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Targeting gut microbiota-derived butyrate improves hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway in late pregnant sows†

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Short chain fatty acids (SCFAs) produced by gut microbiota affected hepatic glucose metabolism *via* the gut–liver axis. The present study aimed to investigate the effects of butyrate produced by gut microbiota on hepatic gluconeogenesis in late-pregnancy sows. A total of 240 primiparous sows in late pregnancy were tested for blood glucose using a glucose meter before feeding and grouped according to their blood glucose level as follows: 0–3.0 mmol L^{-1} (low blood glucose group, LG group) and 3.1–5.0 mmol L^{-1} (normal blood glucose group, NG group). Colonic SCFAs and microbiota, SCFAs in the portal vein and liver, and acetylation and phosphorylation levels in the liver samples were analyzed. Hepatocytes from pregnant sows were examined for the effect of butyrate on hepatic glucose gluconeogenesis. *In vivo* experiments showed that the reproductive performance, serum glucose metabolism index, colonic butyrate and butyrate-producing bacteria decreased in the LG group compared with the NG group. Correlation analysis found a positive correlation among colonic butyrate, butyrate-producing bacteria and the serum glucose metabolism index. Moreover, the hepatic cAMP concentration, PKA activity, GCN5 phosphorylation, and the expression of G6P and PEPCK were decreased and PGC1-α acetylation was increased in the LG group compared with the NG group. *In vitro*, sodium butyrate significantly stimulated the cAMP concentration, PKA activity, GCN5 phosphorylation, and the expression of G6P and PEPCK and inhibited PGC-1α acetylation in the LG group of hepatocytes from late-pregnancy sows. Interestingly, another *in vivo* experiment showed that dietary 1-kestose, a natural regulator of gut bacteria, significantly increased butyrate and butyrate-producing bacteria, and improved the reproductive performance and serum glucose metabolism index in late-pregnancy sows. Taken together, we found that targeting gut microbiota-derived butyrate could improve hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway in late-pregnancy sows.

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1. Introduction

Pregnancy has been described as a special physiological and metabolic process with significant changes in sow nutritional metabolism.[1] Glucose is the most important fuel for fetal growth and development, and late-pregnancy is the rapid growth stage of fetus.[2] The fetus's demand for glucose from the mother increases dramatically, so maternal glucose metabolism will be abnormal in late-pregnancy, causing large fluctuations in blood glucose levels.[3] Besides, maternal fasting blood glucose level is low due to increased energy requirements for rapid fetal growth and development in late pregnancy.[4] As a multiparous animal, the pig has a higher demand for glucose and energy. Thus, a low level of blood glucose causes long delivery and ultimately leads to weak labor and high stillbirth rate, and the maternal fetus presents a low weight status.[5] Therefore, understanding the reason for glucose metabolism disorders in late pregnancy is important for sow reproductive performance and newborn piglet health.

The liver plays a crucial role in maintaining glucose homeostasis *via* its physical function to store and secrete glucose.[6] Gluconeogenesis generates glucose from non-carbohydrate carbon substrates such as pyruvate, glycerin and lactate. Glucose-6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK) are the key enzymes of gluconeogenesis. The expression of G6P and PEPCK is controlled by various transcription factors and signaling pathways that respond to

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changes of nutrient availability. PPAR-γ co-activator 1α (PGC-1α) acts as a key modulator in the regulation of PEPCK and G6P expression,[7,8] which is a determinant for gluconeogenesis. General control non-repressed protein 5 (GCN5, also known as KAT2A) acetylates PGC-1α and inhibits its transcriptional and metabolic function as a coactivator of genes involved in gluconeogenesis.[9] Importantly, a recent study showed that glucose homeostasis is maintained by activating the phosphorylation of GCN5 and thereby inhibiting PGC-1α acetylation.[10,11] Additionally, GCN5 as a nutrient sensor mainly participates in energy metabolism and mitochondrial biogenesis through PGC-1α acetylation.[12] Pregnancy is an extremely complex physiological process, resulting in redistribution of nutrients, and GCN5 may change correspondingly during this special period. However, there is no relevant literature that shows GCN5 regulates hepatic glucose metabolism in pregnant sows.

Recently, numerous studies have shown that intermediates derived from gut microbiota play an important role in energy homeostasis;[13] increasing energy harvest *via* colonic fermentation and short-chain fatty acid (SCFA) production is the most direct.[14] Among the SCFAs, butyrate is a key regulator in mediating microbiota metabolism[15,16] and an energy substrate for both colonocytes and enterocytes.[17] Research has indicated that butyrate promotes hepatic gluconeogenesis to maintain glucose homeostasis in mice.[18] Therefore, studies on regulators targeting butyrate from gut microbiota can be relevant. Recently, 1-kestose has been shown to have impressive potential as a new prebiotic targeting *Faecalibacterium* and *Bifidobacteria* to produce butyrate.[19] However, the effect of dietary supplementation with 1-kestose on hepatic glucose metabolism in late-pregnancy sows has rarely been reported. Herein, the first purpose of this study was to investigate the association of colonic butyrate, butyrate-producing bacteria and hepatic gluconeogenesis in pregnant sows with normal or low blood glucose. A secondary outcome of this study was to evaluate the effect of dietary 1-kestose on hepatic gluconeogenesis through microbiota-derived butyrate *via* the gut–liver axis in late-pregnancy sows.

2. Materials and methods

The experiment was approved by the Animal Care and Use Committee of Huazhong Agricultural University and conformed to the guidelines of the National Research Council for the Care and Use of Laboratory Animals.

2.1 Experimental design and diets

In this study, a total of 240 primiparous sows were herded in Wudang Farmer Animal Husbandry Co., Ltd., located in Fangxian, Shiyan, Hubei Province, China. After insemination, the sows were housed individually in crates (2.4 × 0.7 m) from day 1 to day 100 of pregnancy and then were housed in farrowing crates (2.2 × 1.8 m) until weaning. Throughout the experimental period, there was no antibiotic or probiotic use, and diet intake changed with the body condition of sows (fed at 7:00 and 14:30 each day), and water was provided freely. The sows were fed 2.0 kg of pregnancy diets during 85–104 days of pregnancy, and fed 1.0 kg of pregnancy diets before a week of parturition. After farrowing, the sows were fasted for 12 h to collect samples. The sows in late pregnancy (gestation at 85–100 d) with first litters were tested for blood glucose using a glucose meter before feeding and grouped according to their blood glucose level as follows: 0–3.0 mmol L^{-1} (low blood glucose group, LG group), 3.1–5.0 mmol L^{-1} (normal blood glucose group, NG group), and 5.1–6.0 mmol L^{-1} (high blood glucose group, HG group), which accounts for 29.56%, 67.83%, and 2.61%, respectively.

For further feeding experiments, 100 pregnant sows at 85 d of gestation with second litters were randomly allotted to 5 groups: NG group, LG group, LG + low kes group, LG + med kes group and LG + high kes group, respectively. The NG group and LG group were fed a basal diet, and the LG + low kes group, LG + med kes group and LG + high kes group were fed a basal diet supplemented with 1000 mg kg^{-1}, 3000 mg kg^{-1} and 5000 mg kg^{-1} 1-kestose. 1-Kestose (purified >98%) was purchased from B Food Science Co., Ltd (Chita, Aichi, Japan). The basal diet was formulated based on (NRC, 2012) meeting nutritional requirements, and the ingredients and composition of the basal diet are listed in Table S1.† Housing and breeding management of the experimental animals were carried out as described earlier in detail.[20]

2.2 Sample collection

Farrowing was not induced and no attempts were made to interfere with the natural delivery of the piglets. The reproductive performance of the sows was recorded at delivery, such as the duration of farrowing, litter size and the number of stillbirths, and the number of mummified fetuses (early or middle gestation deaths) was neglected. After farrowing, the average piglet weight/litter was recorded. Then, ten sows per group were deprived of food for 12 h, and blood samples were collected into ordinary vacuum tubes by jugular venipuncture. Sows were euthanized through chemical sedation by Telazol administration (0.1 ml kg^{-1}) *via* intramuscular injection followed by exsanguination. Next, colonic digesta and blood samples from the hepatic portal veins and livers of sows were collected, and the colonic digesta and livers were stored in liquid nitrogen and stored at −80 °C for further analysis. Finally, blood and fecal samples from the subsequent feeding experiment were collected from ten sows per group for further analysis. Blood samples were centrifuged for 15 min at 3000*g* at normal temperature to obtain the serum, and then stored at −80 °C until analysis.

2.3 Serum glucose metabolism index

Serum samples were assayed for glucose, triglyceride, lactate, glycerin, and pyruvate by using commercial kits (Nanjing Jiancheng Bioengineering company, Jiangsu, China) following the manufacturer's procedures using a spectrophotometer (Mepda Instrument Co., Ltd, Shanghai, China).

2.4 16S rRNA gene sequencing and sequence analysis

Colonic samples were extracted using total DNA from each sample (Omega, St Louis, MO, USA). DNA was amplified using primers (forward: 5′-ACTCCTACGGGAGGCAGCA-3′ and reverse: 5′-GGACTACHVGGGTWTCTAAT-3′), targeting the variable 3–4 (V3–V4) region of 16S rRNA. The microbial genomic DNA was used to form a sequencing library (Illumina HiSeq 2500). The original image data files obtained using Illumina HiSeq platforms (Illumina, San Diego, California, USA) were imported into sequenced reads, and the results were stored in the FASTQ file format. The number of reads for 16S rRNA sequencing is shown in Table S2.† The relative abundances of the different groups at the phylum, class, order, family, and genus levels were determined. Rarefaction analysis was performed by determining the alpha diversity indices, which included the calculation of the Chao, ACE, Shannon, and Simpson indices. For beta diversity analysis, principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) were performed using QIIME. The dominant bacterial community difference between groups was detected using LDA effect size.

2.5 SCFAs in the colon and feces

SCFA content was determined using gas chromatography as in a previous study.[21] Colonic contents (50 mg) were added to 1 mL of water, vortexed, and then mixed with 50% sulfuric acid and 1.6 mL of diethyl. SCFAs were extracted by incubation in ice water for 20 min. After centrifugation (8000*g*, 5 min, 4 °C), the supernatant was collected for analysis. An HP-FFAP capillary column (Agilent, Folsom, USA) coupled to a SHIMADZU GC2030-QP2020 NX gas chromatograph–mass spectrometer (Shimadzu, Kyoto, Japan) and flame ionization detector was used. The front inlet purge flow was 3 mL min^{-1}, and the gas flow rate was maintained at 1 mL min^{-1}. The initial temperature was 50 °C for 3 min, and the injector and detector temperatures were 250 and 270 °C, respectively. The mass spectrometry data were obtained in the Scan/SIM mode.

