Nucleotides and young animal health: Can we enhance intestinal tract development and immune function?

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Introduction.

The development of antibiotic resistance in humans has led to a growing interest in antibiotic-free animal production worldwide (Bager et al., 2000). Unconventional management, feeding practices, and additives that act as alternatives to antibiotics have been tested (Turner et al., 2002; Stein, 2002). Research in human nutrition has demonstrated that the inclusion of nucleotides in parenteral formulas and infant milk formulas improve intestinal health and the development of the immune system in infants. Because of their role in maintaining intestinal health, dietary nucleotides may act as an alternative to antibiotics in the feeding of young animals. If this is true it would provide the livestock industry with an alternative to using antibiotics in animal feed. This would be helpful in particular in diets for young animals where in-feed antibiotics are most efficient. However, limited information about the young animals needs for nucleotides exist and only limited information is available about the role of nucleotides in the development of the immune system and intestinal health. The objective of the current contribution is to give an update on the current understanding of the roles and functions of nucleotides in young animal feeding.

Nucleotide biochemistry and nomenclature.

Nucleotides are ubiquitous molecules with considerable structural diversity. They are composed of a nitrogenous base linked to a pentose sugar to which at least one phosphate group is attached (Figure 1). The pentose sugar may either be a ribose for a

ribonucleic acid (RNA) or a 2'-deoxyribose for a deoxyribonucleic acid (DNA). The nitrogenous base can be either a purine or a pyrimidine. Pyrimidine bases are composed of six membered rings and comprise uridine, cytosine, and thymine (Table 1). Purine bases have an additional five membered ring and comprise adenine, guanine, and hypoxanthine. The phosphate group may be in a mono, di, or tri phosphate form, and is commonly esterified to the C-5' hydroxyl group of the pentose sugar (Rudolph, 1994).

When the phosphate group is absent, the compound is known as a nucleoside. Nucleosides are formed by a nitrogenous base attached to a pentose sugar through a glycosidic linkage between the N-1 nitrogen of a pyrimidine or the N-3 nitrogen of a purine, and the C-1' carbon of the pentose sugar (Voet and Voet, 1995). A chain of nucleotides attached together via a phosphodiester linkage at the 3' and 5' positions of neighboring ribose units are called polynucleotides or nucleic acids. Nucleic acids conjugated to proteins are called nucleoproteins.

Sources of nucleotides in food and feed.

Nucleotides, particularly IMP, are mainly found in food rich in protein (Carver and Walker, 1995). Generally, feed or food ingredients containing cellular elements are potential dietary sources of nucleotides in the form of nucleoproteins. Organ meats, poultry, and seafood are good sources of nucleoproteins (Kojima, 1974; Clifford and Story, 1976; Barness, 1994). Single cell proteins, bakers and brewers yeast, and yeast extract are ingredients that have a relatively high concentration of nucleotides (Maloney, 1998; Ingledew, 1999; Tibbets, 2002). Feed ingredients are not routinely analyzed for

their concentrations of nucleotides, but data are available for a few ingredients (Table 2). Most commonly used feed ingredients contain relatively low amounts of nucleotides.

The nucleotide concentration in the milk of lactating mammals is species specific and the concentration of most nucleotides changes during the lactation period (Johke, 1963; Gil and Sanchez-Medina, 1981; Gil and Sanchez-Medina, 1982a; Mateo et al., 2004b). In a recent experiment in our laboratory, we demonstrated that the concentration of 5'AMP, 5'CMP, 5'GMP, 5'IMP, and 5' UMP in porcine milk changed during the initial 2 weeks of lactation, but was relatively constant after that (Mateo et al., 2004b).

Because of the species differences in milk nucleotide concentration, it is possible that the nucleotide requirement may also vary among species, but at this point there are no data available on the nucleotide requirement of animals. The demand for nucleotides increases during periods of stress and rapid growth. Therefore, the requirement may be elevated during the immediate post-weaning period of livestock species. Current research in our laboratory is addressing this hypothesis.

Digestion, absorption, and metabolism, of nucleotides.

Dietary nucleoproteins, nucleic acids, and nucleotides need to be enzymatically hydrolyzed prior to absorption because only nucleosides, bases, and small amounts of nucleotides are absorbed. This process takes place in the small intestine. Endonucleases, phosphodiesterases, and nucleoside phosphorylase are the major enzymes involved in this process (Figure 2). These enzymes originate from the brush border epithelium (Markiewicz, 1983; Morley et al., 1987), pancreatic juice (Weickman et al., 1981), and

bile (Holdsworth and Coleman, 1975). In contrast to adults, the exact metabolism of nucleic acids ingested by infants is unknown (Carver and Walker, 1995). However, an attempt to evaluate the capability of infants to metabolize nucleic acids and nucleotides was made by Thorell et al. (1996) who suggested that enzymes for nucleotide catabolism are present in the fetal small intestine and act on the nucleotide containing substrates.

