Aspects of Nucleotide Nutrition in Pigs

BY

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Aspects of Nucleotide Nutrition in Pigs

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

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A series of studies was conducted with the objective of evaluating the effect of supplementing dietary nucleotides in weanling pig diets. The first study was designed to measure the concentration of nucleotides in the colostrum and milk of sows. The concentrations of 5'AMP, 5'CMP, 5'GMP, and 5'IMP change during the initial week post-partum, but during the last two weeks of a 4-wk lactation period, the concentration is relatively constant. In contrast, the concentration of 5'UMP decreased from d-0 to d-28 of lactation. In colostrum, 5'UMP represented 98% of all 5'monophosphate nucleotides and in milk, 5'UMP accounted for 86-90% of all nucleotides, regardless of d of lactation. In the second study, two experiments were conducted to determine the effects of adding nucleosides to starter diets for weanling pigs. In Exp. 1, pigs allotted to Treatment 1 were fed a corn casein lactose-based basal diet. Pigs allotted to Treatments 2 and 3 were fed the basal diet supplemented with nucleosides in amounts that correspond to 30% and 150% of the quantities found in sow milk on d-14 of lactation, respectively. The concentration of IgG was determined in the serum while microbial concentration was determined in fecal samples on d-0, 7, and 14 post-weaning. Exp. 2 was an in-vitro study that was conducted to determine the antimicrobial and probiotic properties of

nucleosides. Broths containing microflora from pigs were prepared either without nucleosides (control) or with nucleoside supplementation. The bacterial growth in both broths was measured over a 16-h period. The results of the two experiments indicate that nucleoside supplementation during the immediate post-weaning period may positively influence the gastrointestinal microflora by decreasing enterobacteria and increasing *L. acidophilus* and Bifidobacterium species. In the third study, two experiments were conducted to determine the effects of adding nucleotides to starter diets for weanling pigs. In Exp. 1, pigs were allotted to two treatment groups. Pigs allotted to Treatment 1 were fed a conventional starter diet. Pigs allotted to Treatment 2 were fed Diet 1 supplemented with nucleotides in amounts that correspond to 100% of the quantities found in sow milk (DM-basis) on d-14 of lactation. Pig performance, serum IgG concentration, intestinal morphology, intestinal microflora composition, and intestinal tissue DNA, RNA, and protein content were used to determine the effects of nucleotide supplementation on d-14 and d-28 post-weaning. Exp. 2 was an in vitro study that was conducted to determine the antimicrobial and probiotic properties of nucleotides. The results indicate that nucleotide supplementation during the immediate post-weaning period positively influence gastrointestinal morphology and microflora by increasing ileal VH and VH:LPD, and decreasing *Cl. perfringens* count in-vitro. In the final study, the AID and SID coefficients of AA and CP in yeast extract and SDPP by weanling pigs were measured using the difference method. The results indicate that the AID for CP and all AA with the exception of Cys and Ser are similar between yeast extract and SDPP. Likewise, no differences in SID for AA or CP were observed between yeast extract and

SDPP. In addition, yeast extract and SDPP contain protein that is relatively well digested by young pigs.

Key words: Digestibility, Immunoglobulins, Microflora, Morphology, Nucleosides, Nucleotides, Pigs

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LIST OF ABBREVIATIONS

AA	Amino acid
AAd	Amino acid content of dry matter ileal digesta
AAf	Amino acid content of the dry matter feed
ADFI	Average daily feed intake
ADG	Average daily gain
ADP	Adenosine diphosphate
AID	Apparent ileal digestibility coefficient(s)
Ala	Alanine
ALT	Alanine aminotransferase
5’AMP	Adenosine 5’monophosphate
AMPRT	Amidophosphoribosyl-tranferase
ANOVA	Analysis of variance
AOAC	Association of analytical chemists
APRT	Adenosine phosphoribosyltranferase
Arg	Arginine
Asp	Aspartic acid
AST	Aspartate aminotransferase
ATC	Aspartate transcarbamylase
ATCase	Aspartate transcarbomoylase
ATP	Adenosine triphosphate

