Original Research Article

N-Acetyl-D-glicosamine improves the intestinal development and nutrient absorption of weaned piglets via regulating the activity of intestinal stem cells

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A B S T R A C T

Early weaning in piglets can cause a series of negative effects. This causes serious losses to the livestock industry. N-Acetyl-D-glucosamine (D-GlcNAc) plays an important role in regulating the homeostasis of the intestine. This study aimed to investigate the effects of D-GlcNAc on the growth performance and intestinal function of weaned piglets. Twenty-four weaned piglets (Yorkshire × Landrace × Duroc, 6.58 ± 0.15 kg, n = 8) at 21 d old were fed 3 diets supplemented with 0 (control), 1 and 3 g/kg D-GlcNAc. The intestinal organoid model was used to verify the regulatory mechanism of D-GlcNAc on intestinal epithelial cells. On the whole, supplementation of D-GlcNAc in the piglet diet has no significant effect on the growth performance and diarrhea of weaned piglets (P > 0.05). The apparent digestibility of nutrients and mRNA abundance of nutrient transporters in the 1 g/kg D-GlcNAc group were increased significantly (P < 0.05). D-GlcNAc did not affect villus height (VH) and crypt depth (CD) but resulted in a numerically shorter VH and shallower CD, which lead to an increase in ileal VH/CD ratio (P < 0.05). Cell shedding rates in the ileum villi increased (P < 0.05). The relative length and weight of the small intestine of weaned piglets increased (P < 0.05). In vitro studies found that the budding rates of organoids treated with 0.1 mmol/L D-GlcNAc increased on the d 3 and 5 (P < 0.05). The average budding numbers per budding organoid treated with 0.1 and 10 mmol/L D-GlcNAc increased on d 3 (P > 0.05). D-GlcNAc upregulated leucine rich repeat containing G protein-coupled receptor 5 (Lgr5⁺) and Chromogranin A mRNA abundance in organoids (P < 0.05). Micro 2 (Muc2) expression increased when treated with 1 and 10 mmol/L D-GlcNAc (P < 0.05). In conclusion, dietary D-GlcNAc cannot improve the growth performance of weaned piglets. However, it can promote the growth and development of the intestinal tract and improve the digestion and absorption capacity of the intestine, which is achieved by affecting the activity of intestinal stem cells.

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1. Introduction

Early weaned piglets have a series of problems, such as low feed intake, high diarrhoea rate, damaged intestinal mucosa, nutritional diseases, and intestinal microflora imbalance. This stage causes significant economic losses to the livestock husbandry (Moeser et al., 2017). Intestinal epithelial cells play a key role in digesting and absorbing luminal nutrients. Weaning stress usually damages...
the intestinal epithelial cells in weaned piglets, resulting in negative effects such as diarrhoea (Yang et al., 2016c). The renewal of intestinal epithelial cells involves changes in energy metabolism (Yang et al., 2016b, 2016d). The protein expression involved in energy metabolism (monosaccharide catabolism process, lipid catabolism process, and citrate cycle) was down-regulated mainly in jejunal differentiated epithelial cells in post-weaning piglets (Yang et al., 2016a). In pig production, the growth status of piglets directly affects the performance of the entire growth and fattening stage. How to reduce and overcome the weaning stress of piglets has become an important issue in improving the efficiency of pig production.

N-Acetyl-D-glucosamine (D-GlcNAc) is produced following strong mineral-acid hydrolysis at high temperatures from shell waste. D-GlcNAc is an oligosaccharide and can promote the proliferation of intestinal stem cells. Studies have shown that D-GlcNAc is a dietary supplement that can enhance intestinal stem cell division in a model of intestinal atrophy caused by restricted diet and has a positive effect on regulating the intestinal growth and development of animals (Mattila et al., 2018). Moreover, GlcNAc serves as a key sensor in regulating insulin signalling, the cell cycle, and cellular stress response (Zachara and Hart, 2006). D-GlcNAc has many specific biological activities and has been widely used in the pharmaceutical, biomedical, food, and chemical industries (Chen et al., 2010). However, there is little research on D-GlcNAc in weaned piglets. We hypothesized that D-GlcNAc could affect the intestinal growth and development, digestion and absorption of weaned piglets by affecting the activity of stem cells. This study aims to investigate the effects of D-GlcNAc on the growth performance, diarrhoea score, small intestine index, intestinal morphology, crypt division, apparent total tract digestibility of nutrients, abundance of nutrient transporters and intestinal organelles of weaned piglets.

2. Materials and methods

2.1. Animal ethics

The experimental design and procedures in this study were approved by the Animal Care and Use Committee of the Hunan Normal University, Changsha City, Hunan, China (Wang et al., 2020b).

