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Original Research Article

Yeast-based nucleotide supplementation in mother sows modifies the intestinal barrier function and immune response of neonatal pigs

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ABSTRACT

In the present study, we aimed to evaluate the effects of maternal yeast-based nucleotide (YN) supplementation on the intestinal immune response and barrier function in neonatal pigs, as well as the diarrhoea rate and growth performance in suckling piglets. Sixty-four late-gestation sows were assigned to the following groups: the CON (fed a basal diet) and YN groups (fed a basal diet with 4 g YN/kg diet). The experiment started on d 85 of gestation and ended on d 20 of lactation. Diarrhoea rate and average daily gain of the piglets were recorded, and samples of blood and intestines from neonatal piglets were collected before they consumed colostrum during farrowing. Compared with the CON group, maternal YN supplementation increased the weaning weight of litter and decreased the diarrhoea rate ($P < 0.01$). In addition, maternal YN supplementation promoted the ileal villus development in the neonates compared with that in the CON group ($P < 0.01$). Maternal YN supplementation also increased the ileal secretory immunoglobulin A (sIgA) level compared with that in the CON group ($P < 0.05$). The real-time PCR results showed that maternal dietary YN supplementation increased the jejunal and ileal expression of interleukin (*IL*)-*17*, *IL-8*, *IL-1β*, *IL-10* and tumor necrosis factor (*TNF*)- α in the neonates compared with that in the CON group ($P < 0.05$). Overall, maternal nucleotide supplementation improved the villus development and innate immunity of neonatal piglets during late pregnancy. This may be associated with the decrease in diarrhoea and the increase in weaning weight of the litter of suckling piglets.

1. Introduction

Maternal nutrition level is one of the factors programming nutrient partitioning and ultimately regulating the growth and development of foetal tissues and organs (Mcgovern et al., 2014). The fastest growing stage of foetal small intestine is during late pregnancy, and an impaired intestine in foetus can lead to a higher diarrhoea rate and lower growth performance after birth (Che et al., 2016). Chronic diarrhoea in the suckling stage is characterised by small intestinal dysplasia and immunodeficiency (Avila et al., 1989; Bueno et al., 1994). In the pig industry, diarrhoea in suckling piglets is a major concern as it can increase morbidity and decrease the weight gain daily (Kongsted et al., 2014). Thus, it is important to increase the growth and development of foetal intestine during late gestation.

Due to abundant nucleotides in the milk of sows, there are several studies on the importance of nucleotides in the regulation

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of growth performance and intestinal health of pigs (Che et al., 2016; Waititu et al., 2017). Studies on sows have suggested that the growth performance of piglets was improved by maternal yeast culture supplementation during gestation and lactation (Kim et al., 2008; Hung 2015). Furthermore, maternal dietary uridine supplementation reduces diarrhoea incidence in piglets by regulating the intestinal mucosal barrier and cytokine profiles (Wu et al., 2020). In addition, studies on broilers and weaned piglets have suggested that exogenous nucleotides could improve growth performance, intestinal enzyme activity and villus development (Superchi et al., 2012; Daneshmand et al., 2017). On the contrary, there are indications of the protective effects of nucleotide supplementation in infant formulas against diarrhoea and immune benefits (Hess and Greenberg 2012). Dietary nucleotide supplementation can improve immune response and decrease diarrhoea rate in piglets by regulating inflammatory cytokines under unhygienic conditions or weaning stress conditions (Che et al., 2016; Waititu et al., 2017). Hence, nucleotides play an important role in intestinal development and function in animals. However, there is a paucity of studies on the effects of maternal nucleotides supplementation on intestinal immune response and barrier function in neonatal pigs. As a by-product of yeast degradation, yeast-based nucleotide (YN) is rich in nucleotides. It is hypothesised that maternal YN supplementation will enhance intestinal structure and function in neonatal piglets. Thus, the aim of the present study was to evaluate the effects of maternal dietary YN supplementation on the intestinal immune response and barrier function in neonatal piglets.

2. Materials and methods

Animal experiments were approved by the Animal Care Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science, and were approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (2015-8 A).

2.1. Animals, diets and housing

Sixty-four pregnant sows (Large White × Landrace) with similar parity (3 to 6 parities, back-fat thickness 15 to 17 mm) were divided into 2 treatments: the control (CON) and YN groups, there were 32 sows in each group. The experiment began on d 85 of gestation and ended on d 20 of lactation. During this period, sows in the CON group were fed a basal diet and in the YN group were fed a basal diet plus extra 4 g YN/kg diet. The management of breeding and housing was as recently described in detail (Xie et al., 2016; Gao et al., 2019). Experiment was carried out in Henan Guang'an Biology Technology Co. Ltd (Zhengzhou, 450001, China).

YN (Angel Yeast Co., Ltd, Hubei, China) contained crude protein (41.31%), amino acid nitrogen (1.79%), ash (7.38%) and total nucleic acid (10.37%). All nutrients in the basal diet met the nutrient recommendation of the NRC 2012 (Table 1). All sows were fed twice daily at 06:00 and 15:00 and had free access to water during the experiment period. Each sow was fed 2.5 to 3.0 kg/d diet from d 85 to 107 of pregnancy and approximately 1.8 kg/d on d 5 before delivery. On the day of farrowing, the sows initially received approximately 1.0 kg/d of their diets, which was then increased by approximately 0.8 kg/d on d 1 and 2 and by about 1.0 kg/d on d 3 and 4 until reaching at their maximum feed intake.

Thirty sows whose litter size was 10 to 13 were chosen both in CON and YN groups. By exchanging piglets from sows having >11 piglets, litters in each group were standardized to 11 piglets within 24 h after farrowing, and put them in sows having <11 piglets of the same group. The other 4 litters were set as a backup (2 litters in CON and YN groups, respectively), if a piglet in CON or YN group dead within a week, a piglet from standby would be added, but no more than 2 piglets per litter were added (Wu et al., 2012). Piglets were weighed on d 1 (initial weight) and 20 of lactation, in addition, the number of dead piglets during the whole lactation were also recorded to obtain the survival rate and total milk yield.