2.6 SCFAs in the portal vein and liver

The portal vein and liver samples were deproteinized and extracted according to a previous study.[22] 10% (w/v) sample homogenates were prepared by adding 9 times ultrapure water to each tube containing the experimental sample and mixed intermittently using a vortex mixer for 2 min. The supernatant was collected after centrifuging at 4800*g* for 20 min, and then analyzed by injecting into a chromatographic system. Chromatographic analysis was carried out using an Agilent 6890N gas chromatography system equipped with a flame ionization detector and an N10149 automatic liquid sampler (Palo Alto, California, USA). Total SCFA concentrations were analyzed according to a protocol described previously.[23]

2.7 Hepatocyte culture and treatment

Isolation of primary hepatocytes was performed by collagenase perfusion and mechanical destruction in the livers of pregnant sows.[24] Hepatocytes were cultured according to a previous study.[25] For the experiments, hepatocytes of the NG and LG group from late-pregnancy sows were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Millipore, USA) and penicillin/streptomycin at 37 °C under 5% CO_2. Hepatocyte of the LG group from late-pregnancy sows was incubated with or without sodium butyrate (NaB, 100 mg, Sigma-Aldrich), PKA-specific inhibitor H89 (10 μmol, Sigma-Aldrich) and GCN5-specific inhibitor, MB3 (100 μmol, Santa Cruz) for 6 h according to a previous study[18] and was divided further into four subgroups: LG + NaB, LG + H89 + NaB, LG + MB3 and LG + MB3 + NaB groups.

2.8 Cell transfection

Hepatocyte of the LG + NaB group was seeded in 6-cm dishes at 3×10^4 cells and cultured in medium without antibiotics for 24 hours. Chemically modified Stealth small interfering RNA (siRNA) targeting GCN5 and the control siRNA were purchased from Guangzhou RiboBio (RiboBio, Guangzhou, China) and transfected into cells using Lipofectamine 2000 (Invitrogen, Life Technologies, CA, USA) according to the manufacturer's instructions. Hepatocytes were transfected with siRNA at a concentration of 20 nmol L^{-1}. The sequences of siRNA are as follows: siGCN5-1 5′-GGAAAUGCAUCCUGCAGAU-3′ and siGCN5-2 5′-GAGGCCUCAUUGACAAGUA-3′. Thirty-six hours after transfection, hepatocytes were treated with DMSO or 10 μg mL^{-1} bleomycin and incubated for an additional 36 hours. To confirm GCN5 knockdown, western blotting was performed to analyze the GCN5 expression.

2.9 Adenosine triphosphate (ATP) assay

The amount of ATP was measured by the luciferin-luciferase method according to the protocol of the ATP detection kit (Beyotime Institute of Biotechnology, Inc., Haimen, China). Hepatocytes were seeded onto 24-well plates at 2.5×10^5 cells per well. After 24 h, these hepatocytes received the same dexamethasone pre-stimulation as described in the glucose production assay. The hepatocytes were then treated with different concentrations of NaB (0, 0.1, 1, 5 and 10 mmol L^{-1}) for 1 h prior to lysis with lysis buffer (200 μL per well) from the ATP detection kit. After centrifugation at 12 000*g* for 5 min at 4 °C, the supernatant was transferred to a fresh tube for the ATP test. The luminescence of a 20 μL sample was assayed using a luminometer (PerkinElmer, Inc., Waltham, USA) together with 100 μL ATP detection buffer from the ATP detection kit. The protein concentration was determined using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). The concentration of ATP was normalized to that of the protein in the same cell lysate.

2.10 Gluconeogenesis measurements

For the measurements of the gluconeogenesis level, the culture medium was replaced with glucose-free DMEM containing 0.1% BSA. Then, all the groups were incubated for 1.5 h. The culture medium was abandoned and replaced with or without the gluconeogenic substrate (20 mmol L^{-1} sodium

lactate and 2 mmol L^{-1} sodium pyruvate, Sigma-Aldrich) for 4 hours. The glucose concentration was measured using a glucose kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) and normalized to the protein content of the same samples. Hepatocyte gluconeogenesis is determined by containing gluconeogenic substrate minus non-gluconeogenic substrate.

2.11 Glucose production and glycogen measurements

For the measurements of glucose production, all the groups were washed and incubated in glucose production media (glucose- and phenol-free DMEM containing 20 mmol sodium lactate and 2 mmol sodium pyruvate) for 3 h. Glucose released in the media was measured using a glucose assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) and normalized to the protein content of the same samples. For the measurements of the glycogen level, all the groups were measured using a glycogen assay kit (Abcam, Cambridgeshire, UK) according to the manufacturer's instructions.

2.12 ELISA detection of cAMP concentration and PKA kinase activity

After treatment, the levels of cAMP concentration and PKA activity were detected using competitive ELISA kits (Invitrogen, Carlsbad, USA). The cAMP ELISA kit (Cat. EMSCAMPL) and PKA ELISA kit (Cat. EIAPKA) were used in this study according to the manufacturer's instructions. Samples of cell lysates were prepared exactly as described by the manufacturer.

2.13 Quantitative real-time PCR (RT-qPCR) analysis

Total RNA was isolated from the liver homogenized under liquid nitrogen, and mRNA was extracted according to a previous method.[26] The relative mRNA expression levels of β-actin and the primers (ESI Table S3†) of the tested genes were mentioned according to a previous study.[27] Fold changes in the mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Fecal samples were collected for the RT-qPCR assay. The species-specific PCR primers are listed in Table S4.† RT-qPCR was performed using an iCycler IQ real-time detection system using the iCycler optical system interface software version 2.3 (Bio-Rad, Veenendaal, Netherlands) as previously described.[28] Details of the analyses were described previously.[29]

2.14 Western blotting

Total proteins from the liver or hepatocytes were extracted using RIPA lysis buffer. Protein samples were separated using 10% sodium dodecyl sulfate-polyacrylamide and then transferred to PVDF membranes (pore size: 0.45 μm). The membranes were incubated with primary antibodies including GCN5, p-GCN5, PGC-1α, PEPCK, G6P, and β-actin at 4 °C overnight and then washed with TBST [50 mM Tris-HCl (pH 7.5), 150 mmol NaCl and 0.05% Tween 20], which is reported in ESI Table S5.† For determining acetylation of the proteins by western blotting, 50 mM Tris (pH 7.5) with 10% (vol/vol) Tween-20 and 1% peptone (AMRESCO) were used for blocking, and 50 mM Tris (pH 7.5) with 0.1% peptone was used to prepare the primary and secondary antibodies. Finally, the membranes were visualized using an ECL system from Santa Cruz (Dallas, TX). The relative expression of protein was analyzed using ImageJ software according to a previous study.[30]

2.15 Statistical analysis

Data analyses and graph preparation were performed using Excel 2019, SPSS 25 (Chicago, Illinois, USA), R package ggplot2,[31] and GraphPad Prism ver. 8.0 (San Diego, USA). The reproductive performance of pregnant sows, serum glucose metabolism index, colonic SCFAs, and alpha diversity of the microbiota were analyzed by the independent samples *T*-test. The structural variation of the microbial community among samples was analyzed by beta diversity analysis (PERMANOVA).[32] Spearman's correlation analysis between colonic SCFAs, the serum glucose metabolism index and the relative abundance of different microbial genera was performed using the R package.[33] For hepatocyte level analysis, one-way analysis of variance was used (ANOVA, by Duncan and LSD). All data were presented as mean ± standard error of mean (SEM) and considered significant at $P < 0.05$. Trends were identified when $0.05 < P < 0.10$.

3. Results

3.1 Low fasting blood glucose levels affected the reproductive performance in late-pregnancy sows

The productive performance of sows is shown in Table 1. Birth mortality ($P < 0.05$), estrus interval ($P < 0.05$), stillbirth ($0.05 < P < 0.1$) and the duration of farrowing ($P < 0.05$) were increased in the LG group compared with the NG group. The serum glucose metabolism index is shown in Fig. 1. In contrast to the NG group, serum lactate ($P < 0.05$; Fig. 1A), glycerin ($P < 0.05$; Fig. 1C), glucose ($P < 0.01$; Fig. 1E), triglyceride ($P < 0.01$; Fig. 1D), and pyruvate ($P < 0.01$; Fig. 1B) were decreased in the LG group.

3.2 Low fasting blood glucose levels altered colonic SCFAs in late-pregnancy sows

Colonic SCFAs are shown in Table 2. Branched chain fatty acids (BCFAs) including isobutyrate and isovalerate and straight chain fatty acids (SCFAs) including acetate, propionate, butyrate, and valerate were observed. In contrast to the NG group, butyrate ($P < 0.05$), valerate ($P < 0.05$) and BCFAs/SCFAs ($0.05 < P < 0.1$) were decreased in the LG group.

3.3 Low fasting blood glucose levels shaped colonic microbiota in late-pregnancy sows

To analyze the changes of colonic microbial communities between the NG group and the LG group, we evaluated the alpha diversity of the microbial communities. The results of the Chao, Shannon, ACE and Simpson indices are shown in Table 3. In contrast to the alpha diversity indices, however, there were no significant differences between the NG and LG groups.

Table 1 Low fasting blood glucose levels affected the reproductive performance in late-pregnancy sows

Items	NG	LG	*P*-value
Litter size	9.51 ± 0.24	9.93 ± 0.34	0.307
Weak piglets	1.73 ± 0.18	1.57 ± 0.30	0.633
Mummified	1.20 ± 0.20	1.50 ± 0.26	0.443
Stillborn	1.00 ± 0.09	1.33 ± 0.16	0.081
Birth mortality (%)	2.00 ± 0.86^{b}	4.12 ± 1.52^{a}	0.043
Number of litters born alive	8.92 ± 0.44	8.97 ± 0.42	0.985
Estrus interval (d)	5.14 ± 0.36^{b}	6.57 ± 0.35^{a}	0.016
Duration of farrowing (h)	3.10 ± 0.04^{b}	4.13 ± 0.07^{a}	0.021
Mean weight of litters born alive per piglet (kg)	1.47 ± 0.16	1.42 ± 0.12	0.826
Litter (alive) weight at birth per piglet (kg)	12.75 ± 0.29	12.04 ± 0.32	0.889

Data are means of 40 sows per treatment. Values in the same row with different superscripts are significantly different ($P < 0.05$), as determined by the independent samples *T*-test. Abbreviations: NG: normal blood glucose; LG: low blood glucose.

