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Original Research Article

Maternal butyrate supplementation affects the lipid metabolism and fatty acid composition in the skeletal muscle of offspring piglets

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ABSTRACT

Maternal sodium butyrate (SB) intake has important effects on offspring growth and development. This study aimed to investigate the impacts of maternal SB supplementation during gestation and lactation on fatty acid composition and lipid metabolism in the offspring skeletal muscle of pigs. Twenty sows (Yorkshire, parity 2 to 3) were assigned to the control group (diets without SB, $n = 10$) and SB group (diets with 0.1% SB, $n = 10$). The results showed maternal SB supplementation throughout gestation and lactation increased ($P < 0.05$) body weight of offspring piglets at weaning. The concentrations of triglyceride in plasma and milk were enhanced ($P < 0.05$). Maternal SB induced ($P < 0.05$) lipid accumulation with increased expression of peroxisome proliferator activated receptor γ (PPARγ) by enrichment of the acetylation of H3 acetylation K27 (H3K27) in offspring skeletal muscle. Meanwhile, the concentrations of C18:2n-6, C18:3n-3, total polyunsaturated fatty acid (PUFA), n-6 PUFA and n-3 PUFA decreased ($P < 0.05$) in skeletal muscle of weaning piglets derived from SB sows. Together, these results showed that maternal SB supplementation could influence offspring growth performance, lipid metabolism and fatty acid composition of the skeletal muscle.

1. Introduction

Evidence based on the notion of 'metabolic programming' suggests that maternal nutrition during gestation and lactation has impacts on offspring growth and development (Godfrey and Barker, 2001; Mennitti et al., 2015). Maternal dietary fatty acids are not only energy sources, but also they likely influence offspring development by modifying fetal programming and epigenetic regulation (Innis, 2011; Mennitti et al., 2015). Butyrate can be an energy source for supplying energy requirement for ruminants and monogastric animals (Siciliano-Jones and Murphy, 1989; Yen et al., 1991). A previous study has shown that butyrate supplementation in sows during gestation enhanced the growth performance of weaning piglets (Lu et al., 2012). Butyrate could also act as a histone deacetylase (HDAC) inhibitor which plays a role in the epigenetic gene regulation (Davie, 2003; Sekhavat et al., 2007). In our previous studies, the results showed that maternal sodium butyrate (SB) could enhance intramuscular fat deposition (Huang et al., 2017) and lipid accumulation in the liver of offspring in rats (Zhou et al., 2016). Butyrate participating in epigenetic regulation has been wildly documented, and is correlated with lipid metabolism. However, the impact of maternal SB upon offspring lipid metabolism in skeletal muscle of pigs is still largely unclear.

Fatty acids, which play a vital role in regulating kinds of tissues' functions and participate in amounts of metabolic pathways, are indispensable for animals (Wolfrum et al., 2001). A large amounts of diseases such as insulin resistance, obesity (Kraegen and Cooney, 2008; Hafizi Abu Bakar et al., 2015), non-alcoholic fatty liver (Pawlosky and Salem, 2004) have been reported to be associated

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with the changes of fatty acid composition. The link between consumption of dietary fatty acid and human metabolic diseases was well established (Watanabe and Tatsuno, 2020). Fatty acid composition of meat has influences on the health of consumers, and draws a widespread attention (Corpet, 2011). Therefore, investigating the elements, which influence the fatty acid composition of animal skeletal muscle, is of great importance both for animal metabolism and human health.

Thus, in our present study, we added SB into the diet of sows during their gestation and lactation and analyzed the lipid metabolism and fatty acid composition in skeletal muscle of offspring piglets. This study will further clarify the effect of maternal SB supplement on the offspring skeletal muscle and will provide a new perspective for understanding maternal effect on offspring lipid metabolism in pigs.

2. Materials and methods

2.1. Ethics statement

All procedures with animals were approved by the Animal Ethics Committee of Nanjing Agricultural University. The sampling procedures followed the Guidelines on Ethical Treatment of Experimental Animals (2006) No. 398 set by the Ministry of Science and Technology, China.

2.2. Experimental design, treatments, and sample management

The animal experiment was performed at Shanghai Breeding pig Farm Co., Ltd (Shanghai, China). Twenty Yorkshire sows (multiparity, 2 to 3) were artificial insemination with a mixture of semen samples. The chosen sows had similar body weight in our experiments. The sows were housed individually in the same pens (2 m × 2 m) under natural light condition. Feed was provided at 05:00 and 17:00 daily with free access to water.

The sows were assigned to the control group (CON group, $n = 10$) and the sodium butyrate group (SB group, $n = 10$). The CON sows were fed a basal diet, whilst SB sows were fed the basal diet containing 0.1% SB during the whole pregnancy and lactation. The diet composition is shown in Table 1. The litter size was adjusted to 7 to 8 pigs per litter at 24 h post parturition. The newborn piglets were allowed free access to their mothers. Sows and piglets were maintained under identical feeding conditions. Routine farm management procedures were followed.