2.2. Feed intake and back-fat thickness of sows

The feed intake of sows was recorded daily during lactation (d 1 to 20). In addition, back-fat thickness was measured at P2 (6 cm from the mid line at the head of the last rib) with an ultrasonic device (Agroscan A16, France). In briefly, back-fat thickness of each sow both in the left and right sides was measured on d 85 after pregnancy, d 1 and 20 post-farrowing, and the average was calculated.

2.3. Diarrhoea rate of suckling piglets

A daily examination was carried out in all piglets, especially for fecal appearance on rectal swabs. No faeces present (the rectal swab was dry and clean), watery, liquid, creamy and firm or solid (solid bulbs on the rectal swab) were 6 categories during each evaluation (Che et al., 2016; Miao et al., 2019). A piglet was defined as diarrhoeic when those had watery or liquid consistency of faeces on a special day (Adams et al., 2019).

2.4. Litter productive performance

The number and weight of litter on d 1 after cross-fostering and d 20 were recorded; the average daily gain (ADG) and survival rate of piglets were calculated. Also, the ADG of piglets per litter during d 1 to 20 and total milk yield [(weaned litter weight - initial litter weight) × 4] were calculated (Milligan et al., 2002).

2.5. Intestinal samples of neonatal piglets

On the day of birth, one new-born male piglet (1.55 ± 0.270 kg) was randomly selected from 8 of the 32 litters per treatment as soon as they were born, and they were kept apart from sows to avoid ingestion of colostrum (Wan et al., 2018). These piglets were

Table 1
Ingredients and composition of the basal diet (%).

Ingredients	Content	Nutrient levels [2]	Content
Corn	64.35	ME, MJ/kg	13.40
Soybean meal	24.30	DM	86.77
Steam fish meal	3.00	CP	15.00
Soybean oil	2.50	CF	4.50
Glucose	1.00	Ash	5.50
$CaHPO_4$ (16.5%)	1.53	Ca	0.85
Calcium bicarbonate	1.09	Total P	0.66
NaCl	0.50		
L-Threonine (98.5%)	0.07		
DL-Methionine (98.5%)	0.08		
L-lysine HCl (70%)	0.39		
Vitamin-mineral premix [1]	1.20		

[1] The vitamin-mineral premix provided the following per kilogram of diets: antioxidant 100 mg, sweetening agent 200 mg, 6,000 IU of vitamin A, 3,000 IU of vitamin D_3, 20 IU of vitamin E, 1.8 mg of vitamin K_3, 2.0 mg of thiamine, 6.0 mg of riboflavin, 4.0 mg of pyridoxine, 0.02 mg of vitamin B_{12}, 26.0 mg of niacin, 18.0 mg of pantothenic acid, 3.2 mg of folic acid, 0.4 mg of biotin, 20 mg of Cu as $CuSO_4\cdot5H_2O$, 100 mg of Zn as $ZnSO_4\cdot H_2O$, 50 mg of Mn as $MnSO_4\cdot H_2O$, 1.2 mg of I as KI, 0.30 mg of Se as Na_2SeO_3. Feed carrier was zeolite powder.
[2] Nutrient levels were calculated values.

anaesthetised with an intravenous injection of sodium pentobarbital (50 mg/kg BW) and bled by exsanguination (Deng et al., 2009). Middle transections of duodenum, jejunum, ileum (2 cm for each) was collected and fixed with 10% buffer neutral formalin (pH 7.3 ± 0.2) for morphological analyses (Li et al., 2019). Approximately 2 g of duodenum, jejunum, and ileum were collected and immediately frozen in liquid nitrogen, and stored at −80 °C for RNA extraction and gene expression analysis.

2.6. Small intestinal level of secretory immunoglobulin A (sIgA) in neonatal piglets

The intestinal level of sIgA in neonatal piglets was determined using a commercial radioimmunoassay kit (HTA Co., Ltd, Beijing, China) according to the manufacturer's instructions.

2.7. Histomorphology measurements

The formalin-fixed duodenum, jejunum and ileum were processed by using routine histological methods and mounting in paraffin blocks. Experimental methods were as recently described in detail (Wang et al., 2008; Li et al., 2019). A light microscope (Nikon, Japan) was used to examine all specimens, and villus height and crypt depth were measured by an image–analysis system (Yin et al., 2014).

2.8. Real-time PCR

Duodenum, jejunum and ileum were homogenized under liquid nitrogen, and mRNA was extracted as previously described by Xie (Xie et al., 2016). The relative mRNA expression levels of β-actin (reference gene) and related genes were determined by real-time PCR using Luminaries Color HiGreen High ROX (Thermo Scientific) on a Bio-Rad iCycler according to the manufacturer's instructions. The primers used are shown in Table 2. Fold changes in mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.9. Statistical analysis

All data were expressed as means ± standard error of the mean (SEM), and all statistical analyses were performed using the SPSS 17.0 software. Also, all data were evaluated by the normality and homogeneity of variances before analysis. The differences between treatments were evaluated using independent samples *t*-test. Probability values < 0.05 and < 0.01 were considered statistically significant and highly significant, respectively.

3. Results

3.1. Feed intake and back-fat thickness of sows

The average daily feed intake and back-fat thickness per sow on d 85 of gestation and d 1 and 20 post farrowing are summarised in Table 3. The average daily feed intake and back-fat thickness on farrowing and d 20 post farrowing were not influenced by YN supplementation ($P > 0.05$).

3.2. Growth performance of the suckling piglets

The growth performance of suckling piglets is summarised in Fig. 1. The results showed that, compared with the CON group, maternal YN supplementation increased the number of weaning piglets ($P < 0.05$). In addition, the weight of litter, average weight of piglets, and total milk yield in the CON group on d 20 were lower than those in the YN group ($P < 0.05$). Moreover, the ADG of piglets per litter from sow supplemented with YN during d 1 to 20 of lactation exhibited an upward trend compared with that of the CON piglets ($P = 0.098$).

