Animal Nutrition 7 (2021) 111–118

Contents lists available at ScienceDirect

Animal Nutrition

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

Original Research Article

Effects of dietary supplementation of nucleotides from late gestation to lactation on the performance and oxidative stress status of sows and their offspring

Chengquan Tan [a, 1, *], Yongcheng Ji [a, 1], Xichen Zhao [a], Zhongquan Xin [a], Jiaying Li [a], Shuangbo Huang [a], Zhiying Cui [d], Lijun Wen [d], Caihua Liu [e], Sung Woo Kim [c], Jinping Deng [a], Yulong Yin [b, *]

[a] Guangdong Laboratory of Lingnan Modern Agriculture, Guangdong Provincial Key Laboratory of Animal Nutrition Control, National Engineering Research Center for Breeding Swine Industry, College of Animal Science, South China Agricultural University, Guangzhou, 510642, China
[b] National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, 410125, China
[c] Department of Animal Science North Carolina State University, Raleigh, NC, 27695, USA
[d] Guangdong Hinabiotech Co., Ltd., Guangzhou, 510642, China
[e] Hubei Hiyee Biological Technology Co., Ltd., Xiangyang, 510642, China

ARTICLE INFO

Article history:
Received 12 August 2020
Received in revised form
22 October 2020
Accepted 27 October 2020
Available online 28 December 2020

Keywords:
Feed intake
Nucleotide
Oxidative stress
Piglet
Sow

ABSTRACT

Increased metabolic burdens in breeding sows, which are induced by elevated systemic oxidative stress, could increase the need for nucleotides to repair lymphocyte DNA damage; however, de novo synthesis of nucleotides may be insufficient to cover this increased need. This study investigated the effects of dietary nucleotides on milk composition, oxidative stress status, and the reproductive and lactational performance of sows. Forty multiparous sows were assigned to 2 dietary treatments (Control group, and 1 g/kg Nucleotides group) based on a randomized complete block design using their BW at 85 d of gestation as a block. Sows from 2 groups were fed a restricted diet during gestation and ad libitum during lactation. The experiment lasted from 85 d of gestation to 21 d of lactation. The reproductive performance of sows and the growth performance of suckling piglets were measured. Oxidative stress parameters and milk components were also analysed. Data were analyzed using contrasts in the MIXED procedure of SAS. Sows in the Nucleotides group consumed more feed during the first week ($P < 0.01$) and from 1 to 21 d ($P < 0.05$) of lactation than those in Control group. Correspondingly, the litter weight gain of piglets showed a tendency to increase from cross-fostering to 9 d ($P = 0.09$) and from cross-fostering to 20 d ($P = 0.10$) in the Nucleotides group relative to the Control group. Additionally, the Nucleotides group was higher ($P < 0.01$) than the Control group in the concentrations of uridine 5'monophosphate, guanosine 5'monophosphate, inosine 5'monophosphate, adenosine 5'monophosphate and total nucleotides in milk. Furthermore, the Nucleotides group was higher ($P < 0.01$) than the Control group in the serum levels of total antioxidant capacity ($P < 0.01$) for sows at 109 d of gestation and glutathione peroxidase for weaning piglets, but lower at the levels of thiobarbituric acid-reactive substances ($P < 0.05$) in serum of weaning piglets. This study indicated that maternal dietary nucleotides could promote piglet growth, probably due to the higher lactational feed intake and higher concentration of nucleotides in the milk of sows, and lower oxidative stress for both sows and piglets.

* Corresponding authors.
E-mail addresses: tanchengquan@scau.edu.cn (C. Tan), yinyulong@isa.ac.cn (Y. Yin).
[1] These authors contributed equally to this work.
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.2020.10.004

1. Introduction

In the last few decades, genetic selection and management changes have significantly increased the average litter size of sows (Kobek-Kjeldager et al., 2020). This suggests that modern sows need to meet the demands of a large and fast growing litter by producing an appropriate amount of milk, or by improving the quality of milk, through a nutritional strategy (Kim and Easter, 2003). Meanwhile, increased metabolic burdens on sows during late gestation and lactation tend to cause elevated systemic oxidative stress during these important periods (Berchieri-Ronchi et al., 2011; Zhao and Kim, 2020). Therefore, it is important to reduce oxidative stress during the pregnancy and lactation, which could be achieved through dietary manipulation.

Dietary nucleotides are one group of dietary additives that can improve livestock health and productivity, due to their role in cell division, cell growth, the promotion of the growth of nonpathogenic bacteria, and modulation of the immune system (Martinez-Puig et al., 2007; Weaver and Kim, 2014).

In nature, nucleotides can be obtained by either de novo synthesis or salvage. The production of nucleotides in cells requires both time and energy and involves different biochemical processes (Sauer et al., 2011). Therefore, most of tissues tend (Uauy, 1994; Lopez-Navarro et al., 1996; Sauer et al., 2011) to depend on the salvage pathway to obtain exogenous nucleotides from dietary sources, especially during periods of rapid growth, limited feed intake, and stress (Carver and Walker, 1995; Carver, 1999; Sauer et al., 2012).

In previous studies, dietary nucleotides were shown to decrease the prevalence of diarrhea (Martinez-Puig et al., 2007) or improve the intestinal morphology (Jang and Kim, 2019) and growth performance (Moore et al., 2011; Weaver and Kim, 2014) in weaning piglets. There was elevated oxidative DNA damage in lymphocytes throughout the gestational and lactational periods compared to the early gestation period of multiparous sows (Berchieri-Ronchi et al., 2011). Salobir et al. reported that dietary nucleotides could repair the lymphocyte DNA damage caused by oxidative stress induced by high levels of dietary poly-unsaturated fatty acids in piglets (Salobir et al., 2005). This indicates that in the case of oxidative stress, the demand for nucleotides increases over the endogenous supply and that dietary sources are required for piglets. Additionally, a previous study has shown that the 5'monophosphate nucleotide concentration in porcine milk varies during lactation, which suggests that nucleotides from sows' colostrum and milk play a key role in the growth of post-weaning piglets (Mateo et al., 2004). However, very limited information is available about the influence of dietary nucleotides on the performance of sows and their offspring. Therefore, the purpose of this study is to test this hypothesis by evaluating the effects of dietary nucleotides on the reproductive performance, lactational feed intake, and nucleotides in the milk of sows as well as the oxidative stress status of sows and their offspring.