BN broth	Bacto nutrient broth
BW	Body weight
ºC	Degrees celsius
Ca	Calcium
cAMP	Cyclic adenosine monophosphate
CDP-choline	Cytidine diphosphate choline
cGMP	Cyclic guanosine monophosphate
cfu	Colony forming units
cm	Centimeters
5'CMP	Cytidine 5'monophosphate
CoA	Coenzyme A
Con A	Concanavalin A
CP	Crude protein
CPS-II	Carbamyl phosphate synthetase-II
Crd	Chromium content of dry matter in ileal digesta
Crf	Chromium content of dry matter in feed
CTP	Cytidine triphosphate
Cu	Copper
Cys	Cysteine
d	Day
DM	Dry matter
DMI	Dry matter intake

DNA	Deoxyribonucleic acid
DNA:protein	Deoxyribonucleic acid to protein ratio
dUMP	Deoxyuridine monophosphate
dUTP	Deoxyuridine triphosphate
dTMP	Deoxythymidine monophosphate
dTTP	Deoxythymidine triphosphate
EAL	Endogenous amino acid losses
FAD	Flavin adenine dinucleotide
Fe	Iron
g	Grams
G_1	Gap 1
G_2	Gap 2
GDP	Guanosine diphosphate
G:F	Gain to feed ratio
Gln	Glutamine
Gly	Glycine
5’GMP	Guanosine 5’monophosphate
GOT	Glutamic acid oxalacetic transaminase
GPT	Glutamic acid pyruvic transaminase
GTP	Guanosine triphosphate
H	Hydrogen
h	Hour

H and E	Hematoxylin and eosin
HCL	Hydrochloric acid
HGPRT	Hypoxanthine-guanine phosphoribosyltranferase
Hib	*H influenza* type b
His	Histidine
HPLC	High performance liquid chromatography
I	Iodine
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Ile	Isoleucine
5'IMP	Inosine 5'monophosphate
IU	International units
Kcal	Kilocalories
kg	Kilograms
KLH	Keyhole limpet hemocyanin
Leu	Leucine
LPD	lamina propria depth
LPS	Lipopolysaccharides
Lys	Lysine
M	Mitosis
Mcal	Megacalories
ME	Metabolizable energy

Met	Methionine
Mg	Magnesium
mg	Milligrams
MI	Mitotic index
mL	Milliliters
Mn	Manganese
MOF	Multiple organ failure
M-PER	Mammalian protein extraction reagent
MPO	Colonic myeloperoxidase
mRNA	Messenger ribonucleic acid
MRS	De man rogosa sharp
MRS-NNPL	De man rogosa sharp-Neomycin sulfate, nalidixic acid, paromomycin sulfate, and lithium chloride
MSG	Monosodium glutamate
N	Nitrogen
Na	Sodium
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NRC	National research council
OMP	Orotate monophosphate
P	Phosphorus
PCI	Proliferating cell index

Phe	Phenylalanine
PNP	Purine nucleoside phosphorylase
ppm	Parts per million
Pro	Proline
PRPP synthetase	5-Phosphoribosyl-1-pyrophosphate synthetase
PRT	Phosphoribosyltransferase
RC broth	Reinforced clostridial broth
RID	Radial immunodiffusion
RNA	Ribonucleic acid
rRNA	Ribosomal-ribonucleic acid
RNA:DNA	Ribonucleic acid to deoxyribonucleic acid ratio
RNA:protein	Ribonucleic acid to protein ratio
S	Synthesis
SAA	Sulfur containing amino acids
SAS	Statistical analysis software
SCP	Single cell protein
SDPP	Spray dried plasma protein
SDSU	South dakota state university
Se	Selenium
SEM	Standard error of the mean
Ser	Serine
SID	Standardized ileal digestibility coefficient(s)