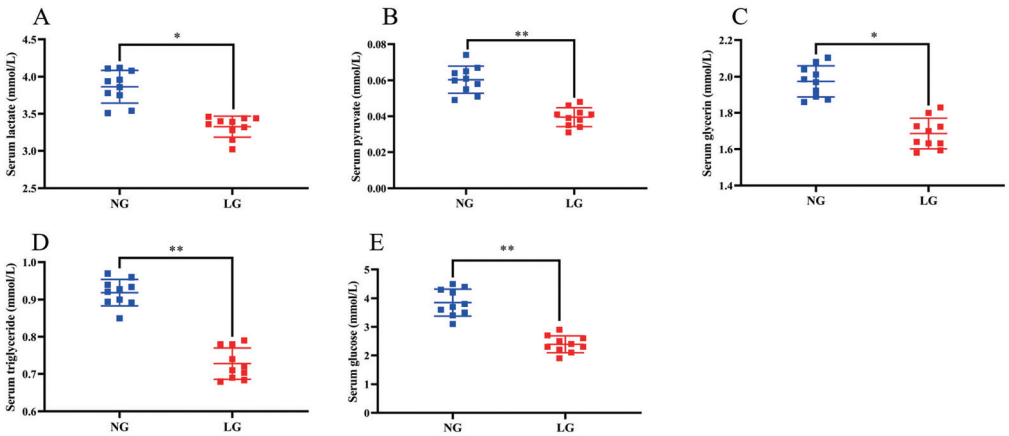

Fig. 1 Low fasting blood glucose levels affected the serum glucose metabolism index in late-pregnancy sows. (A) Serum lactate; (B) serum pyruvate; (C) serum glycerin; (D) serum triglyceride; and (E) serum glucose. Data are means of 10 sows per treatment; *$P < 0.05$ and **$P < 0.01$.

Table 2 Low fasting blood glucose levels altered colonic short chain fatty acids in late-pregnancy sows

Items (mg L^{-1})	NG	LG	*P*-value
Acetate	1145.84 ± 91.13	1214.00 ± 70.09	0.499
Propionate	1076.53 ± 96.39	1171.68 ± 80.53	0.369
Isobutyrate	198.42 ± 16.10	175.40 ± 11.13	0.267
Butyrate	693.29 ± 18.64^{a}	609.36 ± 27.18^{b}	0.029
Isovalerate	213.53 ± 17.36	180.34 ± 14.20	0.170
Valerate	202.91 ± 18.22^{a}	130.64 ± 7.14^{b}	0.004
SCFAs	3118.56 ± 203.25	3125.67 ± 213.56	0.974
BCFAs	411.95 ± 33.38	355.74 ± 24.92	0.207
BCFAs/SCFAs	0.132 ± 0.007	0.113 ± 0.006	0.072
A/P	1.078 ± 0.058	1.036 ± 0.016	0.501
Total short chain fatty acids	3530.51 ± 230.69	3481.41 ± 225.26	0.847

Data are means of 10 sows per treatment. Values in the same row with different superscripts are significantly different ($P < 0.05$), as determined by the independent samples *T*-test. Abbreviations: A/P: acetate/propionate; BCFAs: branched chain fatty acids, including isobutyrate and isovalerate; SCFAs: straight chain fatty acids, including acetate, propionate, butyrate, and valerate; NG: normal blood glucose; LG: low blood glucose.

Table 3 Low fasting blood glucose levels shaped the fecal microbiota community profiles in late-pregnancy sows

Alpha-diversity indices	NG	LG	*P*-value
PD	31.08 ± 0.20	30.71 ± 0.13	0.157
Shannon	5.93 ± 0.19	5.69 ± 0.18	0.392
Simpson	0.93 ± 0.01	0.91 ± 0.01	0.273
ACE	576.19 ± 1.85	580.29 ± 3.29	0.302
Chao	584.84 ± 2.84	587.95 ± 4.38	0.565

Data are means of 10 sows per treatment. Values in the same row with different superscripts are significantly different ($P < 0.05$), as determined by the independent samples *T*-test. The richness estimators (ACE and Chao) and diversity indices (Shannon and Simpson) were calculated using the mothur program. Abbreviations: PD: phylogenetic distance (whole tree); NG: normal blood glucose; LG: low blood glucose.

To evaluate how colonic microbial taxonomic compositions at the phylum and genus levels altered between the NG and LG groups, a Metastat analysis was performed to identify the differentially abundant phyla and genera between the groups. We chose to show the abundance distribution of the first 10 classification units

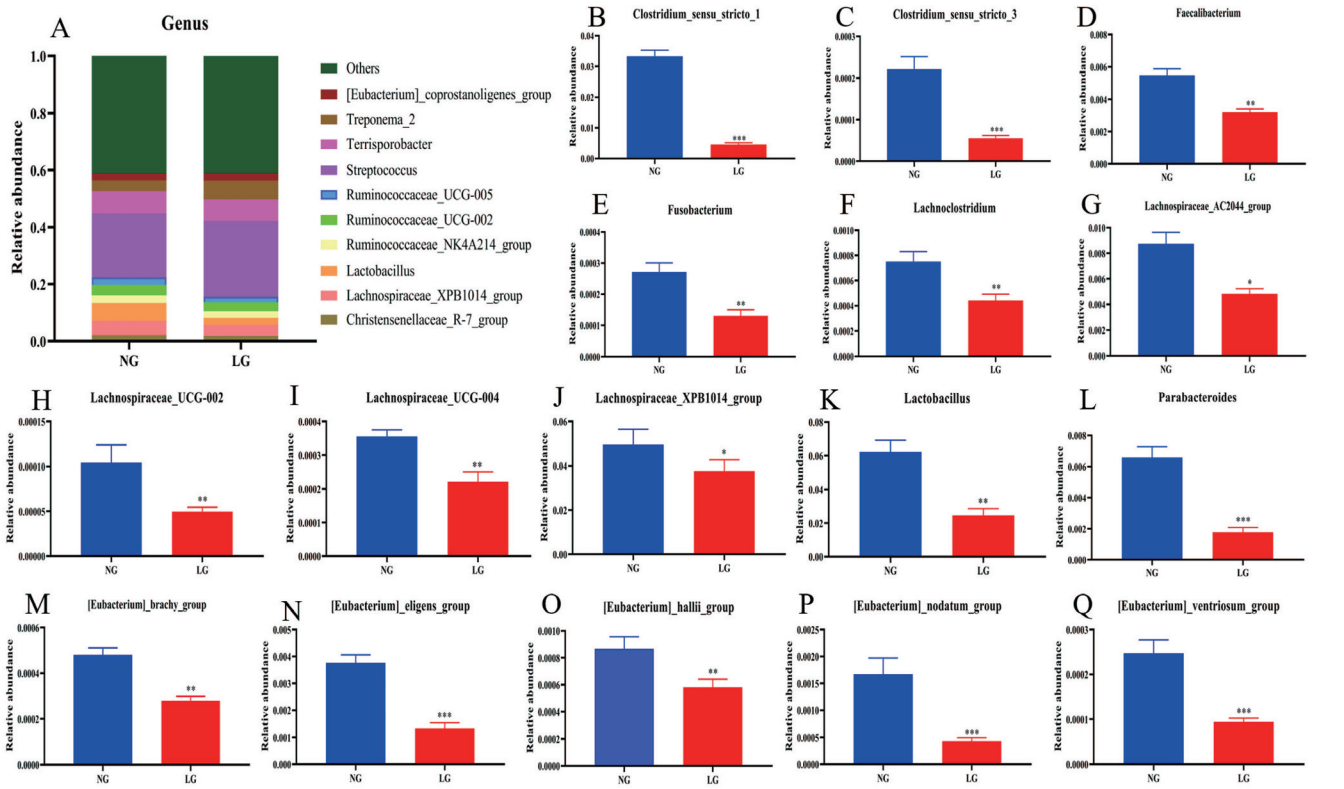

Fig. 2 Low fasting blood glucose levels shaped the colonic microbiota at the genus level in late-pregnancy sows. Relative abundance of the top 10 genera in each group (A) and the significant relative abundance of bacteria that was associated with butyrate and low blood glucose (B–Q). Data are means of 10 sows per treatment; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

with the most significant differences between the NG and LG groups. Then, we picked out the bacteria associated with butyrate and low blood glucose (Fig. 2). The relative abundances of 8 genera (*Clostridium_sensu_stricto_1*, *Clostridium_sensu_stricto_3*, *Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *[Eubacterium]_hallii_group*, *Bifidobacterium*, and *Lachnospiraceae_AC2044_group*) significantly decreased ($P < 0.05$), while the relative abundance of Streptococcaceae increased ($P < 0.05$) in the LG group compared with the NG group. Detailed results of the colonic microbiota changes are available in Fig. S1–4.†

To further understand the differences among the microbiota from the colonic samples, beta diversity was estimated. PCoA was performed for each group, and resampling was performed repeatedly on a subset of the available data of each sample evenly to measure the robustness of individual clusters in the PCoA plots. NMDS can assess the between group distance in the colonic bacterial community structure. The results of both NMDS and PCoA plots showed no significantly different composition of microbiota between the NG and LG groups (Fig. S5†).

3.4 Correlation between colonic microbiota, the serum glucose metabolism index and colonic SCFAs in late-pregnancy sows

Spearman's correlation analysis was performed to evaluate the potential link between the colonic microbiota, SCFAs and the glucose metabolism index. As shown in Fig. 3A, there were positive correlations ($P < 0.05$) between the colonic microbiota and SCFAs, including *Clostridium_sensu_stricto_1*, *Clostridium_sensu_stricto_3*, *Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *[Eubacterium]_hallii_group*, Bifidobacteriaceae, *Lachnospiraceae_AC2044_group* and butyrate, and negative correlations ($P < 0.05$) between Streptococcaceae and butyrate. Moreover, *Lactobacillus* and *[Eubacterium]_ventriosum_group* were positively correlated ($P < 0.05$) with valerate.

The correlation between the colonic microbiota and glucose metabolism index is presented in Fig. 3B. The relative abundances of *Clostridium_sensu_stricto_1*, *Clostridium_sensu_stricto_3*, *Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *[Eubacterium]_hallii_group*, Bifidobacteriaceae, and *Lachnospiraceae_AC2044_group* were positively correlated ($P < 0.05$) with glucose, and Streptococcaceae was negatively correlated ($P < 0.05$) with glucose. Moreover, *Faecalibacterium*, *Lactobacillus*, *Lachnospiraceae_AC2044_group*, *Clostridium_sensu_stricto_1*, *Clostridium_sensu_stricto_3*, Bifidobacteriaceae, and *[Eubacterium]_hallii_group* were positively correlated ($P < 0.05$) with pyruvate, and Streptococcaceae was negatively correlated ($P < 0.05$) with pyruvate. *Clostridium_sensu_stricto_1* and *[Eubacterium]_brachy_group* were positively correlated ($P < 0.05$) with glycerin.

The correlation between colonic SCFAs and the serum glucose metabolism index is presented in Fig. 3C. There were positive ($P < 0.05$) correlations between butyrate and glucose/pyruvate.

