Propionate inhibits fat deposition via affecting feed intake and modulating gut microbiota in broilers

Haifang Li,[†,1] Liqin Zhao,[†,1] Shuang Liu,[*,1] Zhihao Zhang,[†] Xiaojuan Wang,[*] and Hai Lin[*,2]

[*]*College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai'an 271018, China; and*
[†]*College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China*

ABSTRACT As one of the 3 main short-chain fatty acids, the role of propionate in chicken fat metabolism is largely unknown. In this study, we demonstrated that dietary supplementation of coated sodium propionate (**SP**) moderately inhibits fat deposition in broiler chickens, as evidenced by the decreased adipocyte mean area ($P < 0.01$), the lowered triglyceride content in abdominal fat tissue ($P < 0.01$), and the reduced transcription of several lipogenic genes in liver and abdominal fat tissues ($P < 0.05$). Surprisingly, the propionate content was not significantly elevated either in serum or in the cecal chyme by SP administration ($P > 0.05$). However, SP application significantly decreased the average daily feed intake of broilers ($P < 0.05$). In addition, the composition of the cecal microbial communities was altered, with the ratio of *Firmicutes* to *Bacteroidetes* decreasing in particular ($P < 0.05$). At the genus level, SP application increased the richness of *Alistipes*, *Lactobacillus*, and *Bifidobacterium*, while reduced the abundance of *Lachnospiraceae* and *Helicobacter* significantly ($P < 0.05$). Moreover, in vitro experiments indicated that, although physiological concentrations of propionate (0.01 to 0.1 mmol) upregulated or downregulated the transcription of some fat synthesis-associated genes ($P < 0.05$), they did not significantly affect the triglyceride accumulation in hepatocytes and adipocytes ($P > 0.05$). These results suggest that feed supplementation with SP inhibits fat deposition in broilers by reducing feed and caloric intake, but not via direct regulation on hepatic fat synthesis or adipocytic fat deposition. Alteration in the relative populations of the gut microflora suggests that SP may have gut health implications.

Key words: broilers, propionate, feed intake, gut microbiota, fat deposition

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INTRODUCTION

The gastrointestinal tract of animals is densely populated with microorganisms that closely interact with the host (De Vadder et al., 2014; Huang et al., 2018). Gut microbiota is thought to be a key regulator of host metabolism, including fat metabolism (Flint et al., 2012; De Vadder et al., 2014; Deng and Yu, 2014). Studies have indicated that the ratio of 2 main phyla, *Firmicutes* and *Bacteroidetes*, is significantly altered in obese humans and mice (Ley et al., 2005; Parnell et al., 2012). Transplantation of the fecal microbiota of twins discordant for obesity to germ-free mice leads to a similar phenotype in the recipient mice (Ridaura et al., 2013). Modulation of gut microbiomes on fat metabolism is largely dependent on their fermentation metabolites of nondigestible carbohydrates, such as short-chain fatty acids (**SCFA**) (De Vadder et al., 2014; Byrne et al., 2015). Acetate, propionate, and butyrate are the 3 primary SCFA (Brown et al., 2003; Bishehsari et al., 2018). Ridaura et al. (2013) demonstrated that the lean mice have significantly higher cecal propionate and butyrate contents compared with their obese counterparts.

Among the 3 main SCFA, propionate has attracted great attention on regulation of fat metabolism in mammals (Chambers et al., 2015; Cani, 2019). However, inconsistent findings and different mechanisms have been reported (Hong et al., 2005; Li et al., 2014; Chambers et al., 2015; Song et al., 2019). Hong et al. (2005) discovered that 0.1 μmol propionate stimulates adipogenesis and inhibits isoproterenol-induced lipolysis in 3T3-L1 cell lines via free fatty acid receptor 2 (**FFAR2**). In adipogenic differentiation of porcine

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[1]These authors contributed equally to this work.

[2]Corresponding author: hailin@sdau.edu.cn

stromal vascular fractions (**SVF**), propionate higher than 1 mmol enhances mRNA expression of adipocyte markers and the formation of adipocytes (Li et al., 2014). By contrast, feeding propionate can alleviate obesity in both humans and mice, which is associated with an increase in the circulating concentrations of glucagon-like peptide-1 (**GLP-1**) and peptide YY (**PYY**), 2 anorectic gut hormones (Chambers et al., 2015; Psichas et al., 2015). Propionate improves high-fat diet (**HFD**)-induced lipid dysmetabolism by modulating gut microbiota in mice (Song et al., 2019). Weitkunat et al. (2016) confirmed the importance of propionate on repression of lipogenic enzymes expression and triglyceride deposition in livers of HFD-feeding mice. Collectively, the inconsistency of the above reports might be attributable to the differences in experimental models and the varied doses used in each experiment.

For chickens, fat metabolism is different from that in mammals; the liver is the primary site for fat synthesis, whereas the adipose tissues are mainly involved with fat deposition (Brady et al., 1976; Cai et al., 2011). It has been reported that cecal concentration of propionate was positively associated with an increased *Lactobacillus spp.* population when chickens were supplemented with xylo-oligosaccharide (Pourabedin et al., 2015). Moreover, administration of calcium propionate is used as an appetite suppressant in chicken breeding (Sandilands et al., 2005; Tolkamp et al., 2005; Arrazola and Torrey, 2019), to decrease feed intake and avoid obesity-related problems in their health and reproductive performance. Hence, we hypothesized that propionate plays a role in lipid metabolism of broiler chickens.

In the present study, we aimed to elucidate the role and mechanism of propionate in fat metabolism of chickens. We found that dietary supplementation of 0.5 g/kg polyacrylic resin II-coated sodium propionate (**SP**) moderately inhibited fat deposition of broiler chickens. Although the serum and cecal concentrations of propionate were not significantly increased by SP application, the feed intake was suppressed, and the composition of cecal microbial communities was altered significantly. Further in vitro experiments demonstrated that physiological concentrations of propionate did not statistically influence hepatocytic fat synthesis and adipocytic fat deposition. This study suggested the application potential of SP in feed intake limitation, fat deposition suppression, and gut health modification.

MATERIALS AND METHODS

Animals and Experimental Protocol

The animal experiment was performed at the experimental farm of Shandong Agricultural University. All experimental procedures were approved by the Animal Care and Use Committee of Shandong Agricultural University. A total of 120 healthy 1-day-old male broiler chickens (Arbor Acres) were randomly assigned to 2 groups: (1) control, a basal diet supplemented with polyacrylic acid resin II (containing the coating material) and (2) SP, a basal diet supplemented with 0.05% SP coated with polyacrylic acid resin II (70% of SP was protected, Jiafa Granulating Drying Co., Ltd. Changzhou, China). The experimental design was completely randomized design. The housing type of the birds was battery cages. Each 10 birds were placed in one cage. The basal diet was formulated in accordance with the growing stage of broilers: the starter diet with 21% crude protein and 3,100 kcal/kg metabolizable energy from 1 to 21 d of age, and the grower diet with 19% crude protein and 3,300 kcal ME/kg from day 22 to 42 (Chen et al., 2018; Supplementary Table 1). The light regimen was 23 L:1 D and the dark period was from 0:00 to 01:00 am (Zhao et al., 2012; Tang et al., 2019). During the rearing period, the broilers had free access to water and feed. Body weight and feed intake were recorded weekly, and the feed conversion ratio was calculated.

