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# Dietary supplement with nucleotides in the form of uridine monophosphate or uridine stimulate intestinal development and promote nucleotide transport in weaned piglets

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## Abstract

**BACKGROUND: Nucleotides are key constituents of milk, where they are utilized in cell replication, although there are limited studies for weaned piglets. This study evaluated the effects of uridine monophosphate (UMP) with uridine (UR) feed supplementation on the intestinal development and nucleotide transport in weaned piglets.**

**RESULTS: Supplementation with UMP significantly increased ($P < 0.05$) plasma glucose, and UR supplementation significantly reduced ($0.05 < P < 0.10$) the plasma total cholesterol (TC) of piglets when compared with that of the control group, although non-significant difference ($P > 0.05$) in growth performance was observed among three groups. Piglets fed supplementary UR exhibited greater ($P < 0.05$) crypt depth in the duodenum and ileum when compared with those in the supplementary UMP and control groups. Real-time quantitative polymerase chain reaction (RT-qPCR) results revealed that UR supplementation increased ($P < 0.05$) the relative mRNA levels of genes encoding the transmembrane proteins ZO-1 and occludin in the duodenum mucosa, and ZO-1 in the jejunum mucosa ($P < 0.05$). Similarly, UR supplementation increased ($P < 0.05$) expression of solute carriers SLC28A1 and SLC29A1 in the duodenum mucosa. Conversely, claudin-1 expression in the duodenum mucosa was inhibited ($P < 0.05$) by dietary supplementation with UMP or UR.**

**CONCLUSION: Collectively, our data indicated that dietary supplementation with UMP or UR was conducive to stimulating intestinal development and promoting nucleotide transport in weaned piglets.**


**Keywords:** uridine monophosphate; uridine; intestinal development; weaned piglets

## INTRODUCTION

Nucleotides are a group of bioactive agents that play important roles in nearly all biochemical processes, including cellular immune responses,[1] intestine development,[2,3] and nutrients utilization.[4,5] Nucleotides are usually considered to be semi-essential nutrients, their requirements being met via three routes: *de novo* synthesis, salvage pathways and absorption from food. However, under certain conditions, such as stress, limited nutrient intake, immunological challenges and disease states, supply of exogenous nucleotides may become essential nutrients to optimize intestinal and physiological function. Under such circumstances, nucleotides may become limiting because the body cannot afford the cost of *de novo* synthesis or salvage because they may compromise tissue function.[6] Generally, the milk of mammal animals has a higher nucleotide concentration than any other foods. What is more, it has been demonstrated that uridine 5′-monophosphate (5′-UMP) accounts for the vast majority of all 5′-monophosphate nucleotides during the whole lactation period of sows,[7] which is the major difference between milk and creep feed, indicating the high requirement for nucleotides in weaned piglets.[8] Furthermore, intestinal epithelial cells have poor ability to synthesize nucleotides, indicating that supplementation of some nucleotides is important, especially 5′-UMP.[9] Our previous studies showed that uridine monophosphate (UMP) supplementation during the immediate post-weaning improved the growth

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performance of the piglets, and it may have a positive influence on their intestinal morphology development.[10]

As the metabolic product of UMP, uridine (UR) exerts restorative functions in tissues under stress.[11] It has been reported that supplementation with fish oil and UR supports the intestinal physical barrier via up-regulating of the *ZO-1* level.[12] Intra-colonic administration of UR effectively prevented the development and progression of the dextran sulfate sodium (DSS) induced colitis symptoms in mice.[13] Moreover, it has been reported that uridine adenosine tetraphosphate, released from human and murine colon muscles, could be degraded to be ATP and UMP, causing vascular smooth muscle cell proliferation and migration.[14] Nevertheless, UMP and UR have received limited attention in studies of weaned piglets. Therefore, the objective of this study was to assess the effects of dietary supplementation with UMP and UR on growth performance and intestinal barrier of weaned piglets.

## MATERIALS AND METHODS

### Ethics statement

Animal experiments were approved by the Animal Care Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science. Experiments were performed in accordance with regulations and guidelines established by this committee.