All the piglets were individually weighed at 0, 9 and 21 d of age. Then, 1 male piglet closest to the average weight per litter was selected and sacrificed by exsanguination. Colostrum samples were collected immediately after the birth of the first piglet from sows. Colostrum was manually collected from functional teats and stored at −80 °C until analysis. Blood samples were collected into tubes with heparin sodium, and then centrifuged at 900 × *g* for 15 min for separating plasma, which was stored at −80 °C for further analysis. Longissimus dorsi (LD) samples were taken and then rapidly frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.3. Biochemical analysis

The plasma concentrations of glucose (KH674), total cholesterol (KQ436), total triglyceride (AM545), high density lipoprotein-cholesterol (HDL-C) (KP712), low density lipoprotein-cholesterol (LDL-C) (KF253) were measured by a biochemical automatic analyzer (Hitachi 7020, HITACHI, Tokyo, Japan) using commercial assay kits (Wako Pure Chemical Industries, Ltd., Wako, Japan).

Total triglyceride in LD were determined by using the tissue assay kits (E1013; Applygen Technologies, Inc.) following the manufacturer's instructions.

Milk concentrations of cholesterol, triglyceride, protein, and lactose was assayed. The concentrations of cholesterol and triglyceride were measured as with plasma. The total protein concentration was determined by Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). The lactose concentration was measured by using Lactose/Galactose Assay Kit (K-LACGAR 03/14; Megazyme, Bray, Ireland).

Table 1
Ingredients and composition of the experimental diets of sows (as fed basis).

Item	Gestation		Lactation	
	CON	SB	CON	SB
Ingredients, %				
Corn	63.7	63.7	61.4	61.4
Full-fat soybean	–	–	10.0	10.0
Soybean meal	16.9	16.9	14.0	14.0
Fish meal	–	–	1.5	1.5
Fermented soybean meal	–	–	3.0	3.0
Rice bran	15.0	15.0	3.0	3.0
Alfalfa hay	–	–	–	–
Sodium butyrate	–	0.1	–	0.1
Soybean oil	–	–	2.5	2.5
Baking soda	–	–	0.20	0.20
Premix[1]				
Calculated composition, %				
Crude protein	13.96	13.96	17.02	17.02
Crude fiber	2.87	2.87	2.54	2.54
Ca	0.08	0.08	0.17	0.17
AP	0.14	0.14	0.17	0.17
NDF	10.77	10.77	8.81	8.81
ADF	5.29	5.29	4.14	4.14
Digestible energy, MJ/kg	3.18	3.18	3.44	3.44

CON = control; SB = sodium butyrate; AP = calcium hydrophosphate; NDF = neutral detergent fiber; ADF = acid detergent fiber.

[1] Provided the following per kilogram of diet: vitamin A, 160 to 285 kIU; vitamin D_3, 51 to 97 kIU; vitamin E, ≥1,000 mg; vitamin K_3, ≥64 mg; vitamin B_2, ≥137 mg; vitamin B_6, ≥61 mg; vitamin B_{12}, ≥0.3 mg; niacin, ≥700 mg; pantothenic acid, ≥535 mg; folic acid, ≥30 mg; biotin, ≥4.6 mg; choline, ≥20 mg; Mn, 600 to 3,700 mg; Fe, 4,320 to 18,000 mg; Zn, 2,500 to 3,745 mg; I, 16 to 35 mg; Cu, 700 to 873 mg; Se, 5 to16 mg.

2.4. Histopathology of longissimus dorsi

Oil-red O staining of LD were performed by Bioyear Biological technology Co, Ltd. In brief, the LD tissues were cut and fixed with 4% paraformaldehyde, and embedded in paraffin. A 4-μm thick slice was stained with Oil-red O to investigate lipid droplets. Stained slices were scanned using the Pannoramic SCAN II and images were captured with 3DHISTECH software (3DHISTECH Ltd. Budapest, Hungary).

2.5. Fatty acid analysis

The lipids in LD were extracted with a methanol-chloroform (1:2, vol:vol) mixture as previous described. Total fatty acids from LD were methylated with sodium hydroxide methanol solution (Ci et al., 2014). The prepared methyl esters were determined to dissociate the fatty acid by gas chromatography-mass spectrometry (Shimadzu, GC-2010 Plus, Kyoto, Japan). The temperature program was as follows: the initial temperature of oven was set at 100 °C for 5 min and ceaselessly raised to 220 °C (10 °C/min). The parameters

Table 2
Primer sequences.