2. Materials and methods

All procedures involving animals were conducted in line with the protocol approved by the Animal Ethics Committee of South China Agricultural University.

2.1. Animal and experimental designs

This study was conducted in Wens Honghu Lake Original Breeding Pig Farm, Hubei Province, China. A total of 40 multiparous Large White sows, with an average parity of 2.47 ± 0.10, were assigned to 2 dietary treatments based on a randomized complete block design using BW at 85 d of gestation as a block ($n = 20$ per treatment). The sows in the control group received a basal gestation or lactation diet from 85 d of gestation to 21 d of lactation; sows in the nucleotides group were fed a basal diet supplemented with 1 g/kg of pure nucleotides (Nanjing Biotogether Co., Ltd. Nanjing, China). The dietary nucleotides used for this study consisted of 20% adenosine 5'monophosphate (5′AMP), 20% uridylic 5'monophosphate (5′UMP), 20% guanosine 5'monophosphate (5′GMP), 20% cytidine 5'monophosphate (5′CMP), and 20% inosine 5'monophosphate (5′IMP), respectively. All diets were formulated to meet the National Research Council (NRC, 2012) requirements of nutrient standards for gestational and lactational sows. The ingredients and compositions of the basal diet are shown in Table 1.

Sows were housed in individual stalls (2.2 m × 0.6 m) and fed twice (07:00 and 15:00) a day with a constant amount of 3 kg during late gestation. Five days before farrowing, the sows were moved into individual farrowing crates (2 m × 1.5 m), with parturitions being observed frequently in all groups. The farrowing room was strictly controlled, with disturbances avoided as much as possible, and the inner temperature was kept approximately at 20 to 25 °C by air conditioning system. All the farrowing crates were equipped with a feeder and a nipple drinker for sows; a nipple drinker and a heating lamp were provided for suckling piglets. Sows were not provided with feed on their farrowing day but were fed a lactation diet twice a day (06:30 and 17:00) from the next day until weaning. The initial amount of feed was 1.5 kg on the first day postpartum which was increased daily by 0.5 kg until 7 d postpartum, then the sows were fed ad libitum until weaning. The refused feed was weighed and removed every morning, and feed intake was recorded by subtracting the rejected feed from the feed offered. No creep feed was offered to any litters during lactation. All the sows and piglets had free access to water from nipple drinkers throughout the experiment. Sows eliminated from this study, and the reasons for elimination, were recorded in detail (Appendix Table). Other routine management and immunization procedures were carried out according to local large-scale pig farm breeding procedures, including tail breaking, tooth cutting, iron supplementation, castration, etc.

2.2. Sample collection and measurements

Body weight of sows was measured on 84 and 109 d of gestation within 24 h of farrowing and at weaning. Backfat thickness of sows was measured at 65 mm on the right side of the dorsal mid line at the last rib using an ultrasonic backfat scanner (Pig Scan-A-Mode Backfat Scanner, SFK Technology, Herlev, Denmark) by the same skillful person (Tan et al., 2018a). At farrowing, the number and weights of the piglets born, born alive, stillbirths and mummies were recorded. In addition, litters in each group were cross-fostered within 48 h postpartum and the litter size was adjusted to 11.2 ± 0.9 piglets per sow. There were no differences in the number of piglets after cross-fostering between the control and Nucleotides groups (11.23 vs. 11.16). Piglets were weaned at 21 d of lactation. The weight of the piglets was measured after cross-fostering and at 9 and 20 d after farrowing, and the numbers of piglets were also recorded to calculate the litter weight gain and average daily gain (ADG) during lactation.

2.3. Analysis of blood samples

Briefly, 8 sows were selected randomly from each group and 8 mL of blood was collected separately from the ear vein of each

Table 1
Ingredient composition and nutrient levels of the basal diets (as-fed basis).

Item	Gestation	Lactation
Ingredient, %		
Corn	29.80	65.20
Soybean meal (43% CP)	7.10	16.30
Sorghum	30.04	–
Wheat bran	21.70	–
Palm kernel meal	6.00	–
Extruded soybean	–	5.00
Fermented soybean meal	–	4.00
Fish meal (58% CP)	–	2.00
Soybean oil	–	2.60
Limestone	1.61	1.07
Dicalcium phosphate	1.44	1.24
Sodium chloride	0.31	0.41
Potassium chloride	–	0.15
Sodium sulfate	0.30	–
Choline chloride	0.13	0.13
Lysine sulfate (70%)	0.50	0.47
Threonine (98%)	0.18	0.14
Methionine (98%)	0.18	0.07
Tryptophan (20%)	0.06	0.17
Mildewcide[1]	0.05	0.05
Premix[2]	0.60	1.00
Calculated composition[3], %		
NE, Mcal/kg	2.20	2.60
CP	12.99	17.41
SID Lys	0.64	1.05
EE	3.34	6.21
CF	4.52	2.32
NDF	18.77	9.29
Ca	1.00	0.90
ATTD P	0.30	0.31

CP = crude protein, SID = standardized ileal digestible, EE = ether extract, CF = crude fiber, NDF = neutral detergent fiber, ATTD = apparent total tract digestibility.
[1] Mildewcide: potassium propionate.
[2] Provided the following per kilogram of gestation diet: Cu 10.0 mg; Fe 130 mg; Mn 45 mg; Zn 60 mg; I 0.30 mg; Co 0.1 mg; vitamin A 12,000 IU; vitamin D_3 4,800 IU; vitamin E 205 mg; vitamin K 3.6 mg; thiamin 3.6 mg; riboflavin 12 mg; pyridoxine 7.2 mg; niacin 48 mg; folic acid 8.6 mg; vitamin C 200 mg; vitamin B_{12} 0.048 mg; biotin 0.6 mg; pantothenic acid 30 mg.
[3] Calculated chemical concentrations using values for feed ingredients from (NRC, 2012).