SIRS	Systemic inflammatory response syndrome
spp.	Species
SRBC	Sheep red blood cells
Thr	Threonine
TK	Thymidine kinase
TMS	Total milk solids
TPAN	Total potentially available nucleotides
TPN	Total parenteral nutrition
tRNA	Transfer-ribonucleic acid
Trp	Tryptophan
TSC	Tryptose sulfite cycloserine
Tyr	Tyrosine
UDPAG	Uridine diphosphate acetylglucosamine
UDPAGal	Uridine diphosphate acetylgalactosamine
UDPG	Uridine diphosphate glucose
UDPGal	Uridine diphosphate galactose
UDP-hexose	Uridine diphosphate-hexose
UDP-sugars	Uridine diphosphate-sugars
UK	Uridine kinase
5'UMP	Uridine 5'monophosphate
UTP	Uridine triphosphate
Val	Valine

VH	Villus height
VH:LPD	Villus height to lamina propria depth ratio
wk	Week
XO	Xanthine oxidase
Zn	Zinc

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CHAPTER 1

Introduction

For the past two decades, nucleotides and nucleic acids have been studied because of the many functions attributed to them. Nucleotides are important because of their participation in physiological reactions that are essential to the maintenance and propagation of life. Nucleotides are also involved in energy storage and transfer reactions, as well as, decoding of genetic information (Voet and Voet, 1995). Nucleotides optimize the function of rapidly dividing tissues, stimulate growth, and enhance immunity (Uauy, 1994; Cameron et al., 2001).

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 (Pickering et al., 1998). It has been reported that human infants fed nucleotide supplemented infant milk formulas have accelerated physical growth and neurological development, better growth and development of the digestive tract, enhanced immunity, and favorable intestinal microflora associated with a lower rate of diarrhea (Yu, 1998).

The need for nucleotides is elevated during periods of rapid growth, during periods of stress, and in immuno-compromised animals (Carver and Walker, 1995). In newly weaned pigs, all of these factors are present and therefore, it is expected that they have a high tissue requirement for nucleotides during the immediate post-weaning 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 this period. If this is correct, dietary nucleotides are expected to have a growth-promoting and/or health-enhancing effect in newly weaned pigs. However, limited information about the need for nucleotides and about the role of nucleotides in the development of the immune system and the intestinal tissue in young animals exist and only a few studies have been conducted using pigs.

Literature Cited

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

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

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.

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.

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

CHAPTER 2

Nucleotides in young animal nutrition:

Literature review

1. Introduction

The study of nucleotides and nucleic acids has been topics of interest because of the many functions attributed to them. Scientific evidence suggests that nucleotides are important because of their participation in physiological reactions that are essential to the maintenance and propagation of life. The need for nucleotides is elevated during periods of rapid growth, stress, and in immuno-compromised animals (Carver and Walker, 1995).

In newly weaned pigs, all of these factors are present and therefore, it is expected that they have a high requirement for nucleotides during the immediate post-weaning 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 this period. If this were correct, dietary nucleotides would be expected to have a growth promoting and/ or health-enhancing effect on newly weaned pigs.

The majority of research conducted on nucleotide supplementation has been in the field of human nutrition. In contrast, limited information about the need for nucleotides and about the role of nucleotides in the development of the immune system and the intestinal tissue in young animals exist. Also, only a few studies have been conducted using pigs.

2. Nucleotide biochemistry and nomenclature

Nucleotides are ubiquitous molecules with considerable structural diversity and are composed of a nitrogenous base linked to a sugar to which at least one phosphate group is attached (**Figure 1**). When the phosphate group is absent, the compound is known as a nucleoside. Nucleosides are formed from a base and a pentose through a glycosidic bond between the N-1 nitrogen of a pyrimidine or the N-9 nitrogen of a purine and the C-1' carbon of the pentose (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 (**Figure 2**). Carbon and N atoms of the nucleotide bases are derived from AA, CO_2, and N^{10}-Formyl-tetrahydrofolate for purines, and aspartate and carbamoyl phosphate for pyrimidines (Carver and Walker, 1995).

Pyrimidine bases are composed of six membered rings and comprise uridine (U), cytosine (C), and thymine (T). Similarly, purine bases have an additional five membered ring and comprise adenine (A), guanine (G), and hypoxanthine (**Figure 3**). Hypoxanthine is a breakdown product from either inosine or adenosine by the enzymes nucleoside phosphorylase or nucleosidase, respectively. However, it is also converted into inosine 5'monophosphate (IMP), a purine based nucleotide, by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) in a series of reactions known as the Salvage pathway (Carver and Walker, 1995). Adenosine phosphoribosyltransferase (APRT) is the other enzyme involved in the salvage of purines.