Target gene	Sequence (5′ to 3′)	Genebank access
PPARγ	F:GCCATTCGCATCTTTCA R:CAAACCCAGGGATGTTC	NM_214379
C/EBPβ	F:GACAAGCACAGCGACGAGTA R:AGCTGCTCCACCTTCTTCTG	NM_001199889
FAS	F: GTCCTGCTGAAGCCTAACTC R: TCCTTGGAACCGTCTGTG	EF589048
PPARγ 1 (ChIP)	F:AAAAGGCAAGGAAAGTAAGGC R: GAGTTTGAGCAGCAGTTTGG	
PPARγ 2 (ChIP)	F: AGGTGGTAACCATCCTCATAAA R: GCCTTACTTTCCTTGCCTTT	

PPARγ = peroxisome proliferator activated receptor γ; *C/EBPβ* = CCAAT enhancer binding protein β; *FAS* = fatty acid synthetase; *ChIP* = chromatin immunoprecipitation.

Table 3
Characteristics of litter and piglets from sows fed control and sodium butyrate diets.

Item	CON	SB
Litter birth weight, kg	17.23 ± 0.62	19.51 ± 0.48*
Total litter size	13.23 ± 0.42	13.48 ± 0.41
New born piglet BW, kg	1.32 ± 0.04	1.47 ± 0.04*
Piglet BW at 9 d, kg	2.58 ± 0.16	2.92 ± 0.15
Piglet BW at 21 d, kg	6.15 ± 0.16	6.83 ± 0.18*
LD weight at weaning, g	132.57 ± 4.89	132.43 ± 5.52

CON = control; SB = sodium butyrate; LD = longissimus dorsi.
*$P < 0.05$, compared with the control. The values are presented as means ± SEM.

Table 4
Biochemical parameters in plasma of newborn and weaning piglets from sows fed control diet or control diet supplementation with sodium butyrate (mmol/L).

Item	CON ($n = 8$)	SB ($n = 8$)
Newborn piglets		
Glu	3.6 ± 0.64	3.88 ± 0.41
TG	0.09 ± 0.01	0.14 ± 0.02*
CHOL	0.99 ± 0.10	1.10 ± 0.07
HDL-C	0.36 ± 0.04	0.43 ± 0.03
LDL-C	0.46 ± 0.05	0.49 ± 0.04
Weaning piglets		
Glu	7.18 ± 0.22	7.23 ± 0.60
TG	0.72 ± 0.15	0.92 ± 0.20
CHOL	3.91 ± 0.44	3.44 ± 0.20
HDL-C	1.55 ± 0.12	1.44 ± 0.08
LDL-C	2.09 ± 0.37	1.70 ± 0.17

CON = control; SB = sodium butyrate; Glu = glucose; TG = triglyceride; CHOL = cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
*$P < 0.05$, compared with the control. The values are presented as means ± SEM.

were as follows: injector temperature 200 °C, detector temperature 220 °C, the flow rate of the hydrogen carrier gas at 1.0 mL/min with the split ratio at 33:1 for the flame ionization detector. The concentrations of fatty acids were computed according to their peak areas relative to the standard. The fatty acids were quantified as the percentage of total fatty acids in the sample.

Table 5
Milk composition of sows fed control diet or control diet supplementation with sodium butyrate (SB).

Item	CON	SB
Total protein, mg/mL	194.37 ± 8.27	220.47 ± 13.69
Lactose, mg/mL	9.70 ± 0.89	12.70 ± 1.52
TG, mmol/L	29.18 ± 2.10	32.25 ± 1.58*
CHOL, mmol/L	1.21 ± 0.12	1.44 ± 0.12

CON = control; SB = sodium butyrate; TG = triglyceride; CHOL = cholesterol.
*$P < 0.05$, compared with the control. The values are presented as means ± SEM.

2.6. Total RNA isolation and real-time PCR

Total RNA was isolated from LD with TRIzol Reagent (Invitrogen Life Technologies, USA) and reverse transcribed according to the manufacturer's instructions (Takara, Tokyo, Japan). All primers (Table 2) were synthesized by Generay Biotech (Shanghai, China). Peptidyl-prolyl cis–trans isomerase A (PPIA) was chosen as a reference gene. The PCR program was as follows: initial denaturation (5 min at 95 °C), then an amplification stage (10 s at 95 °C, 30 s at 60 °C) was repeated 40 times, final stage (15 s at 95 °C, 60 s at 60 °C, 15 s at 95 °C).

