Animal Nutrition 8 (2022) 10–17

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

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

Zhaobin Wang [a, b], Jie Hu [a], Xinyuan Yang [a], Lanmei Yin [a, b], Min Wang [a, b], Yuebang Yin [b], Jianzhong Li [a], Huansheng Yang [a, b, *], Yulong Yin [a, b]

[a] *Hunan Provincial Key Laboratory of Animal Intestinal Function and Regulation, Hunan International Joint Laboratory of Animal Intestinal Ecology and Health, Laboratory of Animal Nutrition and Human Health, College of Life Sciences, Hunan Normal University, Changsha, Hunan, 410081, China*
[b] *Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Hunan Provincial Key Laboratory of Animal Nutritional Physiology and Metabolic Process, Changsha, Hunan, 410125, China*

ARTICLE INFO

Article history:
Received 23 January 2021
Received in revised form
9 April 2021
Accepted 12 April 2021
Available online 2 October 2021

Keywords:
N-Acetyl-D-glucosamine
Weaned piglet
Growth performance
Intestinal organoid
Intestinal stem cell

ABSTRACT

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 diarrhoea 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 ($P < 0.05$). Mucin 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.

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

* Corresponding author.
E-mail address: yhs@hunnu.edu.cn (H. Yang).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2021.04.008

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 organoids 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, 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 = $\pi \times$ VH $\times$ 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

Table 1
Basic diet composition and nutrition level of weaned piglets (as-fed basis).

Phase 1		Phase 2	
Ingredient	Content, %	Ingredients	Content, %
Corn	33.44	Corn	43.05
Extruded corn	25.00	Extruded corn	20.00
Soy protein concentrate	5.00	Soybean meal (43%)	20.00
Soybean meal (43%)	7.00	Fishmeal (63%)	4.00
Fishmeal (63%)	5.00	Whey powder	5.00
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 premix[1]	0.15
Zinc oxide	0.20	Vitamin premix[2]	0.30
Salt	0.10	L-Lys HCl	0.54
Mineral premix[1]	0.15	DL-Met	0.22
Vitamin premix[2]	0.30	L-Thr	0.17
L-Lys HCl	0.55	L-Trp	0.06
DL-Met	0.33		
L-Thr	0.17		
L-Trp	0.05		
Total	100	Total	100
Calculated nutrition level			
Net energy, Mcal/kg	2.49	Net energy, Mcal/kg	2.46
CP	18.61	CP	18.03
Ca	0.80	Ca	0.70
Available P	0.40	Available P	0.39
Lys[3]	1.34	Lys[3]	1.23
Met + Cys[3]	0.74	Met + Cys[3]	0.68
Thr[3]	0.79	Thr[3]	0.73
Trp[3]	0.22	Trp[3]	0.22

[1] Provide the following minerals per kilogram diet: 100 mg $ZnSO_4$, 30 mg $MnSO_4$, 0.3 mg $CoSO_4$, 150 mg $FeSO_4$, 25 mg $CuSO_4$, 0.3 mg Na_2SeO_3, and 0.5 mg KIO_3.
[2] Provide the following vitamins per kilogram diet: 2,200 IU vitamin A, 17.5 μg vitamin B_{12}, 220 IU vitamin D_3, 16 IU vitamin E, 0.5 mg vitamin K_3, 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.

the statistical results for subsequent analysis was calculated (Chen et al., 2019).