The pentose sugar base may be a ribose or a 2'-deoxyribose for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), respectively (**Figure 4**). The phosphate group may be in a mono, di, or tri phosphate form. The phosphoryl group of nucleotides is commonly esterified to the C-5' hydroxyl group (Rudolph, 1994).

3. Sources of nucleotides

Analysis for nucleotide contents in raw materials in the feed and food industry is not routinely conducted. However, specific studies have identified some ingredient sources containing different concentrations of nucleotides (**Tables 1 and 2**). Carver and Walker (1995) suggested that nucleotides, particularly IMP, are associated with protein rich food. Generally, any feed or food ingredient containing cellular elements are potential dietary sources of nucleotides in the form of nucleoproteins. Nucleoproteins are proteins that are conjugated to nucleic acids.

Muscle protein is a poor source of nucleotides as it is mainly composed of actin-myosin protein (Deveresse, 2000). Other poor sources of nucleotides are oil seeds (i. e., soybeans) and grains (i. e., corn and wheat) that contain less than 5 mg/g of purines and pyrimidines (Deveresse, 2000). Fruits, vegetables, and processed milk products are also poor sources of nucleotides (Barness, 1994).

Organ meats, poultry meat, and seafood, are rich sources of nucleoproteins (Kojima, 1974; Clifford and Story, 1976; Barness, 1994). Single cell proteins (SCP) have nucleic acid levels that are seven times higher than that of meat (Ingledew, 1999). Industrially produced bakers and brewers yeast have a nucleic acid content ranging from

39-95 g/kg dry whole yeast (Maloney, 1998). Yeast extract is a rich source of nucleotides (Tibbets, 2002).

Nucleotides are concentrated in the non-protein nitrogen (NPN) portion of milk (Carver and Walker, 1995; Schlimme et al., 2000). In breast milk, approximately 2-5% of the NPN consists of free and cellular nucleotides (Barness, 1994; Carver and Walker, 1995; Lerner and Shamir, 2000), primarily, adenosine 5'monophosphate (AMP) and cytidine 5'monophosphate (CMP). The concentration of nucleotides present in the milk decreases as lactation advances (Skala et al., 1981; Gil and Sanchez-Medina, 1982a; Schlimme et al., 2000). The nucleotide concentration is high in colostrum and milk samples collected during early lactation (Johke, 1963). Thorell et al. (1996) reported that human milk contained 68 ± 55 μmol/L, 84 ± 25 μmol/L, and 10 ± 2 μmol/L, of nucleic acid, nucleotides, and nucleosides, respectively. These values were expressed as nucleotide equivalents. Leach et al. (1995) collectively called them "total potentially available nucleotides" (TPAN) values. Lerner and Shamir (2000) reported that the overall average of TPAN in human milk is 82-402 μmol/L and the overall mean is 189 μmol/L.

Although some experiments have reported that freezing milk may decrease the nucleotide content (Sanchez-Pozo et al., 1986), others have found that the nucleotide content is not affected by thermization (Paubert-Braquet et al., 1992). A multifold increase in cytidine, guanosine, and inosine concentration was observed in raw milk subjected to thermization and pasteurization (Ott and Schlimme, 1991). However, the authors reported that the adenosine concentration decreased in milk exposed to the same conditions. These results were attributed to the catalytic activity of milk enzymes in the

heating-up phase of milk processing (Schlimme et al., 2000). Gil and Sanchez-Medina (1982b) reported that the process of UHT sterilization, HTST pasteurization, and spray drying lowered CMP, AMP, and orotate concentration of milk. They concluded that the decrease in the amount of nucleotides in milk was more influenced by the time of milk exposure at processing temperature compared to the processing temperature itself.