2.2. Animals and experimental design

This experiment was carried out in Hunan New Wufeng Co., Ltd. Yong’an Branch Pig Farm. Twenty-four weaned piglets [(Yorkshire × Landrace] × Duroc, 6.58 ± 0.15 kg) at 21 d old with similar genetic background and health status were randomly divided into groups for 3 treatments. N-Acetyl-D-glucosamine (A602245, Sangon Biotech Co., Ltd, Shanghai, China) was added to the basal diet of each group at 0 (control group), 1, and 3 g/kg, respectively, with 8 pens per group, 1 piglet per replicate. There were an equal number of barrows and gilts in each group. The piglets were raised in single pens, and each pen was independent of each other. The experimental period lasted 28 d. The basic diet formula (Table 1) was adjusted every 14 d to ensure that the dietary nutrients of weaned piglets met the requirements of NRC (NRC, 2012). During feed replacement, the feed of stage 2 was gradually increased until all the feed of stage 1 was replaced. The transition period lasted 3 d. Then the piglets ate the feed of phase 2 until the end of the experiment. The piglets were allowed to eat and drink freely during the experiment and were vaccinated strictly in accordance with the requirements of the farm. A daily record was maintained of food intake, diarrhoea, illness, death, and any abnormal behaviour. The piglets were weighed every week. The weaned piglets’ average daily gain (ADG), average daily feed intake (ADFI), and ratio of daily gain to daily feed intake (G:F) were recorded and calculated according to the method recommended by Yin et al. during the experiment (Yin et al., 2001).

2.3. Sample collection

Before the weaned piglets were slaughtered, the rectum was stimulated to collect fresh faeces for 3 consecutive days. Freshly collected faeces were mixed with 10% tartaric acid to fix nitrogen. After the experiment, the weaned piglets were euthanized according to the method of Yan et al. (2018). The abdominal cavity of the piglet was dissected and intestinal samples were collected. The weight and length of the small intestine without intestinal contents were measured. After fasting for 8 h, the corresponding organ index was calculated (ratio of small intestinal length or weight to BW) according to the fasting weight of the piglets at the end of experiment. The samples of middle duodenum, middle jejunum, and terminal ileum (about 2 to 3 cm) without intestinal contents were collected and stored in 10% formalin solution. The jejunum was cut longitudinally along the small intestine, and the contents of the intestinal lumen were washed away with a phosphate buffered saline (PBS) precooled at 4 °C. A sterilized glass slide was used to gently scrape the upper mucosa into tin foil. After being snap frozen with liquid nitrogen, the mucosa was stored at −80 °C.

2.4. Faeces score

During the experimental period, the piglets’ diarrhoea was recorded at regular intervals (08:00, 11:00, 14:00, 17:00, and 20:00). Piglet manure was scored on a 5-point scale (1 = dry and hard stool, 2 = wet stool, 3 = mild diarrhoea, 4 = severe diarrhoea, 5 = watery diarrhoea). When the stool score was 3 or more, we considered it diarrhoea (Trevisi et al., 2011).

2.5. The apparent total tract digestibility analysis

According to previous studies, the acid-insoluble ash method (AIA) was used to measure the apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), crude ash, and gross energy (GE) in Phase 2 (McCarthy et al., 1974). Nutrients in feed and faeces were analyzed according to AOAC (AOAC, 2007). The total energy in feed and faeces were measured using a precision automatic calorimeter (5E-C5508, Changsha Kaiyuan Instrument Co., Ltd, China).

2.6. Small intestinal histomorphology

According to the method of Barea et al. (2011), duodenum, jejunum and ileum samples fixed in 10% formalin solution were embedded in paraffin, then cut into 5-μm thick slices and stained with haematoxylin and eosin (H&E). After the stained sections were dried in a fume hood, the intestinal villi and crypts were photographed with a microscope at 40× combined magnification (Leica Microsystems, Cambridge, UK). Image-pro Plus 6.0 software (Media Cybernetics, Inc., US) was used to measure villus height (VH), villus width (VW), and crypt depth (CD). According to the measured results, the ratio of VH to CD (VH:CD) was calculated. The villus surface area (VSA = π × VW × VW) was calculated according to the method recommended by Kisielinski et al. (2002). The villous cell shedding index is the number of villi containing the exfoliated cells divided by the total number of villi (Bullen et al., 2006). At least 30 intact villi and crypts were selected from each piglet’s intestine. The average value of corresponding indicators according to
was diluted 5-fold with sterilized double distilled water and used as the template for the real-time quantitative PCR (RT-qPCR) reaction. Primers were designed using the Primer-BLAST online primer design tool on the NCBI website and then handed over to Beijing Qingke Hunan Branch for synthesis. The primer sequences are shown in Table 2. The final reaction volume of the RT-qPCR instrument (ABI 7900HT Fast Real-Time PCR System, Applied Biosystems, Carlsbad, CA) was 10 μL, which was 5 μL of SYBR Premix Ex Taq II (2 × ), 0.2 μL of forward primer (10 μmol/L), 0.2 μL of reverse primer (10 μmol/L), 0.2 μL of ROX reference Dye II (50 × ), 1 μL of cDNA template, and 3.4 μL of sterilized ddH2O. This kit was purchased from Takara (Code No. RR420A, Dalian, China). Reaction conditions were: 1) Pre-denaturation: 95 °C for 30 s; 2) PCR reaction (40 cycles): 95 °C for 3 s and 60 °C for 30 s. There were 3 repetitions per gene, with each sample corresponding to β-actin as a reference. The target gene mRNA abundance was calculated as 2-ΔΔCt (treatment-control), where -ΔΔCt(treatment-control) = (Ct(target gene - Ct-actin)treatment - (Ct(target gene - Ct-actin)control). The related description can be found in previous research (Chen et al., 2019; Wang et al., 2020b).