3.3. Diarrhoea rate in the suckling piglets

The diarrhoea rate in the suckling piglets is shown in Fig. 2. The results showed that, compared with that in the CON group piglets, maternal YN supplementation significantly decreased the diarrhoea rate in the suckling piglets ($P = 0.002$).

Table 2
Primers used for real-time PCR.

Item	GenBank accession No.	Nucleotide sequence of primers (5′-3′)	Size, bp
IL-6	NM_214399.1	F: TAAGGGAAATGTCGAGGCCG R: TCCACTCGTTCTGTGACTGC	149
IL−1β	XM_021085847.1	F: GCTAACTACGGTGACAACAA R: TCTTCATCGGCTTCTCCACT	196
IL-8	NM_213867.1	F: CTGGCTGTTGCCTTCTTG R: TCGTGGAATGCGTATTTATG	115
IL-17	NM_001005729.1	F: CTCTCGTGAAGGCGGGAATC R: GTAATCTGAGGGCCGTCTGG	137
IL-10	NM_214041.1	F: ATGGGCGACTTGTTGCTGAC R: CACAGGGCAGAAATTGATGACA	217
TNF-α	NM_214022.1	F: ACTGCACTTCGAGGTTATCGG R: GGCGACGGGCTTATCTGA	118
IFN-γ	NM_213948.1	F: TGGTAGCTCTGGGAAACTGAATG R: GGCTTTGCGCTGGATCTG	79
IL-12	NM_214013.1	F: GCCAAGGTTACATGCCACAA R:TAGAACCTAATTGCAGGACACAGATG	108
ZO-1	XM_021098896.1	F: CCAACCATGTCTTGAAGCAGC R: TGCAGGAGTGTGGTCTTCAC	215
Occludin	NC_010458.4	F: TCAGGTGCACCCTCCAGATT R: TATGTCGTTGCTGGGTGCAT	169
Claudin-1	NC_010455.5	F: AAGGACAAAACCGTGTGGGA R: CTCTCCCCACATTCGAGATGATT	247
Beta-actin	NC_010445.4	F: CTGCGGCATCCACGAAACT R: AGGGCCGTGATCTCCTTCTG	147

IL = interleukin; *TNF-α* = tumor necrosis factor-α; *IFN-γ* = interferon γ; *ZO-1* = zonula occludens 1.

Table 3
Effects of maternal supplementation with yeast-based nucleotide (YN) on average daily feed intake and back-fat thickness per sow.[1]

Item	Dietary treatment[2]		SEM	*P*-value
	CON group	YN group		
Daily feed intake, kg/d				
On the farrowing day	1.06	1.00	0.05	0.638
Lactation d 1	1.80	1.81	0.13	0.956
Lactation d 2 to 4	3.62	3.65	0.37	0.979
Lactation d 5 to 20	5.94	5.95	0.24	0.965
Sow back-fat thickness, mm				
Initial d 85	16.30	16.66	0.54	0.755
Lactation d 1	16.64	17.53	0.51	0.402
Lactation d 20	14.00	15.24	0.50	0.232
Back-fat loss (d 1 to 20)	−2.64	−2.28	0.47	0.718

[1] Data are presented as means and SEM, $n = 30$.
[2] CON group, control diet; YN group, control diet supplemented with 4 g YN/kg.

3.4. Small intestinal morphology of neonatal piglets

The intestinal morphology of neonatal piglets is presented in Fig. 3. Our results showed that the average villus height of the ileum significantly increased in neonatal piglets from sows supplemented with YN ($P < 0.05$) compared to the CON group. Furthermore, the villus height-to-crypt depth (V:C) ratio of the ileum in neonatal piglets from sows supplemented with YN significantly increased compared to the CON group ($P < 0.01$).

3.5. Small intestinal sIgA level in neonatal piglets

The small intestinal sIgA level in neonatal piglets is shown in Fig. 4. The ileal sIgA level in neonatal piglets significantly increased in the YN group compared with that in the CON group ($P < 0.05$). In