4. Nucleotides in the food industry: Flavors

In Japan, inosine 5'monophosphate or disodium inosinate (IMP), guanosine 5'monophosphate or disodium guanylate (GMP), and monosodium glutamate (MSG), are three flavor enhancers that contribute to the unique and specific taste of Umami (Nagodawithana, 1995). The sensation of boldness and smoothness in soup is attributed to IMP and glutamic acid (Fuke and Konosu, 1991). Disodium guanylate is approximately twice as effective as disodium inosinate in enhancing flavor. However, a synergistic effect is produced when IMP, GMP, and MSG are combined (Ingledew, 1999). It has been demonstrated that L-glutamate binds preferentially to specific taste receptors and that the presence of nucleotides increase the level of L-glutamate interactions at the receptor surface (Nagodawithana, 1995). Palatability enhancement in animals has also been reported upon addition of 0.3% yeast extract (NuPro™) as a source of nucleotides to diets for felines (W. Hendriks, personal communication).

5. Nucleotides in the milk of different species

The nucleotide concentration has been measured in milk from bovine, caprine, equine, human, ovine, and porcine species (**Table 3**). However, it is difficult to compare and contrast these numbers due to a number of variables such as the time of milk collection, sample handling, and method of analysis. The assays used were post column enzymic characterization of the pooled chromatographic fraction (Gil and Sanchez-Medina, 1981; Schneehagen and Schlimme, 1992; Schlimme et al., 2000), ion exchange chromatography (Johke, 1963; Gil and Medina, 1982a; Schlimme et al., 2000), and high performance liquid chromatography (Thorell et al., 1996).

Gil and Medina (1981) reported that the nucleotide content in the colostrum of cow, sheep, and goat was qualitatively similar, but quantitatively distinct. Colostrum from ruminants had high levels of uridine diphosphate hexose (UDP-hexose) and uridine diphosphate sugars (Johke, 1963). The concentration of CMP, GMP, and AMP were high in sheep (Gil and Medina, 1981). In contrast to the sheep and the goat, orotic acid was high in cows' milk and increased with advancing lactation. However, human milk did not contain detectable orotic acid (Gil and Sanchez-Medina, 1982a; Paubert-Braquet et al., 1992; Carver and Walker, 1995).

Gil and Sanchez-Medina (1982a) suggested that variations in the nucleotide content in human milk might also result from genetic differences, while Lerner and Shamir (2000) indicated that a geographical variation in the nucleotide content exists. Qualitative and quantitative species specificity in nucleotide concentration during lactation may be explained by concepts in milk synthesis and the biosynthesis and control

of nucleotide base derivatives in the mammary gland (Larson, 1969; 1976; Chen and Larson, 1971). Larson (1976) suggested that in the cow, enzymes involved in the synthesis of orotic acid are not inhibited, and orotate accumulates in milk due to the inability of the mammary gland to retain orotate. Because orotic acid is an intermediate in the synthesis of pyrimidines, it may accumulate due to an inability of the orotate phosphoribosyl transferase enzyme to convert it beyond that step in the pathway (Chen and Larson, 1971).

Johke (1963) showed that equine milk had a high concentration of uridine compounds and that uridine diphosphate acetylglucosamine (UDPAG), uridine diphosphate acetylgalactosamine (UDPAGal), uridine diphosphate glucose (UDPG), and uridine diphosphate galactose (UDPGal) were dominant. Total purine compounds were less and an unidentified adenosine nucleotide was present, which differed from cyclic AMP (cAMP) of bovine, caprine, and human milk (Johke, 1963). Both human and equine milk have high lactose and low casein content, but equine milk had a higher nucleotide concentration. Human milk and colostrum contained at least 12 nucleotides predominantly, CMP. Smaller amounts of inosine (Barness, 1994; Schlimme et al., 2000), AMP, cAMP, UMP, and UDP-sugars (Johke, 1963; Gil and Medina, 1982a), were also detected. The most abundant nucleotide in colostrum and milk from lactating sows is UMP. It represented 98% of all 5'monophosphate nucleotides in colostrum, and in milk, UMP accounted for 86-90% of all nucleotides, regardless of d of lactation (Mateo et al., 2004).