sow at 109 d of gestation, farrowing, and day of weaning. Additionally, on weaning day, 8 castrated piglets with a similar average BW were randomly selected out of 8 litters from each group to collect 5-mL blood into sterile vacutainer tubes via jugular vein puncture. Next, the blood samples were stored at room temperature for 60 min to facilitate clot formation, followed by centrifugation at 3,500 × *g* 15 min, and serum partition. Then, they were stored at −80 °C until further analysis of the oxidative parameters, which included lipid peroxidation product malondialdehyde (MDA), total antioxidant capacity (T-AOC), and glutathione peroxidase activity (GSH-Px). The concentration of MDA was determined indirectly by the levels of thiobarbituric acid reactive substances (TBARS) via the reaction with 2-thiobarbituric acid, with the end pink product absorption spectrum at 535 nm. Total antioxidant capacity was measured by the spectrometric method, with one unit of T-AOC being determined as the amount of enzymes required to increase the absorbance by 0.01 per min per mg protein at 37 °C, and with the optimum absorbance at 520 nm. GSH-Px can catalyze the reaction of peroxide (H_2O_2) and reduced glutathione (GSH) to H_2O and oxidized glutathione (GSSG), and its activity can be represented by its enzymatic reaction rate. Hence, the GSH-Px activity can be estimated by measuring the GSH consumption in this enzymatic reaction or its content at the absorbance of 412 nm. Oxidative parameters were determined using the commercial kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.4. Analysis of milk samples

Within 4 h postpartum, 8 sows were chosen randomly for the collection of colostrum, and 20 mL of colostrum was sampled by manual expression into a sterile polypropylene tube (50 mL) per sow from all the functional mammary glands. At 21 d postpartum, 20 mL of mature milk was collected randomly in a sterile polypropylene tube (50 mL) per sow after an injection of 20 IU oxytocin via ear vein with a syringe. The milk samples were immediately frozen at −20 °C. Once the samples were back in the laboratory, original milk samples (10 mL) were taken out and centrifuged at 4 °C, 1,000 × *g* for 10 min to remove the milk fat, then at 6,000 × *g* to collect the whey as previously described (Pinelli-Saavedra et al., 2008). The whey was stored at −80 °C until further analysis. Colostrum and mature milk samples were used to determine the general components by Foss-Milkscan FT+ (CombiFT+200, Denmark).

The concentrations of nucleotides (i.e., 5′CMP, 5′UMP, 5′GMP, 5′IMP, 5′AMP and total nucleotides) in feed, colostrum and milk samples were analyzed by High Performance Liquid Chromatography (Agilent 1200 HPLC, Agilent Technologies Inc., Santa Clara, CA, USA) according to Chinese National Standards GB/T 5413.40-2016. Briefly, aqueous stock solutions of 4, 8, 12, 16, 20 ug/mL were prepared for the nucleotide standards of 5′CMP, 5′UMP, 5′AMP, 5′GMP, 5′IMP. Next, 5.0000 g of each feed sample was dissolved in approximately 0.2 g amylase and 20 mL distilled water at 50 °C, and after cooling to room temperature, the pH of each solution was adjusted to 4.1 with acetic acid. This was followed by diluting the sample to 50 mL with distilled water in a volumetric flask and passing the solution through quantitative filter paper and 0.45 μm nylon filter for HPLC analysis. Finally, the sample solution was injected into HPLC and the nucleotide concentration in each sample solution was measured according to a standard curve of the nucleotide standards.

The levels of MDA, GSH-Px and T-AOC in the whey from the colostrum samples were determined in the same way as described above for the blood analysis.

2.5. Statistical analyses

The sow or litter was the experimental unit for reproductive performance and biomarkers in serum of sows as well as piglets' growth performance. All data were processed preliminary with Excel (2010), and statistical analysis was performed with the general linear model procedures of SAS 8.1 (SAS Inst. Inc, Cary, NC). Prior to analysis, all data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). If the data failed to fit the normal distribution or the homogeneity of variance was not found, the Kruskal–Wallis test was carried out. Differences for the data of piglets' performance, including litter weight, ADG, litter weight gain and average piglet weight at 9 and 20 d of lactation were examined by PROC MIXED (SAS 8.1, Cary, NC). The treatment was specified as a fixed effect and the parity and piglet numbers were considered as random effects. The birth weight or litter weight after cross-fostering, as well as piglet weight or litter weight at 9 d postpartum, were considered as a covariate. Differences for the other data of sows or piglets were tested by PROC ANOVA (SAS 8.1, Inst. Inc, Cary, NC). All data were expressed as means ± SE; significance was assessed by a *P*-value of less than 0.05; $0.05 < P \leq 0.10$ was considered as marginally significant.

3. Results

3.1. Sows' reproductive performance and piglets' growth performance

The amounts of 5′AMP, 5′CMP, 5′UMP, 5′GMP and total nucleotides in diets were elevated in the Nucleotides group compared with the Control group (Table 2). As shown in Tables 3 and 4, dietary treatments had no effect on BW, backfat thickness, litter size of sows and birth weight of piglets ($P > 0.05$). However, the average lactational feed intake was higher in the Nucleotides group than in the Control group during the initial week ($P < 0.01$) and 1 to 21 d ($P < 0.05$) of lactation. Accordingly, the litter weight gain of piglets showed a tendency to increase from cross-fostering to 9 d ($P = 0.09$), and from cross-fostering to 20 d of lactation ($P = 0.10$, Table 5) in the Nucleotides group vs. the Control group. Meanwhile, the Nucleotides group had a tendency to increase over the Control group in the piglets' ADG from cross-fostering to 9 d of lactation ($P = 0.09$).