The duodenum has the greatest absorptive capacity (Bronk and Hastewell, 1987). Differences in the efficiency of uptake among nucleosides have been reported with guanosine being taken up most rapidly (Sanderson and He, 1994). Under physiological conditions, nucleotides have a limited capacity to pass through cell membranes (Sanderson and He, 1994). This may be due to the absence of a nucleotide transport system. Nucleotides also have a high negatively charged phosphate group that hinders absorption. Therefore, the nucleoside form is the major vehicle for the entry of purines and pyrimidines into the epithelial cells. More than 90% of dietary and endogenous nucleosides and bases are absorbed into the enterocyte (Salati et al., 1984; Uauy, 1989). Nucleoside transport into the enterocyte occurs by facilitated diffusion and by specific Na^+-dependent carrier mediated mechanisms (Bronk and Hastewell, 1987).

From the enterocyte, partial metabolic products of dietary and endogenous nucleotides and nucleosides enter the hepatic portal vein. These molecules are carried to the hepatocytes for further metabolism. From the liver, partial metabolic products of dietary and endogenous nucleotides and nucleosides are released into the circulations and enter the muscle tissues. If these products are not reutilized for nucleotide production or not absorbed in a specific tissue, the purine and pyrimidine bases are catabolized into uric acid and ß-alanine or ß-aminoisobutyrate (Rudolph, 1994; Carver and Walker, 1995;

Thorell et al., 1996). In mammals except for primates, uric acid is further catabolized into

allantoin via the enzyme uricase. Allantoin is then excreted into the urine. In avian

species and primates, uric acid is excreted via the urine. The catabolic products of

pyrimidine bases (i. e., ß-alanine and ß-aminoisobutyrate) are further metabolized into

NH_3, CO_2, and Acetyl CoA.

Synthesis of nucleotides.

Humans and animals can synthesize nucleotides de novo via the De novo pathway

provided that the precursors are available. This process takes place in the cytocol of

hepatocytes where all the enzymes for purine and pyrimidine synthesis are available. The

purine IMP is synthesized from α-D-ribose-5-phosphate via a process involving 11

reactions. In the first reaction, α-D-ribose-5-phosphate is phosphorylated at carbon 1 by a

phosphate group donated by ATP to form 5-phosphoribosyl-1-pyrophosphate (PRPP). In

the second reaction, an N-glycosidic bond is formed to synthesize 5-phospho-ß-D-

ribosylamine. Glutamine is the N-donor in this reaction. Glycine, aspartate, and

tetrahydrofolate derivatives are other precursors needed in the synthesis of IMP. Both

AMP and GMP are subsequently formed from IMP via adenylosuccinate and xanthosine

monophoshate, respectively (Rodwell, 2000). The precursors for pyrimidine synthesis are

carbamoyl phosphate and aspartate. The pyrimidine UMP is formed in a process

involving 6 reactions. A dephosphorylation of UMP yields UDP which is subsequently

turned into CMP or TMP. Glutamine and N^5N^{10}-methylene-folate are needed in the

synthesis of CMP and TMP, respectively (Rodwell, 2000). The de novo synthesis of both

purine and pyrimidine nucleotides is a metabolically costly process requiring a significant amount of energy in the form of ATP.

The synthesis of a nucleotide from a nucleoside and an inorganic phosphate group is accomplished via the Salvage Pathway. The nucleosides used in the Salvage Pathway may originate from dietary sources because most dietary nucleotides are changed to nucleosides prior to absorption. The Salvage Pathway may also be used to re-synthesize nucleotides via phosphoribosylation of purines and pyrimidines formed during the catabolism of nucleotides. This pathway may spare energy and allow cells (i. e., leukocytes, erythrocytes, bone marrow cells, intestinal mucosal cells, and lymphocytes), that are incapable of de novo synthesis to maintain their nucleotide pools (Sanderson and He, 1994).

Purines and pyrimidines are synthesized in equal amounts on a molar basis. The synthesis is strictly regulated via feed-back and allosteric regulations (Rodwell, 2000).

Storage of nucleotides.

Nucleotide metabolism is characterized by constant synthesis and catabolism. Tracer studies in animals indicate that 2 to 5% of dietary nucleotides are retained in the small intestine, liver, and skeletal muscle tissue pools (Saviano and Clifford, 1978). Increased tissue retention has been reported in young animals (Kobota, 1969) and during fasting (Saviano and Clifford, 1978; Gross and Saviano, 1991). This may be a manifestation of a physiological requirement. Nucleotide pools are larger in differentiated (i. e., cancerous) cells than in undifferentiated (i. e., nonmalignant) cells (Sanderson and

He, 1994). This suggests that undifferentiated cells are more dependent on the dietary supply of nucleotides.

Physiological roles of nucleotides.

The concentration of ribonucleotides is relatively constant in all cells, while the concentration of deoxyribonucleotides varies with the stage of the cell cycle (Barness, 1994). Nucleotides are the building blocks for nucleic acids (i. e., DNA and RNA). However, nucleotides also have physiological roles in the body such as being a source of energy (i. e., ATP and GTP), cofactors in oxidation and reduction reactions (i. e., FAD, NAD^+, and $NADP^+$), serve as physiological regulators (i. e., cAMP and cGMP), and carry activated intermediates (i. e., UDP-glucose, CMP-sialic acid, and CDP-choline) and acyl groups (i. e., CoA).

Rapidly dividing tissues have increased DNA replication and RNA synthesis (Cory, 1992). Because of the role of nucleotides in RNA synthesis, regeneration of new tissue after injury or relative nutrient deficiency is accelerated (Iwasa et al., 1997; Yamauchi et al., 1998). Replication of DNA occurs during the S phase of the cell cycle, and during this time, the enzyme activity is increased for purine and pyrimidine synthesis and nucleotide interconversion (Carver and Walker, 1995). Therefore, there is an increase in the demand for nucleotides during cell division and growth (Tsujinaka et al., 1999).

