AMERICAN SOCIETY OF ANIMAL SCIENCE

Journal of Animal Science, 2019, 4875–4882

doi:10.1093/jas/skz334
Advance Access publication October 30, 2019
Received: 15 September 2019 and **Accepted:** 24 October 2019
Non Ruminant Nutrition

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Supplemental effects of dietary nucleotides on intestinal health and growth performance of newly weaned pigs

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Abstract

Intestinal challenges upon weaning would increase the needs of nucleotides for enterocyte proliferation, whereas de novo synthesis maybe insufficient. This study aimed to evaluate supplemental effects of dietary nucleotides on intestinal health and growth performance in newly weaned pigs. Fifty newly weaned pigs (19-d-old, 25 barrows and 25 gilts, 4.76 ± 0.42 kg BW) were individually housed and allotted to 5 treatments with increasing nucleotide supplementation (0, 50, 150, 250, and 500 mg/kg) based on a randomized complete block design with the initial BW and sex as blocks. Dietary nucleotides were provided from YT500 (Hinabiotech, Guangzhou, China). Pigs were fed for 21 d based on 2 phases (phase 1: 11 d and phase 2: 10 d) and experimental diets were formulated to meet or exceed nutrient requirements suggested by NRC (2012). Feed intake and BW were recorded. Titanium oxide (0.4%) was added as an indigestible marker from day 17. Plasma collected on day 18 was used to measure tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and malondialdehyde (MDA). Pigs were euthanized on day 21 to collect tissues to evaluate TNF-α, IL-6, MDA, morphology, and crypt cell proliferation rate in the jejunum. Ileal digesta were collected to measure ileal nutrient digestibility. Data were analyzed using contrasts in the MIXED procedure of SAS. Nucleotide supplementation increased ($P < 0.05$) ADFI in phase 1. Nucleotide supplementation at 50 and 150 mg/kg increased ($P < 0.05$) ADG in phase 1, whereas increased ($P < 0.05$) ADFI and tended to increase ($P = 0.082$) ADG in overall. Increasing nucleotide supplementation changed (quadratic, $P < 0.05$) villus height-crypt ratio (at 247 mg/kg) and decreased (linear, $P < 0.05$) crypt cell proliferation rate in the jejunum. Increasing nucleotide supplementation reduced ($P < 0.05$) jejunal IL-6 (at 50 and 150 mg/kg) and tended to change (quadratic, $P = 0.074$) plasma MDA (at 231 mg/kg). Nucleotide supplementation at 50 and 150 mg/kg increased ($P < 0.05$) ileal digestibility of energy and ether extract. In conclusion, nucleotide supplementation at a range of 50 to 250 mg/kg in the diets seems to be beneficial to newly weaned pigs by enhancing growth performance possibly due to reduced intestinal inflammation and oxidative stress as well as improved intestinal villi structure and energy digestibility.

Key words: intestinal health, nucleotides, pigs

Introduction

Weaning is a critical events causing challenges to pigs for their growth and intestinal health (Pluske et al., 2002; Lallès, 2012). After weaning, nutrient digestibility is reduced by the collapse of the intestinal barriers due to intestinal inflammation (Pié et al., 2004; Moeser et al., 2017) and oxidative stress (Chen et al., 2017; Duarte et al., 2019). In addition, weaning can affect the

metabolism and proliferation of enterocytes in nursery pigs (Yang et al., 2016).

Nucleotides in mammalian cells are continuously synthesized and degraded, whereas playing crucial roles in various biological processes (Zaharevitz et al., 1992; Sauer et al., 2011) for transferring energy (Carver and Allan Walker, 1995), RNA transcription (Grimble, 1994), and synthesis of coenzyme component (Cosgrove, 1998). Mateo et al. (2004) described that typical nursery diets severely lack nucleotides compared with those in colostrum and milk consumed before weaning. De novo synthesis of nucleotides in enterocytes and intestinal immune cells is insufficient and thus there is an elevated needs of nucleotides to support rapid turnover of enterocytes from damages after weaning (Uauy et al., 1990; Grimble, 1994; Sauer et al., 2011).