Table 2
Contents of dietary nucleotides in gestation and lactation diets (mg/kg)[1].

Item[2]	Gestation		Lactation	
	Control	Nucleotides[3]	Control	Nucleotides[3]
5′CMP	0	35.1	0	127.4
5′UMP	3.0	16.2	57.7	134.5
5′GMP	16.9	15.2	29.5	43.8
5′IMP	4.4	4.4	9.9	19.0
5′AMP	0	3.6	0	104.7
Total nucleotides	64.4	114.1	186.5	600.7

[1] Values were analyzed according to protocol GB/T23530-2009.
[2] 5′CMP, cytidine 5'monophosphate; 5′UMP, uridine 5'monophosphate; 5′GMP, guanosine 5'monophosphate; 5′IMP, inosine 5'monophosphate; and 5′AMP, adenosine 5'monophosphate.
[3] Nucleotides were supplemented at 1 g/kg.

Table 3
Effects of dietary nucleotides treatment on sows' BW, backfat thickness, and lactational feed intake[1].

Item	Control	Nucleotides[2]	*P*-value
BW[3], kg			
85 d of gestation	264.0 ± 3.8	253.7 ± 4.4	0.11
109 d of gestation	285.4 ± 4.0	278.4 ± 4.6	0.26
BW gain during late gestation[3], kg	21.4 ± 2.0	24.7 ± 1.8	0.24
At farrowing	256.7 ± 4.5	252.7 ± 4.3	0.52
At weaning	229.7 ± 5.7	230.6 ± 5.2	0.91
BW changes during lactation[3], kg	−27.0 ± 2.8	−22.1 ± 2.5	0.20
Backfat thickness[3], mm			
109 d of gestation	18.2 ± 0.6	17.3 ± 0.6	0.26
21 d of lactation	15.7 ± 0.8	15.6 ± 0.5	0.93
Feed intake[4], kg/d			
1st wk of lactation	2.84 ± 0.22	3.74 ± 0.19	<0.01
2nd wk of lactation	4.60 ± 0.22	5.08 ± 0.19	0.12
3rd wk of lactation	5.50 ± 0.16	5.68 ± 0.17	0.70
1 to 21 d of lactation	4.31 ± 0.17	4.83 ± 0.13	0.03

[1] Values are presented as means ± SE.
[2] Nucleotides were supplemented at 1 g/kg.
[3] The numbers of sows in Control and Nucleotides groups were both 17.
[4] The numbers of sows in Control and Nucleotides groups were 13 and 12, respectively.

Table 4
Effects of dietary nucleotides treatment on sows' reproductive performance[1].

Item	Control	Nucleotides[2]	*P*-value
No. of sows	17	17	
No. of pigs per litter			
Total born[3]	12.8 ± 0.8	13.0 ± 0.6	0.82
Born alive	11.5 ± 0.7	11.3 ± 0.5	0.79
Stillborn[3]	1.1 ± 0.3	1.5 ± 0.3	0.34
Mummies[3]	0.2 ± 0.1	0.2 ± 0.1	0.73
Average birth weight, kg	1.46 ± 0.04	1.42 ± 0.06	0.55
Litter weight, kg	18.2 ± 1.0	17.4 ± 0.8	0.54
CV of birth weight[4], %	19.9 ± 1.0	20.6 ± 1.6	0.73

[1] Values are expressed as means ± SE.
[2] Nucleotides were supplemented at 1 g/kg.
[3] The data that does not comply with the normal distribution and thus the significance was tested by Kruskal Wallis test.
[4] CV = coefficient of variation.

3.2. Milk components and nucleotides within colostrum and milk

Table 6 shows the effects of dietary treatment on general milk components of sows. The dietary treatments were shown to produce no difference between the 2 groups in the colostrum and milk composition or in the concentration of nucleotides within colostrum ($P > 0.05$) (Fig. 1). As expected, the results showed that the Nucleotides group was higher than the Control group in the concentrations of 5′UMP ($P < 0.01$), 5′GMP ($P < 0.01$), 5′IMP ($P < 0.01$), and 5′AMP ($P < 0.05$), as well as total nucleotides ($P < 0.01$) within milk. Interestingly, except for the almost constant concentration of AMP during lactation in the Nucleotides group, all of the

Table 5
Effects of dietary nucleotides treatment on litter performance[1].

Item	Control	Nucleotides[2]	*P*-value
No. of sows	13	12	
No. of pigs per litter			
After cross-foster	11.23 ± 0.28	11.16 ± 0.24	0.86
9 d of lactation	10.38 ± 0.29	10.33 ± 0.31	0.90
20 d of lactation	9.38 ± 0.37	9.58 ± 0.23	0.66
Average piglet weight, kg			
After cross-foster	1.99 ± 0.08	1.97 ± 0.10	0.83
9 d of lactation	3.38 ± 0.11	3.48 ± 0.15	0.59
20 d of lactation	6.36 ± 0.18	6.64 ± 0.19	0.28
Piglet ADG, g/d			
Cross-foster to 9 d	231.09 ± 8.62	252.17 ± 11.08	0.14
From 9 to 20 d	269.89 ± 8.44	285.42 ± 6.64	0.17
Cross-foster to 20 d	256.44 ± 8.08	274.63 ± 6.67	0.09
Litter weight, kg			
After cross-foster	22.44 ± 1.23	21.99 ± 1.29	0.80
9 d of lactation	35.10 ± 1.49	35.99 ± 1.91	0.71
20 d of lactation	59.62 ± 2.85	63.70 ± 2.47	0.29
Litter weight gain, kg/d			
Cross-foster to 9 d	12.99 ± 0.61	14.65 ± 0.72	0.09
From 9 to 20 d	27.81 ± 1.35	30.06 ± 0.91	0.19
Cross-foster to d 20	40.80 ± 1.88	44.71 ± 1.40	0.10

[1] Values are presented as means ± SE.
[2] Nucleotides were supplemented at 1 g/kg.