2.7. Real-time quantitative PCR

The collected piglet jejunal mucosa samples were ground into fine particles using liquid nitrogen and quickly transferred into 1.5-mL centrifuge tubes containing RNAiso Plus (Code No. 9109, TaKaRa, Dalian, China). The samples were stood on ice for 1 h and the total RNA was extracted from the sample according to the RNAiso Plus kit instructions. Total RNA samples were detected using a multi-purpose microplate reader and adjusted to the appropriate concentration (700 to 1,000 ng/μL). It was determined whether the $OD_{260/280}$ (1.8 to 2.0) met the experimental requirements. A reverse transcription operation was performed for the total RNA samples that met the requirements in 2 steps according to PrimeScript RT reagent Kit instruction (Code No. RR037A, TaKaRa, Dalian, China): 1) genomic DNA removal reaction; 2) reverse transcription reaction. The reverse transcription conditions in the first step were 42 °C for 2 min. In the second step, they were 37 °C for 15 min and 85 °C for 5 s. The cDNA product obtained by the reverse transcription reaction 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 ddH_2O. This kit was purchased from TaKaRa (Code No. RR420A, Dalian, China). Reaction conditions were: 1) Predenaturation: 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 $2^{-\Delta\Delta Ct(treat-control)}$; $-\Delta\Delta Ct_{(treat-control)} = (Ct_{target\ gene} - Ct_{\beta\text{-}actin})_{treat} - (Ct_{target\ gene} - Ct_{\beta\text{-}actin})_{control}$. 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, SanJose, 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 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, 5% CO_2 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% CO_2. The culture medium was changed every other day. Combined with the concentration of D-GlcNAc 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 applied to the jejunum organoid culture. Images were collected from the visual field of organoids on the third and fifth days after treatment with different concentrations of D-GlcNac 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 2
Primers used for real-time quantitative PCR analysis.

Genes	Primers	Primers sequences (5′ to 3′)	Size, bp	NCBI accession number
PepT1	Forward	CATCGCCATACCCTTCTG	144	NM_214347.1
	Reverse	TTCCCATCCATCGTGACATT		
CAT1	Forward	CAACGACCGGACCAAAACAC	193	NM_001012613.1
	Reverse	CTGGTACACCATGTTCGGCT		
B^0AT1	Forward	CCTGACGCTTATCAACGGGT	137	XM_003359855.4
	Reverse	AGTTCATGTCGCAGGTCTGG		
EAAT3	Forward	GGCACCGCACTCTACGAAGCA	177	NM_001164649.1
	Reverse	GCCCACGGCACTTAGCACGA		
SGLT1	Forward	CGCGTGCTGTTTCCAGATGAT	206	NM_001012297.1
	Reverse	AGGGTGCTGCTGCTGTTAAA		
FATP4	Forward	AGACACACGTTGGACCTTCC	188	XM_021069609.1
	Reverse	GCAGGTTGGTGTTGATGAGC		
Chromogranin A	Forward	ACTCCGAGGAGATGAACGGA	205	NM_001164005.2
	Reverse	CTTGGAGGACGCCTCTTCTG		
Muc2	Forward	GCTCCAGAGAGAAGGCAGAACC	171	XM_021082584.1
	Reverse	CTCAGGTGCACAGCGAACTC		
$Lgr5^+$	Forward	GCCTTTGTAGGCAACCCTTC	121	NM_001315762.1
	Reverse	AGGCACCATTCAAAGTCAGTG		
β-actin	Forward	AGTTGAAGGTGGTCTCGTGG	215	XM_003124280.5
	Reverse	TGCGGGACATCAAGGAGAAG		

PepT1 = peptide transporter 1; *CAT1* = cationic amino acid transporter 1; *B^0AT1* = neutral amino acid transporter; *EAAT3* = neuronal excitatory amino acid transporter; *SGLT1* = sodium glucose cotransporter; *FATP4* = fatty acid transport protein 4; *Muc2* = mucin 2; *$Lgr5^+$* = leucine rich repeat containing G protein-coupled receptor 5.

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 ($P = 0.012$).

Table 3
Effect of dietary D-GlcNAc on growth performance in weaned piglets.