Weaver and Kim (2014) showed that supplementation of 5′inosine monophosphate (5′IMP) increased feed intake, whereas it reduced the intensity of the immune response and measures of oxidative stress in the plasma of newly weaned pigs ameliorating weaning stress. Waititu et al. (2017) also showed that supplementation of dietary nucleotides altered intestinal microbiome by decreasing proliferation of colonic *Enterococcus* spp. and improved proliferation of cecal *Lactobbacillus* spp. Moreover, previous studies found that supplementation of dietary nucleotides in nursery diets enhanced intestinal morphology (Li et al., 2016) and intestinal immunity (Waititu et al., 2017). However, dietary supplemental levels varied among studies with different extents of the results. There is limited information for the optimal supplemental levels of nucleotides in the nursery diets and exploring the sequential responses by dietary nucleotides to intestinal health including inflammatory status, oxidative stress, morphological changes, enterocyte proliferation, nutrient digestion, and finally growth performance as outcomes of these sequential responses. It is, therefore, hypothesized that supplementation of dietary nucleotides may enhance intestinal health and thus growth performance of newly weaned pigs as they have insufficient endogenous nucleotides upon weaning to overcome intestinal challenges upon weaning. To test the hypothesis, the objectives of this study was to evaluate effects of dietary nucleotides at various supplemental levels on intestinal health and growth performance of newly weaned pigs.

Materials and Methods

A protocol of this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University (Raleigh, NC). The experiment was conducted at the North Carolina State University Metabolism Educational Unit (Raleigh, NC).

Animals and Experimental Diets

A total of 50 newly weaned pigs (25 barrows and 25 gilts; initial BW 4.76 ± 0.42 kg) were randomly allotted to 5 treatments based on randomized complete block design with BW and sex as blocks. Each treatment had 10 replicates with 1 pig per pen. Pigs were provided with feed and water ad libitum during the experiment. The experimental period was divided into 2 phases; phase 1 (weaning to day 11) and phase 2 (day 12 to 21). The individual BW of pigs and feed intake were recorded for each phase. Duration of each phase was set based on average body weight of pigs according to NRC (2012).

The experimental diets (Table 1) were mixed at North Carolina Feed Educational Unit (Raleigh, NC). Experimental diets contained low proportion of fish meal and poultry meal to reduce the amount of endogenous nucleotides coming from feed ingredients (Table 2) based on published data from Mateo (2005) and Suresh et al. (2011). The experimental diets were formulated to provide nutrients to meet or exceed requirements suggested by NRC (2012). Diets were supplemented with 0, 50, 150, 250, or 500 mg/kg of dietary nucleotides extracted from yeast cells (YT500; Hinabiotech, Guangzhou, China). The dietary nucleotides used for this study consisted of 33.2% adenosine 5′-monophosphate (5′AMP), 23.3% uridine 5′-monophosphate (5′UMP), 23.3% guanosine 5′-monophosphate (5′GMP), and 19.9% cytidine 5′monophosphate (5′CMP). Crude protein content in the supplement was 55% and replaced equal amounts of soybean meal as supplemented in the diets. On day 17, titanium dioxide

Table 1. Composition of experimental diets (as-fed basis)[1]

Item	Phase 1	Phase 2
Ingredient, %		
Corn, yellow	42.96	54.58
Soybean meal, 48% CP	19.00	23.50
Whey permeate	20.00	10.00
Blood plasma	7.50	4.00
Poultry meal	3.20	2.00
Fish meal	2.00	0.00
L-Lys HCl	0.37	0.41
DL-Met	0.17	0.15
L-Thr	0.10	0.11
Salt	0.22	0.22
Vitamin permix[2]	0.03	0.03
Trace mineral permix[3]	0.15	0.15
Dicalcium phosphate	0.30	0.90
Limestone	1.00	0.95
Poultry fat	2.00	2.00
Calculated composition		
DM, %	90.60	90.03
ME, kcal/kg	3,398	3,371
CP, %	23.40	21.52
SID[4] Lys, %	1.50	1.35
SID Cys + Met, %	0.82	0.73
SID Trp, %	0.26	0.23
SID Thr, %	0.88	0.79
Ca, %	0.85	0.80
STTD[5] P, %	0.44	0.40
Total P, %	0.64	0.63
Nucleotides, mg/kg[6]	112.4	48.6