Dietary nucleotide supplementation has been associated with both humoral and cellular immunity, but the exact mechanism has not been elucidated. Nucleotide deprivation caused the arrest of T-cells in the G phase of the cell cycle, preventing a response to various immunological signals that occur by transition to the S phase

(Kulkarni et al., 1994). Nucleotide deprivations also caused a decrease in phagocytic activity, lymphokine production, and/or inhibited lymphocyte maturation (Paubert-Braquet et al., 1992). Dietary nucleotides contribute to the circulating pool of nucleosides available to stimulate leukocyte production (Kulkarni et al., 1994; Carver and Walker, 1995). Therefore, there is an elevated need for nucleotides during periods of immunological challenges.

Dietary factors play a role in the antibody response to immunization of infants. Infants fed milk formula fortified with nucleotides had better responses to immunization as evidenced by an increase in humoral antibody response (Fanslow et al., 1988; Pickering et al., 1998) and increased cytokine production (Carver et al., 1991). In vivo studies in mice show similar results to nucleotide supplementation (Jyonouchi et al., 1993; Jyonouchi, 1994). Nucleotide-free diets supplemented with single nucleotides (i. e., AMP, GMP, or UMP), have been shown to increase immunoglobulin concentration (Navarro et al., 1996). Dietary supplementation of purified nucleotides to milk replacers of newborn bull calves challenged with lipopolysaccharide (LPS), resulted in calves that tended to have higher mean IgG levels compared to the unsupplemented control calves (Oliver et al., 2003). Nucleotide supplementation also increased lymphocyte stimulation to phytohaemagglutinin and concanavalin-A challenges in weanling piglets by 50 and 30%, respectively (Zomborsky-Kovacs et al., 1998). Similar results were observed to challenges by keyhole limpet hemocyanin (KLH) and non-specific T-cell mitogens in piglets fed yeast RNA (Cameron et al., 2001). Pigs with pathogenic *E. coli* infection fed diets supplemented with yeast extract as a source of nucleotides, have reduced diarrhea and improvement in weight gain and feed conversion compared to pigs fed the control

diet (Maribo, 2003). Results of these studies imply that dietary sources of nucleotides play a role in developing, maintaining, and enhancing the immune system.

Dietary nucleotides enhance intestinal absorption of iron, affect lipoprotein and long chain polyunsaturated fatty acid metabolism, have trophic effects on the intestinal mucosa and liver, and reduce the incidence of diarrhea (Cosgrove, 1998; Schlimme et al., 2000). Fecal flora of infants fed a nucleotide-supplemented commercial milk formula had a predominance of bifidobacteria (Tanaka and Mutai, 1980), while enterobacteria predominated in the fecal flora of infants fed a commercial formula without nucleotide supplementation (Uauy, 1994). These studies suggest that nucleotide supplementation may positively influence the microflora in the gastrointestinal tract, which leads to a lowering of gastric pH and hinders the proliferation of pathogenic bacterial species as evidenced by a lower rate of diarrhea (Yu, 1998).

Nucleotide supplementation increased bacterial resistance of mice inoculated with Candida albicans (Fanslow et al., 1988) and Staphylococcus aureus (Kulkarni et al., 1986; Carver, 1994). Intraperitoneal administration of nucleotides and nucleosides decreased bacterial translocation, number of colony forming units, and increased the survival of mice against methicillin-resistant Staphylococcus aureus (Yamamoto et al., 1997). Therefore, nucleotides are capable of increasing bacterial resistance to a wide range of potentially pathogenic bacteria.

Dietary nucleotides from yeast extract supplemented in calf milk replacers, improved intestinal health as demonstrated by high feacal scores compared to the non-supplemented control group (Oliver et al., 2002). In piglets, nucleotides in concentrations

similar to those in human milk exert a protective effect in the intestinal lumen against an inflammatory response to ischemia-reperfusion (Bustamante et al., 1994).

Dietary nucleosides enhance the growth and maturation of intestinal epithelial cells as evidenced by an increased formation of mucosal protein, DNA, taller villi in the small intestine and increased maltase to lactase enzyme ratio (Uauy et al., 1990; Carver, 1994). Dietary nucleotides may also stimulate enterocyte differentiation (Sanderson and He, 1994). Parenteral supplementation of nucleic acids support mucosal cell proliferation and function as demonstrated by increased mucosal wet weight, protein and DNA contents, villous height, but not crypt depth, and narrower tight junctions of the jejunal mucosa width (Kishibuchi et al., 1997; Tsujinaka et al., 1999). The development of the gastrointestinal tract directly affects the degree of nutrient absorption and ultimately, animal growth. Because of the role of nucleotides in maintaining intestinal morphology and maturation, dietary nucleotides may be needed during the immediate post-weaning period to maintain the structure and growth of the intestinal tract.

Are dietary nucleotides needed in diets for weanling pigs?