Samples were collected at day 21 and day 42. Venous blood was obtained from the main wing veins, and serum was collected after centrifugation at 3,500 $\times$ g for 10 min. After blood collection, broilers were immediately sacrificed by exsanguination after cervical dislocation (Huang et al., 2015). Tissue samples (abdominal fat, liver and the cecal chyme) were collected and snap-frozen in liquid nitrogen and stored at - 80°C for further analysis. The abdominal fat ratio and liver index were calculated based on tissue weight/body weight %.

Histological Analysis

Abdominal fat and liver tissue samples were fixed with formaldehyde, embedded in paraffin, and the paraffin sections were cut into 5 μm thickness. The paraffin sections were deparaffinized with xylene, rehydrated with alcohol and water, and stained with hematoxylin and eosin (**H&E**). The cell morphology in tissues was evaluated and pictured under a microscope (Nikon, Tokyo, Japan). Area with adipocytes in adipose tissues was measured by using image software (Nikon, Tokyo, Japan).

Determination of Serum Parameters, as well as Triglyceride and Total Cholesterol Contents in Tissues

Levels of triglyceride (**TG**) and total cholesterol (**TCH**), as well as alanine aminotransferase activity in the serum were analyzed by an automatic biochemical analyzer (Hitachi7020, Hitachi, Tokyo, Japan), using commercial assay kits (Maccura, Szechwan, China). The contents of TG and TCH in liver and abdominal fat tissues were detected by commercial kits (Nanjing Jiancheng, Nanjing, China) and quantified as mmol/g total protein.

Cecal Microbiome Analysis by 16S rRNA Sequencing

16S rRNA gene sequencing was performed as previously described (Liu et al., 2019). In brief, microbiome

DNA was isolated from cecal chyme samples. The extracted DNA was used as the template to amplify the V3+V4 region of 16S rRNA genes by PCR amplification. After constructing a sequencing library of the V3+V4 regions of the 16S rRNA gene, the purified products were mixed at an equal ratio for sequencing using an Illumina MiSeq system (Illumina Inc., San Diego, CA). Based on the results of OTU clustering analysis, multiple diversity index analysis and sequencing depth can be carried out using line detection. Community structure statistics were evaluated at different classification levels basing on classification information. Histogram and heatmap figures identified bacterial members that markedly different between the 2 groups.

Gas Chromatography-Mass Spectrometer Assay of the SCFA Contents in Serum and Cecal Chyme

Sera and fecal SCFA concentrations were determined using a gas chromatography-mass spectrometer assay. The serum and extracted cecal chyme samples were injected into the GCMS ISQ LT (Thermo Fisher Scientific, Waltham, MA) with the following conditions: column temperature, 100 °C (5 min) − 5 °C/min−150 °C (0 min)–30 °C/min–240 °C (30 min); flow rate, 1 mL/min; split ratio, 75:1; carrier gas, helium; column, TG WAX 30 m × 0.25 mm × 0.25 μm; injector, 240 °C; mass spectrometry, EI source; bombardment voltage, 70 eV; single ion scanning mode, quantitative ions 60,73; ion source temperature, 200 °C; cable temperature, 250 °C. Finally, the external standard curve method was used to quantify the contents of acetate, propionate, and butyrate in each sample.

Isolation and Culture of Hepatocytes and Preadipocytes

Hepatocytes were collected from 17-day-old chicken embryos, as described previously (Cai et al., 2011). Briefly, liver tissues were minced, digested by collagenase IV, filtered, and centrifuged to remove other cell types. Hepatocytes were resuspended in Williams' medium E (HyClone, Logan, UT) containing 10% fetal bovine serum and 1% antibiotic mixture, and cultured in a humidified atmosphere with 5% CO_2 at 37 °C.

Chicken preadipocytes were collected from 17-day-old embryos, in accordance with described protocols (Ramsay and Rosebrough, 2003). Adipose tissues were minced and digested by collagenase I. The digested samples were filtered through a 40-μm mesh filter to remove debris and centrifuged at 600 × *g* for 5 min. The supernatant containing adipocytes was discarded, and the cell pellet was resuspended in DMEM medium (HyClone) containing 10% fetal bovine serum and 1% antibiotic mixture. The preadipocytes were cultured in a humidified atmosphere with 5% CO_2 at 37 °C.

Cell Treatments

To evaluate the effect of propionate on hepatocytic lipogenesis, subconfluent hepatocytes were treated with 0.01 mmol or 0.1 mmol SP for 4 d in the presence of 300 μmol oleic acid.

To induce adipogenic differentiation of preadipocytes, the subconfluent cells were exposed to a cocktail induction medium I containing 5 μg/mL insulin, 1 μmol dexamethasone, 1 μmol rosiglitazone, and 0.5 mmol 3-Isobutyl-1-methylxanthine for the first 2 d, to medium II containing 5 μg/mL insulin, 1 μmol dexamethasone, and 1 μmol rosiglitazone from day 2 to day 4, to medium III with 5 μg/mL insulin and 1 μmol rosiglitazone from day 4 to day 6, and to medium IV with 0.5 μg/mL insulin for the last 2 d. To determine the influence of propionate on adipocytic differentiation, SP at dosages of 0.01 mmol and 0.1 mmol were administrated to the adipogenic cocktail induction medium for 8 d.

Oil Red O and Bodipy Staining

Hepatocytes and adipocytes were stained with oil red O on d 4 and d 8 after treatment, respectively. The cells were washed twice with D-Hank's and subsequently fixed with 4% paraformaldehyde for 1 h at room temperature (**RT**). After fixation, the cells were washed twice with D-Hank's and subsequently stained with 0.6% oil red O solution for 1 h. Hematoxylin staining was performed to visualize the cell nuclei. After washing, the cultures were photographed with an inversion microscope (Olympus, Tokyo, Japan). The stained oil red O was quantified and expressed as nmol/g total protein.

On day 8 after induction of adipocytes, cells grown on slides were washed twice with D-Hank's and subsequently fixed with 4% paraformaldehyde for 1 h at RT. Adipocytes were stained with BODIPY (1 μg/mL) for 30 min, thereafter staining with DAPI (1 μg/mL) for 5 min. The cultures were photographed with a 2-photon laser confocal microscope (Zeiss, Oberkochen, Germany).