Table 6
Effects of dietary nucleotides treatment on general milk components of sows (%)[1].

Item	Control	Nucleotides[2]	*P*-value
Colostrum			
Fat	4.38 ± 0.59	5.37 ± 0.85	0.36
Protein	16.49 ± 1.21	16.38 ± 1.21	0.95
Lactose	4.59 ± 0.18	4.24 ± 0.12	0.12
Nonfat solid	24.99 ± 1.07	24.56 ± 1.17	0.79
Total solids	32.56 ± 0.85	33.29 ± 1.25	0.63
Milk			
Fat	8.56 ± 0.29	8.08 ± 0.33	0.29
Protein	6.55 ± 0.12	6.76 ± 0.07	0.15
Lactose	7.09 ± 0.09	6.98 ± 0.11	0.44
Nonfat solid	17.39 ± 0.14	17.42 ± 0.08	0.88
Total solids	29.83 ± 0.39	29.41 ± 0.32	0.42

[1] Values are presented as means ± SE ($n = 8$).
[2] Nucleotides were supplemented at 1 g/kg.

nucleotides were lower within the milk than in the colostrum in both dietary treatments.

3.3. Oxidative stress parameters of sows and piglets

The oxidative stress parameters of sows and piglets are displayed in Fig. 2. Compared with the Control group, the Nucleotides group exhibited an increase ($P < 0.01$) in the serum level of T-AOC of sows at 109 d of gestation. Despite no difference ($P > 0.05$) in the oxidative stress parameters between the 2 groups in the colostrum of sows, the Nucleotides group was significantly higher ($P < 0.05$) than the Control group in the piglets' serum level of GSH-Px, but lower ($P < 0.01$) in the levels of TBARS.

4. Discussion

Previous studies have shown the beneficial effects of nucleotide supplementation on the growth performance (Moore et al., 2011), intestinal morphology (Martinez-Puig et al., 2007; Godlewski et al., 2009), and immunity (Waititu et al., 2017) of piglets. However,