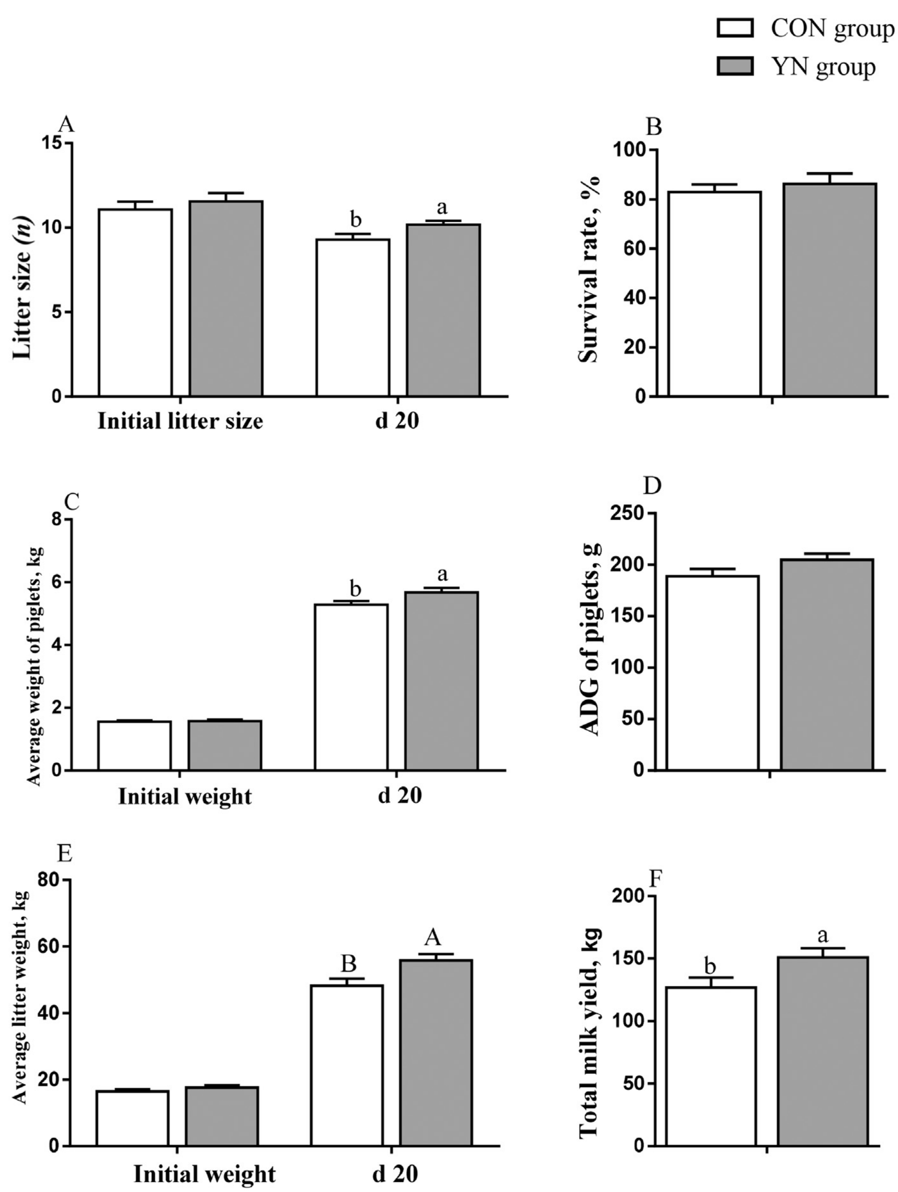

Fig. 1. Effects of maternal supplementation with yeast-based nucleotide (YN) at late pregnancy and lactation on growth performance in suckling piglets. Values are presented as means ± SEM, $n = 30$. [a, b] Means without a common letter indicate a significant difference ($P < 0.05$, *t*-test), and [A, B] means without a common letter indicate a highly significant difference ($P < 0.01$, *t*-test). CON group, control diet; YN group, control diet with 4 g YN/kg. ADG = average daily gain. Survival rate (%) = (The number of survival piglets/Total piglet) × 100; Total milk yield = [(Weaned litter weight - Initial litter weight) × 4].

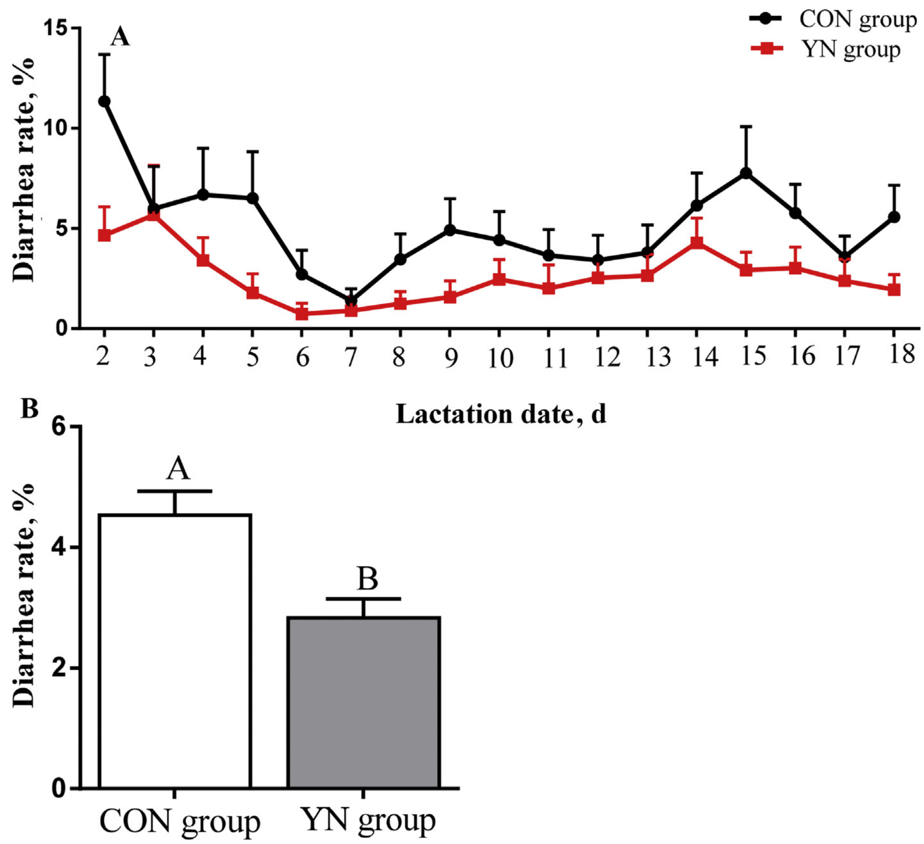

Fig. 2. Effects of maternal supplementation with yeast-based nucleotide (YN) at late pregnancy and lactation on diarrhoea rate in suckling piglets. Values are presented as means ± SEM, $n = 30$. [A, B] Means without a common letter indicate a highly significant difference ($P < 0.01$, t-test). CON group, control diet; YN group, control diet with 4 g YN/kg. Diarrhoea rate (%) = The number of piglets with diarrhoea/The total piglets × 100.

addition, the jejunal sIgA level exhibited an increasing trend in the YN group ($P = 0.097$).

3.6. Gene expression of small intestinal tight junction (TJ) proteins in neonatal piglets

The gene expression of small intestinal TJ proteins in neonatal piglets is shown in Fig. 5. The results showed that, compared with those in the piglets from the CON group, the jejunal and ileal expression of zonula occludens 1 (*ZO-1*) and duodenal and jejunal expression of claudin-1 in neonates from sows supplemented with YN were significantly reduced ($P < 0.05$). In addition, the ileal expression of occludin exhibited a decreasing tendency in the YN group compared with that in the CON group ($P = 0.092$).