Determination of TG Content in Cells

The treated hepatocytes and adipocytes were lysed by RIPA Lysis Buffer. Triglyceride content in the lysed extracts was analyzed using a commercial assay kit (Nanjing Jiancheng, Nanjing, China), which was expressed as mmol/g total protein.

Total RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA from tissues or cells was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA). The extracted RNA was reverse transcribed into cDNA using a PrimeScript TMRT Reagent Kit (Takara, Tokyo, Japan) and processed for qRT-PCR using SYBR Green I labeling (Roche, Basel, Switzerland). The primer sets are listed in Table 1. The reaction was performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for

Table 1. The primers used in this study.

Genes	Sequence 5'to 3′
GAPDH	Forward—CTACACACGGACACTTCAAG
	Reverse—ACAAACATGGGGGCATCAG
PPARG	Forward—AGACACCCTTTCACCAGCATCC
	Reverse—AACCCTTACAACCTTCACAAGCA
Nor	Forward—TCCTTGGTGTTCGTGACG
FAS	Reverse—CGCAGTTTGTTGATGGTGAG
ADPN	Forward—TCACCTACGACCAGTTCCA
	Reverse—CCCGTTGTTGTTGCCCTC
FABP4	Forward—TGAAGCAGGTGCAGAAGT
	Reverse—CAGTCCCACATGAAGACG
LPL	Forward—CAGTGCAACTTCAACCATACCA
	Reverse—AACCAGCCAGTCCACAACAA
FFAR2	Forward—AACGCCAACCTCAACAAGTC
	Reverse—TGGGAGAAGTCATCGTAGCA
FFAR3	Forward—GAAGGTGGTTTGGGAGTGAA
	Reverse—CAGAGGATTTGAGGCTGGAG
ACC1	Forward—AATGGCAGCTTTGGAGGTGT
	Reverse—TCTGTTTGGGTGGGAGGTG
SREBP-1c	Forward—GCCCTCTGTGCCTTTGTCTTC
	Reverse—ACTCAGCCATGATGCTTCTTCC

15 s and 60 °C for 60 s, and followed by a melt curve analysis to ensure only a single product was amplified. The data were analyzed with the $2^{-\Delta\Delta Ct}$ method using GAPDH as a reference.

Western Blot Analysis

Treated hepatocytes and adipocytes were lysed in RIPA Lysis Buffer supplemented with protease inhibitors. The cell lysates were centrifuged at 12,000 × g for 15 min at 4 °C, and the supernatants were collected. Equal amount of proteins was separated by SDS-PAGE and transferred onto nitrocellulose membranes. Immunoblotting was performed using primary antibodies for proteins of interest (ACC1, SREBP-1c, PPARγ, FABP4, and GAPDH), followed by HRP-conjugated secondary antibodies. The protein signals were detected using ECL Plus (Beyotime, Shanghai, China).

Statistical Analysis

Data were expressed as mean ± the standard error of the mean. Results were analyzed using the SAS statistical software (SAS version 8e, SAS Institute, 1998). Student *t*-test was used to analyze differences between independent samples. Differences were considered significant when $P < 0.05$.

RESULTS

Dietary SP Inhibits Feed Intake of Broilers

In this study, low dose of SP treatment (0.5 g/kg diet) effectively reduced the average daily feed intake of broilers during the whole rearing period ($P < 0.05$) (Figure 1A). The average daily gain was also significantly decreased ($P < 0.01$) (Figure 1B), but the feed-to-gain ratio was not statistically changed by dietary SP ($P > 0.05$) (Figure 1C).

Dietary SP Reduces Fat Deposition in Broilers

The abdominal fat ratio and liver index were calculated at both day 21 and day 42. Compared with the control, SP supplementation slightly decreased the abdominal fat ratio at both d 21 and d 42 ($P > 0.05$) (Figure 2A). However, SP treatment extensively decreased the size of the abdominal fat cells at d 42 ($P < 0.05$), as evidenced by H&E staining and adipocyte area measurement (Figures 2C and 2D). With it, the TG content, and the transcription of PPARγ and FABP4 in SP-treated abdominal adipose tissues were inhibited statistically ($P < 0.05$) (Figures 3A and 3C). However, the sera TG and TCH levels were not significantly changed in SP-treated broilers ($P > 0.05$) (Figure 4A).

As shown in Figure 2B, the liver index of SP-treated broilers was not significantly altered either at day 21 or at day 42 ($P > 0.05$). Correspondingly, H&E staining of the liver sections, TG and TCH contents in the liver tissues showed no statistical difference between the 2 treatments (Figure 2E, Figure 3B). By contrast, the transcription of hepatic lipogenic genes, including PPARγ, ACC1, and SREBP-1c, was suppressed by SP ($P < 0.05$) (Figure 3D). The mRNA expression of FFAR2 was reduced as well ($P < 0.01$) (Figure 3D). It was notable that the sera alanine aminotransferase activity was significantly reduced by SP treatment ($P < 0.01$) (Figure 4B).

Dietary SP Supplementation Changes the Population Composition of the Cecal Microbiota

Gut microbiota are closely correlated with fat metabolism, thus we investigated the population composition of the cecal microbiota in the SP and control groups by 16S rRNA gene sequencing. As illustrated in Figure 5A, most of the control and SP-fed chickens presented a distinct clustering of microbial community at the phylum level. Sodium propionate tended to decrease the abundance of *Firmicutes* and increase the richness of *Bacteroidetes,* and reduce the richness of *Proteobacteria* significantly ($P < 0.05$) (Figure 5B). At the genus level, some beneficial bacteria, such as *Alistipes, Lactobacillus,* and *Bifidobacterium* were significantly enriched in the SP groups ($P < 0.05$) (Figures 5C, 5D, and 5E). By contrast, the abundance of *Lachnospiraceae* and *Helicobacter* was strongly decreased in the SP-treated samples ($P < 0.01$) (Figures 5C, 5D, and 5E).