### Materials

UMP-$Na_2$ ($C_9H_{13}N_2O_9P$, MW 368.2 g/mol, purity 990 g/kg) and UR ($C_9H_{12}N_2O_6$, MW 244.2 g/mol, purity 990 g/kg) were provided by Meiya Haian Pharmaceutical Co., Ltd (Hai'an, China).

### Animals and experimental design

After 3 days of accommodation, 108 weaned piglets (Large White × Landrace × Duroc, 7.53 ± 0.31 kg) aged at 21 days were randomly assigned into 18 pens (six piglets per pen). They were fed one of three diets ($n = 6$ pens/diet): (1) piglets from the control group were fed a basal diet formulated to meet nutritional specifications for weaned piglets (NRC 2012); (2) piglets from the UMP group were fed a basal diet containing 0.6 g/kg UMP-$Na_2$; (3) piglets from the UR group were fed a basal diet containing 0.45 g/kg UR (Table 1). All piglets were fed 2–3 times daily and they had free access to water. The experiment lasted for 14 days.

### Growth performance and samples collection

In this study, average daily gain (ADG), average daily food intake (ADFI), feed/gain ratio (*F/G*), and diarrhea incidence of piglets per pen were recorded or determined. At the end of the trial, one piglet per pen with an average weight was randomly selected for sample collection. A blood sample was collected from the jugular vein after 12 h of fasting as described previously.[15] Intestinal segments (5 cm) were obtained from the duodenum, proximal jejunum, and the distal ileum, which were then thoroughly flushed with sterile saline, and immediately frozen in liquid nitrogen. They were stored at −80 °C for later analysis or fixed in 10% neutral buffered formalin for intestinal morphology.[16]

### Plasma biometers

Plasma total protein (TP), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), triglyceride (TG), glucose, total cholesterol (TC), urea, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analyzed using a CX-4 Automatic Biochemical Analyzer (Mairui Inc., Shenzhen, China) and commercial kits (Leadman Biochemistry Technology Company, Beijing, China) according to the manufacturers' instructions.

### Intestinal morphology

For light microscopy observations, jejunum and ileum tissues were sectioned to a thickness of 5 μm and stained with hematoxylin and eosin. Villous lengths and crypt depths were quantified using a fluorescence microscope. The villus length and crypt depth were measured, and then the villus length/crypt depth ratio (VCR) was also calculated. Ten of the longest and straightest villi and their associated crypts from each segment were measured. Mean villus length, crypt depth, and VCR within each segment was calculated for statistical analysis.[17]

### RNA isolation and cDNA synthesis

Total RNA of intestinal tissues was isolated using TRIzol Reagent (Life Technologies, Tokyo, Japan) according to the manufacturer's instructions. Approximately 1.0 μg of total RNA was used to synthesize cDNA using a reverse transcription kit (Takara Biotechnology Co., Ltd, Dalian, China) according to the manufacturers' instructions.

### Real-time quantitative polymerase chain reaction (RT-qPCR)

The relative mRNA expression levels were quantified by SYBR real-time polymerase chain reaction (PCR) (SYBR Premix Ex Taq; Takara Bio Inc., Shiga, Japan). Primers were designed with Primer 5.0 according to published pig gene sequence (http://www.ncbi.nlm.nih.gov/pubmed/) to produce an amplification product (Table 2). Amplification was carried out in an ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster, CA, USA). The relative level of mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method after normalization with *β-actin* as a housekeeping gene.[18]

### Statistical analysis

Statistical analyses of data were carried out using the one-way analysis of variance (ANOVA) with SAS 8.2 software (Cary, NC, USA) followed by a Duncan's multiple comparison test. Differences between significant means were considered as statistically different at $P < 0.05$, and a trend toward significance was $0.05 < P < 0.10$.

## RESULTS

### Growth performance

Growth performance of weaned piglets is presented in Table 1. Non-significant differences were observed for ADFI, ADG, *F/G* and diarrhea incidence ($P > 0.10$).