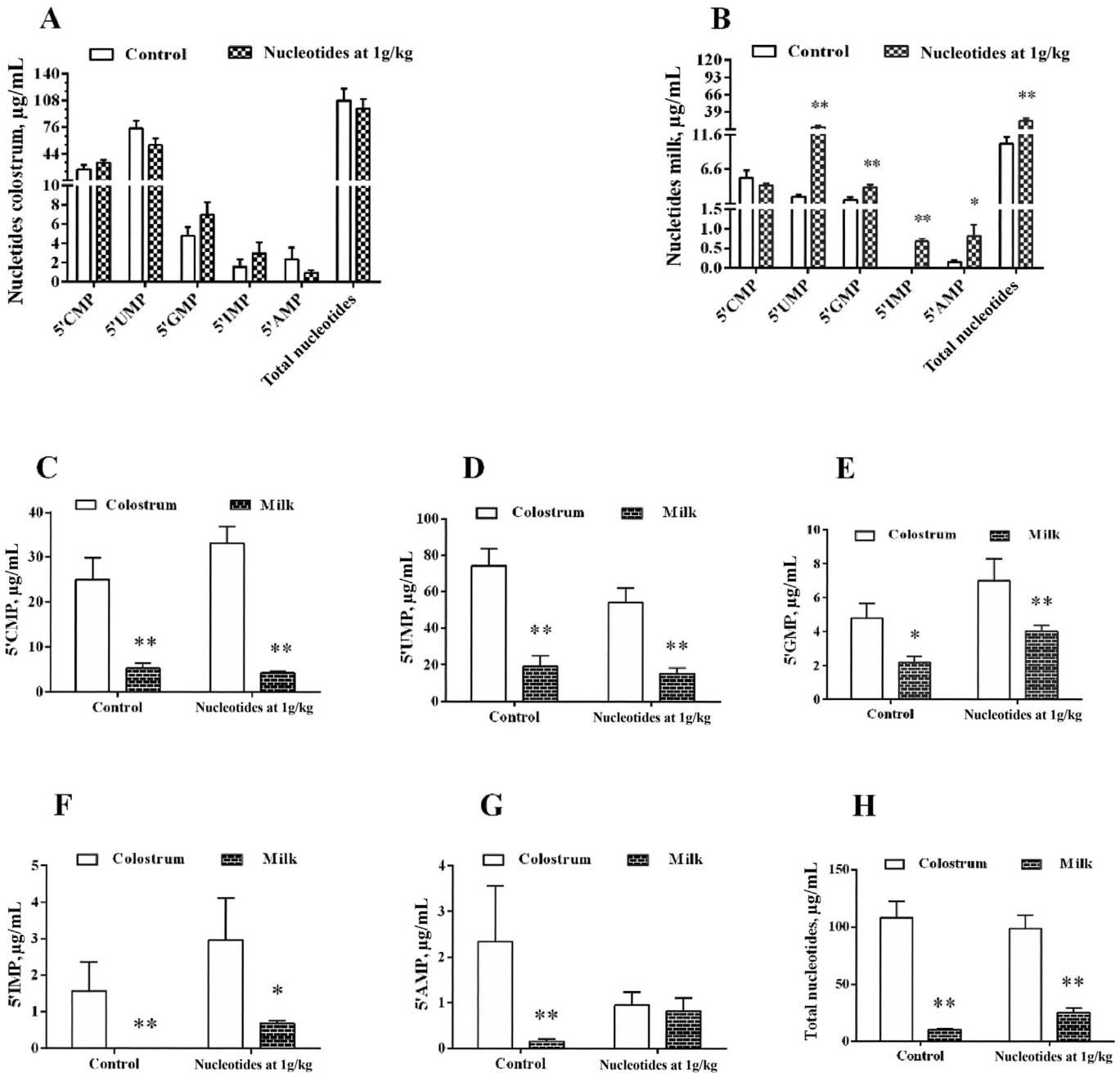

Fig. 1. Effects of dietary nucleotides on the concentration of nucleotides within colostrum and milk ($n = 7$ to 8). The concentration of (A) nucleotides within colostrum and (B) nucleotides within milk, (C) 5′CMP within colostrum and milk, (D) 5′UMP within colostrum and milk, (E) 5′GMP within colostrum and milk, (F) 5′IMP within colostrum and milk, (G) 5′AMP within colostrum and milk, (H) total nucleotides within colostrum and milk. 5′CMP = cytidine 5′monophosphate; 5′UMP = uridine 5′monophosphate; 5′GMP = guanosine 5′monophosphate; 5′IMP = inosine 5′monophosphate; and 5′AMP = adenosine 5′monophosphate. All results are presented as means ± SEM. *, $P < 0.05$ and **, $P < 0.01$ comparison to Control group.

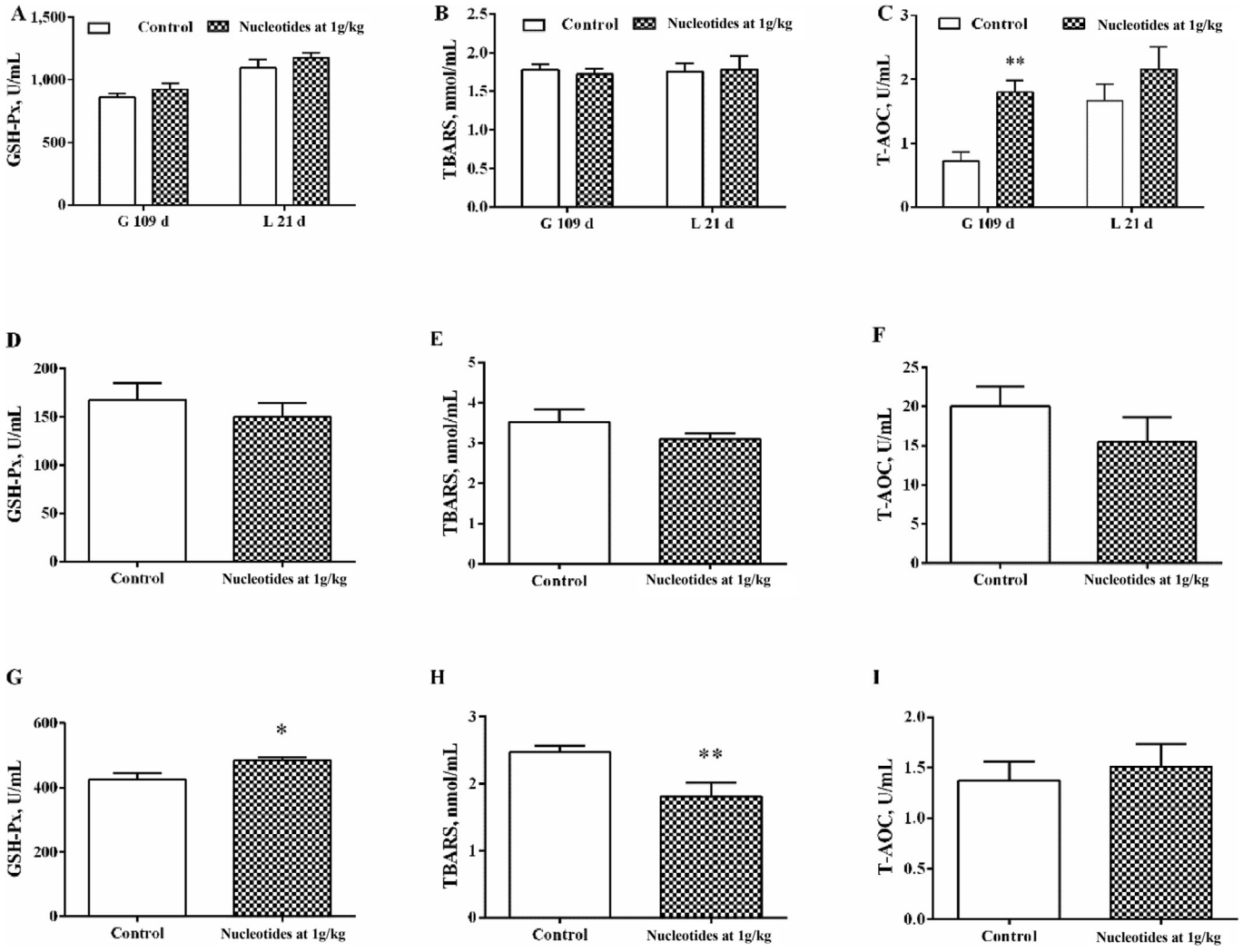

Fig. 2. Effects of dietary nucleotides treatment on the level of oxidative stress parameters in sows and their offspring ($n = 7$ to 8). The serum level of (A) GSH-Px, (B) TBARS, and (C) T-AOC on d 109 of gestation and d 21 of lactation of sows. The level of (D) GSH-Px , (E) TBARS, (F) T-AOC in colostrum. The serum level of (G) GSH-Px, (H) TBARS, and (I) T-AOC in weaning piglets. GSH-Px = glutathione peroxidase activity; TBARS = thiobarbituric acid reactive substances; T-AOC = total antioxidant capacity. All results are presented as means ± SEM. *, $P < 0.05$ and **, $P < 0.01$ comparison to Control group.

there are few reports regarding the use of nucleotides as an additive in sows' diets. In the present study, we showed that the supplementation of nucleotides at 1 g/kg in sows' diets increased their average daily feed intake during the initial lactation period. This was similar to the result of a previous study, which showed that a diet supplemented with 0.1% nucleotides contributed to the feed intake of weanling pigs (Weaver and Kim, 2014). As reported previously, dietary 0.1% to 0.8% 5′AMP increased the feed intake in juvenile red sea bream, *Pagrus major* (Hossain et al., 2016). AMP-activated protein kinase (AMPK), a cellular energy sensor that exists in almost all eukaryotes, can be activated in response to low cellular energy (high AMP-to-ATP ratio) to regulate food intake (Hardie, 2011). It is worth noting that the 5′AMP content was obviously higher in the Nucleotides diet than in the Control diet (Table 2), which might activate the AMPK and then increase the lactational feed intake in the Nucleotides group. However, the dietary supplementation of nucleotides only improved feed intake during the initial phase of lactation, which implies that sows may have developed an adaptation to the taste generated by nucleotides, as flavor is a dynamic event and tends to change during the process of food digestion (Kiyohara et al., 1975). This hypothesis needs to be confirmed in further research.