Item	Control	Dietary D-GlcNAc, g/kg		*P*-value
		1	3	
BW, kg				
Week 0	6.58 ± 0.13	6.58 ± 0.13	6.58 ± 0.15	0.999
Week 1	6.50 ± 0.10	6.56 ± 0.25	6.43 ± 0.19	0.867
Week 2	7.18 ± 0.15	7.41 ± 0.39	6.88 ± 0.22	0.306
Week 3	8.67 ± 0.27	8.86 ± 0.47	8.43 ± 0.27	0.629
Week 4	10.24 ± 0.28	10.16 ± 0.45	9.84 ± 0.32	0.646
ADG, g/d				
Week 1	9.82 ± 21.60	−11.22 ± 19.21	−6.12 ± 16.67	0.685
Week 2	76.79 ± 12.28^{ab}	121.43 ± 21.09^{a}	64.29 ± 16.91^{b}	0.029
Week 3	212.5 ± 22.33	207.14 ± 25.80	239.80 ± 14.61	0.479
Week 4	224.11 ± 23.65	186.74 ± 17.03	201.78 ± 19.36	0.386
Week 1 to 2	43.30 ± 12.34	55.10 ± 19.36	33.16 ± 13.76	0.562
Week 3 to 4	218.31 ± 15.07	196.94 ± 15.56	227.04 ± 11.58	0.272
Week 1 to 4	130.80 ± 12.59	126.02 ± 13.14	116.30 ± 9.84	0.628
ADFI, g/d				
Week 1	146.04 ± 11.03^{a}	139.33 ± 18.93^{a}	93.01 ± 15.02^{b}	0.016
Week 2	329.81 ± 13.37	331.90 ± 30.88	315.25 ± 20.95	0.826
Week 3	472.14 ± 28.83	441.54 ± 28.10	491.89 ± 22.14	0.344
Week 4	581.14 ± 35.26	519.55 ± 41.17	522.95 ± 37.11	0.391
Week 1 to 2	237.92 ± 11.91	235.61 ± 24.53	212.86 ± 20.37	0.553
Week 3 to 4	526.64 ± 24.45	480.55 ± 33.50	520.85 ± 23.87	0.381
Week 1 to 4	382.28 ± 13.07^{a}	304.25 ± 24.39^{b}	196.74 ± 20.95^{b}	0.002
G:F, g/d:g/d				
Week 1	0.25 ± 0.08	−0.06 ± 0.14	−0.12 ± 0.16	0.071
Week 2	0.24 ± 0.05	0.35 ± 0.04	0.21 ± 0.06	0.070
Week 3	0.46 ± 0.05	0.42 ± 0.03	0.47 ± 0.04	0.569
Week 4	0.37 ± 0.03	0.37 ± 0.04	0.39 ± 0.03	0.918
Week 1 to 2	0.20 ± 0.05	0.19 ± 0.07	0.13 ± 0.06	0.658
Week 3 to 4	0.42 ± 0.03	0.42 ± 0.04	0.43 ± 0.03	0.965
Week 1 to 4	0.35 ± 0.03	0.41 ± 0.03	0.40 ± 0.02	0.242

D-GlcNAc = *N*-acetyl-D-glucosamine; 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.
[a, b] Within a same row, means not sharing a same superscript letter are significantly different.

Table 4
Effect of dietary D-GlcNAc on diarrhoea 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.
[1] 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).

3.3. Intestinal histomorphology

Table 6 shows the D-GlcNAc effects on the morphology of the duodenum, jejunum, and ileum of weaned piglets. Our study showed that D-GlcNAc did not affect VH and CD but resulted in numerically shorter VH and shallower CD, which lead to an increase in ileal VH:CD ratio ($P = 0.027$). In addition, the cell shedding rates in the ileum villi were increased ($P < 0.001$).

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 (B^0AT1) ($P = 0.033$), 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.

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 weight[1], g/kg	49.03 ± 1.87^b	50.61 ± 2.05^b	57.03 ± 1.32^a	0.010
Total length, m	11.45 ± 0.35^b	11.52 ± 0.36^b	12.41 ± 0.24^a	0.048
Relative length[2], m/kg	1.12 ± 0.02^b	1.16 ± 0.05^b	1.27 ± 0.03^a	0.012

D-GlcNAc = *N*-acetyl-D-glucosamine.
[a, b] Within a same row, means not sharing a same superscript letter are significantly different.
[1] Relative weight refers to the total weight divided by body weight at 28 d old.
[2] Relative length refers to the total length divided by body weight of 28 d old.

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*, B^0AT1, 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-ClcNAc 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

Table 6
Effect of dietary D-GlcNAc on intestinal histomorphology in weaned piglets.