2.7. Total protein extraction and Western blotting

Total protein was extracted from 100 mg of frozen LD as previously described (Liu et al., 2011). Protein concentration was measured with a Pierce BCA Protein Assay kit (no. 23225, Thermo Scientific). Western blot analysis of peroxisome proliferator activated receptor γ (PPARγ) (AP0686, Bioworld, USA), CCAAT enhancer binding protein β (C/EBPβ) (sc150X, Santa Cruz, USA), G-protein-coupled receptor 43 (GPR43) (sc32906, Santa Cruz, USA), fatty acid transport protein 36 (CD36) (BS7861, Bioword, USA). The glyceraldehyde phosphate dehydrogenase (GAPDH) (MB001H, Bioword, USA) was used as reference for total protein.

2.8. Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation analysis was carried out as in our previous article with several modifications (Sun et al., 2018). In brief, 200 mg of frozen LD was ground in liquid nitrogen and then resuspended using PBS with a protease inhibitor cocktail (Roche, Germany). One percent formaldehyde was used to cross-link protein and DNA, then terminated with 2.5 mol/L glycine. The pellets were flushed with PBS and lysed with sodium dodecyl sulfate lysis buffer containing protease inhibitors. Chromatin was sonicated on ice to 200 to 500 bp length DNA fragments and the protein-DNA complex was diluted using ChIP dilution buffer pre-cleared with salmon sperm DNA/protein G agarose beads (40 μL, 50% slurry, Santa Cruz, USA). The pre-cleared chromatin preparations and 2 μg of primary antibody (H3 acetyl K27, acH3K27, ab4729, Abcam, USA) were incubated overnight at 4 °C. A negative control was included with normal immunoglobulin G (IgG). Protein G agarose beads (40 μL, 50% slurry, Santa Cruz, USA) were added to capture the immunoprecipitated chromatin complexes. Lastly, reverse cross-linking at 65 °C for 1 h was carried out to release DNA fragments from the immunoprecipitated complexes which was then purified. Immunoprecipitated DNA was quantified by real-time PCR as described above with specific primers (Table 2).

2.9. Statistical analysis

All data were checked for normality using exploratory factor analysis. Data were analyzed by One-way ANOVA using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). The statistical model included the fixed effect of SB and the random effects of sows and litter size. For the analysis of colostrum biochemical parameters, sows were considered as the experimental unit. For the analysis of other data, piglets were considered as the experimental unit. The values were presented as the means ± SEM. Differences were considered statistically significant at $P < 0.05$.