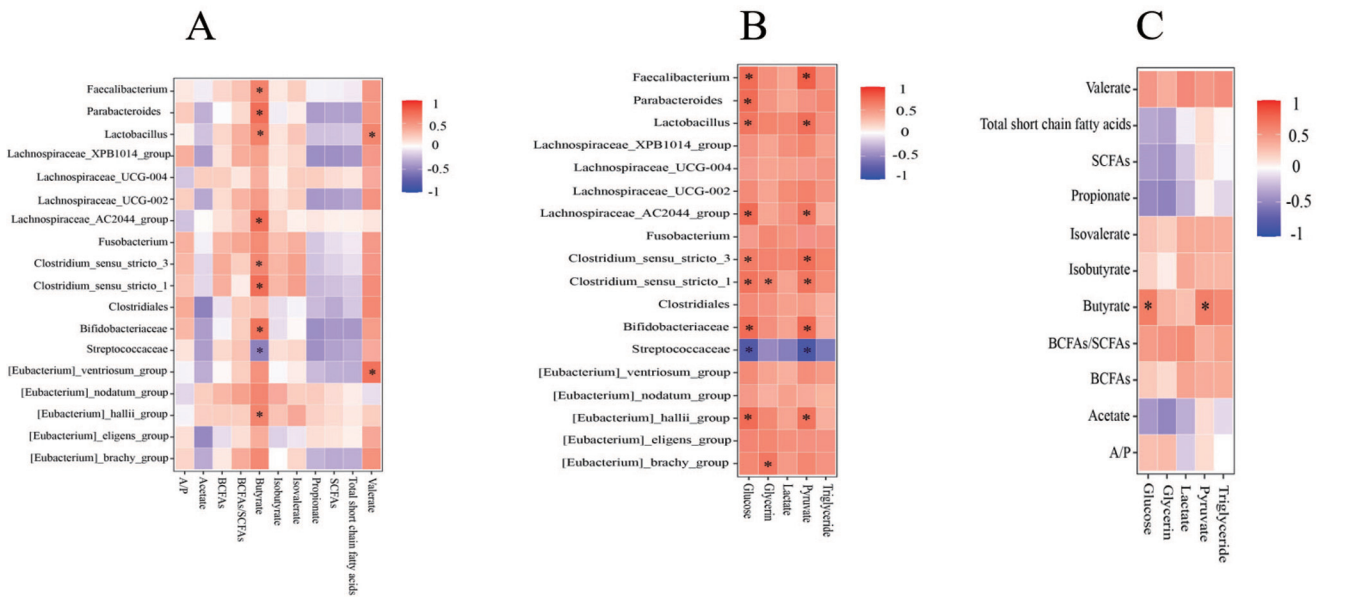

Fig. 3 Correlation between the colonic microbiota, serum glucose metabolism index and colonic short chain fatty acids (SCFAs) in late-pregnancy sows. Colonic microbiota is presented to be correlated with colonic SCFAs (A), the serum glucose metabolism index is presented to be correlated with colonic microbiota (B) and the serum glucose metabolism index is presented to be correlated with colonic SCFAs (C), respectively. A/P: acetate/propionate; BCFAs: branched chain fatty acids, including isobutyrate and isovalerate; SCFAs: straight chain fatty acids, including acetate, propionate, butyrate, and valerate. The color scale represents the strength of correlation, ranging from 1 (strong positive correlation) to –1 (strong negative correlation); *$P < 0.05$.

3.5 Microbiota-derived butyrate altered hepatic gluconeogenesis in late-pregnancy sows

Using metabolomics allowed the identification of gut microbiota derived-SCFAs in the hepatic portal vein and liver. Compared with the NG group, butyrate in the hepatic portal vein and liver was decreased ($P < 0.05$) in the LG group (Table 4). Then, the effect of butyrate on hepatic gluconeogenesis was investigated. The mRNA and protein levels of PEPCK and G6P and the GCN5 phosphorylation level were decreased ($P < 0.05$) and the PGC-1α acetylation level was increased ($P < 0.05$) in the LG group compared with the NG group (Fig. 4C–I). Moreover, the concentration of cAMP and the activity of PKA were decreased ($P < 0.05$) in the LG group compared with the NG group (Fig. 4A and B).

3.6 Butyrate promoted hepatocellular gluconeogenesis in late-pregnancy sows

As ATP is the substrate for cAMP production, the present study first detected the intracellular ATP concentration after primary hepatocytes from the LG group were incubated with various concentrations of NaB for 1 h. Our results showed that NaB treatment resulted in the accumulation of intracellular ATP in a dose-dependent manner, exhibiting a significant effect at the concentration of 1 mmol ($P < 0.05$) (Fig. 5A). Then, we incubated primary hepatocytes from the LG group for 6 h with 5 mmol NaB to investigate the effects of butyrate on hepatic gluconeogenesis. Glucose production and glycogen and gluconeogenesis levels decreased ($P < 0.05$) in the LG group of hepatocytes from late-pregnancy sows; however, NaB treatment increased ($P < 0.05$) glucose production and glycogen and gluconeogenesis levels in the LG group of hepatocytes from late-pregnancy sows (Fig. 5B–D). These results suggested that butyrate increases hepatic gluconeogenesis and promotes the production of glucose, which is ultimately stored in the liver in the form of glycogen. Moreover, the concentration of cAMP and activity of PKA, the GCN5 phosphorylation level, and the protein and mRNA levels of PEPCK and G6P were decreased ($P < 0.05$) and the PGC-1α acetylation level was increased ($P <$

Table 4 Low fasting blood glucose levels altered short chain fatty acids in the hepatic portal vein and liver in late-pregnancy sows

	Hepatic portal vein (μmol L^{-1})			Liver (mmol L^{-1})		
	NG	LG	*P*-value	NG	LG	*P*-value
Acetate	156.37 ± 9.23	160.03 ± 8.76	0.892	9.34 ± 0.21	9.27 ± 0.31	0.773
Propionate	14.40 ± 1.05	14.05 ± 1.14	0.725	11.14 ± 0.13	11.15 ± 0.12	0.897
Butyrate	15.04 ± 1.16[a]	11.13 ± 1.02[b]	0.031	7.37 ± 0.14[a]	5.82 ± 0.08[b]	0.029

Data are means of 10 sows per treatment. Values in the same row with different superscripts are significantly different ($P < 0.05$), as determined by the independent samples *T*-test. Abbreviations: NG: normal blood glucose; LG: low blood glucose.

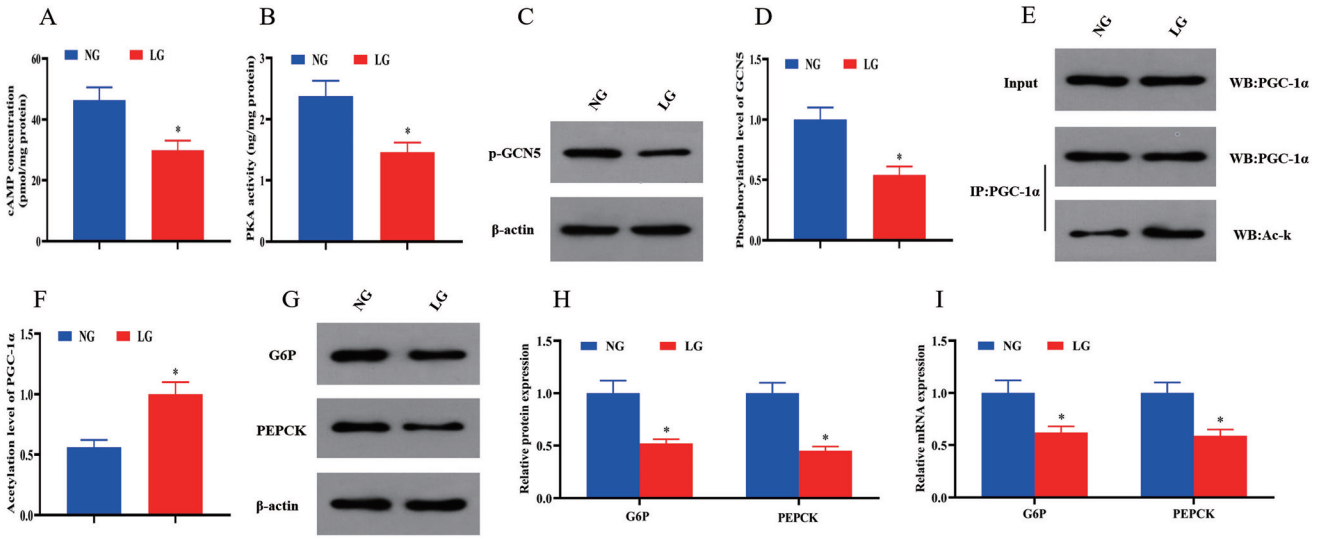

Fig. 4 Butyrate altered hepatic gluconeogenesis-related genes and proteins in late-pregnancy sows. (A) cAMP concentration; (B) PKA activity; (C and D) GCN5 phosphorylation; (E and F) PGC-1α acetylation; and (G–I) gluconeogenic mRNA and protein expression. p-GCN5: phosphorylation of general control non-repressed protein 5; PGC-1α: PPAR-γ co-activator 1α; G6P: glucose-6-phosphatase; PEPCK: phosphoenolpyruvate carboxykinase. Data are means of 10 sows per treatment; *$P < 0.05$.