### Plasma biochemical indices

Plasma biochemical indices of piglets are listed in Table 3. In this study, UMP supplement significantly increased ($P < 0.05$) plasma glucose, and UR supplementation showed a trend ($0.05 < P < 0.10$) of reducing the plasma TC of piglets when compared to those in the other two groups. However, non-significant differences were observed for other plasma biochemical indices ($P > 0.10$).



  

**Table 1.** Growth performance of weaned piglets affected by uridine monophosphate (UMP) and uridine (UR) supplementation[a]

| Items | Dietary treatment | | | SEM | *P*-Value |
|---|---|---|---|---|---|
| | Control | UMP | UR | | |
| Initial body weight (kg) | 7.53 | 7.53 | 7.54 | 0.13 | 0.97 |
| Final body weight (kg) | 10.83 | 10.79 | 10.91 | 0.27 | 0.90 |
| ADFI (g/d) | 380.6 | 381.2 | 383.6 | 2.31 | 0.99 |
| ADG (g/d) | 236.2 | 233.0 | 240.8 | 2.21 | 0.90 |
| *F/G* | 1.62 | 1.66 | 1.60 | 0.15 | 0.75 |
| Diarrhea incidence (%) | 0.13 | 0.17 | 0.14 | 0.10 | 0.58 |

[a] $n = 6$.
Note: SEM, standard error of the mean; ADFI, average daily food intake; ADG, average daily gain; *F/G*. feed/gain ratio.

**Table 2.** Primers used in this study

| Gene | Accession number | Sequence | Product length (bp) |
|---|---|---|---|
| Claudin-1 | NM_001244539.1 | F: CTCAATACAGGAGGGAAGCCA<br>R: ATATTTAAGGACCGCCCTCTCC | 91 |
| ZO-1 | XM_013993251.1 | F: ACCATGTCTTGAAGCAGCCA<br>R: GCCTGTCTCTCCATGTACGG | 103 |
| Occludin | NM_001163647.2 | F: TCAGGTGCACCCTCCAGATT<br>R: TGTCGTTGCTGGGTGCATAA | 167 |
| SLC28A1 | NM_214112.1 | F: TTTGTGAGTCAGACCGAGGC<br>R: ATCACCGAGGCAGCAATCAA | 173 |
| SLC29A1 | XM_005666066.2 | F: GGGTCACAGGCGCCTTTATT<br>R: CGTCGCCATTGTCCAGAAAC | 236 |
| *β*-Actin | XM_003357928.2 | F: CGTTGGCTGGTTGAGAATC<br>R: CGGCAAGACAGAAATGACAA | 132 |

### Intestinal morphology

Intestinal morphology is the major indicator of intestinal health and also reflects the maturation rate of enterocytes.[16] UR supplementation increased ($P < 0.05$) crypt depth in the duodenum and ileum, whereas the villus length and the VCR did not reach the significant difference level (Fig. 1). These results suggested that UR supplementation improved intestinal development, but UMP did not.

### Relative mRNA expression of tight junction proteins and nucleotide transporters in the small intestine

Our data showed that, compared with the control group, dietary supplementation with UR improved ($P < 0.05$) the relative levels of ZO-1 in the duodenum and jejunum. Supplementation of both UR and UMP increased ($P < 0.05$) the relative level of occludin in the duodenum when compared with that in the control group. Interestingly, UMP or UR supplementation inhibited ($P < 0.05$) the expression of Claudin-1 in the duodenum, which needs further study to verify. We did not find any significant difference in the expression of tight junction proteins in the ileum among the three groups **(**Fig. 2**)**.

In addition, UR supplementation increased ($P < 0.05$) the mRNA levels of *SLC28A1* and *SLC29A1* in the duodenum when compared with those in the control group, whereas UMP supplement inhibited ($P < 0.05$) the mRNA expression of *SLC29A1* in the duodenum, jejunum and ileum. The mRNA level of *SLC28A1* in the jejunum and ileum did not show significant difference (Fig. 3).