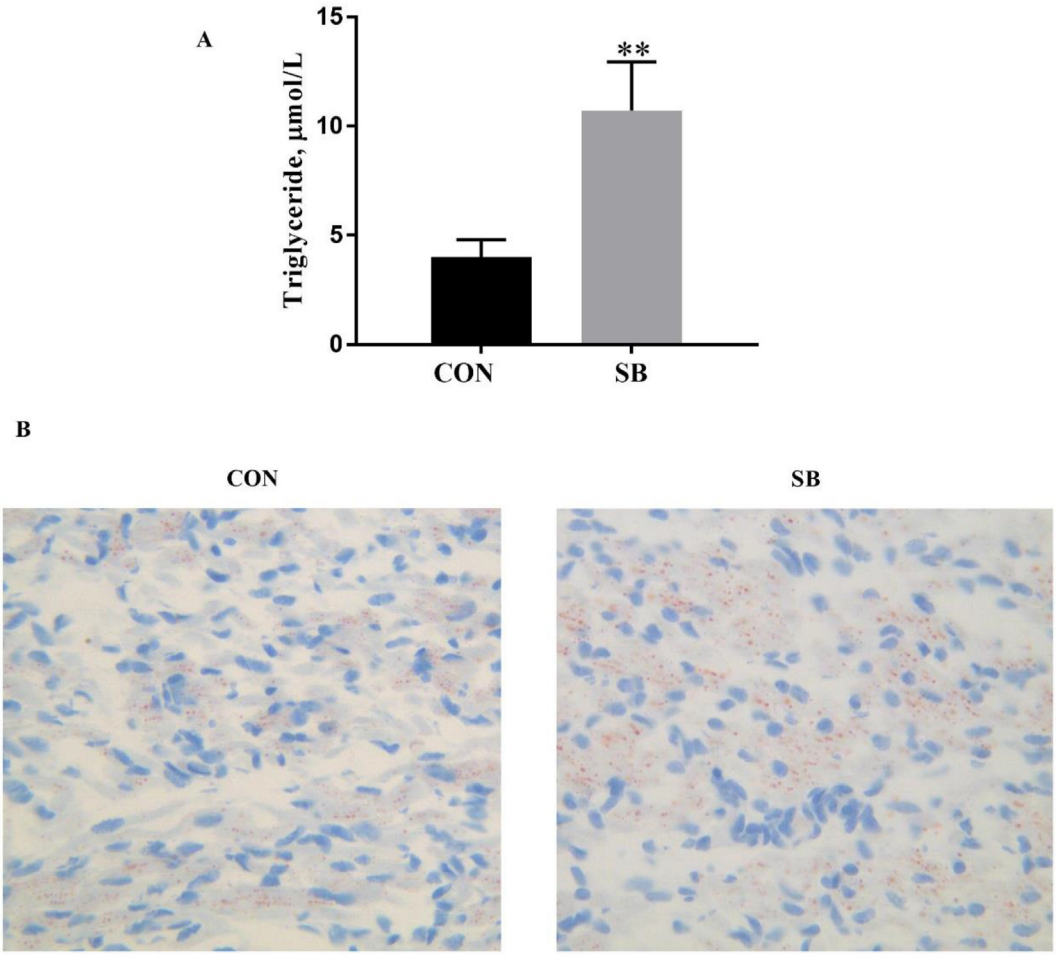

Fig. 1. The concentration of total triglyceride in longissimus dorsi of weaning piglets. (A) Intramuscular triglyceride. The values are presented as means ± SEM. $^{**}P < 0.01$, compared with the control. (B) Oil-red O staining. CON = control group; SB = sodium butyrate group.

3. Results

3.1. Offspring growth performance

In newborn piglets, the experiment butyrate diet affected ($P < 0.05$) the litter weight and body weight at birth (Table 3). However, there was no effect of gestation diet on total litter size. Piglets' body weight was influenced ($P < 0.05$) by sows' dietary treatment at 21 d, but no significant difference was observed at 9 d. Longissimus dorsi weight had no significant difference in weaning piglets between 2 groups.

3.2. Biochemical parameters in milk and plasma in newborn and weaning piglets

Maternal SB intake significantly increased ($P < 0.05$) the triglyceride level in plasma of newborn piglets but did not markedly alter the triglyceride level in plasma of weaning piglets compared with CON group. No significant effects were detected on the concentration of glucose, cholesterol, HDL-C and LDL-C both in newborn and weaning piglets between 2 groups (Table 4).

The milk from SB group contained higher ($P < 0.05$) triglyceride compared with CON group. However, the concentrations of total protein, lactose and cholesterol were not affected by the dietary treatment (Table 5).

3.3. Total triglyceride concentration in longissimus dorsi of weaning piglets

The concentration of triglyceride in LD was higher ($P < 0.05$) in SB group than in CON group (Fig. 1A). Oil-red O staining showed the abundance of intramuscular adipocytes in LD cross sections of SB group but not in that of CON group (Fig. 1B).

3.4. The lipid metabolism gene and protein expression in longissimus dorsi of weaning piglets

Both mRNA and protein expression of PPARγ in LD of weaning piglets were significantly ($P < 0.05$) increased (Fig. 2A and B), but *C/EBPβ* and fatty acid synthetase (*FAS*) gene expression did not exhibit any difference with butyrate supplementation (Fig. 2A). In addition, the protein expression of *GPR43* and *CD36* showed no marked change with maternal butyrate supplementation (Fig. 2B).

The enrichment of acH3K27 on the *PPARγ* promoter was determined with ChIP assay. H3 acetylation K27 on *PPARγ* promoter was significantly up-regulated ($P < 0.05$) in SB group (Fig. 3B).

3.5. The fatty acid composition in longissimus dorsi of weaning piglets

The fatty acid composition of LD at weaning stage was explored. The concentrations of C18:2n-6, C18:3n-3, total PUFA, n-6 PUFA and