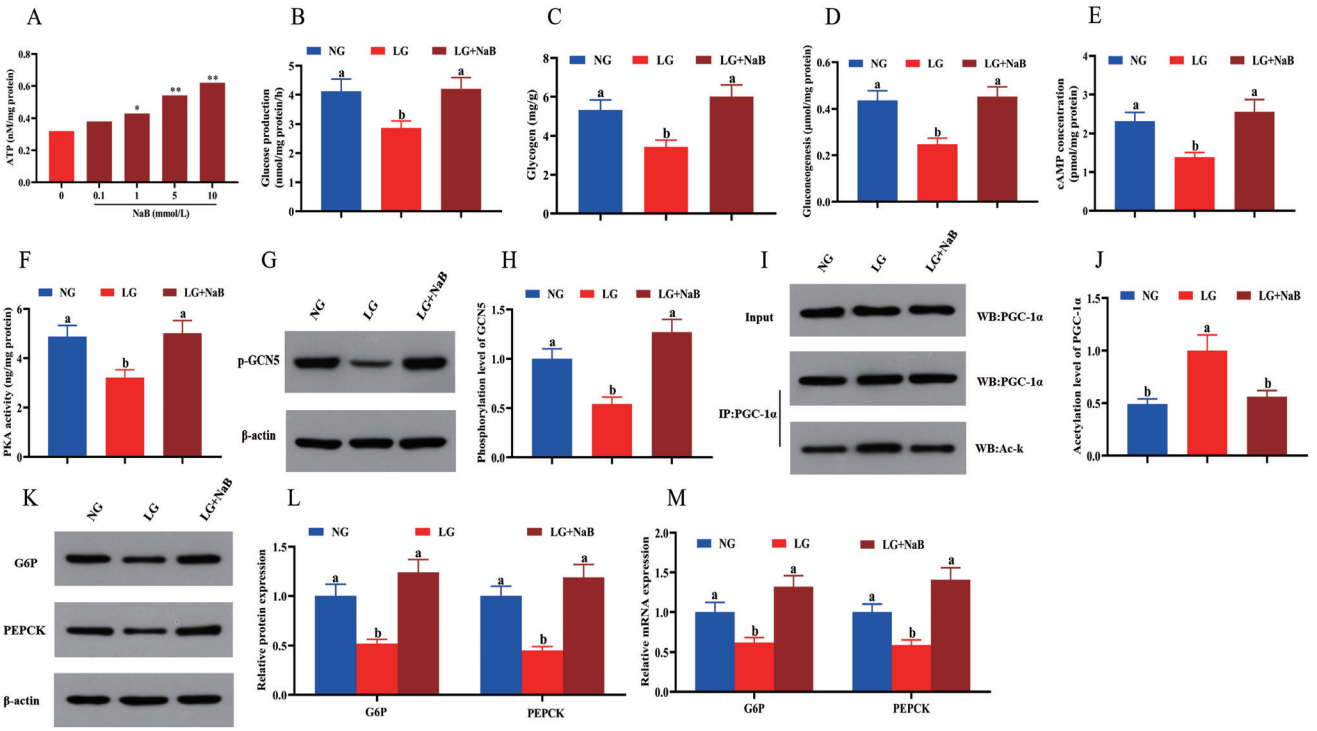

Fig. 5 Butyrate regulated gluconeogenesis *via* the cAMP-PKA-GCN5 pathway in the hepatocytes from late-pregnancy sows. (A) ATP; (B) glucose production; (C) glycogen; (D) gluconeogenesis; (E) cAMP concentration; (F) PKA activity; (G and H) GCN5 phosphorylation; (I and J) PGC-1α acetylation; and (K–M) gluconeogenic mRNA and protein expression. ATP: adenosine triphosphate; p-GCN5: phosphorylation of general control non-repressed protein 5; PGC-1α: PPAR-γ co-activator 1α; G6P: glucose-6-phosphatase; PEPCK: phosphoenolpyruvate carboxykinase. Data are means of 10 sows per treatment. Different letters indicate statistical differences between groups ($P < 0.05$). *$P < 0.05$ and **$P < 0.01$.

0.05) in the hepatocytes from the LG group of late-pregnancy sows ($P < 0.05$). Furthermore, NaB treatment increased ($P < 0.05$) the concentration of cAMP, the activity of PKA, the GCN5 phosphorylation level, and the protein and mRNA levels of PEPCK and G6P and decreased ($P < 0.05$) the PGC-1α acetylation level in the hepatocytes from the LG group of late-preg-

nancy sows (Fig. 5E–M). These results suggested that butyrate can promote hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway.

3.7 Mechanisms of butyrate improved gluconeogenesis *via* the cAMP-PKA-GCN5 pathway in hepatocytes from late-pregnancy sows

Given that NaB activated the concentration of cAMP and activity of PKA, it was expected that H89, an inhibitor of PKA, inhibited the concentration of cAMP and activity of PKA in the NaB treatment of hepatocytes from late-pregnancy sows (Fig. 6A and B). Also, H89 treatment decreased ($P < 0.05$) the GCN5 phosphorylation level and the protein and mRNA levels of PEPCK and G6P and increased ($P < 0.05$) the PGC-1α acetylation level in the NaB treatment of hepatocytes from late-pregnancy sows (Fig. 6C–I). Overall, these results indicated that H89 inhibited butyrate from improving hepatic gluconeogenesis *via* the cAMP-PKA-GCN5 pathway.

To confirm the phosphorylation of GCN5 mediated the acetylation of PGC-1α. We verified the effect of GCN5 on PGC-1α acetylation by over-expressing, knocking down GCN5 and using GCN5 inhibitor in NaB treatment of hepatocytes from late-pregnancy sows, respectively. Western blot analysis results showed that MB3 or siGCN5 treatment decreased ($P < 0.05$) the GCN5 expression and the GCN5 phosphorylation level in the NaB treatment of hepatocytes from late-pregnancy sows (Fig. 7A–E). Moreover, MB3 or siGCN5 treatment increased ($P < 0.05$) the PGC-1α acetylation level and decreased ($P < 0.05$) the protein and mRNA levels of PEPCK and G6P in the NaB treatment of hepatocytes from late-pregnancy sows (Fig. 7F–J). Intriguingly, NaB treatment increased ($P < 0.05$) the GCN5 phosphorylation level and the protein and mRNA levels of PEPCK and G6P and decreased ($P < 0.05$) the PGC-1α acetylation level in the hepatocytes with GCN5 overexpression (Fig. 7K–Q). These data indicated that butyrate inhibits the acetylation of PGC-1α through the phosphorylation of GCN5 to promote hepatic gluconeogenesis.

3.8 Effects of dietary 1-kestose on the reproductive performance and fecal butyrate-producing bacteria in late-pregnancy sows

The reproductive performance of sows is shown in Table 5. Compared with the LG group, dietary supplementation with 1-kestose decreased ($P < 0.05$) the estrus interval, duration of farrowing, stillbirth and birth mortality, and increased ($P < 0.05$) the number of litters born alive. The serum glucose metabolism index is shown in Fig. 8. In contrast to the LG group, dietary supplementation with 1-kestose increased ($P < 0.05$) serum lactate (Fig. 8A), pyruvate (Fig. 8B), glycerin (Fig. 8C), and glucose (Fig. 8D).

Using quantitative real-time PCR analysis allowed identification of butyrate-producing bacteria in the feces in late-pregnancy sows. The results showed that compared with the LG group, dietary supplementation with 1-kestose increased ($P < 0.05$) the number of Bifidobacteriaceae (Fig. 9A), *Faecalibacterium* (Fig. 9D), *Lachnospiraceae_AC2044_group* (Fig. 9E), *Lactobacillus* (Fig. 9F), *Parabacteroides* (Fig. 9G), and *[Eubacterium]_hallii_group* (Fig. 9I) in the feces. Simultaneously, dietary supplementation with 1-kestose

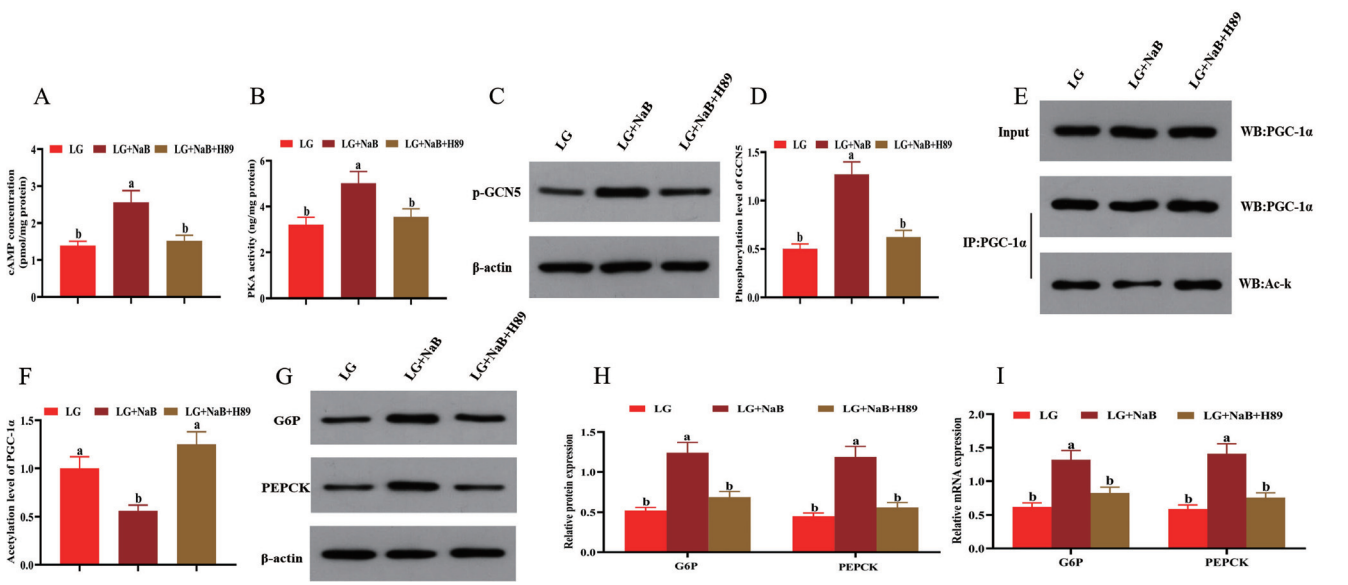

Fig. 6 Suppression of PKA caused low fasting blood glucose levels in late-pregnancy sows. (A) cAMP concentration; (B) PKA activity; (C and D) GCN5 phosphorylation; (E and F) PGC-1α acetylation; and (G–I) gluconeogenic mRNA and protein expression in the hepatocytes from late-pregnancy sows. p-GCN5: phosphorylation of general control non-repressed protein 5; PGC-1α: PPAR-γ co-activator 1α; G6P: glucose-6-phosphatase; PEPCK: phosphoenolpyruvate carboxykinase. Data are means of 10 sows per treatment. Different letters indicate statistical differences between groups ($P < 0.05$).