2.8. Intestinal organoid culture

The organoid isolation and culture in this study were performed in accordance with the previous methods of our research group (Wang et al., 2020b). A 24-well plate was prepared, with 4 °C pre-cooled PBS solution and thawed Matrigel (BD Biosciences, San Jose, CA) in advance. Seven-day-old suckling piglets were euthanized and the abdominal cavity was dissected along the midline of the abdomen to get the middle jejunum samples. After washing with PBS, a glass slide was used to gently scrape the villus and fat was removed as much as possible. The tissue was then washed with PBS, and cut into small pieces (about 0.2 cm), and transferred to a 50-mL centrifuge tube, and then washed with PBS 3 to 4 times. The tissue was mixed with 2 mmol/L ethylenediaminetetraacetic acid (Sigma–Aldrich) at 4 °C and incubated for 30 min to facilitate epithelial separation. After the incubation was completed, the centrifuge tube was shaken for 5 min until high purity crypts were obtained, and then filtered through a 70-μm cell strainer. Ten percent (vol/vol) fetal calf serum was added to the crypt suspension, and centrifuged at 300 × g for 5 min. The supernatant was discarded, and 15 mL of advanced DMEM-GF (Gibco, Grand Island, NY) was added to the centrifuge tube to resuspend the crypts. Then, the resuspended liquid was centrifuged at 150 × g for 2 min. The supernatant continued to be discarded. The crypts were suspended in Matrigel. Then, 50 μL of Matrigel-crypt mixture was added to the centre of each well of the 24-well plate, and placed in a 37 °C incubator for 15 min to solidify. A volume of 500 μL of culture medium was added to each well containing growth factors including Wnt3a, R-Spondin 1-conditioned medium, Noggin, N2 supplement (Invitrogen, Carlsbad, CA), B27 supplement (Invitrogen), N-acetyl cysteine (Sigma–Aldrich), glutamine (SigmaAldrich), nicotinamide (Sigma–Aldrich), recombinant murine epidermal growth factor (PeproTech, Rocky Hill, NJ), and SB202190 (Sigma–Aldrich). The organoids were maintained at 37 °C in an incubator containing 5% CO2. The culture medium was changed every other day. Combined with the concentration of D-Glucose and piglet weight in vivo, it was converted into the required amount of substance concentration (0 as control, 0.1, 1 and 10 mmol/L) and added to the jejunal organoid culture. Images were collected from the visual field of organoids on the third and fifth days after treatment with different concentrations of D-Glucose under a microscope in a 20× combined magnification (Version 4.12, Leica Microsystems, Wetzlar, Germany). The budding rates and bud numbers of budding organoids were counted on the third and fifth days. The mRNA abundance of leucine rich repeat containing G protein-coupled receptor 5 (Lgr5), Chromogranin A and mucin 2 (Muc2) was detected by RT-qPCR.