Neither Hepatocytic Fat Synthesis nor Adipocytic Fat Deposition is Directly Affected by Physiological Concentrations of Propionate

The propionate content in the serum was numerically but not significantly increased by dietary SP

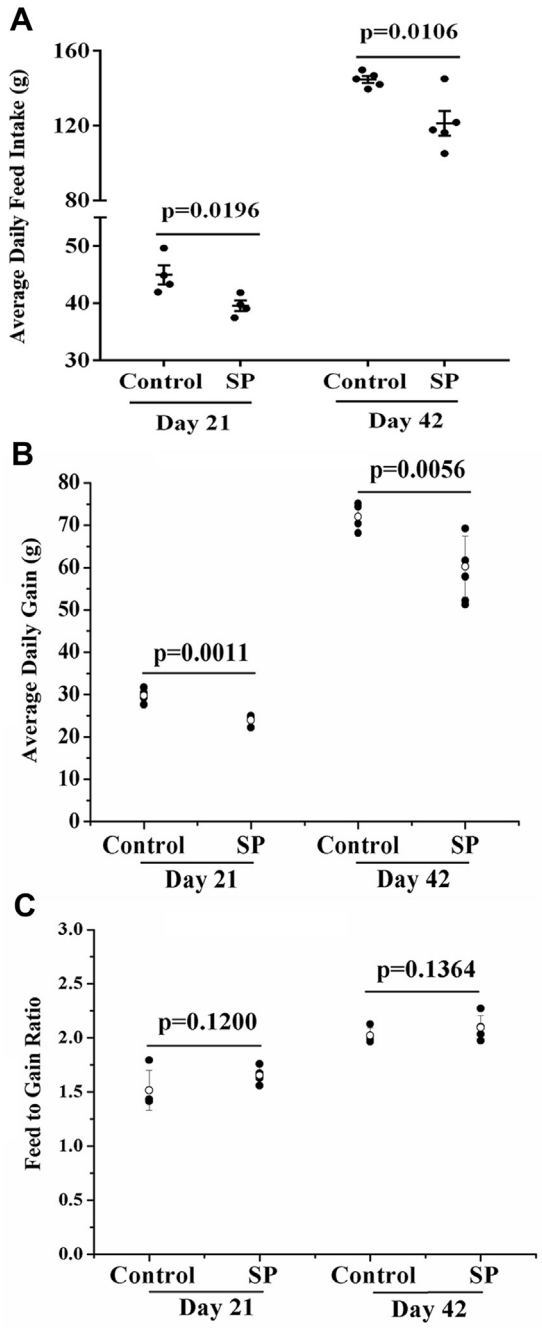

Figure 1. Effect of dietary SP on average daily feed intake (A), average daily gain (B), and feed to gain ratio of broilers. Data are mean ± SEM. *P* values are shown after comparing with the control. Abbreviation: SP, sodium propionate.

administration, and its level in the lower digestive tract was not changed (Table 2). The physiological concentration of propionate in cecal chyme ranged from 1.720 mmol to 4.623 mmol, whereas it was between 0.007 mmol and 0.095 mmol in the serum (Table 2).

After absorption, propionate can be delivered to other tissues through the blood stream (Brown et al., 2003; Byrne et al., 2015). To test if propionate could regulate fat synthesis or fat deposition at physiological concentrations, we treated hepatocytes and adipocytes in vitro with 0.01 mmol and 0.1 mmol SP, respectively. Notably, although the mRNA levels of some lipogenic markers in hepatocytes were altered, neither the protein expression degree nor the deposited TG content was significantly influenced by SP (Figure 6). Similarly, in spite of down-regulating the transcription of some adipogenic genes ($P < 0.05$), SP at dosages of 0.01 mmol and 0.1 mmol did not statistically affect adipocytic fat accumulation, as evidenced by oil red O/BODIPY staining, TG content determination, and the protein expression of adipogenic markers (Figure 7).

DISCUSSION

In the present study, we demonstrated that dietary supplementation of low-dose coated SP strongly inhibited feed intake and moderately suppressed fat deposition in broiler chickens. Further investigation showed that the propionate content only numerically increased in the serum and not changed in the cecal chyme of SP-treated broilers. This may be due to the low supplementation dosage (0.5 g/kg diet) and being a small water-soluble molecule, the absorption rate is likely to be rapid. We speculated that the coated SP in the diet may be digesting and releasing after eating by chickens, and a proportion of it reaches the intestines. The SP that reached the intestines is likely to take action on stimulating the secretion of appetite-suppressing peptides by intestinal L cells, thereby inhibiting the feed intake. Previous studies have reported that propionate would stimulate PYY and GLP-1 secretion from intestinal L cells (Kaji et al., 2011), thereby inhibiting appetite and further reducing fat accumulation in mammals (Chambers et al., 2015; Psichas et al., 2015). Our recent study (Zhang et al., 2019) also found propionate induces GLP-1 secretion in cultured intestinal epithelial cells, and GLP-1 suppresses hepatocytic lipogenesis in vitro. The appetite-suppressing peptides might be involved in the inhibition of oral propionate administration on chicken fat deposition, as the average daily feed intake of broilers was significantly reduced. It is notable that smaller doses of propionate may have a greater effect on appetite suppression and feed intake in chickens than larger doses needed by humans and rodents (Chambers et al., 2015; Psichas et al., 2015).

In addition, the inhibition of SP on chicken fat deposition was mainly observed in adipose tissue, as opposed to the liver. Our findings fit well with previous reports in which oral administration or colonic infusion of SP could reduce fat accumulation and weight gain in mice and humans (Chambers et al., 2015; Song et al., 2019). Alteration of gut microbiota after dietary treatments has been established to play a key role in regulating fat metabolism (Wu et al., 2019; Zhang et al., 2019). To test whether dietary supplementation of SP suppressed chicken fat deposition via gut microbiota, we performed high-throughput sequencing on hypervariable region of the 16S rRNA genes of cecal bacteria. Supplemental SP decreased the ratio of *Firmicutes* to *Bacteriodetes*. The reduction in fat deposition in our study was also associated with increased *Bacteriodetes* and reduced *Firmicutes* populations in other work (Ley et al., 2005;

