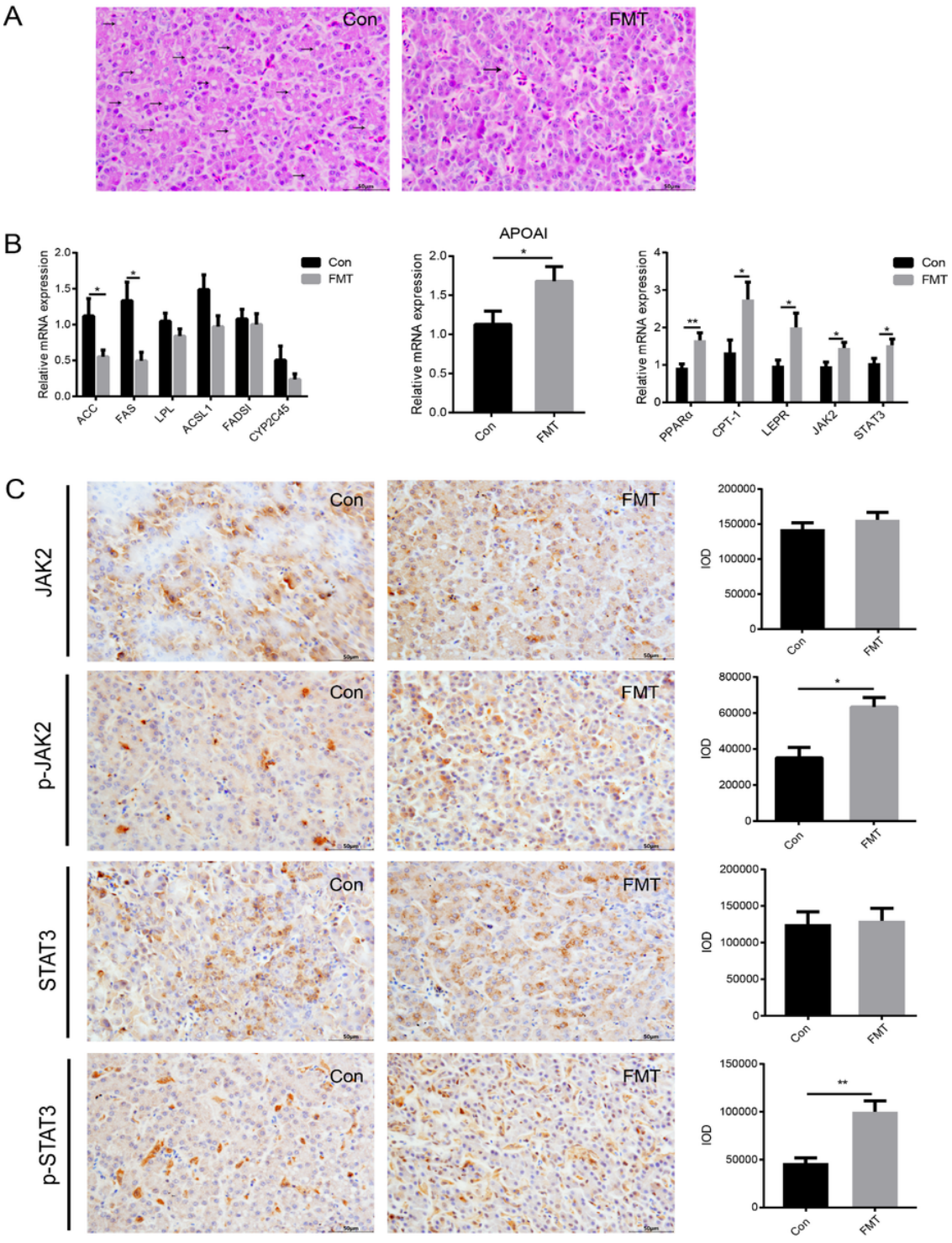

Figure 11

FMT changed the fat metabolism in the liver of chickens

The comparison of fat metabolism levels in the chicken liver between the control and the FMT groups. (A) HE staining sections of fat content in hepatocytes of the chickens. (B) The comparison of relative mRNA expression of fat synthesis related genes, fat transport related genes, and fat catabolism related genes (q-PCR). (C) The protein distribution and the expression levels of JAK2, p-JAK2, STAT3, and p-STAT3 (IHC). Scale bars =

50 μm. Con represents the control group, and FMT represents the fecal microbiota transplantation group. All data were presented as mean ± SEM. * $p < 0.05$ **$p < 0.01$.

Supplementary Files

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- FigureS1.tif
- FigureS2.tif
- FigureS3.tif