[1]Diets in each phase supplemented with increasing supplementation of 0, 0.005, 0.015, 0.025, and 0.05% nucleotides extracted from yeast cell (YT500, Hinabiotech, Guangzhou, China) for a total of 5 diets per phase.
[2]The vitamin premix provided per kilogram of complete diet: 6,614 IU of vitamin A as vitamin A acetate, 992 IU of vitamin D3, 19.8 IU of vitamin E, 2.64 mg of vitamin K as menadione sodium bisulfate, 0.03 mg of vitamin B12, 4.63 mg of riboflavin, 18.52 mg of D-pantothenic acid as calcium panthonate, 24.96 mg of niacin, and 0.07 mg of biotin.
[3]The trace mineral premix provided per kilogram of complete diet: 33 mg of Mn as manganous oxide, 110 mg of Fe as ferrous sulfate, 110 mg of Zn as zinc sulfate, 16.5 mg of Cu as copper sulfate, 0.30 mg of I as ethylenediamine dihydroiodide, and 0.30 mg of Se as sodium selenite.
[4]SID, standardized ileal digestible.
[5]STTD P, standardized total tract digestible phosphorus.
[6]Data from Mateo (2005) and Suresh et al. (2011).

Table 2. Nucleotides composition in feed ingredients and experimental diets

Item	Nucleotides, mg/kg					
	AMP	CMP	GMP	IMP	UMP	Total
Feed ingredients						
Corn[1]	2	3	3	1	0	9
Barley[1]	1	2	1	1	0	5
Soybean meal[1]	8	16	3	2	0	29
Fish meal (anchovy)[2]	312	28	83	1,150	32	1,605
Poultry meal[2]	127	60	41	88	45	361
Protein plasma spray dried[1]	2	2	2	1	0	7
Whey powder[1]	19	270	0	4	1	294
Calculated nucleotides composition						
P1 diet	17	61	5	27	2	112
P2 diet	8	34	3	3	1	49

[1]Data from Mateo (2005).
[2]Data from Suresh et al. (2011).

(0.4%) was added as an indigestible marker in all diets and these diets were fed to pigs for 5 d to provide sufficient time before the sampling of ileal digesta.

Sample Collection

Blood samples were collected (10 mL) using vacutainer tubes containing dipotassium EDTA (BD, Franklin Lakes, NJ) from the jugular vein of pigs in all pens on day 18. The tubes were centrifuged at 3,000 × *g* for 15 min and then plasma aliquoted to 1.5 mL tubes and stored at −80 °C until analysis. Blood sampling was done 5 d before the last day of the experiment to avoid impacts of potential stress from blood sampling on the final sampling. At the last day of experiment, all pigs were euthanized by the penetration of a captive bolt followed by exsanguination. After euthanasia, jejunal mucosa, jejunal tissues, and ileal digesta were collected. Jejunal tissues (15 cm) were obtained and flushed with saline solution to remove digesta. The first 10 cm was used to collect jejunal mucosa by scraping of the mucosal layer in jejunum using glass microscope slide and the remaining 5 cm was stored in 10% formalin buffer for histological evaluation. Plasma and jejunal mucosa samples were used to measure tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) as indicators of inflammatory status and malondialdehyde (MDA) as one of the indicators of oxidative stress status. Representing pro-inflammatory cytokines (TNF-α and IL-6) were selected to evaluate inflammatory status in the intestinal epithelium (Chen et al., 2017; Duarte et al., 2019) and MDA was selected as one of the biomarkers to indicate oxidative stress status in the intestinal epithelium (Shen et al., 2014; Park et al., 2018).

ELISA for TNF-α, IL-6, and MDA

Jejunal mucosa was weighed to 0.5 g and homogenized (Tissuemiser, Thermo Fisher Scientific Inc., Rockford, IL) on ice in 2 mL PBS. Sample preparation for analysis was followed as previously described by Chen et al. (2017). Homogenized samples were centrifuged at 15,000 × *g* for 30 min and then the supernatant was collected. The protein amount in supernatant was measured using Pierce BCA Protein Assay Kit (23225#, Thermo Fisher Scientific Inc., Rockford, IL) to determine concentrations of TNF-α, IL-6, and MDA per mg of protein in the jejunal mucosa.