## DISCUSSION

Nucleotides, including their metabolic products, such as coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), are essential to many biological processes.[19] Nucleotides have essential roles in the maintenance of the nucleic acids, lipid metabolism, and hepatic function.[20,21] Provision of extra nucleotides in the diet improved the stress-coping abilities of weaned piglets, thus enhancing the growth performance.[22] Nucleotide supplements increase ADG, ADFI, and *F/G* of piglets when concentrations reach up to 1 g/kg.[23] In contrast, neither UMP nor UR had significant effects on the growth performance of weaned piglets tested. This may be explained by the different techniques we used to supplement the nucleotides as the poor oral bioavailability may limit the use of UR as an effective oral therapeutic agent.[24] However, further studies are required to confirm this hypothesis.

Nucleosides play important roles in cell physiology, both as nutrients and as modulators of cellular homeostasis, both of which are implicated in crucial processes such as cell signaling and metabolic regulation related to adenosine triphosphate, as messengers and coenzymes in metabolic pathways, and in nucleic acid production and salvage.[25] Moreover, gut epithelial cells have limited capacity for *de novo* nucleotides synthesis. Hence, external provision of nucleotides is essential to gut development.[26–28] Nucleoside transporters are integral membrane proteins implicated in the salvage of natural nucleobases and nucleosides for nucleic acid synthesis.[29] Nucleosides belong to solute carrier families 28 and



  

**Table 3.** Plasma indices of weaned piglets affected by uridine monophosphate (UMP) and uridine (UR) supplementation[1]

| Items | Dietary treatment | | | SEM | *P*-Value |
|---|---|---|---|---|---|
| | Control | UMP | UR | | |
| TP (g/L) | 39.66 | 37.30 | 42.88 | 0.99 | 0.32 |
| Glucose (mmol/L) | 4.04[b] | 6.55[a] | 4.51[b] | 0.41 | 0.01 |
| ALT (U/L) | 65.42 | 62.16 | 68.27 | 1.56 | 0.79 |
| AST (U/L) | 68.23 | 59.08 | 61.70 | 1.62 | 0.59 |
| ALP (U/L) | 373.8 | 386.4 | 449.3 | 4.62 | 0.60 |
| TC (mmol/L) | 2.52[a] | 2.23[ab] | 2.16[b] | 0.20 | 0.07 |
| TG (mmol/L) | 0.39 | 0.38 | 0.48 | 0.14 | 0.33 |
| HDL-C (mmol/L) | 0.91 | 0.83 | 0.91 | 0.14 | 0.43 |
| LDL-C (mmol/L) | 1.25 | 1.14 | 1.08 | 0.19 | 0.38 |
| LDH (U/L) | 602.1 | 607.6 | 638.8 | 3.60 | 0.70 |
| Urea (mmol/L) | 4.19 | 3.60 | 4.02 | 0.31 | 0.27 |

Data are mean value of three replicates ± standard error of the mean (SEM). Means in a row with a superscript without a common letter indicate significant difference ($P < 0.05$).
[1] $n = 6$.
Note: TP, total protein; ALT, alanine transaminase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDH, lactate dehydrogenase.