The need for nucleotides is elevated during periods of rapid growth, during periods of stress, and in immuno-compromised animals. In newly weaned pigs, all of these factors are present – therefore, it is expected that they have a high requirement for nucleotides during this period. Because nucleotide synthesis is an energy- and glutamine-requiring process and because newly weaned pigs are often deficient in both energy and glutamine, it is possible that pigs are not able to synthesize sufficient quantities of nucleotides during the immediate post-weaning period. If this is correct, dietary

nucleotides have to be supplied. In a typical starter diet for weanling pigs, the concentration of 5'CMP is close to the concentration found in the DM of sow's milk during the last half of lactation, but the concentration of 5'AMP, 5'GMP, 5'IMP, and 5'UMP is much lower than in sow's milk (Table 4). Assuming that the concentration of nucleotides in sow's milk are representing of the requirement of the pigs, it is easily concluded that a starter diet for young pigs is deficient in four of the five nucleotides. It may, therefore, be beneficial to add additional nucleotides to such diets. In a recent experiment in our laboratory, we added nucleosides to a deficient starter diet. We used nucleosides rather than nucleotides because dietary nucleotides need to be digested to nucleotides before absorption. Blood samples and fecal samples were collected from weanling pigs on the day of weaning, on day 7 post-weaning and on day 14 post weaning. Blood samples were analyzed for IgG concentrations, while the microbial flora was quantified in the samples. Results of the experiment showed that fecal counts of *Cl. perfringens* was reduced in samples collected from pigs fed a nucleoside supplemented diet as compared to the non-supplemented control diet. On day 14 post-weaning, the fecal counts of *L. acidophilus* and *Bifidobacterium spp.* also were higher in pigs fed the nucleoside containing diet (Mateo et al., 2004a). These results indicate that nucleoside supplementation during the immediate post-weaning period may positively influence the gastrointestinal microflora by decreasing *Cl. perfringens* and increasing *L. acidophilus* and *Bifidobacterium spp.* This observation was subsequently confirmed in an in-vitro experiment that showed that nucleotides have antibacterial properties against *Cl. perfringens* and *e.coli*. The implication of this finding is that pigs fed diets supplemented with nucleosides may have improved intestinal health, less scoring, and improved

performance. Although no improvement in serum IgG was observed in the above experiment, the improved intestinal health may also lead to an improvement in the immune system of animals fed diets supplemented with nucleotides. A reduced concentration of enterobacteria and an increased number of probiotic bacteria (i.e. *L. acidophilus* and *Bifidobacterium spp.)* may also result in improved intestinal morphology and improved nutrient uptake. This hypothesis is being tested in a current research project at South Dakota State University.

Conclusion

Nucleotides are molecules with considerable structural diversity. They are composed of a nitrogenous base linked to a pentose sugar to which at least one phosphate group is attached. Feed or food ingredients containing cellular elements are potential sources of nucleotides. Nucleotides have many important physiological, gastrointestinal, and immunological functions in the body. The exact metabolism of nucleic acids ingested by young animals is unknown. Synthesizing nucleotides de novo is metabolically costly compared to synthesis via the Salvage pathway. During periods of rapid growth and development, disease challenges, injury or stress, dietary nucleotide supplementation may be beneficial because of the role of nucleotides in developing and enhancing immunity, maintaining intestinal health, and preserving energy. However, more research is needed to verify this hypothesis.

References

Bager, F., F. M. Aarestrup, and H. G. Wegner. 2000. Dealing with antimicrobial resistance- the Danish experience. Can. J. Anim. Sci. 80:223-228.

Barness, L. 1994. Dietary source of nucleotides-from breast milk to weaning. J. Nutr. 124:128-130.

Bronk, J. R., and J. G. Hastewell. 1987. The transport of pyrimidines into tissue rings cut from rat small intestine. J. Physiol. 382:475-488.

Bustamante, S. A., N. Sanchez, J. Crosier, D. Miranda, G. Colombo, and M. J. S. Miller. 1994. Dietary nucleotides: effects on the gastrointestinal system in swine. J. Nutr. 124:149-156.

Cameron, B. F., C. W. Wong, G. N. Hinch, D. Singh, J. V. Nolan, and I. G. Colditz. 2001. Effects of nucleotides on the immune function of early-weaned pigs. Pages 66-68 in Digestive Physiology of Pigs. Proc. 8th Symposium. J. E. Lindberg and B. Ogle, eds. CABI Publishing New York, NY.

Carver, J. D., B. Pimentel, W. I. Cox, and L. A. Barness. 1991. Dietary nucleotide effects upon immune function in infants. Pediatrics. 88:359-363.

Carver, J. D. 1994. Dietary nucleotides: cellular immune, intestinal and hepatic system effects. J. Nutr. 124: 144-148.

Carver, J. D., and W. A. Walker 1995. The role of nucleotides in human nutrition. Nutr. Biochem. 6:58-72.

Clifford, A. J., and D. L. Story. 1976. Levels of purines in foods and their metabolic effect in rats. J. Nutr. 106:435-442.

Cory, J. G. 1992. Purine and pyrimidine nucleotide metabolism. Pages 529-571 in Textbook of Biochemistry. T. M. Devlin, ed. Wiley-Liss, Inc., New York, NY.

Cosgrove, M. 1998. Perinatal and infant nutrition. Nucleotides. Nutr. 14:748-51.

Fanslow, W. C., A. D. Kulkarni, C. T. Van Buren, F. B. Rudolph. 1988. Effect of nucleotide restriction and supplementation on resistance to experimental murine candidiasis. J. Parenter. Enteral. Nutr. 12:49-52.