3.7. Gene expression of small intestinal cytokine in neonatal piglets

The gene expression of cytokines in the small intestine of neonatal piglets is shown in Fig. 6. Maternal YN supplementation increased the jejunal expression of interleukin (*IL*)*-17*, *IL-6*, *IL-8*, *IL-1β*, *IL-10*, *IL-12*, *IFN-γ*, and tumor necrosis factor (*TNF*)*-α* ($P < 0.05$) compared with the CON group. Furthermore, the ileal expression of *IL-17*, *IL-8*, *IL-1β*, *IL-10*, and *TNF-α* was significantly up-regulated in the neonates from sows that received the YN diet ($P < 0.05$) compared with the CON group.

4. Discussion

Maternal YN supplementation during late pregnancy and lactation increased the average weaning individual weight and weaning number in piglets. There are only a few studies on nucleotides by using sow as a model, although, there are many studies using weaned piglet as a model. On one hand, Kim et al. (2008) and Hung (2015) reported that nucleotide supplementation to sows resulted in some differences in the growth performance of offspring between the experimental and control groups (Kim et al., 2008; Hung 2015). On the other hand, another study has suggested that the supplementation of NuPro (a source of yeast-derived proteins, with no uridine 5′-monophosphate, UMP) in the diet of lactating sows had no beneficial effects on the growth performances of piglets (Plante et al., 2011). Uridine 5′-monophosphate is the most abundant nucleotide in sow milk, and the level of UMP is approximately 555.6 μmol/100 mL in colostrum and 104 μmol/100 mL in milk on d 28 (Mateo et al., 2004). These results showed that UMP may play an important role in the growth performance of piglets, and may account for different results between the 2 studies. On the contrary, experiments on weaned piglets have shown that dietary nucleotide improved their growth performance (Superchi et al., 2012). In addition, a study on broiler chickens suggested that pyrimidine nucleosides increased the ADG in broilers (Daneshmand et al., 2017). Thus, pyrimidine nucleosides, especially UMP, may have a growth-promoting effect. The results of the present study demonstrated that maternal YN supplementation may contribute to the better growth performance of suckling piglets.

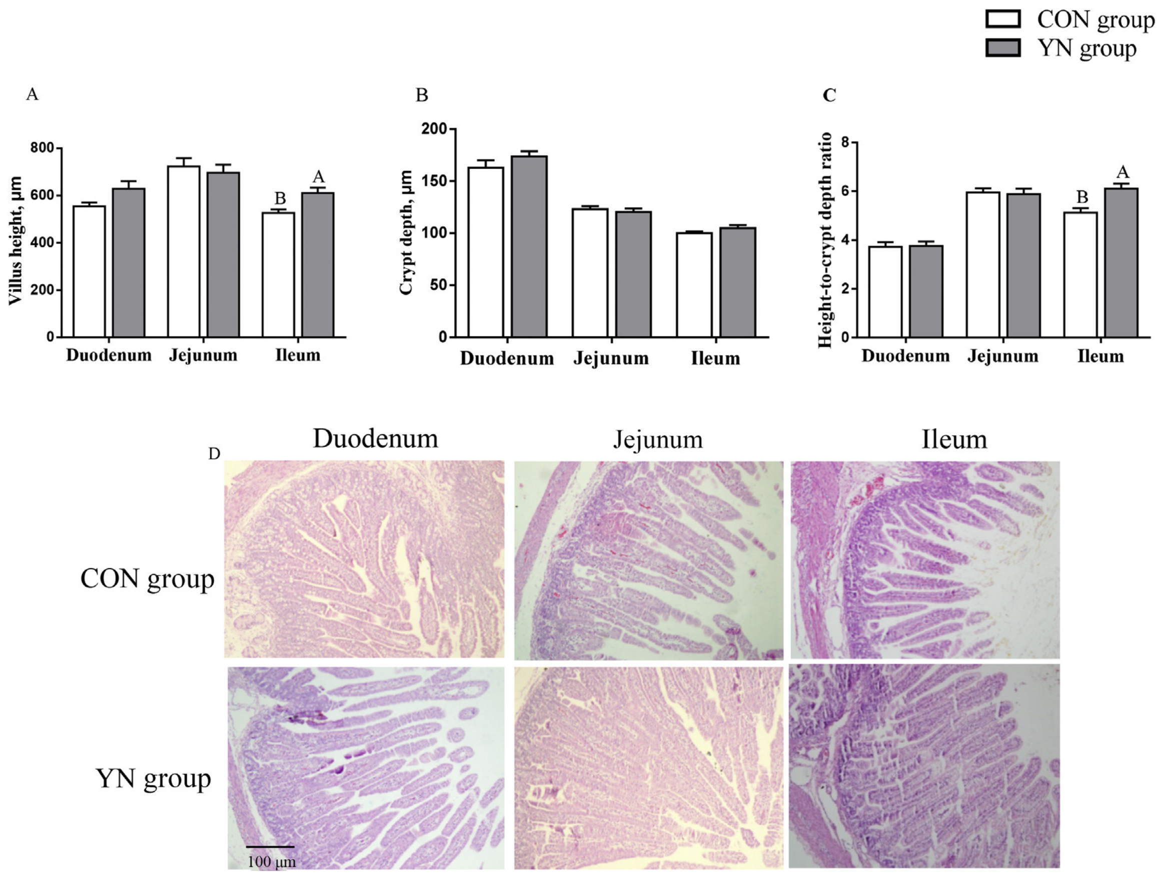

Fig. 3. Effects of maternal yeast-based nucleotide (YN) supplementation at late pregnancy on villous morphology in the small intestine of neonatal piglets. Values are presented as means ± SEM, $n = 8$. [A, B] Means without a common superscript indicate a highly significant difference ($P < 0.01$, *t*-test). CON group, control diet; YN group, control diet with 4 g YN/kg.