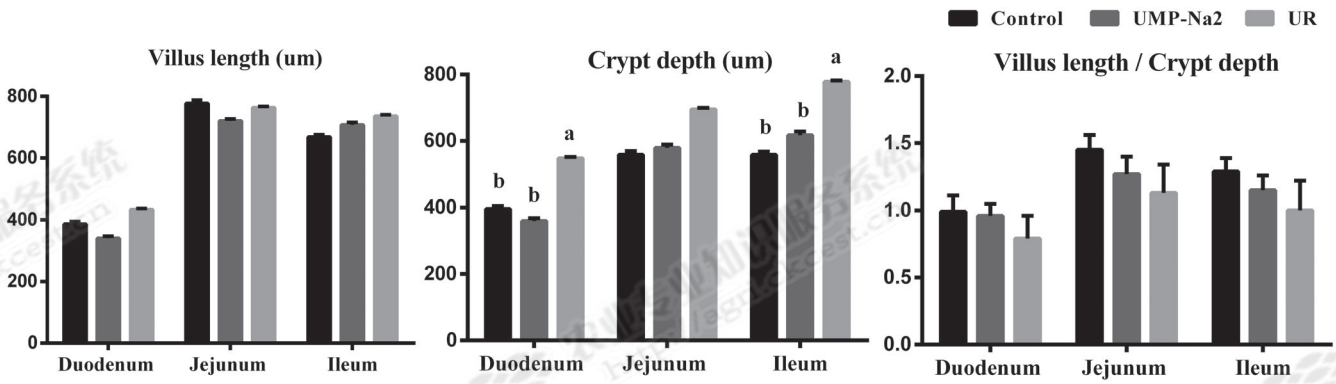


**Figure 1.** Intestinal morphology of weaned piglets affected by uridine monophosphate (UMP) and uridine (UR) supplements. Values are means plus standard error of the mean (SEM), $n = 6$. Means with a superscript without a common letter indicate significant difference ($P < 0.05$).

29 (SLC28 and SLC29), which encode human concentrative nucleoside transporters and equilibrate nucleoside transporter proteins, respectively.[30] In our assay, UR supplementation improved the mRNA expression of *SLC28A1* and *SLC29A1* in the duodenum, suggesting that UR may improve the bioavailability or action of nucleotides by providing the additional carrier proteins.[31] However, the effect of UMP on the nucleoside transport in the small intestine requires further investigation.

As a precursor to nucleic acid synthesis, nucleotides are important for intestinal epithelial cell division.[9] As mentioned earlier, dietary nucleotides increased mucosal protein and villus height of weaned rats, whereas nucleotides restriction decreased mucosal height and gut wall thickness.[4,32] Exogenously-supplied purines and pyrimidines increased mucosal wet weights in the jejunum and the ileum.[33] In the present study, dietary supplementation with UR increased the crypt depth in the duodenum and ileum, which was consistent with previous studies that showed nucleotides were important nutrients for intestinal repair and the intestinal development and repair in pigs,[3] whereas neither UR nor UMP had significant effect on intestinal length.

Besides the intestinal morphology, epithelial tight junctions are critical physical barrier to the permeation of proinflammatory molecules, such as pathogens, toxins, and antigens, from the luminal environment into the mucosal tissues and circulatory system. Tight junctions are multi-protein complexes composed of transmembrane proteins, such as claudins, ZO-1, occludin, and a wide spectrum of cytosolic proteins.[34] Nucleotide supplementation for infants less than six-months old decreased the severity and incidence of diarrhea, due to effects on the intestinal integrity and stimulation of repair function.[35] Interestingly, it has been shown that ZO-1-deficient cells exhibited a clear delay in the assembly of other tight junction proteins including occludin and claudins, indicating ZO proteins have an important role in the regulation of tight junction assembly.[36] In the present study, we found that UR supplementation enhanced ZO-1 gene transcription in the duodenum and jejunum, and occludin accumulation in the duodenum, which indicated UR contributed to maintaining intestinal integrity of weaned piglets.

Notably, we found dietary UMP significantly increased the plasma glucose levels of weaned piglets, which was an unexpected finding. It is well known that adequate uptake of glucose from the plasma is a key factor for maintaining normal brain function and piglets survival.[37] This confirms our previous study that showed that weaning process could suppresses glucose metabolism in the liver of piglets.[38] These findings strongly



  