| Table 1 | Basic diet composition and nutrition level of weaned piglets (as-fed basis, %). |
|---|---|
| Phase 1 | Content | Phase 2 | Content |
| Ingredients | Ingredients |
| Corn | 33.44 | Corn | 43.05 |
| Extruded corn | 25.00 | Extruded corn | 20.00 |
| Soy protein concentrate | 5.00 | Soybean meal (43%) | 20.00 |
| Fishmeal (63%) | 7.00 | Fishmeal (63%) | 4.00 |
| Yeast hydrolysate | 3.00 | Dextrose Monohydrate | 3.00 |
| Whey powder | 14.00 | Soybean oil | 1.50 |
| Sucrose | 2.00 | Stone powder | 0.58 |
| Dextrose monohydrate | 2.00 | Dicalcium phosphate | 0.88 |
| Stone powder | 0.94 | Choline chloride | 0.10 |
| Dicalcium phosphate | 0.32 | Antioxidants | 0.05 |
| Choline chloride | 0.10 | Acidifiers | 0.30 |
| Antioxidants | 0.05 | Salt | 0.10 |
| Acidifiers | 0.30 | Mineral premix1 | 0.15 |
| Zinc oxide | 0.20 | Vitamin premix2 | 0.30 |
| Salt | 0.10 | L-Thr | 0.17 |
| Mineral premix1 | 0.15 | DL-Met | 0.22 |
| Vitamin premix2 | 0.30 | L-Lys HCl | 0.55 |
| DL-Met | 0.33 | L-Trp | 0.05 |
| L-Thr | 0.17 | L-Trp | 0.05 |
| Total calculated nutrition level | 100 | Total calculated nutrition level | 100 |
| Net energy, Mcal/kg | 2.49 | Net energy, Mcal/kg | 2.43 |
| CP | 18.61 | CP | 18.03 |
| Ca | 0.80 | Ca | 0.70 |
| Available P | 0.40 | Available P | 0.39 |
| phosphorus | 1.34 | Lys | 1.23 |
| Met + Cys2 | 0.74 | Met + Cys3 | 0.68 |
| Thr1 | 0.79 | Thr1 | 0.73 |
| Trp1 | 0.22 | Trp1 | 0.22 |

1. Provide the following minerals per kilogram diet: 100 mg ZnSO4, 30 mg MnSO4, 0.3 mg CoSO4, 150 mg FeSO4, 25 mg CuSO4, 0.3 mg Na2SeO3, and 0.5 mg KIO3.
2. Provide the following vitamins per kilogram diet: 2,200 IU vitamin A, 17.5 mg vitamin D3, 220 IU vitamin E, 0.5 mg vitamin K3, 3.5 mg riboflavin, 30 mg niacin, 10 mg D-pantothenic acid, 0.05 mg biotin, 0.3 mg folic acid, 1.0 mg thiamine, 7 mg pyridoxine, and 4.0 mg ethoxyquin.
3. Standardized ileal digestible.
2.9. Statistical analysis

All data in this study were analysed by an ANOVA with polynomial contrasts using the GLM procedure of SAS software (Version 9.4; SAS Institute, Inc., Cary, NC, USA). Data were presented as means ± SEM. P < 0.05 was considered significant and 0.05 < P < 0.1 was considered a statistically significant trend.

3. Results

3.1. Growth performance

Table 3 shows that the diet supplemented with 1 g/kg D-GlcNAc significantly increased the ADG of the second week (P = 0.029). The G:F of the second week had an upward trend (P = 0.070). Compared to the control group, weaned piglets supplemented with D-GlcNAc had a lower feed intake (P = 0.002), which was particularly evident during the first week of post-weaning (P = 0.016). Throughout the experimental period, there was no significant difference in the growth performance of weaned piglets (P > 0.05). Table 4 shows the results of diarrhoea scores in weaned piglets throughout the experimental period. This study has shown that D-GlcNAc supplementation in the diet has no significant effect on diarrhoea scores in weaned piglets (P > 0.05).

3.2. The development of small intestine

Table 5 shows the effect of D-GlcNAc on the development of the small intestine in weaned piglets. Diets supplemented with 3 g/kg D-GlcNAc tended to increase the total intestinal weight (P = 0.087) and relative weight (P = 0.010) of weaned piglets, accompanied by a significant increase in length (P = 0.048) and relative length (P = 0.012).

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### Table 2

| Genes          | Primers         | Primers sequences (5’ to 3’) | Size, bp | NCBI accession number |
|----------------|-----------------|------------------------------|----------|-----------------------|
| **PepT1**      | Forward         | CATCCGATACCTCCCCTTCG         | 144      | NM_214347.1           |
|                | Reverse         | TTCCCATCCATCTATGCAATTT       |          |                       |
| **CAT1**       | Forward         | CAAGCAGGCAGAAGAAGAAGAAGAAGA | 193      | NM_001012613.1        |
|                | Reverse         | CTGTCACACATCTTCGCGG          |          |                       |
| **B^AT1**      | Forward         | CTTGACCGCTTATCAACGGC cott     | 137      | XM_003359855.4        |
|                | Reverse         | AGTCACTGCGACGGCGTGG          |          |                       |
| **EAT3**       | Forward         | GGACACCGACCTTAAAGGAAG       | 177      | NM_001164649.1        |
|                | Reverse         | GCCACCGACCTTAAAGGAAC         |          |                       |
| **SGT1**       | Forward         | CCACACGTCTTTCGAGTAT          | 206      | NM_001012297.1        |
|                | Reverse         | AGGGTGTCTCCTTGCTGTTAAA       |          |                       |
| **FATP4**      | Forward         | AGACACACGTGACCTCTCC          | 188      | XM_021069609.1        |
|                | Reverse         | GCAAGTTGTGTTGATGACCG         |          |                       |
| **Chromogranin A** | Forward     | ACTCGAGGATCATGAAAGGA         | 205      | NM_001164005.2        |
|                | Reverse         | CTCCAGAGACCTGCTTCTG          |          |                       |
| **Muc2**       | Forward         | GCTCCAGAGAGAGAAGGAGAGAGGAGG | 171      | XM_021082584.1        |
|                | Reverse         | CTCAAGTGACAGAGGACCTC          |          |                       |
| **Lgr5**       | Forward         | GCCCTTGTAGGCAACCCCTCT        | 121      | NM_00115762.1         |
|                | Reverse         | AGGACACATCTAAAAGGACCTG       |          |                       |
| **β-actin**    | Forward         | ACTGGAAGGTTGCTGTTCTGG        | 215      | XM_003124280.5        |
|                | Reverse         | TCCGGGACATCAACACAGGAG         |          |                       |