Gil, A., and F. Sanchez-Medina. 1981. Acid soluble nucleotides of cow's, goat's and sheep's milks at different stages of lactation. J. Dairy Sci. 48:35-44.

Gil, A., and F. Sanchez-Medina. 1982a. Acid soluble nucleotides of human milk at different stages of lactation. J. Dairy Res. 49:301-307.

Gross, C. J., and D. A. Saviano. 1991. The effect of nutritional state and allopurinol on nucleotide formation in enterocytes from the guinea pig small intestine. Biochem. Biophys. Acta. 1073:260-267.

Holdsworth G., and R. Coleman. 1975. Enzyme profiles of mammalian bile. Biochem. Biophys. Acta 389:47-50.

Ingledew, W. M. 1999. Yeast-could you base a business on this bug? Pages 27-47 in Biotechnology in the Feed Industry. Proc. of Alltech's 15th Annual Symposium. T. P. Lyons and K. A. Jacques, eds. Nottingham University Press, Nottingham, UK.

Iwasa, Y., M. Iwasa, Y. Omori, T. Toki, A. Yamamoto, H. Maeda, M. Kume, and S. Ogoshi. 1997. The well-balanced nucleoside-nucleotide mixture "OG-VI" for special medical purposes. Nutr. 13:361-364.

Johke, T. 1963. Acid soluble nucleotides of colostrum, milk, and mammary gland. J. Biochem. 54:388-397.

Jyonouchi, H., L. Zhang, and Y. Tomita. 1993. Immunomodulating actions of RNA and nucleotides on murine lymphocytes in vitro. Augmentation of antibody production to T-dependent antigens and expansion of T-helper cells. J. Nutri. Immunol. 22:5-24.

Jyonouchi, H. 1994. Nucleotide actions on humoral immune responses. J. Nutr. 124:138-143.

Kishibuchi, M., T. Tsujinaka, M. Yano, T. Morimoto, S. Iijima, A. Ogawa, H. Shiozaki, and M. Monden. 1997. Effects of nucleoside and a nucleotide mixture on gut mucosal barrier function on parenteral nutrition in rats. J. Parenter. Enteral Nutr. 21:104-111.

Kobota, A. 1969. Nutritional study of nucleotide components in the milk. Acta Paediatr. Jap. 73:197-209.

Kojima, K. 1974. Safety evaluation of disodium 5'-inosinate, disodium 5'-guanylate and disodium 5'-ribonucleate. Toxicology 2:185-206.

Kulkarni, A. D., W. C. Fanslow, F. B. Rudolph, and C. T. Van Buren. 1986. Effect of dietary nucleotides on response to bacterial infections. J. Parenter. Enteral Nutr. 10:169-171.

Kulkarni, A. D., F. B. Rudolph, and C. T. Van Buren. 1994. The role of dietary sources of nucleotides in immune function: a review. J. Nutri. 124:1442-1446.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Maloney, D. 1998. Yeasts. Pages 761-788 in Kirk-Othmer Encyclopedia of Chemical Technology. 4th, ed. J. I. Kroschwitz and M. Howe-Grant, eds. John Wiley and Sons, Inc., New York, NY.

Maribo, H. 2003. Weaning pigs without antibiotic growth promoters: strategies to improve health and performance. Pages 179-184 in Nutritional Biotechnology in the Feed and Food Industries. Proc. of Alltech's 19th International Symposium. T. P. Lyons and K. A. Jacques, eds. Nottingham University Press, Nottingham, UK.

Markiewicz A., M. Kaminski, W. Chocilowski, T. Gomoluch, H. Boldys, and B. Skrzypek. 1983. Circadian rhythms of four marker enzymes activity of the jejunal villi in man. Acta Histochem. 72:91-99.

Mateo, C. D., R. Dave, and H. H. Stein. 2004a. Effects of supplemental nucleosides for newly weaned pigs. J. Anim. Sci. 82(Suppl. 2):XX (Abstr.) In Press.

Mateo, C. D., D. N. Peters, and H. H. Stein. 2004b. Nucleotides in sow colostrum and milk at different stages of lactation. J. Anim. Sci. 82:xxxx. In Press.

Morley, D. J., D. M. Hawley, T. M. Ulbright, L. G. Butler, J. S. Culp, and M. E. Hodes. 1987. Distribution of phosphodiesterase I in normal human tissues. J. Histochem. Cytochem. 35:75-82.

Navarro, J., A. R. Barvo, M. J. Valera, and A. Gil 1996. Modulation of antibody-forming cell and mitogen-driven lymphoproliferative responses by dietary nucleotides in mice. Immunology Letters 53:141-145.

Oliver, C. E., M. L. Bauer, J. W. Schroeder, W. L. Keller, and C. S. Park. 2002. Dietary nucleotides enhance calf immune function. FASEB J. 16:A 985 (Abstr.).

Oliver, C. E., M. L. Bauer, C. M. D. J. Arias, W. L. Keller, and C. S. Park. 2003. Influence of dietary nucleotides on calf health. J. Anim. Sci. 81(Suppl. 1): 136 (Abstr.).