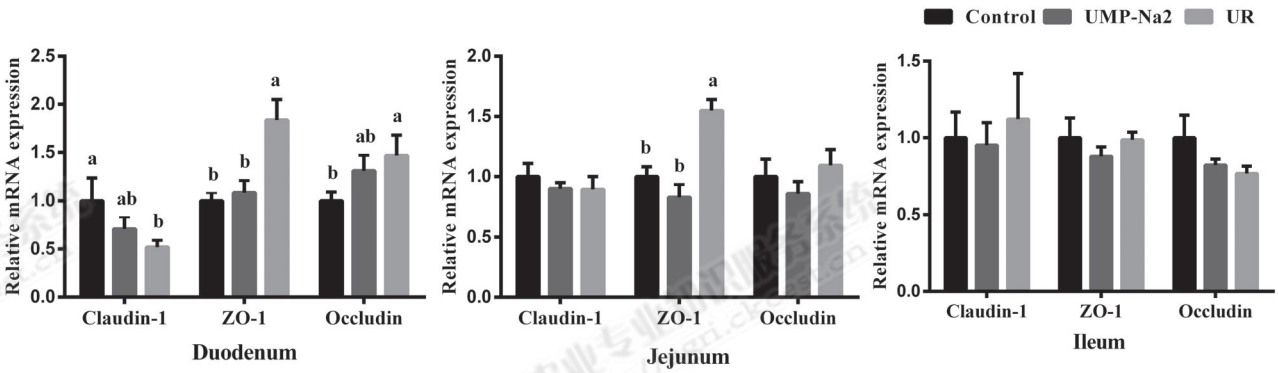


**Figure 2.** The relative mRNA level of tight junction proteins in the small intestine of weaned piglets. Values are means plus SEM, $n = 6$. Means with a superscript without a common letter indicate significant difference ($P < 0.05$).

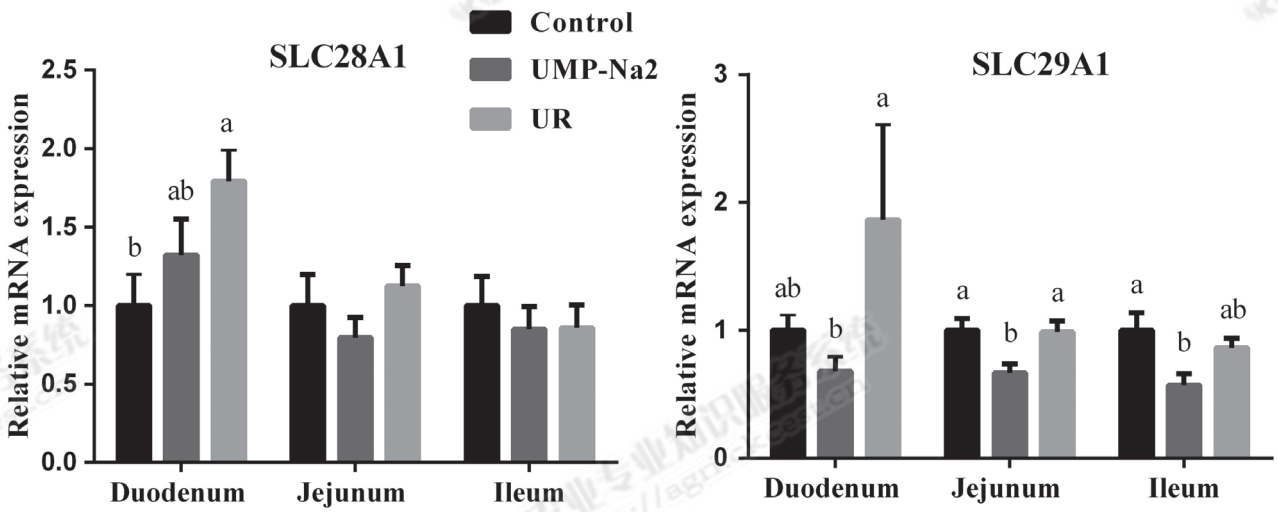


**Figure 3.** The relative mRNA level of nucleotide transporters in the small intestine of weaned piglets. Values are mean plus SEM, $n = 6$. Means with a superscript without a common letter indicate significant difference ($P < 0.05$).

suggested that UMP supplementation is crucial to maintaining normoglycemia in weaned piglets.

## CONCLUSIONS

Taken altogether, we found that dietary UR, more than UMP, improved the intestinal barrier and nucleotide transport in weaned piglets. These findings would provide more evidence for the importance of supplementation of dietary nucleotides in the diets of weaned piglets.