PepT1 – peptide transporter 1; CAT – cationic amino acid transporter 1; B^AT1 – neutral amino acid transporter; EAT3 – neuronal excitatory amino acid transporter; SGT1 – sodium glucose cotransporter; FATP4 – fatty acid transport protein 4; Muc2 – mucin 2; Lgr5 – leucine rich repeat containing G protein-coupled receptor 5.

### Table 3

| Item          | Control | Dietary D-GlcNAc, g/kg | P-value |
|---------------|---------|------------------------|---------|
| BW, kg        |         |                        |         |
| Week 0        | 6.58 ± 0.13 | 6.58 ± 0.13       | 0.999   |
| Week 1        | 6.50 ± 0.10 | 6.56 ± 0.25         | 0.867   |
| Week 2        | 7.18 ± 0.15 | 7.41 ± 0.39         | 0.306   |
| Week 3        | 8.67 ± 0.27 | 8.86 ± 0.47         | 0.629   |
| Week 4        | 10.24 ± 0.28 | 10.16 ± 0.45       | 0.646   |
| ADG, g/d      |         |                        |         |
| Week 1        | 9.82 ± 21.60 | −11.22 ± 19.21     | 0.685   |
| Week 2        | 76.79 ± 12.28b | 121.43 ± 21.09b   | 0.029   |
| Week 3        | 212.5 ± 22.33 | 207.14 ± 25.80    | 0.479   |
| Week 4        | 224.11 ± 23.65 | 186.74 ± 17.03   | 0.386   |
| Week 1 to 2   | 43.30 ± 12.34 | 55.10 ± 19.36     | 0.562   |
| Week 3 to 4   | 218.31 ± 15.07 | 196.94 ± 15.56   | 0.272   |
| Week 1 to 4   | 130.80 ± 12.59 | 126.02 ± 13.14   | 0.628   |
| ADFI, g/d     |         |                        |         |
| Week 1        | 146.04 ± 11.03a | 139.33 ± 18.93a   | 0.016   |
| Week 2        | 329.81 ± 13.37 | 311.90 ± 30.88    | 0.826   |
| Week 3        | 472.14 ± 28.83 | 441.54 ± 28.10   | 0.344   |
| Week 4        | 581.14 ± 35.26 | 519.59 ± 41.17    | 0.391   |
| Week 1 to 2   | 237.02 ± 11.91 | 235.61 ± 24.53    | 0.553   |
| Week 3 to 4   | 256.64 ± 24.45 | 248.05 ± 33.50    | 0.381   |
| Week 1 to 4   | 382.28 ± 13.07a | 304.25 ± 24.39b  | 0.002   |
| G:F, g/d/g/d  |         |                        |         |
| Week 1        | 0.25 ± 0.08 | −0.06 ± 0.14       | 0.016   |
| Week 2        | 0.24 ± 0.05 | 0.35 ± 0.04        | 0.070   |
| Week 3        | 0.46 ± 0.05 | 0.42 ± 0.03        | 0.569   |
| Week 4        | 0.37 ± 0.03 | 0.37 ± 0.04        | 0.018   |
| Week 1 to 2   | 0.20 ± 0.05 | 0.19 ± 0.07        | 0.016   |
| Week 3 to 4   | 0.42 ± 0.03 | 0.42 ± 0.04        | 0.004   |
| Week 1 to 4   | 0.35 ± 0.03 | 0.41 ± 0.03        | 0.059   |

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = the ratio of average daily gain to average daily feed intake.