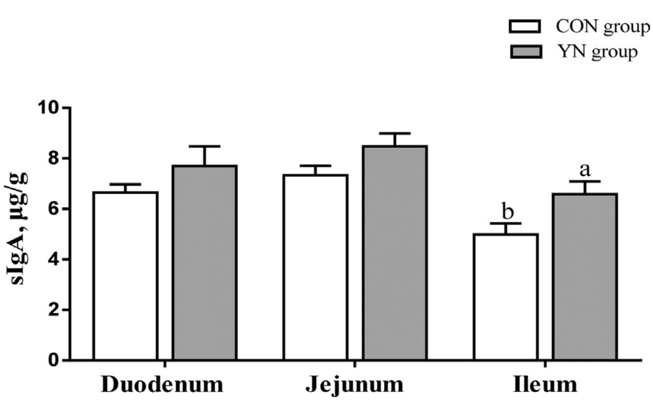

Fig. 4. Effects of maternal supplementation with yeast-based nucleotide (YN) at late pregnancy on sIgA levels in the small intestine of neonatal piglets. Values are presented as means ± SEM, $n = 8$. [a, b] Means without a common letter indicate a significant difference ($P < 0.05$, *t*-test). CON group, control diet; YN group, control diet with 4 g YN/kg. sIgA = secretory immunoglobulin A.

Further, the increased milk yield with maternal YN supplementation may be a reason for the better growth performance.

In the present study, maternal YN supplementation during late pregnancy and lactation significantly decreased the diarrhoea rate. As physiological factors, intestinal development, milk nutrients, stress factors, and feeding management could cause diarrhoea in piglets; thus, it is important to reduce diarrhoea in piglets by improving intestinal development. The results demonstrated dietary supplementation of uridine to sows could reduce the incidence of diarrhoea in suckling piglets (Wu et al., 2020). A study on infants showed that nucleotide supplementation in infant formulas has anti-diarrhoeal effect and immune benefits (Hess and Greenberg 2012). A study on weaned piglets has shown that nucleotides have a beneficial effect on the development of small intestine and intestinal repair after diarrhoea (Mashiko et al., 2009). Furthermore, dietary nucleotides promote small intestinal repair and decrease diarrhoea in weaned rats (Bueno et al., 1994). Thus, nucleotides have a positive effect on diarrhoea in mammals. The lower diarrhoea rate is partially associated with the observed increase in growth performance in piglets.

Nucleotides are considered conditionally essential nutrients, as their endogenous synthesis might be insufficient to maintain a normal demand under certain circumstances, such as the rapid growth piglets or breastfed infants (Mateo et al., 2004; Bustamante et al., 1994). On the contrary, the salvage pathway utilising exogenous nucleotides is particularly important in small intestinal epithelial cells because of the limited capacity for the de novo synthesis of nucleotides (Sanderson and He 1994; Deng et al., 2017). Several studies on weaned pigs have showed that dietary nucleosides can increase intestinal villus height and V:C ratio (Daneshmand et al., 2017; Waititu et al., 2017), as the growth and development of intestinal epithelial cells can be enhanced by dietary nucleosides; furthermore, mucosal protein and DNA, and small intestinal villi are improved by nucleosides (Uauy et al., 1990). To the best of our knowledge, this is the first study on the effects of maternal supplementation of nucleotides during late pregnancy on small intestinal development in neonatal piglets. Therefore, it was