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## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

## REFERENCES

1 Adjei AA, Jones JT, Enriquez FJ and Yamamoto S, Dietary nucleosides and nucleotides reduce *Cryptosporidium parvum* infection in dexamethasone immunosuppressed adult mice. *Exp Parasitol* **92**:199–208 (1999).
2 Tsujinaka T, Kishibuchi M, Iijima S, Yano M and Monden M, Nucleotides and intestine. *JPEN J Parenter Enteral Nutr* **23**:S74–S77 (1999).
3 Che L, Hu L, Liu Y, Yan C, Peng X, Xu Q *et al.*, Dietary nucleotides supplementation improves the intestinal development and immune function of neonates with intra-uterine growth restriction in a pig model. *PLoS One* **11**:e0157314 (2016).
4 Carver JD, Dietary nucleotides: cellular immune, intestinal and hepatic system effects. *J Nutr* **124**:144S–148S (1994).
5 Novak DA, Carver JD and Barness LA, Dietary nucleotides affect hepatic growth and composition in the weanling mouse. *JPEN J Parenter Enteral Nutr* **18**:62–66 (1994).
6 Carver JD and Walker WA, The role of nucleotides in human-nutrition. *J Nutr Biochem* **6**:58–72 (1995).
7 Mateo CD, Peters DN and Stein HH, Nucleotides in sow colostrum and milk at different stages of lactation. *J Anim Sci* **82**:1339–1342 (2004).
8 Mateo CD, Dave RI and Stein HH, Effect of supplemental nucleosides for newly weaned pigs. *J Anim Sci* **82**:71–71 (2004).
9 Sanderson IR and He Y, Nucleotide uptake and metabolism by intestinal epithelial cells. *J Nutr* **124**:131S–137S (1994).



  