Nucleotides in colostrum and milk are important for the rapid growth of pigs due to their various functions mentioned above. The present study showed that dietary supplementation of nucleotides can improve the content of 5'monophosphate nucleotides in the milk. The changes in the concentration of nucleotides within colostrum or milk are consistent with a previous study that reported an increase of nucleotides during the first week after farrowing, followed by a decrease (Mateo et al., 2004). This suggests that the contents of nucleotides within colostrum or milk found in this research are representative and acceptable. It is worth noting that the contents of 5'monophosphate nucleotides in colostrum are nearly the same in all the groups, due to the supplementation of nucleotides in the late gestation period. A possible explanation is that the reaction time of the nucleotides supplemented to the diet

was not long enough to cause changes in their content in the 2 groups, leading to the similar reproductive performance of sows in the groups.

Piglets are mainly dependent on the sows' milk for nutrients to reach adequate BW and growth rate before weaning (Tan et al., 2018b). Numerous studies have shown that increased milk production is the result of an increased lactational feed intake of sows (Van den Brand et al., 2000; Tan et al., 2015b). In the present study, the increased growth of piglets in the Nucleotides group during the suckling phase can be regarded as the result of a higher feed intake of sows. Aside from the milk yield, milk quality is also a main contributor to litter performance. In the current study, despite no differences in the 2 groups in general milk composition, the Nucleotides group was higher than the Control group in the concentrations of 5′ monophosphate nucleotides except 5′CMP and total nucleotides within milk. Interestingly, the concentrations of 5′ monophosphate nucleotides and total nucleotides were noticeably lower in milk than in colostrum in the 2 groups, except for 5′AMP, which remained nearly unchanged during lactation. Previous studies have shown that 5′AMP is involved in various physiological roles, such as gut development and repair, skeletal muscle development, and immune response (Yang et al., 2010; Hossain et al., 2016). Consequently, increasing the content of nucleotides in the milk may contribute to improved milk quality, which in turn promotes the growth of piglets.

In previous studies, sows were shown to suffer increased systemic oxidative stress during gestation and lactation, which could not be fully recovered until weaning (Tan et al., 2015a, 2016). In the present study, dietary supplementation of nucleotides was shown to alleviate oxidative stress of sows and their offspring. This finding agreed with a previous report which showed that nucleotides may prevent oxidative stress by promoting the synthesis of RNA for enzymes involved in oxidative stress regulation (Salobir et al., 2005). For years, nucleotides have not been considered essential for humans and animals because they can be synthesized de novo from amino acids and glucose. De novo pathways are metabolically costly (Yu, 2002) and are limited in some tissues with a high replication rate (Pérignon et al., 1987), such as intestinal mucosa and bone marrow. Currently, nucleotides are becoming essential nutrients involved in health and productive performance of animals, especially during the periods of rapid growth, sanitary challenge, injury, and stress (Uauy, 1994; Carver, 1999; Sauer et al., 2012). This suggests that both sows and suckling piglets need more nucleotides to meet their physiological needs.

5. Conclusion

In this study, we demonstrated that the supplementation of nucleotides at 1 g/kg in sows' diets from late gestation to the lactation stage improved the performance and alleviated the oxidative stress of both sows and piglets. These beneficial effects on sows and their offspring could be attributed to the increased lactational feed intake, promoted by the umami taste of dietary nucleotides and elevated level of nucleotides in mature milk of sows. These results indicate that supplementation of nucleotides in sows' diets can serve as a dietary strategy to improve the performance of sows and their offspring.

Author contributions

Yulong Yin and Chengquan Tan designed research; Yongcheng Ji, Xichen Zhao, Shuangbo Huang, Jiaying Li conducted research; Zhongquan Xin, Jinping Deng and Chengquan Tan analyzed data; Chengquan Tan and Xichen Zhao wrote the paper. Zhiying Cui, Caihua Liu, Sung Woo Kim revised the paper.

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.

Acknowledgments

This work was supported by Project of National Natural Science Foundation of China (No. 31872985) and National Key R & D Program of China (No. 2018YFD0500600). Guangdong Provincial Promotion Project on Preservation and Utilization of Local Breed of Livestock and Poultry. And, we thank the Guangdong Wens Foodstuff Group Co., Ltd. for providing the pig farm.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2020.10.004.