Paubert-Braquet, M., C., Dupont, N., Hedef, and S. Picquot. 1992. Quantification of nucleotides in human milk and their effects on cytokine production by murine fibroblasts, J774A1 macrophages and human monocytes. Foods, Nutrition and Immunity. 1:22-34.

Pickering, L. K., D. M. Granoff, J. R. Erickson, M. L. Masor, C. T. Cordle, J. P. Schaller, T. R. Winship, C. L. Paule, and M. D. Hilty. 1998. Modulation of the immune system by human milk and infant formula containing nucleotides. Pediatrics. 101:242-249.

Quan, R. and Uauy, R. 1991. Nucleotides and gastrointestinal development. Sem. Pediatr. Gastr. Nutr. 2:3-11.

Rodwell, V. W. 2000. Metabolism of purine and pyrimidine nucleotides. P. 386-401 in Harpers Biochemistry, 25th edition. Murray, R. K., D. K. Granner, P. A. Mayes, and V. W. Rodwell (Eds.). Appleton and Lange, Stanford, CT.

Rudolph, F. B. 1994. The biochemistry and physiology of nucleotides. J. Nutr. 124:124-127.

Salati, L. M., C. J. Gross, L. M. Henderson, and D. A. Saviano. 1984. Absorption and metabolism of adenine, adenosine-5'-mono-phosphate, adenosine and hypoxanthine by the isolated vascularly perfused rat small intestine. J. Nutr.114:753-760.

Sanderson, I. R., and H. E. Youping. 1994. Nucleotide uptake and metabolism by intestinal epithelial cells. J. Nutr. 124:131-137.

Saviano, D. A., and Clifford, A. J. 1978. Absorption, tissue incorporation and excretion of free purine bases in the rat. Nutr. Rep. Int. 17:551-556.

Schlimme, E., D. Martin, and H. Meisel. 2000. Nucleosides and nucleotides: natural bioactive substances in milk and colostrum. Br. J. Nutr. 84:59-68.

Stein, H. H. 2002. Experience of feeding pigs without antibiotics - a European perspective. Anim. Biotechnol, 13:85-95.

Tanaka, R., and M. Mutai. 1980. Improved medium for selective isolation and enumeration of Bifidobacterium. Appl. Environ. Microbiol. 40:866-869

Thorell, L., Sjoberg, L. B., and O. Hernell. 1996. Nucleotides in human milk: sources and metabolism by the newborn infant. Pediatr. Res. 40:845-852.

Tibbets, G. W. 2002. Nucleotides from yeast extract: potential to replace animal protein sources in food animal diets. Pages 435-443 in Nutritional Biotechnology in the Food and Feed Industries. Proc. Alltech's 18th Annual Symposium. T. P. Lyons and K. A. Jacques, eds. Nottingham University Press, Nottingham, UK.

Tsujinaka, T., M. Kishibuchi, S. Iijima, M. Yano, and M. Monden. 1999. Nucleotides and intestine. J. Parenter. Enteral Nutr. 23:74-77.

Turner, J. L., S. S. Dritz, and J. E. Minton. 2001. Review: Alternatives to conventional antimicrobials in swine diets. Prof. Anim. Sci. 17:217-226.

Uauy, R. 1989. Dietary nucleotides and requirements in early life. Pages 265-280 in Textbook of Gastroenterology and Nutrition in Infancy. E. Lebenthal, ed. Raven Press, Ltd. New York, NY.

Uauy, R., G. Stringel, R. Thomas, and R. Quan. 1990. Effect of dietary nucleosides of growth and maturation of the developing gut in rat. J. Pediatr. Gastroenterology Nutr. 10:497-503.

Uauy, R. 1994. Nonimmune system responses to dietary nucleotides. J. Nutr. 124:157-159.

Voet D., and J. G. Voet. 1995. Nucleotide Metabolism. Pages 795-797 in Biochemistry. 2nd ed., N. Rose, ed. John Wiley and Sons, Inc. New York, NY.

Weickman, J. L., M. Elson, and D. G. Glitz. 1981. Purification and characterization of human pancreatic ribonuclease. Biochem. 20:1272-1278.

Yamamoto, S., M. F. Wang, A. A. Adjei, and C. K. Ameho. 1997. Role of nucleotides and nucleosides in the immune system, gut reparation after injury, and brain function. Nutr. 13:372-374.

Yamauchi, K., A. A. Adjei, C. K. Ameho, S. Sato, K. Okamoto, S. Kakinohana, and S. Yamamoto. 1998. Nucleoside-nucleotide mixture increases bone marrow cell number and small intestine RNA content in protein deficient mice after an acute bacterial infection. Nutr. 14:270-275.

Yu, V. Y. 1998. The role of dietary nucleotides in neonatal and infant nutrition. Sing. Med. J. 39:145-150.

Zomborsky-Kovacs, M., S. Tuboly, H. Biro, L. Bardos, P. Soos, A. Toth, G. Tornyos. 1998. The effect of b-carotene and nucleotide base supplementation on haematological, biochemical and certain immunological parameters in weaned pigs. J. Anim. and Feed Sci. 7:245-251.