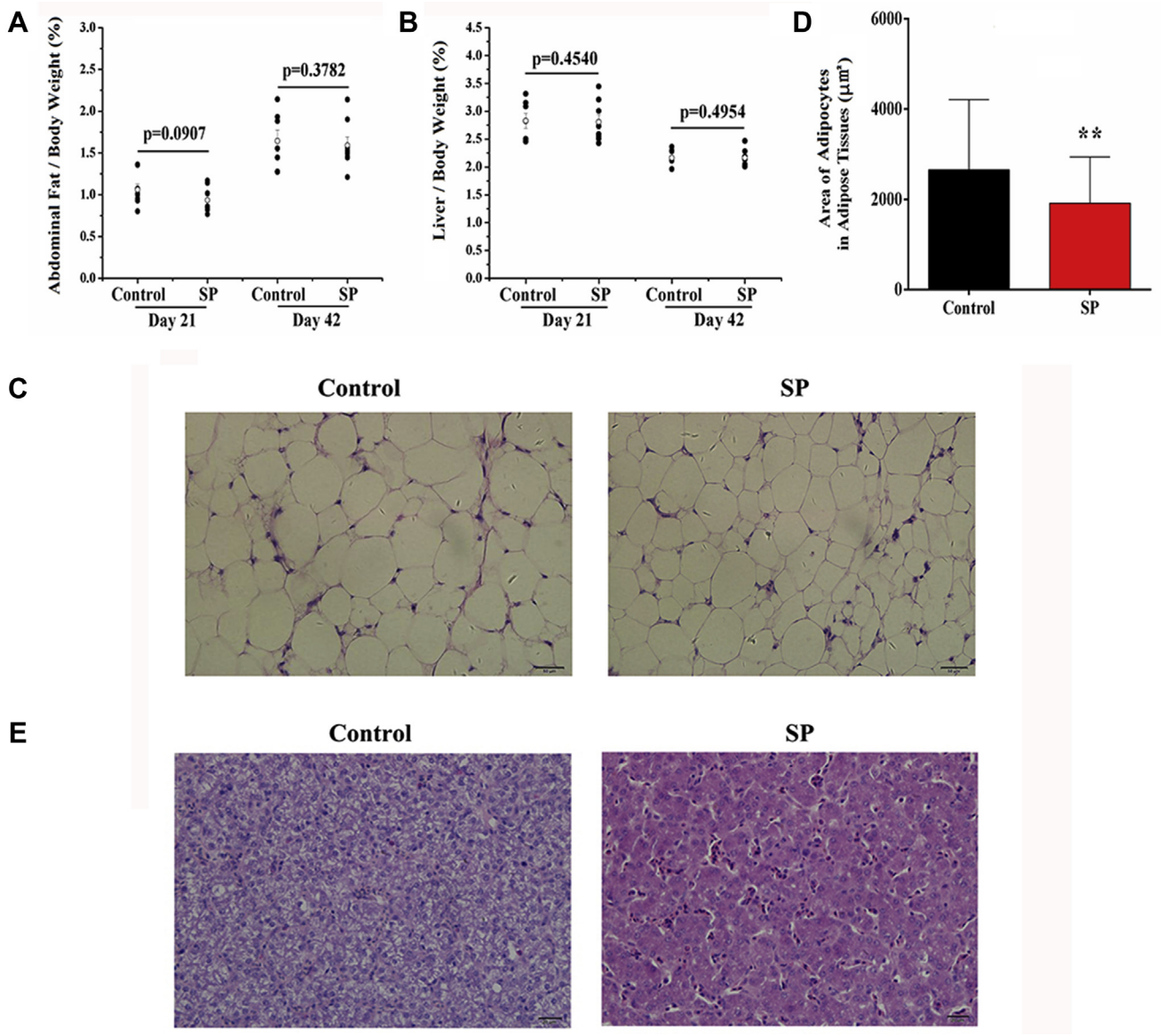

Figure 2. Effect of dietary SP on fat deposition in broilers. (A) Abdominal fat ratio in the control and SP-treated broilers at d 21 and d 42. (B) Liver index in the control and SP-treated broilers at d 21 and d 42. (C) Representative H&E staining pictures of the abdominal fat slides of 42-day-old broilers. Scale bar is 50 μm. (D) Quantification of the adipocytes' area in adipose tissue sections. (E) Representative H&E staining pictures of the liver sections of 42-day-old broilers. Scale bar is 20 μm. Data are presented as the mean ± SEM. $^{**}P < 0.01$ compared with the control. Abbreviations: H&E, hematoxylin and eosin; SP, sodium propionate.

Parnell et al., 2012). In addition, the SP group showed higher abundance of *Proteobacteria* colonies than the control group. At the genus level, the abundance of *Alistipes, Lactobacillus,* and *Bifidobacterium* was increased, whereas *Lachnospiraceae* and *Helicobacter* were decreased in response to SP supplementation. *Alistipes, Lactobacillus,* and *Bifidobacterium* are considered as beneficial bacteria, which have anti-inflammation, immunity-regulation, intestinal health-improvement, and fat metabolism–modulation capabilities (Nagai et al., 2010; Yoshitaka et al., 2010; Singh et al., 2017; Kim et al., 2019). By contrast, *Proteobacteria, Lachnospiraceae,* and *Helicobacter* are often associated with intestinal inflammation and gastrointestinal diseases (Kang et al., 2019; Bakhti et al., 2020; Zeng et al., 2020). We speculated that SP supplementation results in the growth of some beneficial bacteria, while suppressing the growth of harmful species. These results demonstrate that the reduction of SP on chicken fat deposition is at least partially dependent on alterations in relative population densities of gut microbiome. The small amount of SP reached to the lower digestive tract is likely to affect the intestinal microcircumstances, such as lowering the pH, which leads to alteration of the composition of gut bacterial communities. This also indicates the implication of propionate on gut health, being consistent with previous studies of which showing the beneficial role of butyrate and propionate in gut health of both humans and other animals (Bedford and Gong, 2018; Blaak et al., 2020).

Sodium propionate is such a small molecule that oral administration would be absorbed and metabolized