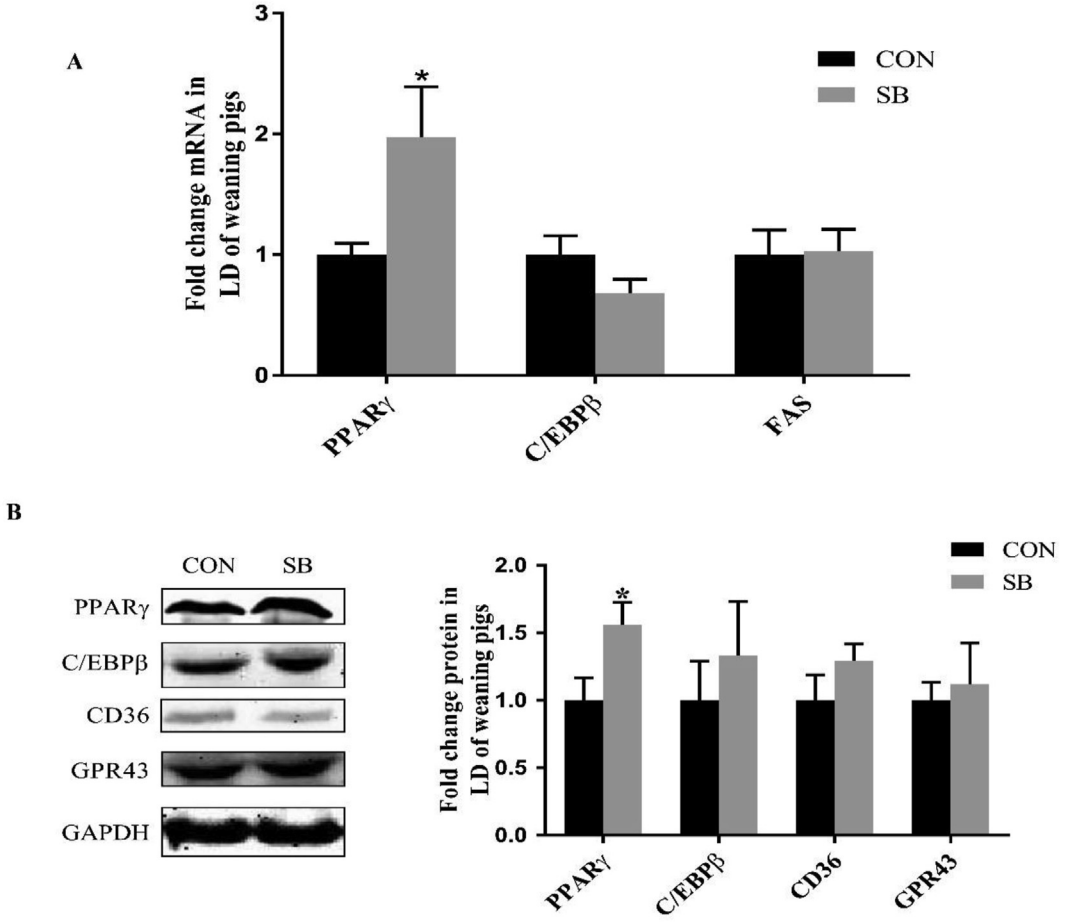

Fig. 2. The lipid metabolism in longissimus dorsi (LD) of weaning piglets. (A) The mRNA expression of lipid metabolism genes. (B) The protein expression of genes related to lipid metabolism. GAPDH was used for normalization. The values are presented as means ± SEM. *$P < 0.05$, compared with the control. CON = control group; SB = sodium butyrate group; PPARγ = peroxisome proliferator activated receptor γ; C/EBPβ = CCAAT enhancer binding protein β; FAS = fatty acid synthetase; CD36 = fatty acid transport protein; GPR43 = G-protein-coupled receptor 43; GAPDH = glyceraldehyde phosphate dehydrogenase.

n-3 PUFA exhibited a markedly decrease ($P < 0.05$) with maternal SB supplementation compared with CON group (Table 6).

4. Discussion

Maternal nutrients could influence the growth performance of their offspring in the early stage (Pantaleão et al., 2013; Max et al., 2014). A recent study demonstrated that maternal butyrate supplementation could enhance the growth rate of piglets (Karimi et al., 2016). In our study, we observed that the litter weight and body weight of piglets were significantly increased with maternal SB treatment though the total litter size did not show any significant differences.

The present study clarified that the plasma triglyceride concentration in SB group was significantly higher than that in CON group in newborn piglets. This result is consistent with the increased maternal milk triglyceride, and it may be inferred that the improved maternal milk triglyceride increased the transport plasma triglyceride in the offspring. A previous report demonstrated that maternal SB supplementation can increased the fatty acid transport in the placenta (Díaz et al., 2015). Short chain fatty acids (SCFA) such as butyrate, are precursors to lipogenesis used by the mammary gland (Theil et al.). We supplemented SB during the gestation and lactation period, and we infer that the maternal milk triglyceride was transported through the milk. How SB regulates triglyceride in the milk and plasma of sow is warranted further investigation.

The level of intramuscular triglyceride reflects meat quality such as meat tenderness, juiciness, flavor and taste (Wood et al., 2008). To further expound the impact of maternal butyrate supplementation on offspring lipid metabolism, we measured the triglyceride in the LD of offspring piglets. Our results showed that the concentration of triglyceride was significantly higher in butyrate supplementation group, which was consistent with oil-red O staining result. These results were associated with our previous study that maternal butyrate supplementation increased lipid accumulation in the skeletal muscle of weaning offspring rats (Huang et al., 2017). It is possible that the elevated offspring intramuscular lipid deposition in SB group resulted from the increased intramuscular lipid uptake from the milk.