Item	Control	Dietary D-GlcNAc, g/kg		*P*-value
		1	3	
Villus height, μm				
Duodenum	381.13 ± 13.21	368.46 ± 21.98	375.30 ± 12.94	0.865
Jejunum	433.42 ± 10.23	403.09 ± 11.09	425.45 ± 22.77	0.389
Ileum	364.85 ± 15.33	343.74 ± 14.94	390.88 ± 17.04	0.132
Crypt depth, μm				
Duodenum	321.70 ± 12.88	296.83 ± 21.63	333.04 ± 28.60	0.485
Jejunum	259.83 ± 12.85	252.59 ± 11.50	243.18 ± 15.08	0.677
Ileum	221.15 ± 15.71	201.41 ± 9.42	204.32 ± 5.01	0.405
Villus width, μm				
Duodenum	156.92 ± 8.88	140.93 ± 7.71	150.38 ± 6.50	0.365
Jejunum	134.35 ± 5.05	132.39 ± 3.26	137.65 ± 2.00	0.645
Ileum	140.00 ± 6.36	138.31 ± 4.16	148.70 ± 4.29	0.314
Villus surface area, mm^2				
Duodenum	0.19 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.515
Jejunum	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.513
Ileum	0.16 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.119
VH:CD, μm:μm				
Duodenum	1.19 ± 0.03	1.26 ± 0.07	1.15 ± 0.07	0.438
Jejunum	1.69 ± 0.09	1.62 ± 0.08	1.79 ± 0.14	0.524
Ileum	1.58 ± 0.06^{b}	1.73 ± 0.09^{ab}	1.92 ± 0.08^{a}	0.027
Cell shedding rates in the villus, %				
Duodenum	4.96 ± 1.70	8.76 ± 0.83	8.29 ± 1.51	0.153
Jejunum	16.94 ± 3.00	16.46 ± 2.93	22.85 ± 2.71	0.241
Ileum	10.63 ± 1.17^{c}	16.44 ± 1.90^{b}	23.44 ± 1.75^{a}	<0.001

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
Effect of dietary D-GlcNAc on apparent total tract digestibility of nutrients in weaned piglets (%).

Item	Control	Dietary D-GlcNAc, g/kg		*P*-value
		1	3	
DM	95.99 ± 0.20^{b}	97.03 ± 0.14^{a}	95.75 ± 0.20^{b}	<0.001
CP	83.64 ± 1.01^{ab}	87.37 ± 1.48^{a}	80.70 ± 1.70^{b}	0.012
Crude ash	61.23 ± 1.20^{b}	72.16 ± 1.21^{a}	60.56 ± 0.96^{b}	<0.001
GE	89.17 ± 0.39^{b}	92.45 ± 0.64^{a}	87.98 ± 0.87^{b}	<0.001

D-GlcNAc = *N*-acetyl-D-glucosamine; DM = dry matter; CP = crude protein; GE = gross energy.
[a, b] Within a same row, means not sharing a same superscript letter are significantly different.

intestinal villi. In general, the stress response caused by weaning can damage the intestinal villi of piglets (Beers-Schreurs van et al., 1998). An abnormal intestinal morphological structure usually affects the growth and development of weaned piglets. When intestinal villi are shortened, resulting in a reduced contact surface between nutrients and villi, this causes absorption and utilization of fewer nutrients by piglets, which in turn reduces growth performance (Wang et al., 2020a). In this study, 3 g/kg D-GlcNAc did not affect VH and CD but produced numerically shorter VH and shallower CD, which led to an increase in ileal VH:CD ratio. Crypts are areas where numerous stem cells exist and have strong self-renewal capabilities (Verdile et al., 2019). Intestinal epithelial cells migrate from the crypt to the villi, and then shed into the intestinal cavity. Intestinal epithelial cells are constantly renewed. 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

Table 8
Effect of dietary D-GlcNAc on mRNA abundances of the jejunal nutrient transporters in weaned piglets.