Within a same row, means not sharing a same superscript letter are significantly different.
3.4. Apparent nutrient digestibility and mRNA abundances of nutrient transporters

Table 7 shows that the apparent digestibility of DM (P < 0.001), CP (P = 0.012), crude ash (P < 0.001), and GE (P < 0.001) in the diet supplemented with 1 g/kg D-GlcNAc significantly increased. The mRNA abundances of the jejunal nutrient transporters are presented in Table 8. Peptide transporter 1 (PepT1) (P = 0.042), neutral amino acid transporter (B₀AT₁) (P = 0.033), and sodium glucose cotransporter (SGLT₁) (P = 0.034) mRNA abundance increased significantly in the 1 g/kg D-GlcNAc group. mRNA abundance increased significantly in the 1 g/kg D-GlcNAc group (P < 0.05). Neuronal excitatory amino acid transporter (EAAT₃) (P = 0.036) and fatty acid transport protein 4 (FATP₄) (P = 0.040) mRNA abundance increased linearly with the increase in dietary D-GlcNAc contents. However, there was no significant difference in cationic amino acid transporter 1 (CAT₁) expression of the jejunal mucosa (P > 0.05).

3.5. Growth of jejunum organoids and expression of related markers

Fig. 1 includes images of jejunum organoids on the third and fifth days after treatment with different concentrations of D-GlcNAc. Figs. 2 and 3 are the statistical results of organoid budding rates and the average budding numbers per budding organoid, respectively. The budding rates of organoids treated with 0.1 mmol/L D-GlcNAc both increased significantly on the third and fifth days (P < 0.05). Compared with the control group, the average budding numbers per budding organoid treated with 0.1 and 10 mmol/L D-GlcNAc increased significantly on the third day (P < 0.05). The average budding numbers per budding organoid treated with 10 mmol/L D-GlcNAc were significantly lower than those of the control group by the fifth day (P < 0.05). Fig. 4 shows the mRNA abundance of Lgr5⁺, Chromogranin A, and Muc2 in the organoids collected on the fifth day. This study showed that organoids treated with D-GlcNAc significantly upregulated the expression of 2 genes, Lgr5⁺ and Chromogranin A (P < 0.05). Compared with the control group, the mRNA abundances of Muc2 genes in organoids treated with 1 and 10 mmol/L D-GlcNAc also increased significantly.

4. Discussion

This study focused on the effects of dietary D-GlcNAc on the growth performance and intestinal function in weaned piglets. Weaning is one of the most stressful events in a pig’s life. It can cause intestinal and immune system dysfunction and lead to reduced pig growth and feed intake (Campbell et al., 2013). Nutrition interventions in post-weaning are important factors in regulating piglet intestinal development and maturity (Xiong et al., 2019). It was found that supplementation with chito-oligosaccharides in weaned piglet diets can significantly increase the average daily gain and feed efficiency (Chen et al., 2009). Another study showed that chito-oligosaccharides in diets can significantly increase the growth rates of broilers (Li et al., 2007). However, our research shows that D-GlcNAc as a feed additive does not seem to improve the growth performance of piglets. The weaned piglets in this experiment were reared in a single pen. Due to the small number of piglets in each group, there were large variations in the feed intake and daily gain of each piglet, resulting in non-significant growth performance results. Interestingly, the apparent digestibility of DM, CP, crude ash, and GE in the diet supplemented with 1 g/kg D-GlcNAc was higher than that in other groups. This study used naturally occurring acid-insoluble ash as a marker. The digestibility may be overestimated due to the lower acid-insoluble ash content in the samples (Naranjo, 1947). Still, differences were observed in this study. Subsequently, the expression of the transporters for small peptides, amino acids, glucose, and fatty acids in the jejunal mucosa were examined. The study found that supplementation with 1 g/kg D-GlcNAc caused a high expression of PepT1, B₀AT₁, and SGLT₁ in the piglet jejunum. Moreover, further increasing the concentration of D-GlcNAc could significantly increase the expression of EAAT₃ and FATP₄ in terms of RNA level, an increase in mRNA abundance caused an increase in the efficiency of nutrient absorption and utilization. Since piglets are susceptible to psychological, nutritional and environmental factors during the weaning stage, the homeostasis of the intestine is easily disrupted (Moeser et al., 2017). D-GlcNAc can quickly respond to weaning stress during the weaning period of piglets, and D-GlcNAc assists piglets resist the threats caused by weaning and greatly improves their digestive ability. This is extremely important for improving the growth of pigs in later stages, including restoring growth performance. In the future, the author plans to verify this hypothesis on the basis of larger-scale experiments.

The small intestine is the main site for digestion and absorption of nutrients. Weaning affects the intestinal function of piglets. The intestinal morphology can well reflect the intestinal function (Pluske et al., 1996). The intestinal villus plays an important role in the process, as piglets absorb nutrients mainly through the