Concentration of TNF-α, IL-6, and MDA in plasma and jejunal mucosa were measured using ELISA kits for TNF-α and IL-6 (R&D Systems, Minneapolis, MN) and Thiobarbituric Acid Reactive Substance (TBARS) Assay Kit for MDA (Cell Biolabs, Inc., San Diego, CA) following the manufacturer's protocols. These kit analysis was followed as previously described by Chen et al. (2017).

Histology and Immunohistochemistry

Jejunal tissue samples were fixed in 10% formalin buffer for 3 wk and sent to the Histology Laboratory of North Carolina State University (Raleigh, NC) for hematoxylin and eosin staining as well as immunohistochemistry for detecting Ki67+ antibody as a biological maker for measuring the proliferation of enterocytes. A total of 15 villus and 15 crypt in each slide were selected to measure villus height (VH), villus width (VW), crypt depth (CD), and percent of Ki67+ enterocyte using a microscope (Olympus CX31 microscope). The ratio of villus height to crypt depth (VH:CD) was calculated. The measuring histomorphology was followed as previously described by Duarte et al. (2019).

Chemical Analysis

Ileal digesta were stored at −20 °C and freeze-dried (24D 48, Virtis, Gardiner, NY). The diets and freeze-dried digesta samples were ground and analyzed for dry matter concentration (method 930.15; AOAC Int., 2007), titanium dioxide concentration following the methodology described by Short et al. (1996), nitrogen using TruSpec N Nitrogen Determinator (LECO Corp., St. Joseph, MI) to calculate crude protein (6.25*N), ether extract (method 2003.06; AOAC Int., 2007), and gross energy using a calorimeter (Model 6200, Parr Instrument Company). Apparent ileal digestibility of gross energy (GE), ether extract (EE), and crude protein (CP) were calculated using titanium concentration in the diets and digesta followed as previously described by Chen et al. (2017). Measuring AID of nutrients using T-cannulation could be an effective method (Fuller, 1988; Donkoh et al., 1994) compared with the current method (Li et al., 2007; He et al., 2016) but due to the complication of multiple samplings, the current method was used.

Statistical Analysis Data

A randomized complete block design was used in this study with sex and initial BW as blocking criteria. In the model, a fixed effect was the nucleotide supplementation and random effects were sex and initial BW. The number of replications was determined based on a power test (Martin et al., 1987) to determine the effects

of increasing supplemental levels of dietary nucleotides. The data were analyzed using Mixed procedure of SAS (version 9.4, SAS Inst., Inc., Cary, NC). A pen was the experimental unit. Contrasts were preplanned to determine the effects of dietary supplemental nucleotides for the 1) linear responses, 2) quadratic responses, and 3) responses at 50 + 150 mg/kg which was determined based on previous studies (Shen et al., 2009; Sauer et al., 2011). Preplanned contrasts were determined using the Interactive Matrix Language (IML) procedure of SAS to generate coefficients for the unevenly spaced orthogonal contrasts. These coefficients generated by the IML procedure were then used in the Mixed procedure for contrasts. Contrasts were for linear effects of the *P* values less than 0.05 were considered statistically significance and between 0.05 and 0.10 were considered tendency.

Results

Growth Performance

Dietary nucleotide supplementation at 50 and 150 mg/kg tended to increase BW on day 11 ($P = 0.063$) and on day 21 ($P = 0.075$) compared with no nucleotide supplementation (Table 3). During the phase 1, dietary nucleotide supplementation at 50 and 150 mg/kg tended to increase ($P = 0.059$) ADG and increased ($P < 0.05$) ADFI compared with no nucleotide supplementation. Dietary nucleotide supplementation increased ($P < 0.05$) ADFI compared with no nucleotide supplementation. During the phase 2, dietary nucleotide supplementation at 50 and 150 mg/kg tended to increase ($P = 0.059$) ADFI compared with no nucleotide supplementation. During the overall period, dietary nucleotide supplementation at 50 and 150 mg/kg tended to increase ($P = 0.067$) ADG and increased ($P < 0.05$) ADFI compared with no nucleotide supplementation. The G:F was not influenced by dietary nucleotide supplementation.