PPARγ, a considered master regulator of adipogenesis, has been shown to be essential for adipogenesis both in vitro and in vivo (Tontonoz et al., 1994; Barak et al., 1999). In the previous study, dietary supplementation with SCFA prevented and reversed metabolic abnormalities induced by a high fat diet in mice by reducing the expression and activity of PPARγ (den Besten et al., 2015). In the present study, the increased levels of PPARγ and total triglyceride were found with maternal SB supplementation. Histone deacetylation was mediated by classical zinc-dependent histone deacetylase (HDAC) (Seto and Yoshida, 2014). Previous

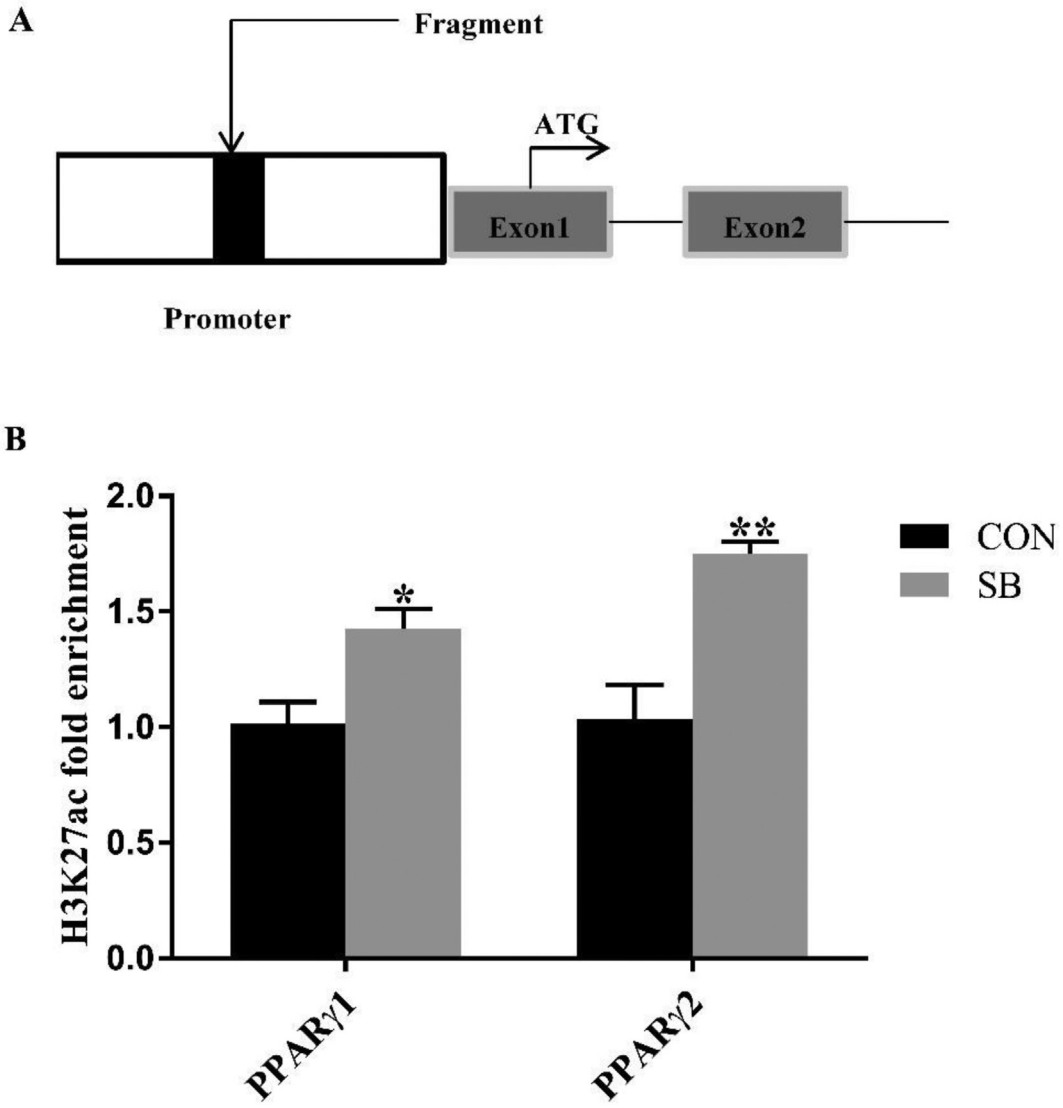

Fig. 3. Histone acetylation of H3K27 on the promoter of PPARγ. (A) The schematic diagram of ChIP assay. (B) Histone modifications on the gene promoter of PPARγ. The values are presented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the control. ATG is the DNA sequence corresponding to initiation codon. CON = control group, SB = sodium butyrate group; H3K27 = H3 acetyl K27; PPARγ = peroxisome proliferator activated receptor γ; ChIP = chromatin immunoprecipitation.

researches indicated butyrate regulated lipid metabolism and adipogenesis with inhibition of HDAC expression or activation of short chain fatty acid receptors (Samuel et al., 2008; Hong et al., 2016; Zhou et al., 2016). Our results supported that butyrate supplementation of mother could induce lipid accumulation in offspring skeletal muscle with increasing levels of the acetylation of H3 acetylation K27 (H3K27) on the promoters of *PPARγ* without change of *GPR43*. The mRNA and protein of *C/EBPβ* and *CD36* protein expressions did not show any changes with maternal SB diet. However, it should be mentioned that SB may affect other HDAC and change the acetylation condition of other histones apart from H3K27 detected in this study.