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Table 4
Effect of dietary D-GlcNAc on diarrhea in weaned piglets.¹

| Item                          | Control | Dietary D-GlcNAc, g/kg | P-value |
|-------------------------------|---------|-----------------------|---------|
|                               |         | 1                     |         | 3               |
| Fecal score                   |         |                       |         |                 |
| Week 1                        | 2.05 ± 0.31 | 1.41 ± 0.21 | 1.52 ± 0.24 | 0.131 |
| Week 2                        | 1.73 ± 0.31 | 1.59 ± 0.16 | 2.05 ± 0.25 | 0.374 |
| Week 3                        | 1.54 ± 0.24 | 1.47 ± 0.22 | 2.00 ± 0.28 | 0.211 |
| Week 4                        | 1.30 ± 0.13 | 1.18 ± 0.12 | 1.54 ± 0.21 | 0.256 |
| Week 1 to 2                   | 1.89 ± 0.21 | 1.50 ± 0.14 | 1.79 ± 0.14 | 0.193 |
| Week 3 to 4                   | 1.42 ± 0.15 | 1.33 ± 0.16 | 1.77 ± 0.22 | 0.158 |
| Week 1 to 4                   | 1.66 ± 0.13 | 1.41 ± 0.11 | 1.78 ± 0.15 | 0.130 |

D-GlcNAc = N-acetyl-D-glucosamine.

Table 5
Effect of dietary D-GlcNAc on small intestinal index in weaned piglets.

| Item                          | Control | Dietary D-GlcNAc, g/kg | P-value |
|-------------------------------|---------|-----------------------|---------|
|                               |         | 1                     |         | 3               |
| Small intestine               |         |                       |         |                 |
| Total weight, g               | 503.63 ± 28.33 | 495.64 ± 15.17 | 559.25 ± 15.07 | 0.087 |
| Relative weight¹, g/kg        | 49.03 ± 1.87  | 50.61 ± 2.05     | 57.03 ± 1.32  | 0.010 |
| Total length, m              | 11.45 ± 0.35b | 11.52 ± 0.36b    | 12.41 ± 0.24b | 0.048 |
| Relative length², m/kg        | 1.12 ± 0.02b | 1.16 ± 0.05b     | 1.27 ± 0.03b  | 0.012 |

D-GlcNAc = N-acetyl-D-glucosamine.

¹ Within a same row, means not sharing a same superscript letter are significantly different.
² Relative length refers to the total length divided by body weight of 28 d old.
³ Relative weight refers to the total weight divided by body weight of 28 d old.

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The shedding rates of epithelial cells in the villi can reflect the barrier function of the intestine (Watson et al., 2005). Moreover, this mechanism may be caused by apoptosis (Iwanaga et al., 1993; Hall et al., 1994). Due to weaning stress, the piglet's intestinal epithelium is damaged and the homeostasis of intestinal epithelial cells is destroyed. Supplementing 3 g/kg D-GlcNAc promoted the shedding of crypt cells, accelerated the renewal process of intestinal epithelial cells, and improved the turnover rate.

The intestinal epithelium has a high self-renewal capacity in mammals (Scheper et al., 2011). Thus, the villi and crypt structures of the intestinal epithelium always maintain a dynamic growth process. Most intestinal crypts can be cultured in 3D intestinal organoid models, which include the complete intestinal structure of the crypt, villi, and intestinal lumen. This is a good in vitro model for studying the growth of intestinal stem cells (Sato et al., 2009, 2011). It can be seen from the data in vivo that the length and weight of the small intestine in weaned piglets treated with D-GlcNAc increased linearly. It is hypothesized that this may be related to the regulation of intestinal stem cells. The piglet jejunum

Table 6

| Item          | Control         | Dietary D-GlcNAc, g/kg | P-value |
|---------------|-----------------|------------------------|---------|
| Villus height, μm |                |                        |         |
| Duodenum      | 381.13 ± 13.21  | 368.46 ± 21.98         | 0.865   |
| Jejunum       | 432.42 ± 10.23  | 403.09 ± 11.09         | 0.389   |
| Ileum         | 364.85 ± 15.33  | 343.74 ± 14.94         | 0.132   |
| Crypt depth, μm |                |                        |         |
| Duodenum      | 321.70 ± 12.88  | 296.83 ± 21.63         | 0.485   |
| Jejunum       | 259.83 ± 12.85  | 252.59 ± 11.50         | 0.677   |
| Ileum         | 221.15 ± 15.71  | 201.41 ± 9.42          | 0.405   |
| Villus surface area, mm² |        |                        |         |
| Duodenum      | 156.92 ± 8.88   | 140.93 ± 7.71          | 0.365   |
| Jejunum       | 134.85 ± 5.05   | 132.39 ± 3.26          | 0.645   |
| Ileum         | 140.00 ± 6.36   | 138.31 ± 4.16          | 0.314   |

D-GlcNAc = N-acetyl-D-glucosamine; VH:CD = the ratio of villus height to crypt depth.

a, b Within a same row, means not sharing a same superscript letter are significantly different.