Table 1. Nucleotide nomenclature

Base	Product:	Nucleoside	Ribo-nucleotide[a]	Deoxyribo-nucleotide[b]	Diphosphate nucleotide[c]	Triphosphate nucleotide[d]
Purines						
Adenine		Adenosine	AMP	dAMP	ADP/ dADP	ATP/ dATP
Guanine		Guanosine	GMP	dGMP	GDP/ dGDP	GTP/ dGTP
Hypoxanthine		Inosine	IMP	-	-	-
Pyrimidines						
Cytosine		Cytidine	CMP	dCMP	CDP/ dCDP	CTP/ dCTP
Uracil		Uridine	UMP	dUMP	UDP/ dUDP	UTP
Thymine		Thymidine		dTMP	dTDP	dTTP

[a]AMP = adenosine 5'-monophosphate; GMP = guanosine 5'-monophosphate; IMP = inosine 5'-monophosphate; CMP = cytidine 5'-monophosphate; UMP = uridine 5'-monophosphate

[b]dAMP = deoxyadenosine 5'-monophosphate; dGMP = deoxyguanosine 5'-monophosphate; dCMP = deoxycytidine 5'-monophosphate; dUMP = deoxyuridine 5'-monophosphate; dTMP = deoxythymidine 5'-monophosphate

[c]ADP = adenosine 5'-diphosphate; dADP = deoxyadenosine 5'-diphosphate; GDP = guanosine 5'-diphosphate; dGDP = deoxyguanosine 5'-diphosphate; CDP = cytidine 5'-diphosphate; dCDP = deoxycytidine 5'-diphosphate; UDP = uridine 5'-diphosphate; dUDP = deoxyuridine 5'-diphosphate; dTDP = deoxythymidine 5'diphosphate

[d]ATP = adenosine 5'-triphosphate; dATP = deoxyadenosine 5'-triphosphate; GTP = guanosine 5'-triphosphate; dGTP = deoxyguanosine 5'-triphosphate; CTP = cytidine 5'-triphosphate; dCTP = deoxycytidine 5'-triphosphate; UTP = uridine 5'-triphosphate; dTTP = deoxythymidine 5'-triphosphate

Table 2. Nucleotide concentration in some commonly used feed ingredients (as is basis)[a]

Ingredient Nucleotide:	Nucleotide (mg/g)				
	5'CMP	5'AMP	5'GMP	5'UMP	5'IMP
Barley	0.002	0.001	0.001	0.000	0.001
Casein	0.001	0.000	0.000	0.000	0.000
Corn	0.003	0.002	0.003	0.000	0.001
Fish meal	0.026	0.011	0.002	0.001	0.035
Naked oats	0.003	0.003	0.003	0.001	0.001
Protein plasma, spray dried	0.002	0.002	0.002	0.000	0.001
Red blood cells, spray dried	0.000	0.044	0.003	0.002	0.006
Soybean meal, 44 %	0.016	0.008	0.003	0.009	0.002
Soy Protein Concentrate	0.000	0.001	0.002	0.000	0.001
Whey, dried	0.270	0.019	0.000	0.001	0.004

[a] Data from Mateo et al. (2004a)

Table 3. Concentration of adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), inosine 5'-monophosphate (IMP), citidine 5'-monophosphate (CMP), and uridine 5'-monophosphate (UMP) in milk from different species. (μmoles/100ml)[ab]

Nucleotide	Milk	Day of lactation					
		5-7	8	10-11	14-15	21	28-31
AMP	Human	2.24[e]	-	-	2.60[e]	-	2.02[e]
	Bovine	3.15[d]	1.80[c]	-	2.91[d]	1.81[d]	-
	Caprine	11.00[d]	6.30[d]	12.20[c]	2.79[d]	-	4.07[d]
	Ovine	-	15.67[d]	-	11.87[d]	-	8.47[d]
	Equine	-	-	0.50[c]	-	-	-
	Porcine	12.80[f]	-	-	6.80[f]	4.30[f]	3.00[f]
CMP	Human	3.10[e]	-	-	2.64[e]	-	1.87[e]
	Bovine	3.02[d]	6.20[c]	-	4.90[d]	4.12[d]	-
	Caprine	8.07[d]	5.86[d]	-	2.28[d]	-	3.55[d]
	Ovine	-	23.30[d]	-	7.17[d]	-	8.70[d]
	Equine	-	-	1.50[c]	-	-	-
	Porcine	7.10[f]	-	-	3.50[f]	2.30[f]	2.50[f]
GMP	Human	0.50[e]	-	-	-	-	0.32[e]
	Bovine	0.83[d]	-	-	-	-	
	Caprine	-	-	1.70[c]	0.99[d]	-	0.70[c]

	Ovine	-	1.50[d]	-	-	-	-
	Equine	-	-	-	-	-	-
	Porcine	14.00[f]	-	-	10.20[f]	6.00[f]	7.10[f]
IMP	Porcine	2.60[f]	-	-	1.40[f]	0.90[f]	0.40[f]
UMP	Bovine	2.87[d]	1.30[d]	-	-	-	-
	Caprine	12.37[d]	12.59[d]	5.90[c]	16.08[d]	-	12.64[d]
	Ovine	-	65.16[d]	-	20.07[d]	-	26.08[d]
	Equine	-	-	7.70[c]		-	-
	Porcine	263.10[f]	-	-	144.00[f]	122.80[f]	104.00[f]

[a] Number of samples analyzed varied between 4 and 12

[b] Nucleotide analysis via enzymatic analysis or HPLC.