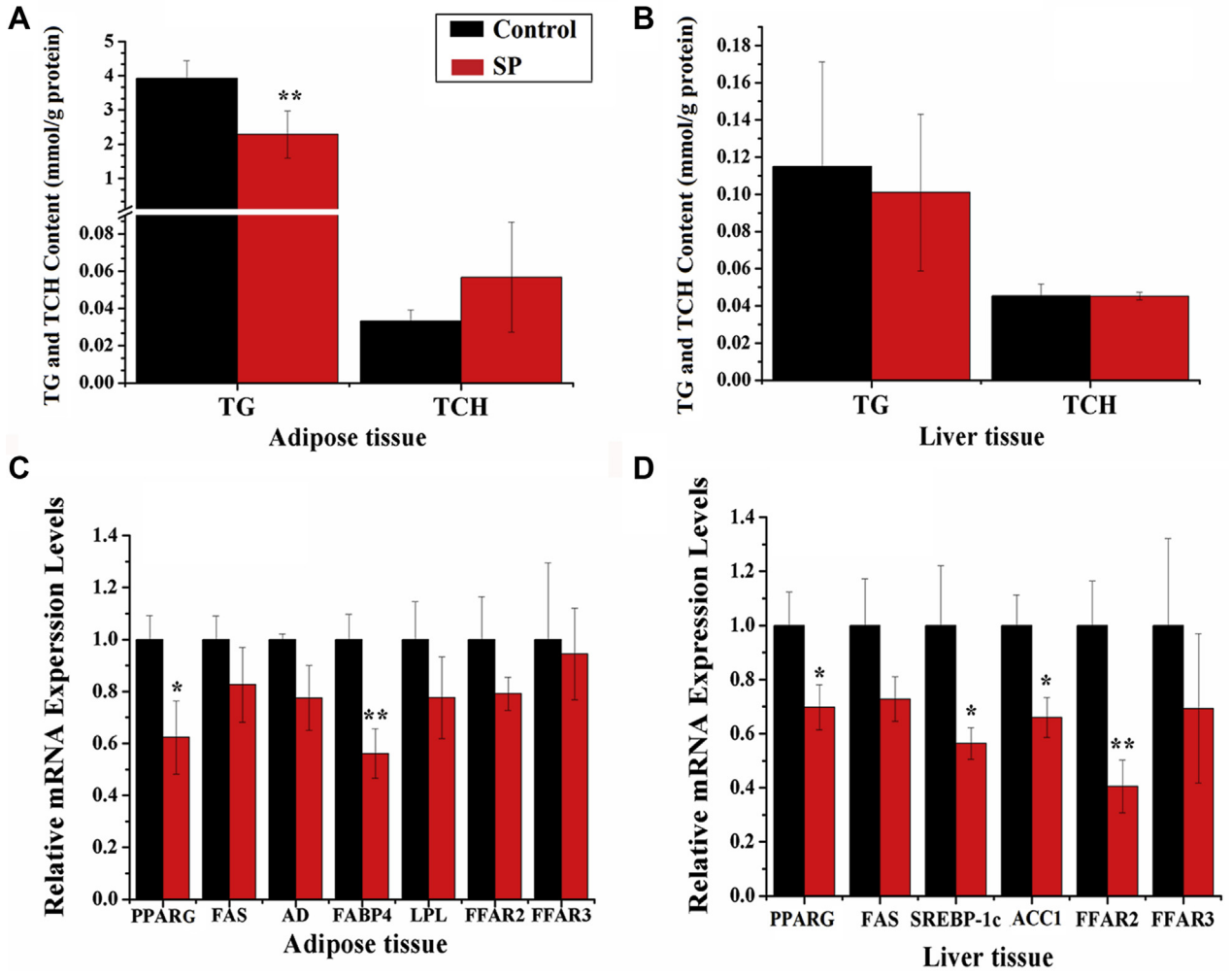

Figure 3. Effect of dietary SP on the contents of TG and TCH, and the expression of genes in abdominal fat and liver tissues of broilers. (A) The content of TG and TCH in adipose tissue of 21-day-old broilers. Data are expressed as mmol/g total protein. (B) The TG and TCH contents in liver of 21-day-old broilers. Data are expressed as mmol/g total protein. (C) The relative mRNA levels of fat deposition associated genes in adipose tissue of 21-day-old broilers. (D) The relative mRNA levels of fat synthesis–associated genes in liver tissue of 21-day-old broilers. Data are presented as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control. Abbreviations: SP, sodium propionate; TG, triglyceride; TCH, total cholesterol.

quickly to permit a numerical elevated content of propionate in serum as observed in this study. To analyze whether physiological concentrations of propionate directly affects hepatocytic and adipocytic fat accumulation, in vitro experiments were conducted by using cultured hepatocytes and adipocytes. Results indicated that neither the hepatocytic fat synthesis nor the adipocytic fat deposition was statistically affected by

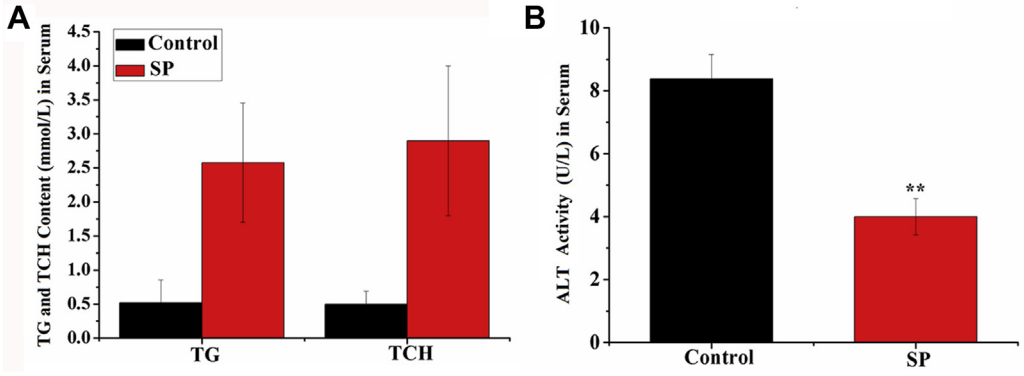

Figure 4. Effect of dietary SP on sera parameters of broilers. (A) The content of TG and TCH in serum of 21-day-old broilers. (B) The ALT activity in serum of 21-day-old broilers. Data are presented as the mean ± SEM. $^{**}P < 0.01$ compared with the control. Abbreviations: ALT, alanine aminotransferase; SP, sodium propionate; TG, triglyceride; TCH, total cholesterol.