Jejunal Morphology and Crypt Cell Proliferation

Villus height and crypt depth were not influenced by dietary nucleotide supplementation (Table 4). Villus height to crypt depth ratio showed a quadratic change ($P < 0.05$) as dietary nucleotide supplementation increased. A maximum villus height to crypt depth ratio (2.2) was observed when dietary nucleotides were supplemented at 247 mg/kg (Fig. 1). The proportion of newly proliferating enterocytes to mature enterocytes in the crypts was linearly decreased ($P < 0.05$) as dietary nucleotide supplementation increased.

TNF-α, IL-6, and MDA

In the jejunal mucosa, dietary nucleotide supplementation at 50 and 150 mg/kg decreased ($P < 0.05$) IL-6 compared with no nucleotide supplementation, whereas plasma inflammatory cytokines (TNF-α and IL-6) was not influenced by dietary nucleotide supplementation (Table 5). Plasma MDA tended to show a quadratic change ($P = 0.074$) as dietary nucleotide supplementation increased (Fig. 2). A minimum plasma MDA concentration (12.9 μM/mL) was observed when dietary nucleotides were supplemented at 231 mg/kg. The jejunal mucosa MDA was not influenced by dietary nucleotide supplementation.

Apparent Ileal Digestibility

Dietary nucleotide supplementation tended to increase ($P = 0.072$) energy digestibility (Table 6). Dietary nucleotide supplementation at 50 and 150 mg/kg increased ($P < 0.05$) energy digestibility and tended to increase ($P = 0.083$) ether extract digestibility compared with no nucleotide supplementation. The crude protein digestibility was not influenced by dietary nucleotide supplementation.

Discussion

In this study, the results indicate that the supplementation of dietary nucleotides can mitigate the negative effects induced by weaning stress in pigs. Previous studies described that weaning stress causes BW loss and reduced feed intake with reduced nutrient digestibility as well as diarrhea (Campbell et al., 2013). In terms of intestinal health, nursery pigs with weaning challenge

Table 3. Growth performance of nursery pigs fed diets with increasing levels of nucleotides (NUC) on day 21

							P-value			
	Nucleotides[1], mg/kg						NUC		0 vs.	
Item	0	50	150	250	500	SEM	Linear	Quadratic	NUC	50 + 150
BW, kg										
Day 0	4.8	4.8	4.8	4.8	4.8	0.2	0.778	0.676	0.963	0.720
Day 11	6.9	7.4	7.4	6.8	7.1	0.4	0.919	0.894	0.195	0.063
Day 21	10.6	11.6	11.6	10.8	10.5	0.7	0.230	0.310	0.336	0.075
ADG, g/d										
Phase 1	190	233	239	189	215	25	0.968	0.815	0.182	0.059
Phase 2	378	424	425	397	350	32	0.208	0.248	0.589	0.206
Overall	280	324	323	288	272	25	0.244	0.290	0.340	0.067
ADFI, g/d										
Phase 1	218	276	283	231	250	24	0.997	0.418	0.049	0.015
Phase 2	464	532	538	486	450	36	0.259	0.195	0.313	0.059
Overall	335	398	404	359	346	28	0.449	0.165	0.124	0.023
G:F										
Phase 1	0.85	0.84	0.87	0.82	0.84	0.05	0.778	0.908	0.873	0.949
Phase 2	0.82	0.79	0.78	0.82	0.78	0.03	0.620	0.888	0.453	0.386
Overall	0.83	0.81	0.81	0.80	0.79	0.03	0.350	0.864	0.390	0.565

[1]Nucleotides were extracted from yeast cell and provided from Hinabiotech (Guangzhou, China).