Item	Control	Dietary D-GlcNAc, g/kg		*P*-value
		1	3	
PepT1	0.94 ± 0.13^{b}	1.74 ± 0.23^{a}	1.38 ± 0.22^{ab}	0.042
B^0AT1	0.93 ± 0.07^{b}	1.36 ± 0.12^{a}	1.08 ± 0.12^{ab}	0.033
CAT1	0.94 ± 0.06	1.31 ± 0.18	1.15 ± 0.24	0.392
EAAT3	0.69 ± 0.13^{b}	0.92 ± 0.08^{ab}	1.06 ± 0.14^{a}	0.036
SGLT1	0.95 ± 0.11^{b}	1.50 ± 0.19^{a}	1.20 ± 0.15^{ab}	0.034
FATP4	0.82 ± 0.04^{b}	1.02 ± 0.08^{ab}	1.10 ± 0.08^{a}	0.040

D-GlcNAc = *N*-acetyl-D-glucosamine; *PepT1* = peptide transporter 1; *B^0AT1* = neutral amino acid transporter; *CAT1* = cationic amino acid transporter 1; *EAAT3* = neuronal excitatory amino acid transporter; *SGLT1* = sodium glucose cotransporter; *FATP4* = fatty acid transport protein 4.
[a, b] Within a same row, means not sharing a same superscript letter are significantly different.

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 (Schepers 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

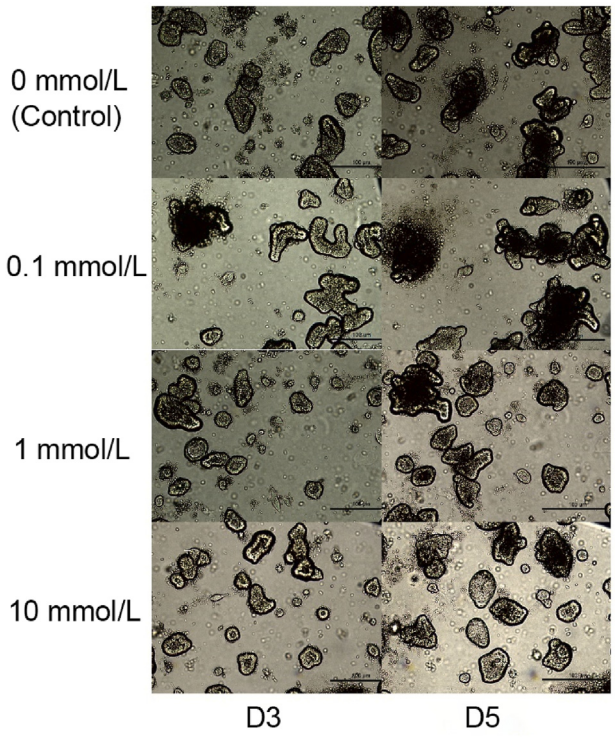

Fig. 1. The organoid images observed under a microscope in a 20× combined magnification and image processing and analysis system. 0 (control), 0.1, 1, and 10 mmol/L D-GlcNAc were used to culture jejunal organoids of piglets in vitro. The budding rates and bud numbers of budding organoids were counted on the third (D3) and fifth (D5) days of culture. Scale bar, 100 μm. D-GlcNAc = *N*-acetyl-D-glucosamine.

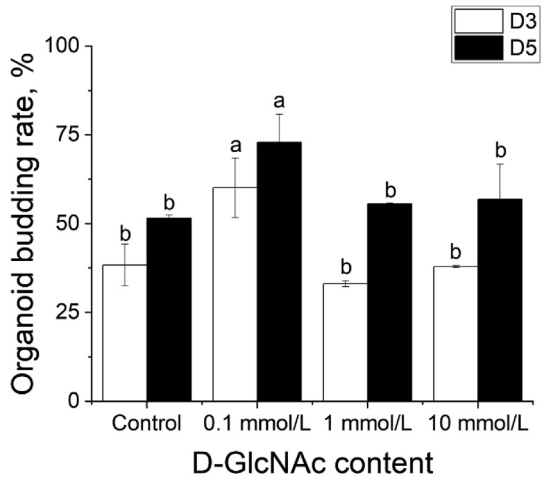

Fig. 2. The organoid budding rates on the third (D3) and fifth (D5) days treated with 0 (control), 0.1, 1, and 10 mmol/L D-GlcNAc. [a, b] Different letters indicate statistical significance ($P < 0.05$). D-GlcNAc = *N*-acetyl-D-glucosamine.