[c] Data from Johke (1963)

[d] Data from Gil and Sanchez-Medina (1981)

[e] Data from Gil and Sanchez-Medina (1982a)

[f] Data from Mateo et al. (2004b)

Table 4. Calculated nucleotide concentration of a starter diet for weanling pigs [a]

		Nucleotide (ppm)				
Item	Nucleotide:	CMP	AMP	GMP	UMP	IMP
Total in starter diet [b]		58.99	6.46	2.03	1.00	4.33
Sows milk [c]		56.00	117.50	185.5	2334.50	23.5
Difference		2.99	-111.04	-183.47	-2333.50	-19.17

[a] Data from Mateo et al. (2004a)

[a] Diet formulated to contain the following feed ingredients: Corn, 49.32%; Whey powder, 20%, Soybean meal, 8%; Fish meal, 8%; spray dried protein plasma, 7.5%, vitamins, minerals, oil, and crystalline amino acids, 7.18%.
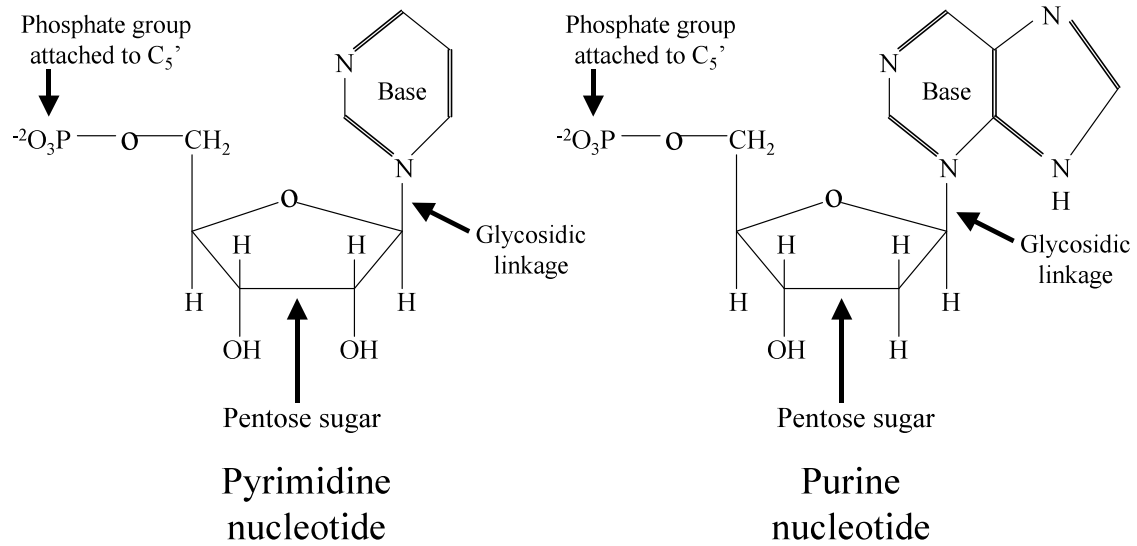
[c] Data from Mateo et al. (2004b)

Figure 1. Structure of a nucleotide. C = carbon atom, H = hydrogen atom, O = oxygen atom, and N = nitrogen atom.
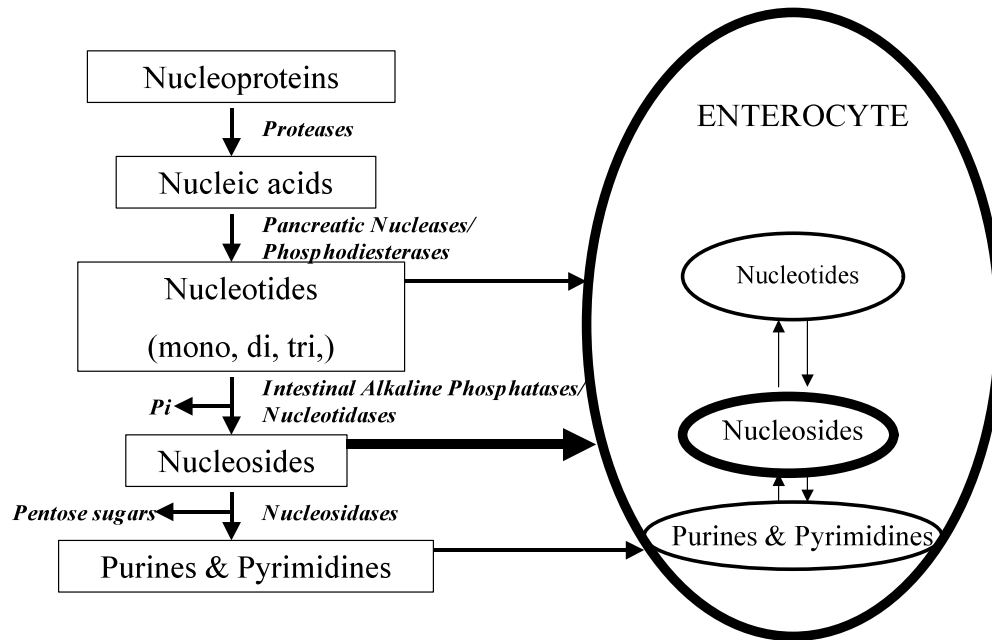
Figure 2**.** Digestion and absorption of nucleic acids and their related products. Adapted

from Quan and Uauy, 1991