Table 4. Jejunal morphology and crypt cells proliferation of weaned pigs fed diets with increasing levels of nucleotides (NUC) on day 21

							P-value			
	Nucleotides[1], mg/kg						NUC		0 vs.	
Item	0	50	150	250	500	SEM	Linear	Quadratic	NUC	50 + 150
Jejunum										
Villus height, μm	474	462	525	511	469	36	0.993	0.137	0.622	0.602
Villus width, μm	98	100	97	101	103	4	0.298	0.874	0.532	0.882
Crypt depth, μm	263	245	262	261	258	13	0.830	0.816	0.628	0.494
VH:CD[2]	2.00	2.11	2.21	2.17	2.01	0.09	0.712	0.024	0.144	0.140
Crypt proliferation, %	20.0	19.8	18.7	19.6	17.1	1.1	0.032	0.602	0.265	0.476

[1]Nucleotides were extracted from yeast cell and provided from Hinabiotech (Guangzhou, China).
[2]Villus height to crypt depth ratio.

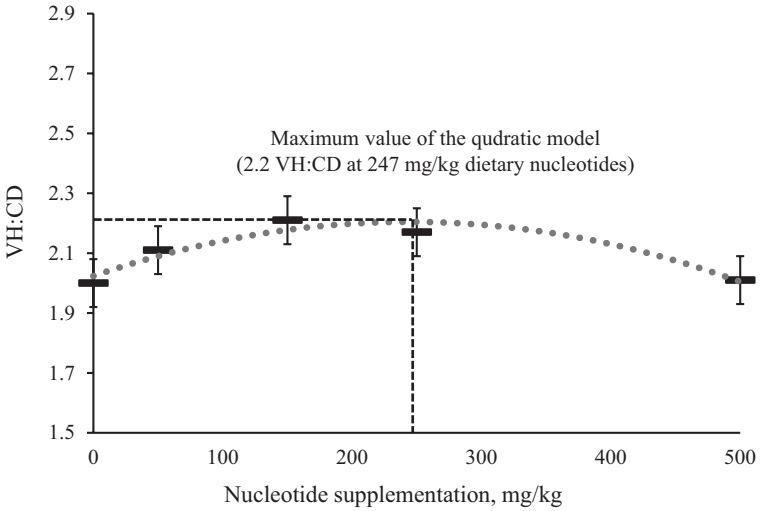

Figure 1. VH:CD of nursery pigs fed diets with increasing levels of nucleotide on day 21. The nucleotide supplementation that maximized VH:CD was calculated to be 247 mg/kg; the quadratic model: Eq. $Y = -3{\cdot}10^{-6}{\cdot}X^2 - 15{\cdot}10^{-4}{\cdot}X + 2.022$: X = nucleotide supplementation (mg/kg); Y = VH:CD; $P < 0.05$; $R^2 = 0.91$.

Table 5. Inflammatory cytokines and MDA of weaned pigs fed diets with increasing levels of nucleotides (NUC) on day 21

							P-value			
	Nucleotides[1], mg/kg						NUC		0 vs.	
Item[2]	0	50	150	250	500	SEM	Linear	Quadratic	NUC	50 + 150
Plasma										
TNF-α, pg/mL	186.1	144.7	174.6	187.8	165.5	47.1	0.996	0.640	0.418	0.226
IL-6, pg/mL	29.3	30.1	34.6	31.9	33.7	2.5	0.232	0.453	0.237	0.212
MDA, μM/mL	17.9	13.6	14.0	13.0	18.6	3.5	0.486	0.074	0.271	0.181
Jejunal mucosa										
TNF-α, pg/g of protein	3.84	3.48	3.17	3.20	5.47	0.85	0.124	0.132	0.998	0.324
IL-6, pg/g of protein	3.08	2.63	2.34	3.35	2.76	0.33	0.935	0.921	0.394	0.040
MDA, μM/g of protein	0.40	0.42	0.37	0.43	0.39	0.09	0.982	0.946	0.912	0.993

[1]Nucleotides were extracted from yeast cell and provided from Hinabiotech (Guangzhou, China).
[2]TNF-α, tumor necrosis factor- α; IL-6 interleukin-6; MDA, malondialdehyde.

also shows increased pro-inflammatory cytokines (Pié et al., 2004), increased oxidative stress (Shen et al., 2015; Xiong et al., 2019), and increased proliferation of enterocytes (Duarte et al., 2019) with impaired intestinal histomorphology. Interestingly, this study showed that the supplementation of dietary nucleotides reduced negative responses induced by weaning on growth performance and intestinal histomorphology with alleviating inflammatory response and oxidative stress.