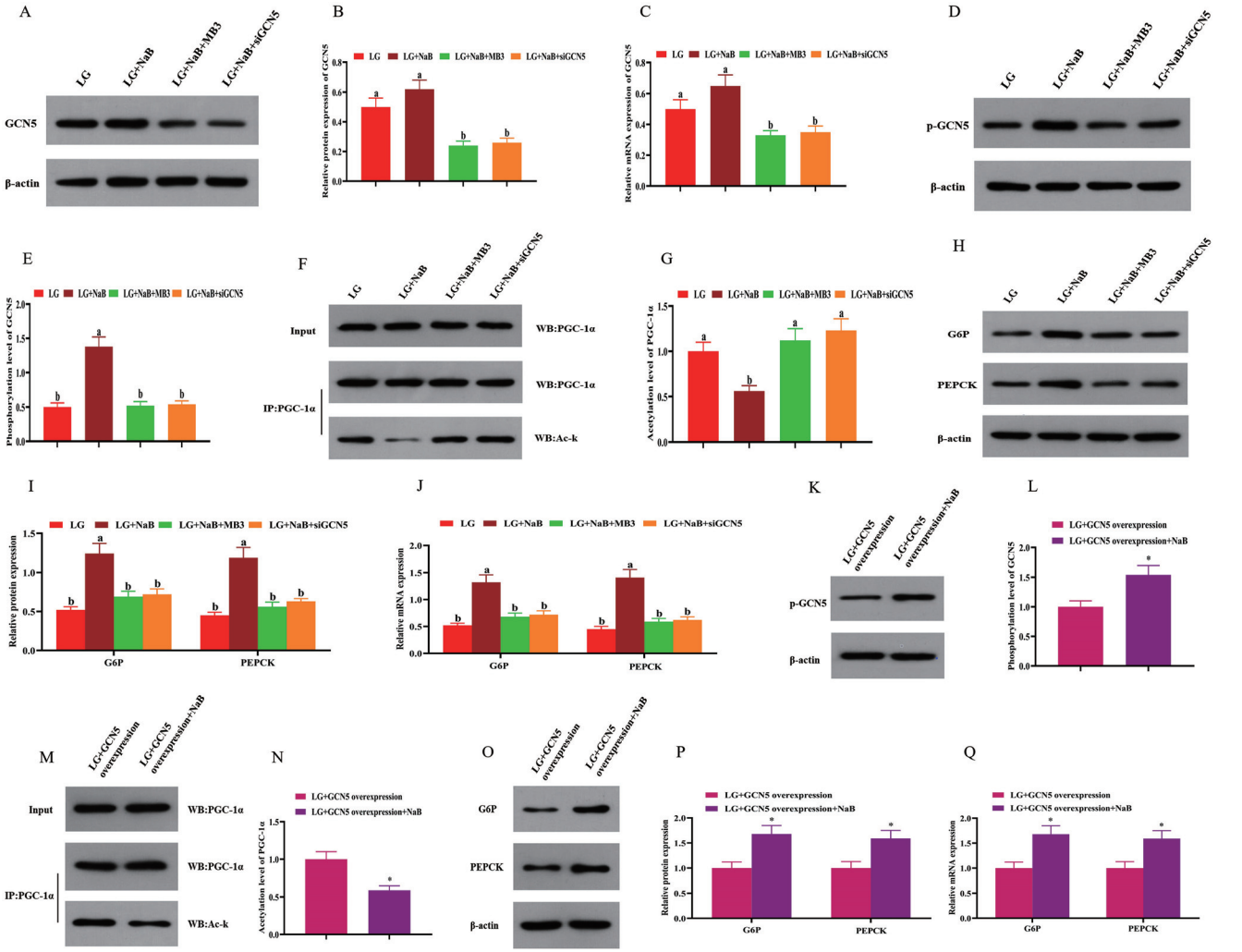

Fig. 7 Suppression of hepatic GCN5 phosphorylation resulted in low fasting blood glucose levels in late-pregnancy sows. (A–C) GCN5 expression; (D and E and K and L) GCN5 phosphorylation; (F and G and M and N) PGC-1α acetylation; and (H–J and O–Q) gluconeogenic mRNA and protein expression in the hepatocytes in late-pregnancy sows. p-GCN5: phosphorylation of general control non-repressed protein 5; PGC-1α: PPAR-γ co-activator 1α; G6P: glucose-6-phosphatase; PEPCK: phosphoenolpyruvate carboxykinase. Data are means of 10 sows per treatment. Different letters indicate statistical differences between groups ($P < 0.05$); *$P < 0.05$.

increased ($P < 0.05$) the content of butyrate in the feces (Fig. 9J).

4. Discussion

Here, we describe the novel aspects of the gut–liver interplay, namely that the SCFA butyrate modulates hepatic gluconeogenesis and is additionally able to maintain glucose homeostasis in late pregnancy by shaping the gut microbiota. It has been indicated that a glucose level of <54 mg dL^{-1} (3.0 mmol L^{-1}) is serious, clinically important hypoglycemia.[34] In pigs, hypoglycemia accounts for the second highest mortality in swine production farms.[35,36] We found that blood glucose level <3.0 mmol L^{-1} caused higher birth mortality, estrus interval and stillbirth due to longer duration of farrowing and weak labor, which correlated with the disturbance of the gut microbiota. Lack of gut microbiota-derived butyrate leads to insufficient butyrate and attenuates hepatic gluconeogenesis.

Emerging evidence has demonstrated that the gut microbiota plays an important role in the maintenance of glucose homeostasis through microbial metabolites.[37] SCFAs, one of the most representative gut microbial metabolites, have been shown to exert beneficial regulatory effects on glucose metabolism.[38] In the present study, the relative abundance of Streptococcaceae was increased, and the relative abundance of Lachnospiraceae and Bifidobacteriaceae was decreased in the colon in the low blood glucose state, which is consistent with a previous study.[39] This was so because Lachnospiraceae and Bifidobacteriaceae are well-known butyrate-producing bacteria;[37,40] the deficiency of these two bacteria has a potential link to low blood glucose. Moreover, our results showed

Table 5 Effects of dietary 1-kestose on the reproductive performance in late-pregnancy sows

Items	NG	LG	LG + low kes	LG + med kes	LG + high kes	*P*-value
Litter size	9.94 ± 0.73	9.78 ± 0.82	10.02 ± 0.88	10.84 ± 1.04	10.66 ± 0.98	0.157
Weak piglets	1.85 ± 0.19	1.94 ± 0.21	1.59 ± 0.17	1.49 ± 0.15	1.41 ± 0.14	0.634
Mummified	1.37 ± 0.15	1.68 ± 0.20	1.20 ± 0.22	1.09 ± 0.14	1.10 ± 0.12	0.428
Stillborn	1.21 ± 0.09^{ab}	1.65 ± 0.16^{a}	1.32 ± 0.14^{ab}	0.91 ± 0.09^{b}	0.89 ± 0.09^{b}	0.037
Birth mortality (%)	2.12 ± 0.23^{b}	3.98 ± 0.39^{a}	1.87 ± 0.21^{b}	1.72 ± 0.19^{b}	1.69 ± 0.15^{b}	0.039
Number of litters born alive	9.07 ± 0.68^{ab}	8.72 ± 0.79^{b}	9.47 ± 0.86^{ab}	10.68 ± 0.90^{a}	10.49 ± 0.82^{a}	0.041
Estrus interval (d)	5.26 ± 0.47^{b}	6.97 ± 0.52^{a}	6.38 ± 0.70^{ab}	4.98 ± 0.47^{b}	5.02 ± 0.51^{b}	0.022
Duration of farrowing (h)	3.28 ± 0.24^{ab}	4.42 ± 0.36^{a}	3.98 ± 0.32^{ab}	2.85 ± 0.32^{b}	2.79 ± 0.28^{b}	0.034
Mean weight of litters born alive per piglet (kg)	1.46 ± 0.12	1.39 ± 0.11	1.49 ± 0.13	1.55 ± 0.15	1.52 ± 0.17	0.726
Litter (alive) weight at birth per piglet (kg)	13.38 ± 1.02	13.26 ± 0.98	13.72 ± 1.02	14.61 ± 1.34	14.58 ± 1.33	0.562

Data are means of 20 sows per treatment. Values in the same row with different superscripts are significantly different ($P < 0.05$), as determined by the independent samples *T*-test. Abbreviations: NG: normal blood glucose; LG: low blood glucose; low kes: basal diet supplemented with 1000 mg kg^{-1} 1-kestose; med kes: basal diet supplemented with 3000 mg kg^{-1} 1-kestose; high kes: basal diet supplemented with 5000 mg kg^{-1} 1-kestose.

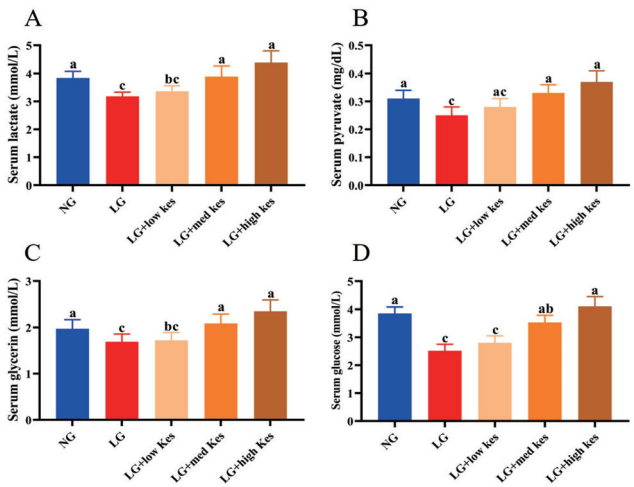

Fig. 8 Effects of dietary 1-kestose on serum glucose metabolism index in late-pregnancy sows. (A) Serum lactate; (B) serum pyruvate; (C) serum glycerin; (D) serum glucose. Data are means of 10 sows per treatment, different letters indicated statistical differences between groups ($P < 0.05$).

that other butyrate-producing bacteria, such as *Clostridium*, *Eubacterium*, *Faecalibacterium*, *Lactobacillus* and *Parabacteroides*, were significantly decreased in the low blood glucose state. In addition, colonic butyrate was markedly decreased in the low blood glucose state along with a decrease in butyrate-producing bacteria in late-pregnancy sows. With all the results together, we inferred that the deficiency of butyrate-producing bacteria and butyrate production were associated with impaired glucose homeostasis.

SCFAs are absorbed *via* intestinal epithelial cells and travel through the portal vein to the liver.[41] Using metabolomics allowed the identification of gut microbial derived-SCFAs in the hepatic portal vein and liver. SCFAs act as signal molecules to maintain hepatic energy balance through mediating glucose homeostasis.[42] Our results showed that butyrate in the hepatic portal vein and liver was decreased in the low blood glucose state, indicating that gut microbiota-derived butyrate can reach the liver and affect hepatic glucose metabolism. PGC-1α is a pivotal transcription factor,

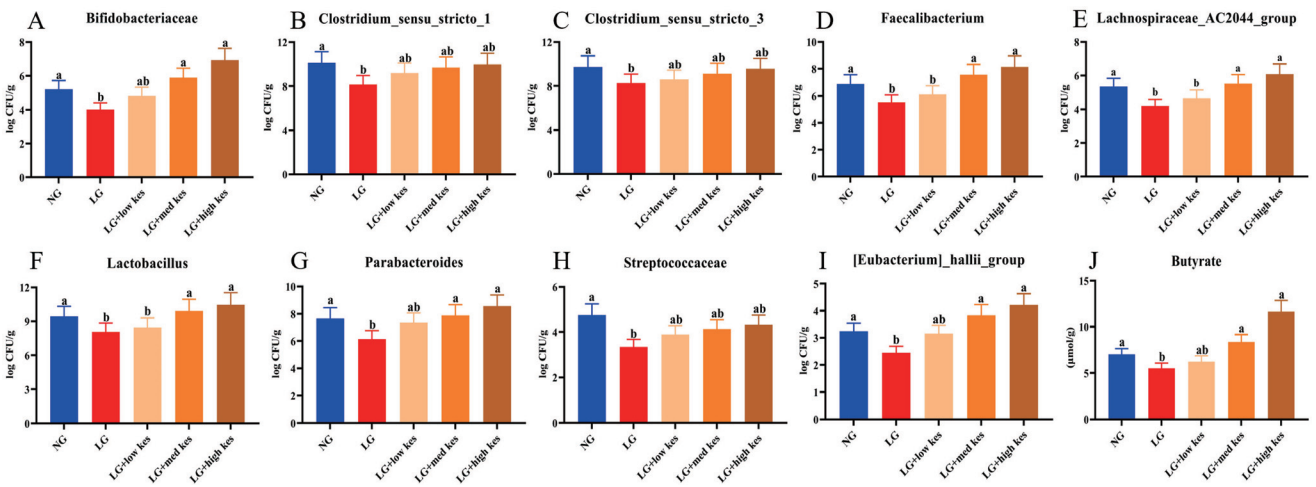

Fig. 9 Effects of dietary 1-kestose on fecal butyrate-producing bacteria and butyrate in late-pregnancy sows. (A–I) Fecal butyrate-producing bacteria and (J) fecal butyrate content. Data are means of 10 sows per treatment. Different letters indicated statistical differences between groups ($P < 0.05$).