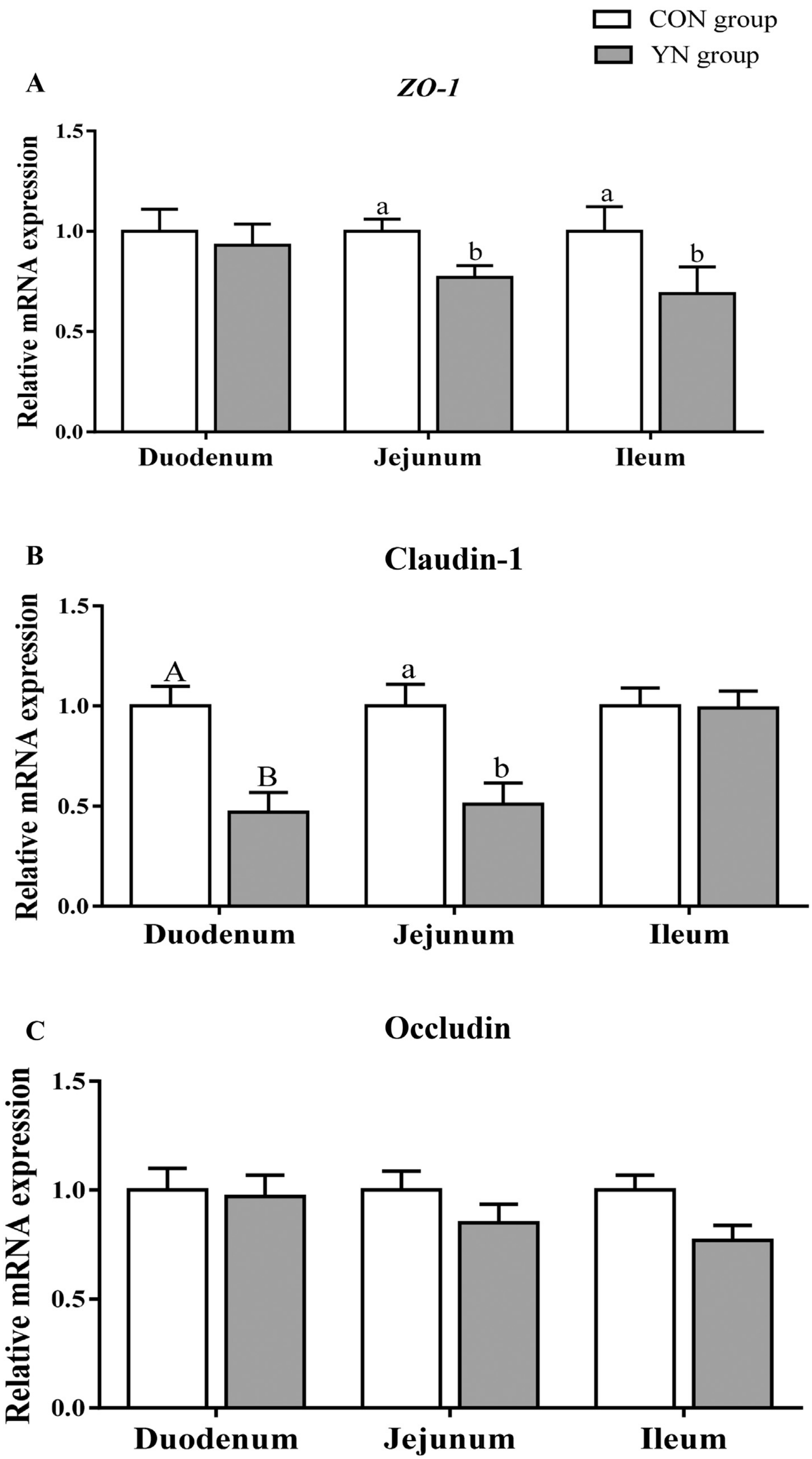

Fig. 5. Effects of maternal yeast-based nucleotide (YN) supplementation on relative mRNA expression of tight junction proteins in the small intestine of neonatal piglets. Values are presented as means ± SEM, $n = 8$. [a, b] Means without a common letter indicate a significant difference ($P < 0.05$, *t*-test), and [A, B] means without a common letter indicate a highly significant difference ($P < 0.01$, *t*-test). CON group, control diet; YN group, control diet with 4 g YN/kg. *ZO-1* = zonula occludens 1.

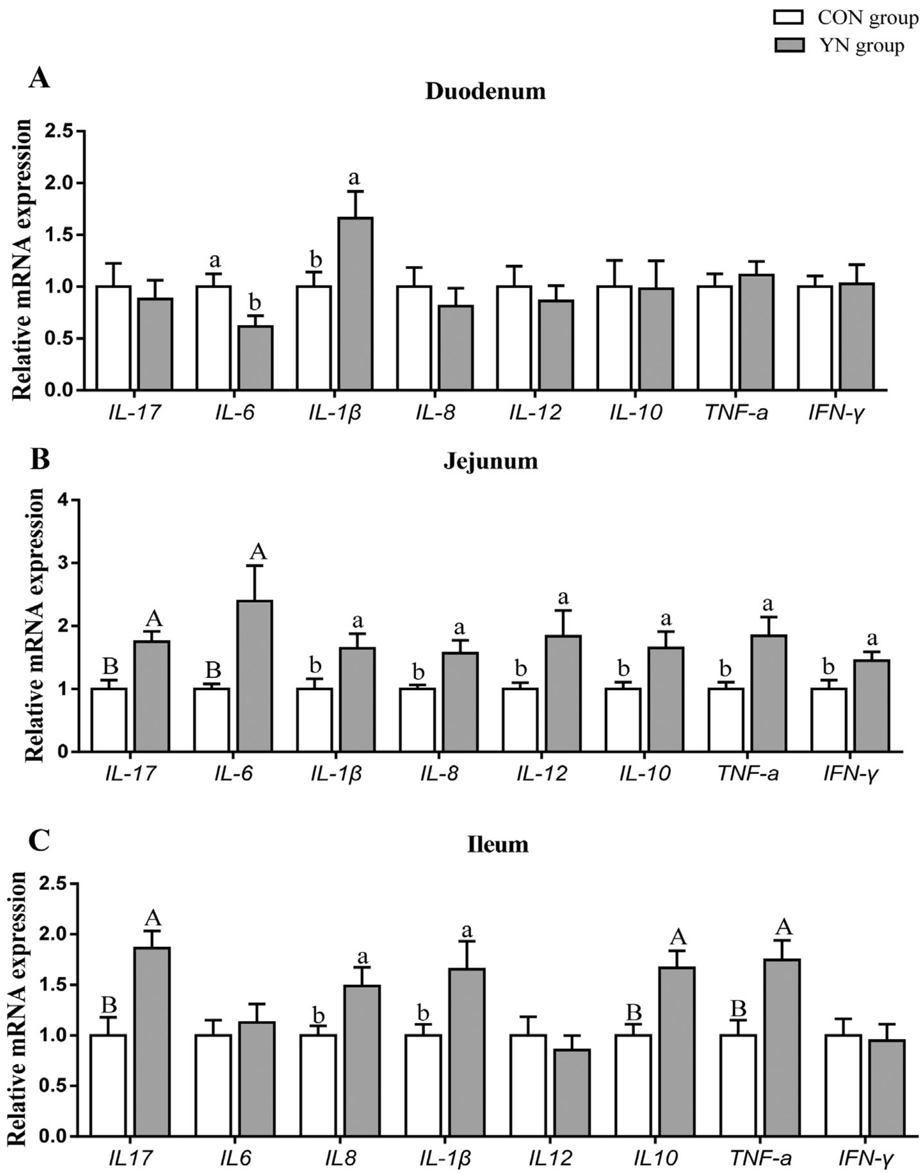

Fig. 6. Effects of maternal yeast-based nucleotide (YN) supplementation at late pregnancy on relative mRNA expression of cytokine in the intestinal epithelial cells of neonatal piglets. (A) Duodenum. (B) Jejunum. (C) Ileum. Values are presented as means ± SEM, $n = 8$. [a, b] Means without a common letter indicate a significant difference ($P < 0.05$, *t*-test), and [A, B] means without a common letter indicate a highly significant difference ($P < 0.01$, *t*-test). CON group, control diet; YN group, control diet with 4 g YN/kg. *IL* = interleukin; *TNF*-α = tumor necrosis factor-α; *IFN*-γ = interferon γ.

speculated that nucleotides could regulate homeostasis between intestinal epithelium renewal and apoptosis in neonates, because nucleotides play important roles in the proliferation, maturation and apoptosis of intestinal enterocytes (Sato et al., 1999; Li et al., 2019). Thus, small intestinal villi and V:C ratio of neonatal piglets significantly improved in the YN group compared with that in the CON group. Diarrhoea is always due to intestinal dysfunction, and the decrease of V:C ratio associated with intestinal dysfunction has been reported (Hu et al., 2013). These results implied that maternal nucleotide supplementation may be beneficial to the development of the gut structure, which was partially associated with the observed decrease in diarrhoea rate in pigs (Mccracken et al., 1999).