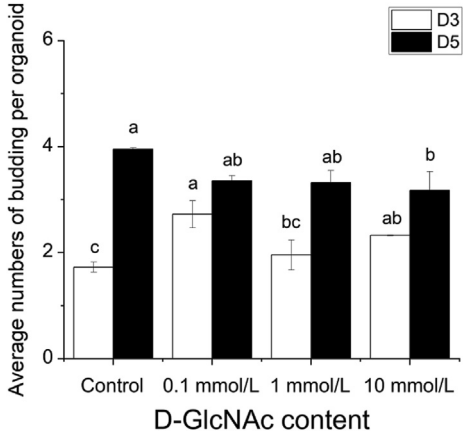

Fig. 3. The average bud numbers per budding organoid on the third (D3) and fifth (D5) days treated with 0 (control), 0.1, 1, and 10 mmol/L D-GlcNAc. [a, b] Different lowercase letters indicate statistical significance ($P < 0.05$). D-GlcNAc = *N*-acetyl-D-glucosamine.

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 represents an increase in the number of crypts. It is reported that there are numerous undifferentiated intestinal stem cells in the crypt (Barker et al., 2007). Our study found that organoids treated with D-GlcNAc upregulated *Lgr5*$^+$ expression. It is known that *Lgr5*$^+$ is an important marker of intestinal stem cells (Barker et al., 2007; Yan et al., 2012). Numerous stem cells in the small intestine will continue to differentiate into various mature intestinal cells to regulate intestinal function. Chromogranin A and *Muc2* are 2 markers of intestinal cell differentiation (Wang et al., 2020b). By detecting differentiation markers in organoids, the abundance of Chromogranin A and *Muc2* both increased significantly. The renewal and differentiation of intestinal epithelial cells promoted the development of the small intestine. A previous study has reported that intestinal length is positively correlated with digestion

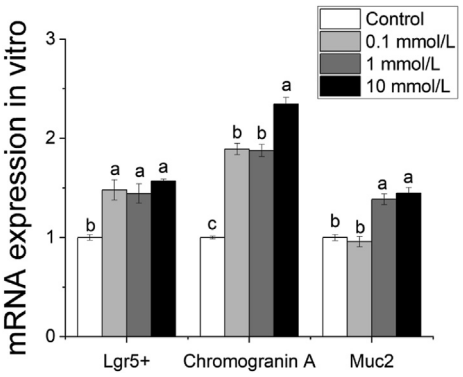

Fig. 4. The mRNA abundance of *Lgr5*$^+$, Chromogranin A and *Muc2* in organoids treated with 0 (control), 0.1, 1, and 10 mmol/L D-GlcNAc. [a, b] Different lowercase letters indicate statistical significance ($P < 0.05$). D-GlcNAc = *N*-acetyl-D-glucosamine; *Lgr5*$^+$ = leucine rich repeat containing G protein-coupled receptor 5; *Muc2* = mucin 2.

and absorption capacity, which is beneficial to the growth of weaned piglets (Wang et al., 2019). This provides a reasonable explanation for the increase in length of the small intestine and the improvement of intestinal digestion and absorption in vivo. D-GlcNAc is an important regulator of intestinal crypt cell differentiation in weaned piglets and provides an important measure for maintaining cell homeostasis in animals.

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.

Acknowledgements

This work was supported by Hunan Province Key Field R&D Program (2019NK2193), Key Programs of Frontier Scientific

Research of the Chinese Academy of Sciences (QYZDY-SSW-SMC008), Natural Science Foundation of Hunan Province (2017JJ1020), Young Elite Scientists Sponsorship Program by CAST (YESS20160086).

References

AOAC. Official methods of analysis. 18th ed. Gaithersburg, MD: Association of Official Analytical Chemists; 2007.