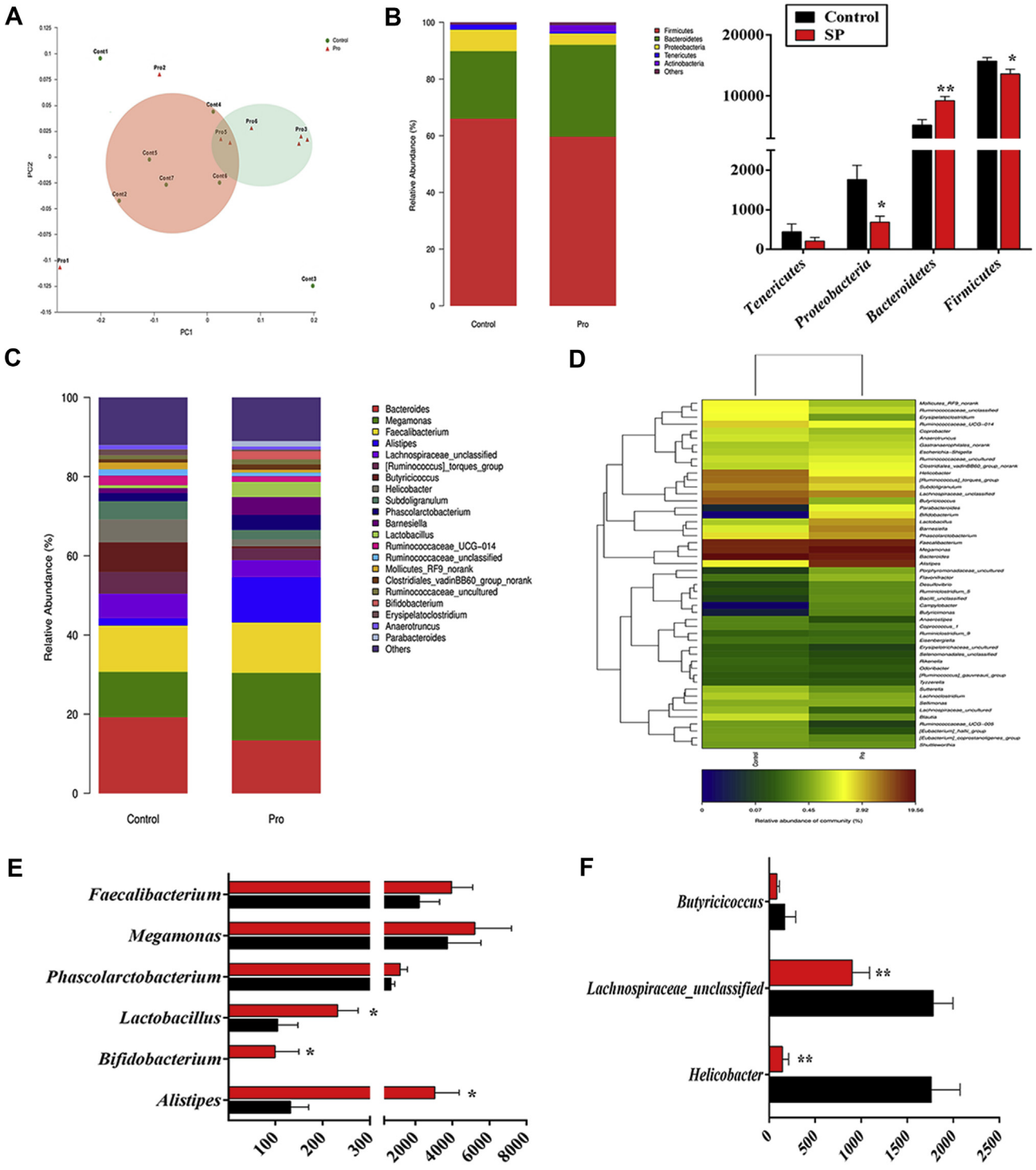

Figure 5. Alteration of dietary SP on the population composition of cecal microbiota. (A) PCoA analysis of the cecal microbiome at the phylum level among samples of different treatments. (B) The mean relative abundance of cecal bacteria (≥1% relative abundance) in the control and SP groups at the phylum level. (C) The mean relative abundances of cecal bacteria (≥1% relative abundance) in the control and SP groups at the genus level. (D) Heatmap showed the abundance of cecal bacteria in the control and SP groups at the genus level. (E and F) Comparison of the extensively increased (E) and decreased (F) bacteria by SP at the genus level. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control. Abbreviation: SP, sodium propionate.

physiological concentrations of propionate. This is not consistent with some in vitro investigations, in which propionate exerts stimulation on adipogenesis of 3T3-L1 cells (Hong et al., 2005) or inhibition on adipogenic differentiation of porcine SVF (Li et al., 2014). A note of caution should be used, considering that the effect of propionate on fat accumulation is dose-dependent. Another reason may be that the chicken hepatocytes and adipocytes are not as sensitive as mammalian cells in response to propionate.

In conclusion, oral SP administration reduces fat deposition in broiler chickens by affecting feed intake and altering

Table 2. Short-chain fatty acid concentrations in the cecal chyme and serum of control and SP-treated broilers.

Samples	Items	Control (mmol)	SP (mmol)	*P*-value
Cecal chyme	Acetate	6.25 ± 0.92	6.07 ± 1.13	0.41
	Propionate	3.40 ± 1.33	2.49 ± 0.42	0.14
	Butyrate	4.31 ± 1.20	4.32 ± 1.27	0.49
Serum	Acetate	0.016 ± 0.005	0.017 ± 0.002	0.39
	Propionate	0.012 ± 0.004	0.022 ± 0.020	0.13
	Butyrate	0.006 ± 0.004	0.007 ± 0.005	0.41

the composition of gut microbiome communities, but not through direct regulation on hepatic fat synthesis or adipocytic fat deposition. These findings indicate that SP could be used as a feed additive to modify gut microbiota and inhibit fat deposition in chickens. Supplementation with SP could also place limits on feed intake.

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DISCLOSURES

The authors declare no conflict of interests.