Previous studies described that growth retardation induced by weaning stress is related to intestinal inflammatory responses (Pié et al., 2004; Moeser et al., 2017). This study shows that pigs fed with dietary nucleotides had lower concentration

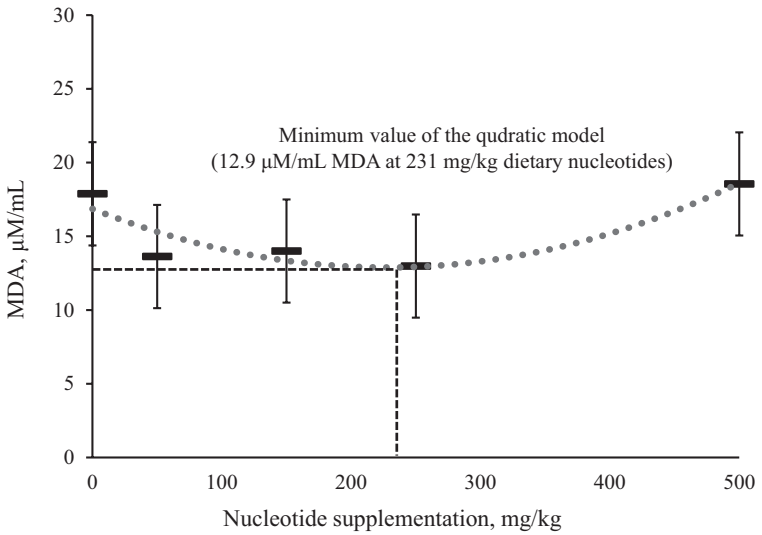

Figure 2. Malondialdehyde (MDA) in the plasma of nursery pigs fed diets with increasing levels of nucleotides on day 21. The nucleotide supplementation that minimized MDA was calculated to be 231 mg/kg; the quadratic model: Eq. $Y = 7{\cdot}10^{-5}{\cdot}X^2 - 0.034X + 16.842$: X = nucleotide supplementation (mg/kg); Y = amount of MDA in the plasma (μM/mL); $P = 0.074$; $R^2 = 0.84$.

Table 6. Apparent ileal digestibility of weaned pigs fed diets with increasing levels of nucleotides (NUC) on day 21

							P-value			
	Nucleotides[1], mg/kg						NUC		0 vs.	
Item	0	50	150	250	500	SEM	Linear	Quadratic	NUC	50 + 150
GE, %	70.9	76.1	76.2	75.5	73.6	2.9	0.923	0.179	0.072	0.040
EE, %	73.5	78.9	77.4	73.8	74.9	2.6	0.640	0.779	0.247	0.083
CP, %	77.6	78.6	79.4	74.3	79.4	2.1	0.849	0.297	0.854	0.458

[1]Nucleotides were extracted from yeast cell and provided from Hinabiotech (Guangzhou, China).

of IL-6 in the jejunum, which is a mediator for the intestinal inflammatory response (McCracken et al., 1999; Zhang and An, 2007). Previous studies showed that an increase in local and circulatory IL-6 concentrations is associated with local and systemic inflammation (Dienz et al., 2009; Tanaka et al., 2014). According to in vitro studies, supplemental nucleotide showed anti-inflammatory effects by downregulating pro-inflammatory cytokines involved in macrophages and T-cell functions (Deguchi et al., 1998; Haskó et al., 2000). Moreover, dietary nucleotides can also be involved in enhancing immune cell proliferation and differentiation as de novo synthesis of nucleotides is insufficient under stressful conditions (Elías and Fló, 2002; Hess and Greenberg, 2012). This study indicates that supplementation of dietary nucleotides helped the recovery of small intestine from weaning stress and modulated immune cells to attenuate inflammatory response and oxidative stress response (Hess and Greenberg, 2012).