Fatty acid composition has a significant effect on the quality and nutritional value of meat. It is recommended to choose meat with a higher content of intramuscular fat and a balanced composition of fatty acids (DeVol et al., 1988). The benefits of polyunsaturated fatty acid (PUFA) over saturated fatty acid (SFA) are well recognized by the Food and Agriculture Organization. As well, decreasing the intake of SFA (known to increase total cholesterol and LDL-C) and increasing the intake of n-3 PUFA is highly encouraged. A previous study showed that the proportions of C18:2n-6 and C18:3n-3 were dramatically higher in pigs fed a high fiber diet during the growing phase (Gondret et al., 2014). Our current study indicated lower C18:2n-6, C18:3n-3, total PUFA, n-6 PUFA and n-3 PUFA concentrations in LD with maternal SB supplementation, which was not beneficial for consumers. We inferred that maternal SB supplementation may influence the activity of related enzyme, such as stearoyl-CoA desaturase, and further reduced the PUFA concentrations in the skeletal muscle of offspring. The underlying mechanism has been rarely reported. The muscle fatty acid composition at the finishing stage after maternal butyrate supplementation still needs further investigation.

Table 6
Fatty acid composition (percentage of total fatty acids) in longissimus dorsi of weaning piglets.

Item	Con	SB
C10:0	0.02 ± 0.00	0.02 ± 0.00
C14:0	1.55 ± 0.09	1.50 ± 0.09
C15:0	0.45 ± 0.10	0.62 ± 0.15
C16:0	26.19 ± 0.68	26.65 ± 0.49
C18:0	6.39 ± 0.35	7.10 ± 0.34
C20:0	0.07 ± 0.01	0.07 ± 0.01
C16:1	5.80 ± 0.44	5.68 ± 0.46
C17:1	0.33 ± 0.06	0.36 ± 0.04
C18:1	30.43 ± 1.66	30.30 ± 1.15
C20:1	0.34 ± 0.03	0.37 ± 0.02
C18:2n-6	21.19 ± 0.41	19.33 ± 0.60*
C18:3n-3	1.22 ± 0.09	0.96 ± 0.05*
C20:3n-6	0.17 ± 0.02	0.20 ± 0.02
C20:5n-3	0.04 ± 0.01	0.04 ± 0.01
C20:3n-3	0.12 ± 0.00	0.11 ± 0.00
C20:4n-6	1.02 ± 0.17	0.90 ± 0.36
SFA[1]	34.68 ± 1.09	35.95 ± 0.64
MUFA[2]	36.90 ± 1.57	36.71 ± 1.33
PUFA[3]	23.54 ± 0.60	21.54 ± 0.60*
n-6 PUFA[4]	21.19 ± 0.41	19.33 ± 0.60*
n-3 PUFA[5]	1.51 ± 0.08	1.27 ± 0.05*
n-6:n-3	14.20 ± 0.69	15.35 ± 0.63

CON = control group; SB = sodium butyrate group; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.
*$P < 0.05$, compared with the control. The values are presented as means ± SEM.
[1] SFA = C10:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0.
[2] MUFA = C16:1 + C17:1 + C18:1 + C20:1.
[3] PUFA = C18:2n-6*trans* + C18:2n-6*cis* + C18:3n-3 + C20:3n-6 + C20:5n-3 + C20:3n-3 + C20:4n-6.
[4] n-6 PUFA = C18:2n-6*trans* + C18:2n-6*cis*.
[5] n-3 PUFA = C18:3n-3 + C20:3n-6 + C20:3n-3.

5. Conclusion

Maternal SB supplement induced lipid accumulation with an increased expression of *PPARγ* by enhancing enrichment of the acetylation of H3K27 in offspring skeletal muscle at weaning age. Regarding fatty acid composition, the concentrations of C18:2n-6, C18:3n-3, total PUFA, n-6 PUFA and n-3 PUFA significantly decreased with maternal SB supplementation in LD of weaning piglets.

Author contributions

Yongsen Zhao contributed to biochemical parameters, data analysis and drafting of the manuscript. **Danping Wang** contributed to fatty acid assays. **Yanping Huang** contributed to lipid metabolism related genes and proteins analysis. **Dangdang Wu** and **Xiaoming Ji** were responsible for animal care, breeding and sampling. **Xiaobing Zhou** provided technical support. **Dong Xia** and **Xiaojing Yang** contributed to conception, experimental design, data interpretation and critical revision of the manuscript.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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