and is modified by apparent acetylation that plays a decisive role in gluconeogenesis.[43] In order to further investigate the effect of butyrate on hepatic gluconeogenesis, the PGC-1α acetylation level was determined as butyrate activates PGC-1α transcriptional activity to maintain glucose homeostasis.[44] We found that hepatic PGC-1α acetylation level was increased in the low blood glucose state. Notably, PGC-1α acetylation reduced the expression of gluconeogenic genes and glucose output.[45] Our results showed that the mRNA and protein levels of G6P and PEPCK were decreased in the liver in the low blood glucose state. Inhibition of PEPCK and G6P, the predominant hexokinases, may cause hypoglycemia because of its decisive role in maintaining glucose homoeostasis.[43] GCN5 as a nutrient sensor mainly participates in energy metabolism to maintain glucose homeostasis through PGC-1α acetylation.[12] However, little is known about GCN5 regulating hepatic glucose gluconeogenesis in pregnant sows. In this study, we found that the GCN5 phosphorylation level was decreased in the low blood glucose state, suggesting that phosphorylation of GCN5 can inhibit PGC-1α acetylation. In addition, cAMP, the classical second messenger, relies on its main effector, protein kinase A (PKA),[46] and phosphorylates GCN5 to regulate cellular energy metabolism, exerting a stimulatory effect on glucose utilization.[11,47] Our results showed that the cAMP concentration and PKA activity were decreased in the liver in the low blood glucose state. It could be specified that butyrate suppresses hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway in the low blood glucose state.

We have studied the molecular mechanism of butyrate on hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway. To this end, the hepatocytes were treated with NaB and the role of PGC-1α was observed in the transcriptional activity of G6P and PEPCK. The results in our study showed that the mRNA and protein levels of G6P and PEPCK were decreased in the hepatocytes from late-pregnancy sows in the low blood glucose state. Inhibition of G6P and PEPCK is due to the activation of PGC-1α acetylation. This was so because PGC-1α acetylation reduced the expression of gluconeogenic genes, which was consistent with the *in vivo* experiments. Pregnancy is associated with deregulated activity and the expression of enzymes (*eg*, G6P and PEPCK) in gluconeogenesis that control the production of glucose, thus, leading to deficient gluconeogenesis and glucose output in the liver, resulting in fasting hypoglycemia.[48,49] However, NaB treatment increased the mRNA and protein levels of G6P and PEPCK, suggesting that butyrate improves hepatic gluconeogenesis to maintain glucose homoeostasis.[15] The PGC-1α acetylation level was decreased and the GCN5 phosphorylation level was increased in NaB-treated hepatocytes from late-pregnancy sows in the low blood glucose state, indicating that butyrate can promote hepatic gluconeogenesis through activating the phosphorylation of GCN5 and thereby inhibiting PGC-1α acetylation. In addition, the cAMP concentration and PKA activity were increased in NaB-treated hepatocytes from late-pregnancy sows in the low blood glucose state, suggesting that butyrate can promote hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway.

We next sought to investigate whether butyrate promoted gluconeogenesis through the hepatic cAMP-PKA-GCN5 pathway. To uncover this, H89, a kind of PKA inhibitor was co-treated with NaB to verify whether butyrate promotes hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway. Indeed, H89 dissects the cAMP-PKA-GCN5 pathway, indicating that the cAMP-PKA-GCN5 pathway is an essential element in maintaining glucose homoeostasis induced by butyrate against low blood glucose. In order to comprehensively understand butyrate regulates PGC-1α through cAMP-PKA-GCN5 pathway. We evaluated the effect of GCN5 on PGC-1α acetylation by over-expressing, knocking down GCN5 and using GCN5 inhibitor in NaB treatment of hepatocytes. Our results showed that when GCN5 was inhibited or knockdown, the phosphorylation level of GCN5 and the expression of PEPCK and G6P were repressed and the acetylation level of PGC-1α was activated in the NaB treatment of hepatocytes from late-pregnancy sows in the low blood glucose state, indicating that inhibition of GCN5 expression suppressed gluconeogenesis, causing severe hypoglycemia. It has been established that depletion of GCN5 or the expression of the phosphorylation-defective mutant GCN5 in mouse liver with obesity and type 2 diabetes suppressed gluconeogenesis and thereby improved glycemia, suggesting that inhibition of GCN5 expression or phosphorylation may ameliorate diabetes.[11] Contrarily, when GCN5 was overexpressed, the phosphorylation level of GCN5 and the expression of PEPCK and G6P were activated and the acetylation level of PGC-1α was repressed in NaB-treated hepatocytes from late-pregnancy sows in the low blood glucose state, suggesting that butyrate inhibited the acetylation of PGC-1α through the phosphorylation of GCN5 to promote hepatic gluconeogenesis. In this study, we prefer to focus on the key role of butyrate, as a regulator of cAMP, that can increase the cAMP level.[50] It has been reported that cAMP-PKA can activate the phosphorylation of GCN5,[11] which inhibits PGC-1α acetylation to promote hepatic gluconeogenesis. Indeed, butyrate acts as an HDAC inhibitor which may contribute to PGC-1 regulation, which promotes hepatic gluconeogenesis,[44] and as a ligand for GPR43, it can also promote blood glucose homeostasis *via* the GPR43-AKT-GSK3 signaling pathway.[30] Thus, butyrate serves as a key regulator in hepatic gluconeogenesis.

In order to solve the issue of low fasting blood glucose in late pregnancy, we look for regulators through the molecular mechanism, targeting butyrate produced by the gut microbiota to improve hepatic gluconeogenesis. 1-Kestose is a non-digestible oligosaccharide consisting of glucose linked to two fructose units. It has been established that dietary 1-kestose increased the levels of *Faecalibacterium* and *Bifidobacteria* in humans,[19] and increased butyrate production in the ceca of rats.[51] Our results showed that dietary 1-kestose increased the levels of *Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *[Eubacterium]_hallii_group*, Bifidobacteriaceae, and *Lachnospiraceae_AC2044_group* to produce butyrate. This was

so because 1-kestose is not digested in the small intestines of mammals; it is fermented in the cecum and colon due to its chemical structure and water solubility.[52] In addition, dietary 1-kestose improved the reproductive performance and serum glucose metabolism index in late-pregnancy sows.

5. Conclusion

In conclusion, we found in the present study that low fasting blood glucose levels affected the reproductive performance and serum glucose metabolism index in late-pregnancy sows. Our *in vivo* and *in vitro* experiments demonstrated that butyrate serves as a key regulator of hepatic gluconeogenesis in late-pregnancy sows. Moreover, dietary 1-kestose, a natural regulator of gut bacteria, significantly increased butyrate and butyrate-producing bacteria, and improved the reproductive performance and serum glucose metabolism index of sows in late pregnancy. We found that targeting gut microbiota-derived butyrate could improve hepatic gluconeogenesis through the cAMP-PKA-GCN5 pathway in late-pregnancy sows.

Author contributions

Feiruo Huang, Longshan Qin and Tongxin Wang designed the study; Longshan Qin, Taimin Jin and Baoyin Guo performed the experiment; Weilei Yao, Tongxin Wang, and Shu Wen conducted the experiment and analyzed the data; Longshan Qin wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There are no financial or personal conflicts of interest to report.

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References

1 D. Newbern and M. Freemark, Placental hormones and the control of maternal metabolism and fetal growth, *Curr. Opin. Endocrinol., Diabetes Obes.*, 2011, **18**, 409–416.
2 J. Henrichs, J. J. Schenk, C. S. Barendregt, H. G. Schmidt, E. A. Steegers, A. Hofman, V. W. Jaddoe, H. A. Moll, F. C. Verhulst and H. Tiemeier, Fetal growth from mid- to late pregnancy is associated with infant development: the generation R study, *Dev. Med. Child Neurol.*, 2010, **52**, 644–651.
3 D. Ježková and M. Smrčková, Changes in blood glucose and lactic acid in pregnant sows and their fetuses and in sows and their piglets up to ten days after birth, *Vet. Med.*, 1990, **35**, 613–620.
4 T. Rogne and G. W. Jacobsen, Association between low blood glucose increase during glucose tolerance tests in pregnancy and impaired fetal growth, *Acta Obstet. Gynecol. Scand.*, 2015, **93**, 1160–1169.
5 Y. Yang, M. Deng, J. Chen, X. Zhao and C. Tan, Starch supplementation improves the reproductive performance of sows in different glucose tolerance status, *Anim. Nutr.*, 2021, **7**, 1231–1241.
6 A. K. Madiraju, D. M. Erion, Y. Rahimi, X. M. Zhang, D. T. Braddock, R. A. Albright, B. J. Prigaro, J. L. Wood, S. Bhanot and M. J. Metformin, suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase, *Nature*, 2014, **510**, 542–546.
7 J. C. Yoon, P. Pulgserver, G. Chen, J. Donovan, Z. Wu, J. Rhee, G. Adelmant, J. Stafford, C. R. Kahn and D. K. Granner, Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1, *Nature*, 2001, **413**, 131–138.
8 S. Herzig, F. Long, U. S. Jhala, S. Hedrick, R. Quinn, A. Bauer, D. Rudolph, G. Schutz, C. Yoon and P. Puigserver, CREB regulates hepatic gluconeogenesis through the coactivator PGC-1, *Nature*, 2001, **413**, 179–183.
9 K. Sharabi, L. Hua, C. Tavares, J. E. Dominy and J. P. Camporez, Selective chemical inhibition of PGC-1a gluconeogenic activity ameliorates type 2 diabetes, *Cell*, 2017, **169**(1), 148–160.
10 M. E. Haque, M. Jakaria, M. Akther, D. Y. Cho and D. K. Choi, The GCN5: Its biological functions and therapeutic potentials, *Clin. Sci.*, 2021, **135**, 231–257.
11 M. Sakai, T. Tujimura-Hayakawa, T. Yagi, H. Yano, M. Mitsushima, H. Unoki-Kubota, Y. Kaburagi, H. Inoue, Y. Kido and M. Kasuga, The GCN5-CITED2-PKA signalling module controls hepatic glucose metabolism through a cAMP-induced substrate switch, *Nat. Commun.*, 2016, **7**, 13147.
12 B. Mutlu and P. Puigserver, GCN5 acetyltransferase in cellular energetic and metabolic processes, *Biochim. Biophys. Acta, Gene Regul. Mech.*, 2020, **1864**, 194626.
13 C. S. Byrne, E. S. Chambers, D. J. Morrison and G. Frost, The role of short chain fatty acids in appetite regulation and energy homeostasis, *Int. J. Obes.*, 2015, **39**(9), 1331–1338.
14 P. Turnbaugh, An obesity-associated gut microbiome with increased capacity for energy harvest, *Nature*, 2006, **444**, 1027–1031.
15 F. D. Vadder, P. Kovatcheva-Datchary, D. Goncalves, J. Vinera and G. Mithieux, microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits, *Cell*, 2014, **156**, 84–96.