Weaning stress can also affect the intestinal histomorphology with increased inflammatory response (Pluske et al., 1997; Shen et al., 2009) and reduces the energy utilization for growth (Chen et al., 2017; Huntley et al., 2018). In this study, supplementation of dietary nucleotides in nursery diets increased villus height to crypt depth ratio with increased ADG, indicating that dietary nucleotides reduced mucosal inflammatory response induced by weaning stress with improved growth performance and intestinal histomorphology. In contrast, enterocytes proliferation was decreased by the addition of dietary nucleotides, indicating that nucleotides may induce increased enterocyte turnover rate faster for recovery from weaning stress (Kuhn et al., 2014). With maintaining healthy intestinal histomorphology, the nutrients and energy would be more absorbed and directly used for growth instead of enterocyte proliferation to recover from intestinal damages (Buccigrossi et al., 2010; Wang et al., 2018). This study showed that supplementation of dietary nucleotides reduced oxidative stress of newly weaned pigs. Reduction in oxidative stress by dietary nucleotides can be associated with improved health status and growth performance in nursery pigs (Lykkesfeldt and Svendsen, 2007; Shen et al., 2014). The concentration of MDA could have been broadly used as indicator of lipid peroxidation (Shen et al., 2012; Weaver et al., 2014). Salobir et al. (2005) suggested that dietary nucleotides could improve synthesis of RNA involved in the enzymes related to reduced oxidative stress. Some studies showed that dietary nucleotides could reduce cellular damage in newly weaned pigs (Godlewski et al., 2009; Weaver and Kim, 2014). Moreover, this study indicates that specific supplemental levels of dietary nucleotides at 247 and 231 mg/kg showed improved histomorphology in the jejunum and minimized concentration of MDA in the plasma, respectively.

Ohyanagi et al. (1989) found that excessive addition of dietary nucleotides at 3,350 mg/L reduced the synthesis of their DNA and RNA in liver cells and lower level of dietary nucleotides at 335 mg/L enhanced the growth of liver cells of rats under in vitro experiments. Dose–response of nucleotides has been shown in

fish production (Li and Gatlin, 2006) and overdose of nucleotides was related to reduced growth, impaired immune function, and reduced health in channel catfish (Welker et al., 2011), rainbow trout (Tacon and Cooke, 1980), and malabar grouper (Lin et al., 2009). Causes of negative impact of overdosing nucleotides could be related to unbalance among nucleotides in the body (Lane and Fan, 2015) which warrant further investigation of proper mechanisms. This study indicates that the beneficial effects of dietary nucleotides are dose dependent and thus evaluation of an optimal supplemental level of dietary nucleotides seems to be important in the application.

Previous studies described that reduction of feed intake is one of the reasons for growth retardation upon weaning (Campbell et al., 2013). This study shows that dietary nucleotides improved feed intake of nursery pigs immediately after weaning. Supplementation of dietary nucleotides could be related to increased feed intake due to reduction in intestinal inflammation upon weaning. Weaver and Kim (2014) also demonstrated increased feed intake and reduced systemic inflammation by dietary supplementation of nucleotides. Reduced inflammatory response may potentially affect the secretion of cholecystokinin (CCK) and intestinal peptides enhancing the appetite in pigs fed with dietary nucleotides (Moeser et al., 2017; Rehfeld, 2017).

However, some of previous nucleotide studies showed that dietary nucleotides did not affect the growth performance of nursery pigs (Andrés-Elias et al., 2007; Superchi et al., 2012; Waititu et al., 2017). These variable outcomes in the growth performance were related to the type of source (either purified or yeast extraction) and composition of dietary nucleotides. In this study, pigs were fed diets with dietary nucleotides having 33.2% 5′AMP, 23.3% 5′UMP, 23.3% 5′GMP, and 19.9% 5′CMP. These nucleotides were extracted from yeast cells. The source, composition, and level effects might imply a complex mechanism due to the change in needs of nucleotides for enterocytes depending on the specific conditions.

In conclusion, nucleotide supplementation seems to be beneficial at a range of 50 to 250 mg/kg to newly weaned pigs by enhancing growth performance possibly due to reducing intestinal inflammation and oxidative stress as well as enhancing intestinal villi structure and energy digestibility.

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