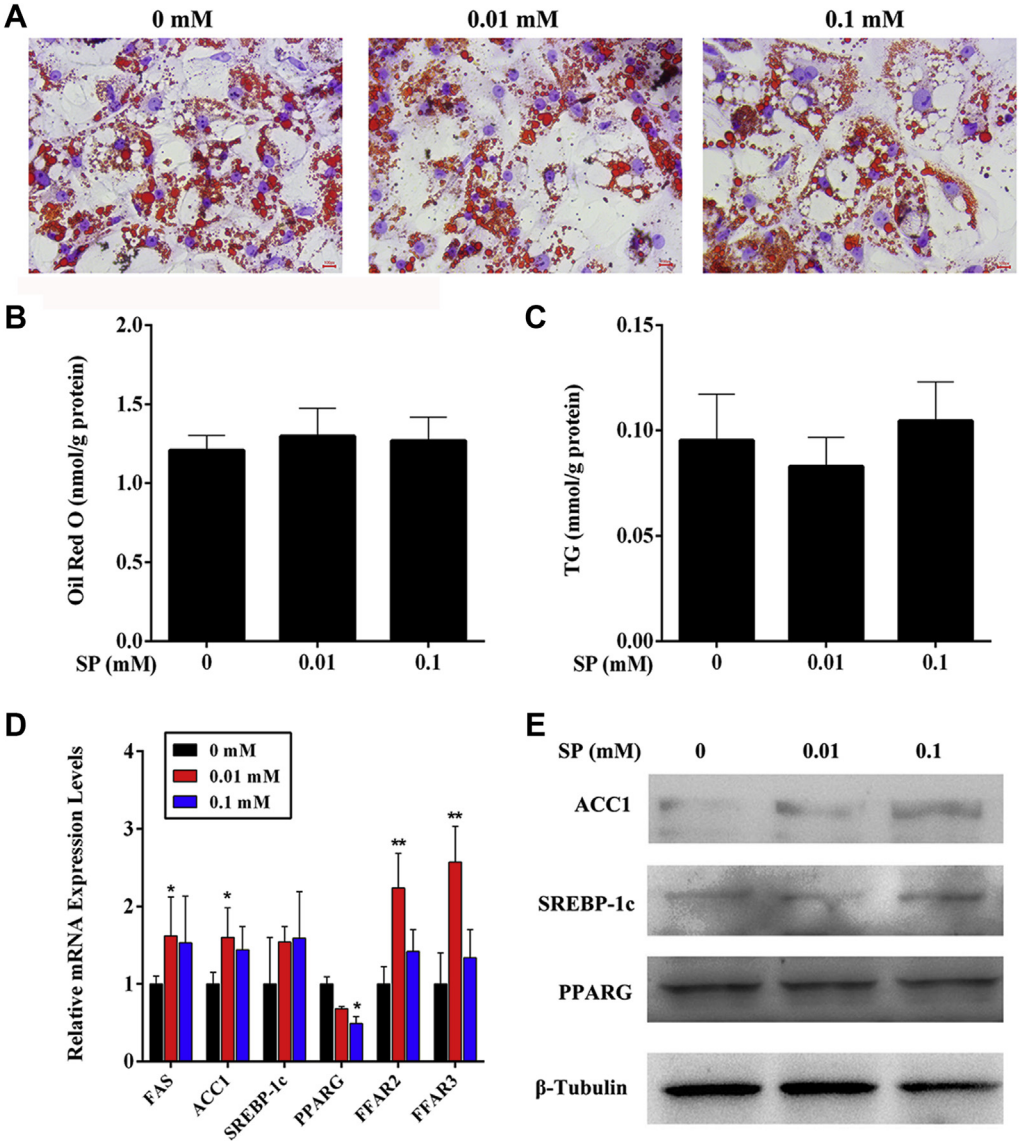

Figure 6. Effect of physiological concentrations of propionate on hepatocytic fat synthesis. (A) Oil red O staining of hepatocytes on treating with different concentrations of SP. Nuclei were stained with hematoxylin (purple). (B) The stained oil red O was quantified after isopropanol extraction, which was shown as nmol/mg total protein. (C) The accumulated TG content in hepatocytes, which was quantified on the basis of the same content of protein. (D) The relative mRNA levels of FAS, ACC1, SREBP-1c, PPARγ, FFAR2, and FFAR3 in hepatocytes after treating with different concentrations of SP, which took GAPDH as an internal reference. (E) Western blot analysis showing the protein expression of fat synthesis-related markers at d 4 of treatment, which took β-Tubulin as an internal reference. The experiment was repeated at least 3 times. Values are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control. Abbreviation: SP, sodium propionate; TG, triglyceride.

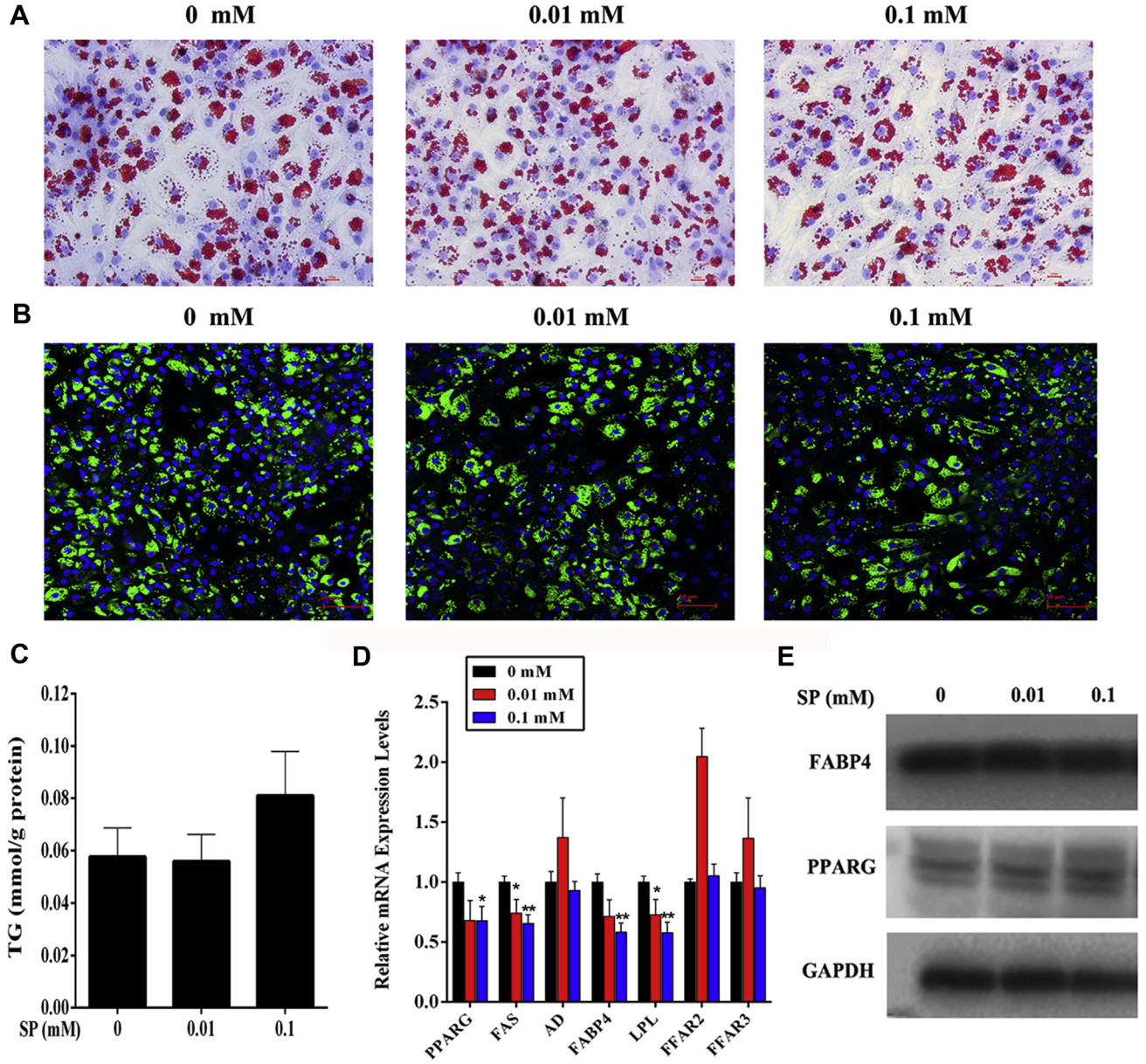

Figure 7. Effect of physiological concentrations of propionate on fat accumulation in chicken adipocytes. (A) Oil red O staining pictures of adipocytes after treating with different concentrations of SP at d 8. Nuclei were stained by hematoxylin (purple). (B) Bodipy staining pictures of adipocytes after treating with different concentrations of SP at d 8 (Green). Nuclei were stained with DIPA (blue). (C) The accumulated TG content in adipocytes, which was quantified on the basis of the same content of protein. (D) The relative mRNA levels of PPARγ, FAS, AD, FABP4, LPL, FFAR2, and FFAR3 in adipocytes treated with different concentrations of SP, which took GAPDH as an internal reference. (E) Western blot analysis showing the protein expression of adipogenic markers at d 8 of treatment. The experiment was repeated at least 3 times. Values are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control. Abbreviation: SP, sodium propionate; TG, triglyceride.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.10.009.

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