Barea R, Nieto R, Vitari F, Domeneghini C, Aguilera J. Effects of pig genotype (Iberian v. Landrace × Large White) on nutrient digestibility, relative organ weight and small intestine structure at two stages of growth. Animal 2011;5(4):547–57. https://doi.org/10.1017/S1751731110002181.

Barker N, Es van J, Kuipers J, Kujala P, Born van den M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449(7165):1003–7. https://doi.org/10.1038/nature06196.

Beers-Schreurs van H, Nabuurs M, Vellenga L, Valk H, Wensing T, Breukink H. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J Nutr 1998;128(6):947–53.

Bullen T, Forrest S, Campbell F, Dodson A, Hershman M, Pritchard D, et al. Characterization of epithelial cell shedding from human small intestine. Lab Invest 2006;86(10):1052–63.

Campbell J, Crenshaw J, Polo J. The biological stress of early weaned piglets. J Anim Sci Biotechnol 2013;4(1):19.

Chen C, Wang Z, Li J, Li Y, Huang P, Ding X, et al. Dietary vitamin E affect small intestinal histomorphology, digestive enzyme activity and the expression of nutrient transporters by inhibiting proliferation of intestinal epithelial cells within jejunum in weaned piglets. J Anim Sci 2019;97(3):1212–21. https://doi.org/10.1093/jas/skz023.

Chen J, Shen C, Liu C. N-acetylglucosamine: production and applications. Mar Drugs 2010;8(9):2493–516.

Chen Y, Kim I, Cho J, Yoo J, Wang Y, Huang Y, et al. Effects of chitooligosaccharide supplementation on growth performance, nutrient digestibility, blood characteristics and immune responses after lipopolysaccharide challenge in weanling pigs. Livest Sci 2009;124(1–3):255–60.

Hall P, Coates P, Ansari B, Hopwood D. Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. J Cell Sci 1994;107(12):3569.

Iwanaga T, Han H, Adachi K, Fujita T. A novel mechanism for disposing of effete epithelial cells in the small intestine of Guinea pigs. Gastroenterology 1993;105(4):1089–97. https://doi.org/10.5555/uri:pii:001650859390953A.

Kisielinski K, Willis S, Prescher A, Klosterhalfen B, Schumpelick V. A simple new method to calculate small intestine absorptive surface in the rat. Clin Exp Med 2002;2(3):131–5. https://doi.org/10.1007/s102380200018.

Li X, Piao X, Kim S, Liu P, Wang L, Shen Y, et al. Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. Poultry Sci 2007;86(6):1107–14.

Mattila J, Kokki K, Hietakangas V, Boutros M. Stem cell intrinsic hexosamine metabolism regulates intestinal adaptation to nutrient content. Dev Cell 2018;47(1):112–21. https://doi.org/10.1016/j.devcel.2018.08.011. e113.

McCarthy J, Aherne F, Okai D. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can J Anim Sci 1974;54(1):107–9. https://doi.org/10.4141/cjas74-016.

Moeser A, Pohl C, Rajput M. Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs. Anim Nutr 2017;3(4):313–21. https://doi.org/10.1016/j.aninu.2017.06.003.

Naranjo V. Evaluation of novel dietary feed additives for nursery and growing/finishing pigs. LSU Master's Theses 1947;Etd-11092008-134145. https://digitalcommons.lsu.edu/gradschool_theses/1947.

NRC. Nutrient requirements of swine. 11th revised edition. Washington, DC: National Academy Press; 2012.

Pluske J, Williams I, Aherne F. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim Sci 1996;62(1):131–44.

Sato T, Es van J, Snippert H, Stange D, Vries R, Born van den M, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2011;469(7330):415–8. https://doi.org/10.1038/nature09637.

Sato T, Vries R, Snippert H, Wetering van de M, Barker N, Stange D, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459(7244):262–5. https://doi.org/10.1038/nature07935.

Schepers A, Vries R, Born van den M, Wetering van de M, Clevers H. Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. EMBO J 2011;30(6):1104–9. https://doi.org/10.1038/emboj.2011.26.