10 Li B, Zhou H, Wu X, Chen Z, Yao J and Yin Y, Effects of dietary supplementation with uridine monophosphate on performance and intestinal morphology of weanling piglets. *J Anim Sci* **94**:82–86 (2016).
11 Urasaki Y, Pizzorno G and Le TT, Chronic uridine administration induces fatty liver and pre-diabetic conditions in mice. *PLoS One* **11**:e0146994 (2016).
12 Perez-Pardo P, Dodiya HB, Broersen LM, Douna H, van Wijk N, Lopes da Silva S *et al.*, Gut-brain and brain-gut axis in Parkinson's disease models: effects of a uridine and fish oil diet. *Nutr Neurosci* **21**:391–402 (2018).
13 Jeengar MK, Thummuri D, Magnusson M, Naidu VGM and Uppugunduri S, Uridine ameliorates dextran sulfate sodium (DSS)-induced colitis in mice. *Sci Rep* **7**:3924 (2017).
14 Wiedon A, Tolle M, Bastine J, Schuchardt M, Huang T, Jankowski V *et al.*, Uridine adenosine tetraphosphate (Up(4)A) is a strong inductor of smooth muscle cell migration via activation of the P2Y(2) receptor and cross-communication to the PDGF receptor. *Biochem Biophys Res Commun* **417**:1035–1040 (2012).
15 Wu X, Xie C, Long C, Li J, Zhou X, Fan Z, Blachier F, Yin Y. Effects of a daily 3-meal pattern with different dietary protein contents on pig growth performance, carcass and muscle quality traits. *Journal of the Science of Food and Agriculture* **98**:415–421 (2018).
16 He L, Yang H, Hou Y, Li T, Fang J, Zhou X *et al.*, Effects of dietary L-lysine intake on the intestinal mucosa and expression of CAT genes in weaned piglets. *Amino Acids* **45**:383–391 (2013).
17 Touchette KJ, Carroll JA, Allee GL, Matteri RL, Dyer CJ, Beausang LA *et al.*, Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. *J Anim Sci* **80**:494–501 (2002).
18 Xie C, Wu X, Long C, Wang Q, Fan Z, Li S *et al.*, Chitosan oligosaccharide affects antioxidant defense capacity and placental amino acids transport of sows. *BMC Vet Res* **12**:243 (2016).
19 Pollak N, Dolle C and Ziegler M, The power to reduce: pyridine nucleotides – small molecules with a multitude of functions. *Biochem J* **402**:205–218 (2007).
20 Uauy R, Quan R and Gil A, Role of nucleotides in intestinal development and repair: implications for infant nutrition. *J Nutr* **124**:1436S–1441S (1994).
21 Mateo CD, Aspects of Nucleotide Nutrition in Pigs. PhD thesis, South Dakota State University, Brookings, SD (2005).
22 Superchi P, Saleri R, Borghetti P, Angelis ED, Ferrari L, Cavalli V *et al.*, Effects of dietary nucleotide supplementation on growth performance and hormonal and immune responses of piglets. *Animal* **6**:902–908 (2012).
23 Weaver CA and Kim SW, Supplemental nucleotides high in inosine 5′-monophosphate to improve the growth and health of nursery pigs. *J Anim Sci* **92**:645–651 (2014).
24 Pizzorno G, Cao D, Leffert JJ, Russell RL, Zhang D and Handschumacher RE, Homeostatic control of uridine and the role of uridine phosphorylase: a biological and clinical update. *Biochim Biophys Acta* **1587**:133–144 (2002).
25 Cacho NT and Lawrence RM, Innate immunity and breast milk. *Front Immunol* **8**:584 (2017).
26 Kim SR, Saito Y, Maekawa K, Sugiyama E, Kaniwa N, Ueno H *et al.*, Thirty novel genetic variations in the SLC29A1 gene encoding human equilibrative nucleoside transporter 1 (hENT1). *Drug Metab Pharmacokinet* **21**:248–256 (2006).
27 Cano-Soldado P, Lorráyoz IM, Molina-Arcas M, Casado FJ, Martinez-Picado J, Lostao MP *et al.*, Interaction of nucleoside inhibitors of HIV-1 reverse transcriptase with the concentrative nucleoside transporter-1 (SLC28A1). *Antivir Ther* **9**:993–1002 (2004).
28 Badagnani I, Chan W, Castro RA, Brett CM, Huang CC, Stryke D *et al.*, Functional analysis of genetic variants in the human concentrative nucleoside transporter 3 (CNT3; SLC28A3). *Pharmacogenomics J* **5**:157–165 (2005).
29 Pastor-Anglada M and Perez-Torras S, Nucleoside transporter proteins as biomarkers of drug responsiveness and drug targets. *Front Pharmacol* **6**:13 (2015).
30 Murata A, Amano R, Yamada N, Kimura K, Yashiro M, Nakata B *et al.*, Prognostic predictive values of gemcitabine sensitivity-related gene products for unresectable or recurrent biliary tract cancer treated with gemcitabine alone. *World J Surg Oncol* **11**:117 (2013).
31 Minuesa G, Huber-Ruano I, Pastor-Anglada M, Koepsell H, Clotet B and Martinez-Picado J, Drug uptake transporters in antiretroviral therapy. *Pharmacol Ther* **132**:268–279 (2011).
32 Uauy R, Stringel G, Thomas R and Quan R, Effect of dietary nucleosides on growth and maturation of the developing gut in the rat. *J Pediatr Gastroenterol Nutr* **10**:497–503 (1990).
33 Tsujinaka T, Iijima S, Kido Y, Homma T, Ebisui C, Kan K *et al.*, Role of nucleosides and nucleotide mixture in intestinal mucosal growth under total parenteral nutrition. *Nutrition* **11**:532 (1993).
34 Suzuki T, Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci* **70**:631–659 (2013).
35 Brunser O, Espinoza J, Araya M, Cruchet S and Gil A, Effect of dietary nucleotide supplementation on diarrhoeal disease in infants. *Acta Paediatr* **83**:188–191 (1994).
36 Umeda K, Matsui T, Nakayama M, Furuse K, Sasaki H, Furuse M *et al.*, Establishment and characterization of cultured epithelial cells lacking expression of ZO-1. *J Biol Chem* **279**:44785–44794 (2004).
37 Sprague JE and Arbelaez AM, Glucose counterregulatory responses to hypoglycemia. *Pediatr Endocr Rev P* **9**:463–473; quiz 474–465 (2011).
38 Xie CY, Wang QH, Wang J, Tan B, Fan ZY, Deng ZY *et al.*, Developmental changes in hepatic glucose metabolism in a newborn piglet model: a comparative analysis for suckling period and early weaning period. *Biochem Biophys Res Commun* **470**:824–830 (2016).




  