The prenatal intestine of piglet has the ability to absorb and utilise large molecules during the final weeks of gestation, and this ability that appears at around 2 wk before delivery and stops within 1 to 2 d after birth is called 'intestinal closure' (Sangild et al., 2002). Maternal nutrition might affect intestinal closure in foetuses via the placenta (Mehrazar et al., 1993), and the absorption of maternal IgG ceases in neonates when the intestinal permeability decreases (Telemo et al., 1987). As a major determinant of epithelial barrier function, TJ act as a selective permeability barrier between epithelium and endothelium (Schneeberger and Lynch 1984). Tight junctions play an important role in the small intestines via passive and active transport of nutrients during pregnancy and lactation in animals (Mcgovern et al., 2014). Studies have showed that the strengthening of TJ has a negative effect in the paracellular transport of nutrients (Madara, 1986; Fihn et al., 2000), and the size of TJ could be regulated by small intestinal TJ proteins, including ZO-1, occludin and claudins (Ikari et al., 2004; Hou, 2005; Angelow et al., 2007). Overall, prenatal intestinal permeability of piglets is very important for the absorption of large molecules and nutrients. Furthermore, our previous studies demonstrated that nucleotides, such as UMP and uridine, increased the relative expression of *ZO-1* or *occludin-1* in the small intestine of weaned piglets (Li et al., 2019; Xie et al., 2019; Wu et al., 2020). In the present study, maternal YN supplementation decreased the relative expression of *claudin-1* in the duodenum and jejunum, and *ZO-1* in the jejunum and ileum in neonates. The results suggested that maternal YN supplementation during late pregnancy increased foetal or neonatal intestinal permeability to a certain extent, indicating a promoting effect of YN on the absorption of macromolecular substances and nutrients (Sureda et al., 2017).

T lymphocytes are the most abundant intestinal epithelial cells and participate in the regulation of the immune response (Xiao et al., 2013). Further, nucleotides are continuously required by pigs, especially in systems that present a high rate of cell turnover, such as the immune system. Nucleotides in infant formulas have immune benefits (Hess and Greenberg 2012) and can improve the IgA activity in the small intestine (Daneshmand et al., 2017). Studies on weaned piglets have suggested that dietary nucleotides or nucleotide-rich yeast extract supplementation before weaning or under unhygienic conditions could affect the mRNA expression of *IL-6*, *IL-1β*, *IL-10*, and *TNF-α* (Superchi et al., 2012; Waititu et al., 2017). In addition, dietary nucleotides considerably increased the serum IL-1β level in a model of piglets with intrauterine growth restriction (Che et al., 2016). In line with the findings of previous studies, the present study confirmed that maternal YN supplementation during late pregnancy increased the small intestinal sIgA activity in neonatal piglets, indicating the level of intestinal immune system. We also observed that the *IL-17*, *IL-8*, *IL-6*, *IL-1β*, *IL-10*, and *TNF-α* genes in the small intestine of neonatal piglets were up-regulated in the YN group compared with that in the CON group, indicating that nucleotides promoted the secretion of cytokines in the intestines during late pregnancy. The pro-inflammatory cytokines, such as IL-1β, TNF-α, IL-8, IL-6, and IL-17, and anti-inflammatory cytokines, such as IL-10, are crucial in mediating host defence via neutrophil mobilisation (Kruse et al., 2008; Severino et al., 2011). In addition, intestinal sIgA represents an important mucosal surface defines for resisting their entry into the lumen (Zhu and Wang, 2017). Nucleotides are important for the development of immune organs (Li et al., 2019), and late pregnancy is an important period for the development of immune cells and system in foetus (Heidenreich et al., 1981). Thus, maternal YN supplementation during late pregnancy may be beneficial for the development of immune cells and system in neonatal piglets, and in enhancing the expression of pro-inflammatory and anti-inflammatory cytokines in piglets before birth (Pié et al., 2004). Furthermore, the labour stress may be another reason for the higher expression of cytokines (Chen et al., 1998). The higher expression of pro-inflammatory cytokines may regulate the growth of small intestine, as cytokines can regulate cellular migration along the intestinal crypt–villus axis and pro-inflammatory cytokines can promote enterocyte renewal (Julissa et al., 2003). These results indicate that maternal YN supplementation during late pregnancy may be beneficial in resisting stress and pathogens, and may be crucial for the growth and mitigating diarrhoea in suckling piglets.

5. Conclusion

Maternal YN supplementation during late pregnancy improved the development of small intestinal villus, and gene expression of pro-inflammatory and anti-inflammatory cytokines, and secretory IgA in neonatal piglets, and these may prevent diarrhoea and increase the weaning weight of piglets to a certain extent. These results indicate that nucleotides are beneficial in improving the reproductive performance of sows.

Author contributions

L. Gao: formal analysis; data curation; writing- original draft preparation; C. Xie: investigation; writing-reviewing and editing; X. Liang: investigation and resources; Z. Li: resources; B. Li: resources; X. Wu: methodology, data curation; project administration; funding acquisition; Y. Yin: conceptualization; supervision; funding acquisition.

Conflict of interest

We declare that we have no financial or personal relationships with other people or organizations that might 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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