16 Z. Li, C. X. Yi, S. Katiraei, S. Kooijman, E. Zhou, C. K. Chung, Y. Gao, D. Van, O. C. Meijer and J. F. P. Berbée, Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit, *Gut*, 2018, **67**, 1269–1279.
17 D. R. Donohoe, N. Garge, X. X. Zhang, W. Sun, T. M. O'Connell, M. K. Bunger and S. J. Bultman, The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon, *Cell Metab.*, 2011, **13**, 517–526.
18 G. D. Besten, A. Bleeker, R. Havinga, A. Gerding, K. V. Eunen, A. K. Groen, B. M. Bakker and D. J. Reijngoud, Gut-derived acetate and butyrate increase gluconeogenesis and decrease lipogenesis via the hepatic cAMP-PKA- CREB protein signaling pathway, University of Groningen, Groningen, 2014.
19 T. Tochio, Y. Kadota, Y. Tanaka and Y. Koga, 1-Kestose, the smallest fructooligosaccharide component, which efficiently stimulates Faecalibacterium prausnitzii as well as Bifidobacteria in humans, *Foods*, 2018, **7**(9), 140.
20 L. M. Gao, X. Lin, C. Y. Xie, T. Y. Zhang and Y. L. Yin, The time of calcium feeding affects the productive performance of sows, *Animals*, 2019, **9**, 337.
21 X. N. Liu, X. Li, B. Xia, X. Jin, Q. H. Zou, Z. H. Zeng, W. Y. Zhao, S. K. Yan, L. Li and S. F. Yuan, High-fiber diet mitigates maternal obesity-induced cognitive and social dysfunction in the offspring via gut-brain axis, *Cell Metab.*, 2021, **33**(5), 923–938.
22 D. J. Morrison, K. Cooper, S. Waldron, C. Slater, L. T. Weaver and T. Preston, A streamlined approach to the analysis of volatile fatty acids and its application to the measurement of whole-body flux, *Rapid Commun. Mass Spectrom.*, 2010, **18**, 2593–2600.
23 E. Boets, V. S. Gomand, L. S. Deroover, T. Preston, K. Vermeulen, V. D. Preter, H. M. Hamer, G. V. Mooter, L. D. Vuyst and C. M. Courtin, Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study, *J. Physiol.*, 2017, **595**(2), 541–555.
24 S. A. Gradilone, F. I. Carreras, G. L. Lehmann and R. A. Marinelli, Phosphoinositide 3-kinase is involved in the glucagon-induced translocation of aquaporin-8 to hepatocyte plasma membrane, *Biol. Cell*, 2012, **97**, 831–836.
25 T. X. Wang, W. L. Yao, J. Li, Q. Y. He and F. R. Huang, Acetyl-CoA from inflammation-induced fatty acids oxidation promotes hepatic malate-aspartate shuttle activity and glycolysis, *Am. J. Physiol.: Endocrinol. Metab.*, 2018, **315**(4), E496–E510.
26 L. S. Qin, W. Ji, B. Li, J. L. Wang, J. P. Hu and X. Wu, Effects of dietary supplementation with yeast glycoprotein on growth performance, intestinal mucosal morphology, immune response and colonic microbiota in weaned piglets, *Food Funct.*, 2019, **10**, 2359–2371.
27 L. H. Sun, N. Y. Zhang, M. K. Zhu, L. Zhao, J. C. Zhou and D. S. Qi, Prevention of aflatoxin b1 hepatoxicity by dietary selenium is associated with inhibition of cytochrome P450 isozymes and up-regulation of 6 selenoprotein genes in chick liver, *J. Nutr.*, 2016, **146**, 655–661.
28 H. Namkung, J. G. Li, M. H. Yu, M. Cottrill and C. F. M. De Lange, Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs, *Can. Vet. J.*, 2004, **84**, 697–704.
29 T. X. Wang, W. L. Yao, J. Li, Y. F. Shao and F. R. Huang, Dietary garcinol supplementation improves diarrhea and intestinal barrier function associated with its modulation of gut microbiota in weaned piglets, *J. Anim. Sci. Biotechnol.*, 2020, **11**, 12.
30 W. Q. Zhang, T. T. Zhao, D. K. Gui, C. J. Gao, J. L. Gu, W. J. Gan, W. Huang, Y. Xu, H. Zhou and W. N. Chen, Sodium butyrate improves liver glycogen metabolism in type 2 diabetes mellitus, *J. Agric. Food Chem.*, 2019, **67**(27), 7694–7705.
31 J. Maag, Gganatogram: An R package for modular visualisation of anatograms and tissues based on ggplot2, *F1000Research*, 2018, **7**, 1576.
32 B. H. McArdle and M. J. Anderson, Fitting multivariate models to community data: a comment on distance & based redundancy analysis, *Ecology*, 2001, **82**, 290–297.
33 J. R. White, N. Nagarajan and M. Pop, Statistical methods for detecting differentially abundant features in clinical metagenomic samples, *PLoS Comput. Biol.*, 2009, **5**(4), e1000352.
34 A. Nakhleh and N. Shehadeh, *Hypoglycemia in diabetes*, 2018.
35 S. K. Mondal, U. K. De, G. K. Das, A. M. Powde and A. K. Verma, Pattern of mortality of crossbred pigs in an organized swine production farm, *J. Anim. Sci.*, 2012, **3**, 37–44.
36 P. R. English and V. Morrison, Causes and prevention of piglet mortality, *Pig News Information*, 1984, **5**, 369–376.
37 T. Zhu and M. O. Goodarzi, Metabolites linking the gut microbiome with risk for type 2 diabetes, *Curr. Nutr. Rep.*, 2020, **9**, 83–93.
38 E. Canfora, J. W. Jocken and E. Blaak, Short-chain fatty acids in control of body weight and insulin sensitivity, *Nat. Rev. Endocrinol.*, 2015, **11**, 577–591.
39 S. Quercia, S. Turroni, J. Fiori, M. Soverini, S. Rampelli, E. Biagi, A. Castagnetti, C. Consolandi, M. Severgnini and M. Pianesi, Gut microbiome response to short-term dietary interventions in reactive hypoglycemia subjects, *Diabetes/Metab. Res. Rev.s*, 2017, **33**, e2927.
40 H. Gamage, S. G. Tetu, R. Chong, J. Ashton, N. H. Packer and I. T. Paulsen, Cereal products derived from wheat, sorghum, rice and oats alter the infant gut microbiota in vitro, *Sci. Rep.*, 2017, **7**, 14312.
41 S. M. Zhang, J. W. Zhao, F. Xie, H. X. He, L. J. Johnstone, X. F. Dai, C. D. Wu and X. Ma, Dietary fiber-derived short-chain fatty acids: A potential therapeutic target to alleviate obesity–related nonalcoholic fatty liver disease, *Obes. Rev.*, 2021, **22**, e13316.
42 J. M. Hu, S. L. Lin, B. D. Zheng and P. C. K. Cheung, Short-chain fatty acids in control of energy metabolism, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**(8), 1243–1249.

43 C. Sun, M. H. Wang, X. Y. Liu, L. Luo, K. X. Li, S. Q. Zhang, Y. J. Wang, Y. M. Yang, F. Ding and X. S. Gu, PCAF improves glucose homeostasis by suppressing the gluconeogenic activity of PGC-1α, *Cell Rep.*, 2014, **9**, 2250–2262.

44 Z. G. Gao, J. Yin, Z. Jin, R. E. Ward, R. J. Martin, M. Lefevre, W. T. Cefalu and J. P. Ye, Butyrate improves insulin sensitivity and increases energy expenditure in mice, *Diabetes*, 2009, **58**, 1509–1517.

45 J. Dominy, Y. Lee, M. Jedrychowski, H. Chim, M. Jurczak, J. Camporez, H. B. Ruan, J. Feldman, K. Pierce and R. Mostoslavsky, The deacetylase sirt6 activates the acetyltransferase GCN5 and suppresses hepatic gluconeogenesis, *Mol. Cell*, 2012, **48**, 900–913.

46 K. Lefkimmiatis, D. Leronni and A. M. Hofer, The inner and outer compartments of mitochondria are sites of distinct cAMP/PKA signaling dynamics, *J. Cell Biol.*, 2013, **202**, 453–462.

47 H. Osawa, R. L. Printz, R. R. Whitesell and D. K. Granner, Regulation of hexokinase II gene transcription and glucose phosphorylation by catecholamines, cyclic AMP, and insulin, *Diabetes*, 1995, **44**, 1426–1432.

48 H. Masuyama, T. Mitsui, J. Maki, K. Tani, K. Nakamura and Y. Hiramatsu, Dimethylesculetin ameliorates maternal glucose intolerance and fetal overgrowth in high-fat diet-fed pregnant mice via constitutive androstane receptor, *Mol. Cell. Biochem.*, 2016, **419**, 185–192.

49 K. L. Franko, A. J. Forhead and A. L. Fowden, Effects of stress during pregnancy on hepatic glucogenic capacity in rat dams and their fetuses, *Physiol. Rep.*, 2017, **5**(11), e13293.

50 X. Y. Ji, F. Y. Zhou, Y. Q. Zhang, R. Y. Deng, W. Xu, M. Y. Bai, Y. Liu, L. Shao, X. Wang and L. Zhou, Butyrate stimulates hepatic gluconeogenesis in mouse primary hepatocytes, *Exp. Ther. Med.*, 2019, **17**, 1677–1687.

51 T. Tochio, Y. Kitaura, S. Nakamura, C. Sugawa and Y. Shimomura, An alteration in the cecal microbiota composition by feeding of 1-kestose results in a marked increase in the cecal butyrate content in rats, *PLoS One*, 2016, **11**, e0166850.

52 A. Watanabe, Y. Kadota, H. Yokoyama, S. Tsuruda, R. Kamio, T. Tochio, Y. Shimomura and Y. Kitaura, Experimental determination of the threshold dose for bifidogenic activity of dietary 1-kestose in rats, *Foods*, 2019, **9**, 4.