Table 7

| Item          | Control         | Dietary D-GlcNAc, g/kg | P-value |
|---------------|-----------------|------------------------|---------|
| Item          | Control         | Dietary D-GlcNAc, g/kg | P-value |
| Villus height, μm |                |                        |         |
| Duodenum      | 381.13 ± 13.21  | 368.46 ± 21.98         | 0.865   |
| Jejunum       | 432.42 ± 10.23  | 403.09 ± 11.09         | 0.389   |
| Ileum         | 364.85 ± 15.33  | 343.74 ± 14.94         | 0.132   |
| Crypt depth, μm |                |                        |         |
| Duodenum      | 321.70 ± 12.88  | 296.83 ± 21.63         | 0.485   |
| Jejunum       | 259.83 ± 12.85  | 252.59 ± 11.50         | 0.677   |
| Ileum         | 221.15 ± 15.71  | 201.41 ± 9.42          | 0.405   |
| Villus surface area, mm² |        |                        |         |
| Duodenum      | 156.92 ± 8.88   | 140.93 ± 7.71          | 0.365   |
| Jejunum       | 134.85 ± 5.05   | 132.39 ± 3.26          | 0.645   |
| Ileum         | 140.00 ± 6.36   | 138.31 ± 4.16          | 0.314   |

D-GlcNAc = N-acetyl-D-glucosamine; VH:CD = the ratio of villus height to crypt depth.

a, b Within a same row, means not sharing a same superscript letter are significantly different.

Table 8

| Item         | Control         | Dietary D-GlcNAc, g/kg | P-value |
|--------------|-----------------|------------------------|---------|
| Item         | Control         | Dietary D-GlcNAc, g/kg | P-value |
| Item         | Control         | Dietary D-GlcNAc, g/kg | P-value |
| Villus height, μm |                |                        |         |
| Duodenum      | 381.13 ± 13.21  | 368.46 ± 21.98         | 0.865   |
| Jejunum       | 432.42 ± 10.23  | 403.09 ± 11.09         | 0.389   |
| Ileum         | 364.85 ± 15.33  | 343.74 ± 14.94         | 0.132   |
| Crypt depth, μm |                |                        |         |
| Duodenum      | 321.70 ± 12.88  | 296.83 ± 21.63         | 0.485   |
| Jejunum       | 259.83 ± 12.85  | 252.59 ± 11.50         | 0.677   |
| Ileum         | 221.15 ± 15.71  | 201.41 ± 9.42          | 0.405   |
| Villus surface area, mm² |        |                        |         |
| Duodenum      | 156.92 ± 8.88   | 140.93 ± 7.71          | 0.365   |
| Jejunum       | 134.85 ± 5.05   | 132.39 ± 3.26          | 0.645   |
| Ileum         | 140.00 ± 6.36   | 138.31 ± 4.16          | 0.314   |

D-GlcNAc = N-acetyl-D-glucosamine; VH:CD = the ratio of villus height to crypt depth.

a, b Within a same row, means not sharing a same superscript letter are significantly different.
is the main site for digestion and absorption of nutrients. The jejunum was used for crypt organoid culture treated with D-GlcNAc. The study found that the organoid budding rate, when treated with 0.1 mmol/L D-GlcNAc increased significantly. On the third day of organoid culture, the average number of budding organoids per budding organoid in the 0.1 and 10 mmol/L groups were significantly higher than that in the control group. The increase in the budding rate and the average bud number of budding organoids treated with 0.1 mmol/L D-GlcNAc increased significantly. The in-vitro further found that D-GlcNAc is an important regulator of intestinal crypt cell differentiation in weaned piglets. D-GlcNAc can improve the intestinal development and nutrient absorption of weaned piglets via regulating the activity of intestinal stem cells. In the long run, the improvement of the piglet’s intestinal digestive function during weaning may have a positive impact on the growth performance of pigs. This conjecture will be verified based on more experiments in the future.

5. Conclusions

On the whole, D-GlcNAc will not improve the growth performance of weaned piglets. However, 3 g/kg D-GlcNAc can promote the intestinal growth of weaned piglets. Supplementation of 1 g/kg D-GlcNAc is beneficial to the improvement of total tract nutrient digestibility and the expression of nutrient transporters. Experiments in vitro further found that D-GlcNAc is an important regulator of intestinal crypt cell differentiation in weaned piglets. D-GlcNAc can improve the intestinal development and nutrient absorption of weaned piglets via regulating the activity of intestinal stem cells. In the long run, the improvement of the piglet's intestinal digestive function during weaning may have a positive impact on the growth performance of pigs. This conjecture will be verified based on more experiments in the future.

Author contributions

Huansheng Yang: Conceptualization, Supervision, Funding acquisition, Methodology, Writing — review & editing. Zhaobin Wang: Investigation, Resources, Writing — original draft preparation. Xinyuan Yang: Visualization. Lanmei Yin: Data curation. Min Wang: Validation. Yuebang Yin: Format modification. Jie Hu: Formal analysis. Jianzhong Li: Project administration. Yulong Yin: Writing — review & editing, Funding acquisition.

Declaration of competing 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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