Trevisi P, Casini L, Coloretti F, Mazzoni M, Merialdi G, Bosi P. Dietary addition of Lactobacillus rhamnosus GG impairs the health of Escherichia coli F4-challenged piglets. Animal 2011;5(9):1354–60. https://doi.org/10.1017/S1751731111000462.

Verdile N, Mirmahmoudi R, Brevini T, Gandolfi F. Evolution of pig intestinal stem cells from birth to weaning. Animal 2019;13(12):2830–9. https://doi.org/10.1017/s1751731119001319.

Wang M, Yang C, Wang Q, Li J, Huang P, Li Y, et al. The relationship between villous height and growth performance, small intestinal mucosal enzymes activities and nutrient transporters expression in weaned piglets. J Anim Physiol Anim Nutr 2020a;104(2):606–15. https://doi.org/10.1111/jpn.13299.

Wang M, Yang C, Wang Q, Li J, Li Y, Ding X, et al. The growth performance, intestinal digestive and absorptive capabilities in piglets with different lengths of small intestines. Animal 2019:1–8. https://doi.org/10.1017/S175173111900288X.

Wang Z, Li J, Wang Y, Wang L, Yin Y, Yin L, et al. Dietary vitamin A affects growth performance, intestinal development, and functions in weaned piglets by affecting intestinal stem cells. J Anim Sci 2020b;98(2):skaa020. https://doi.org/10.1093/jas/skaa020.

Watson A, Chu S, Sieck L, Gerasimenko O, Bullen T, Campbell F, et al. Epithelial barrier function in vivo is sustained despite gaps in epithelial layers. Gastroenterology 2005;129(3):902–12. https://doi.org/10.1053/j.gastro.2005.06.015.

Xiong X, Tan B, Song M, Ji P, Kim K, Yin Y, et al. Nutritional intervention for the intestinal development and health of weaned pigs. Front Vet Sci 2019;6(46). https://doi.org/10.3389/fvets.2019.00046 (Review).

Yan K, Chia L, Li X, Ootani A, Su J, Lee J, et al. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci Unit States Am 2012;109(2):466–71. https://doi.org/10.1073/pnas.1118857109.

Yan S, Long L, Zong E, Huang P, Li J, Li Y, et al. Dietary sulfur amino acids affect jejunal cell proliferation and functions by affecting antioxidant capacity, Wnt/beta-catenin, and the mechanistic target of rapamycin signaling pathways in weaning piglets. J Anim Sci 2018;96(12):5124–33. https://doi.org/10.1093/jas/sky349.

Yang H, Xiong X, Wang X, Tan B, Li T, Yin Y. Effects of weaning on intestinal upper villus epithelial cells of piglets. PLoS One 2016a;11(3):e0150216. https://doi.org/10.1371/journal.pone.0150216.

Yang H, Wang X, Xiong X, Yin Y. Energy metabolism in intestinal epithelial cells during maturation along the crypt-villus axis. Sci Rep 2016b;6:31917. https://doi.org/10.1038/srep31917.

Yang H, Xiong X, Wang X, Li T, Yin Y. Effects of weaning on intestinal crypt epithelial cells in piglets. Sci Rep 2016c;6:36939. https://doi.org/10.1038/srep36939.

Yang H, Xiong X, Yin Y. Metabolomic analysis of intestinal epithelial cell maturation along the crypt–villus axis. RSC Adv 2016d;6(33):27566–74. https://doi.org/10.1039/C5RA27722A.

Yin Y, Baidoo S, Schulze H, Simmins P. Effects of supplementing diets containing hulless barley varieties having different levels of non-starch polysaccharides with β-glucanase and xylanase on the physiological status of the gastrointestinal tract and nutrient digestibility of weaned pigs. Livest Prod Sci 2001;71(2):97–107. https://doi.org/10.1016/S0301-6226(01)00214-7.

Zachara N, Hart G. Cell signaling, the essential role of O-GlcNAc! Biochim Biophys Acta Mol Cell Biol Lipids 2006;1761(5):599–617. https://doi.org/10.1016/j.bbalip.2006.04.007.