References

Berchieri-Ronchi C, Kim S, Zhao Y, Correa C, Yeum K-J, Ferreira A. Oxidative stress status of highly prolific sows during gestation and lactation. Animal 2011: 1774–9.
Carver J. Dietary nucleotides: effects on the immune and gastrointestinal systems. Acta Paediatr 1999;88:83–8.
Carver JD, Walker WA. The role of nucleotides in human nutrition. J Nutr Biochem 1995;6:58–72.
Godlewski MM, Bierła JB, Strzałkowski A, Martinez-Puig D, Pająk B, Kotunia A, Chetrit C, Zabielski R. A novel cytometric approach to study intestinal mucosa rebuilding in weaned pigs fed with dietary nucleotides. Livest Sci 2009;123: 215–20.
Hardie DG. Sensing of energy and nutrients by AMP-activated protein kinase. Am J Clin Nutr 2011;93:891S–6S.
Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM. Dietary effects of adenosine monophosphate to enhance growth, digestibility, innate immune responses and stress resistance of juvenile red sea bream, Pagrus major. Fish Shellfish Immunol 2016;56:523–33.
Jang KB, Kim SW. Supplemental effects of dietary nucleotides on intestinal health and growth performance of newly weaned pigs. J Anim Sci 2019;97:4875–82.
Kim S, Easter R. 12 amino acid utilization for reproduction in sows. Amino Acids Animal Nutri 2003:203.
Kiyohara S, Hidaka I, Tamura T. The anterior cranial gustatory pathway in fish. Experientia 1975;31:1051–3.
Kobek-Kjeldager C, Moustsen VA, Theil PK, Pedersen LJ. Effect of litter size, milk replacer and housing on production results of hyper-prolific sows. Animal 2020;14:824–33.
Lopez-Navarro A, Ortega MA, Peragon J, Bueno JD, Gil A, Sanchez-Pozo A. Deprivation of dietary nucleotides decreases protein synthesis in the liver and small intestine in rats. Gastroenterology 1996;110:1760–9.
Martinez-Puig D, Manzanilla E, Morales J, Borda E, Pérez J, Piñeiro C, Chetrit C. Dietary nucleotide supplementation reduces occurrence of diarrhoea in early weaned pigs. Livest Sci 2007;108:276–9.
Mateo C, Peters D, Stein H. Nucleotides in sow colostrum and milk at different stages of lactation. J Anim Sci 2004;82:1339–42.
Moore K, Mullan B, Pluske J, Kim J, D'Souza D. The use of nucleotides, vitamins and functional amino acids to enhance the structure of the small intestine and circulating measures of immune function in the post-weaned piglet. Anim Feed Sci Technol 2011;165:184–90.
NRC. Nutrient requirements of swine. Washington, DC: Natl. Acad. Press; 2012.
Pérignon J-L, Bories DM, Houllier A-M, Thuillier L, Cartier PH. Metabolism of pyrimidine bases and nucleosides by pyrimidine-nucleoside phosphorylases in cultured human lymphoid cells. Biochim Biophys Acta Mol Cell Res 1987;928: 130–6.
Pinelli-Saavedra A, Calderón de la Barca AM, Hernández J, Valenzuela R, Scaife JR. Effect of supplementing sows' feed with alpha-tocopherol acetate and vitamin C on transfer of alpha-tocopherol to piglet tissues, colostrum, and milk: aspects of immune status of piglets. Res. Vet. Sci. 2008 Aug;85(1):92–100.

Salobir J, Rezar V, Pajk T, Levart A. Effect of nucleotide supplementation on lymphocyte DNA damage induced by dietary oxidative stress in pigs. ANIMAL SCIENCE-GLASGOW THEN PENICUIK- 2005;81:135.
Sauer N, Mosenthin R, Bauer E. The role of dietary nucleotides in single-stomached animals. Nutr Res Rev 2011;24:46–59.
Sauer N, Eklund M, Bauer E, Gänzle M, Field C, Zijlstra R, Mosenthin R. The effects of pure nucleotides on performance, humoral immunity, gut structure and numbers of intestinal bacteria of newly weaned pigs. J Anim Sci 2012;90:3126–34.
Tan C, Wei H, Ao J, Long G, Peng J. Inclusion of konjac flour in the gestation diet changes the gut microbiota, alleviates oxidative stress, and improves insulin sensitivity in sows. Appl Environ Microbiol 2016;82:5899–909.
Tan C, Wei H, Sun H, Ao J, Long G, Jiang S, Peng J. Effects of dietary supplementation of oregano essential oil to sows on oxidative stress status, lactation feed intake of sows, and piglet performance. BioMed Res Int 2015a;2015.
Tan C, Wei H, Sun H, Long G, Ao J, Jiang S, Peng J. Effects of supplementing sow diets during two gestations with konjac flour and Saccharomyces boulardii on constipation in peripartal period, lactation feed intake and piglet performance. Anim Feed Sci Technol 2015b;210:254–62.
Tan C, Sun H, Wei H, Tan J, Long G, Jiang S, Peng J. Effects of soluble fiber inclusion in gestation diets with varying fermentation characteristics on lactational feed intake of sows over two successive parities. Animal: Int J Animal Biosci 2018a;12:1388–95.
Tan C, Zhai Z, Ni X, Wang H, Ji Y, Tang T, Ren W, Long H, Deng B, Deng J. Metabolomic profiles reveal potential factors that correlate with lactation performance in sow milk. Sci Rep 2018b;8:1–9.
Uauy R. Nonimmune system responses to dietary nucleotides. J Nutr 1994;124:157S–9S.
Van den Brand H, Heetkamp M, Soede N, Schrama J, Kemp B. Energy balance of lactating primiparous sows as affected by feeding level and dietary energy source. J Anim Sci 2000;78:1520–8.
Waititu S, Yin F, Patterson R, Yitbarek A, Rodriguez-Lecompte J, Nyachoti C. Dietary supplementation with a nucleotide-rich yeast extract modulates gut immune response and microflora in weaned pigs in response to a sanitary challenge. Animal: Int J Animal Biosci 2017;11:2156.
Weaver A, Kim S. Supplemental nucleotides high in inosine 5′-monophosphate to improve the growth and health of nursery pigs. J Anim Sci 2014;92:645–51.
Yang Y, Zhou S, Qiao J. Effects of adenosine 5′ monophosphate and guanosine 5′ monophosphate on the growth performance and antioxidant capability in weaned piglets. J Fujian Agric For Univ (Nat Sci Ed) 2010;39:63–6.
Yu V. Scientific rationale and benefits of nucleotide supplementation of infant formula. J Paediatr Child Health 2002;38:543–9.
Zhao Y, Kim SW. Oxidative stress status and reproductive performance of sows during gestation and lactation under different thermal environments. Asian-Australas J Anim Sci 2020;33:722–31.