6. Metabolism

6.1 Nucleotide metabolism in infants

The exact metabolism of nucleic acids ingested by breast fed infants is unknown (Carver and Walker, 1995). Thorell et al. (1996) attempted to evaluate the capability of infants to metabolize nucleic acids and nucleotides. They used 22-week-old fetal small intestines and analyzed them for specific digestive enzymes. Their findings showed that fetal small intestines were able to digest RNA to cytidine, uridine, and uric acid in vivo. They also found that fetal small intestine homogenate increased purine and pyrimidine content when incubated with human milk. However, this result was not seen when homogenates were incubated with infant formula that was devoid of nucleotides. In vivo, digestive enzymes for different substrates (i. e., nucleotides) originate from the brush border epithelium (Markiewicz, 1983; Morley et al., 1987), pancreatic juice (Weickman et al., 1981), and bile (Holdsworth and Coleman, 1975). Sanderson and He (1994) reported that nucleotides enhanced the expression of brush border enzyme activity of sucrase, lactase, and alkaline phosphatase in carcinoma cells during glutamine deprivation. They further suggested that nucleotides may also affect enterocyte differentiation.

Carver and Walker (1995) summarized the overall digestion of nucleic acids and reported that the endonucleases, phosphodiesterases, and nucleoside phosphorylase are involved in the process. Nucleoproteins are degraded into nucleic acids in the intestinal tract by proteolytic enzymes (Carver and Walker, 1995). Endonucleases degrade RNA and DNA into oligonucleotides, phosphodiesterases degrade these oligonucleotides into

free nucleosides, and nucleoside phosphorylases degrade nucleosides into bases and ribose-1-P (**Figure 5**). If these degradation products are not utilized, the purine bases are further degraded into uric acid and the pyrimidine bases are degraded to ß-alanine, ß-aminoisobutyrate, NH_3, and CO_2 (Rudolph, 1994; Carver and Walker, 1995; Thorell et al., 1996).

6.2 Absorption

The upper small intestine has the greatest absorptive capacity (Bronk and Hastewell, 1987). 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 high negatively charged phosphate groups that hinder absorption. Therefore, the nucleoside form is the major vehicle for the entry of purines and pyrimidines into the epithelial cells. However, differences in the efficiency of uptake among nucleosides have been reported with guanosine being the one taken up most rapidly (Sanderson and He, 1994). Nucleoside transport into the enterocyte occurs both by facilitated diffusion and specific Na^+-dependent carrier-mediated mechanisms (Bronk and Hastewell, 1987). Generally, nucleosides are transported across intestinal epithelial cells and then partially metabolized. Over 90% of dietary and endogenous nucleosides and bases are absorbed into the enterocyte (Salati et al., 1984; Uauy, 1989). Metabolites are transported in a form that is available for the salvage pathway of other cell types (Sanderson and He, 1994). The salvage pathway is a series of reactions that result in the re-synthesis of nucleotides

from nucleosides and inorganic phosphates. The nucleosides used in this reaction originate from nucleotide catabolism or from dietary sources.

6.3 Storage

Sanderson and He (1994) reported that nucleotides may be stored in enterocytes available for various physiological functions. They also suggested that nucleotide pools are larger in differentiated (i. e., cancerous) cells than in undifferentiated (i. e., nonmalignant) cells. This would suggest that undifferentiated cells would be more dependent on the dietary supply of nucleotides. Catabolic enzymes such as purine nucleoside phosphorylase (PNP), xanthine oxidase (XO), and uridine kinase (UK), thymidine kinase (TK), etc., predominate anabolic enzymes such as 5-phosphoribosyl-1-pyrophosphate synthetase (PRPP synthetase), amidophosphoribosyl-tranferase (AMPRT), and carbamylphosphate synthetase-II (CPS-II), aspartate transcarbamylase (ATC), etc., for purines and pyrimidines, respectively (Imondi et al., 1969). Therefore, it is presumed that exogenous nucleotides are not nutritionally significant (Carver and Walker, 1995). However, tracer studies in animals indicate that 2 to 5% of dietary nucleotides are incorporated into the small intestinal, liver, and skeletal muscles tissue pools (Saviano and Clifford, 1978). Tissue retention has been reported to be elevated in young animals (Kobota, 1969) and during fasting (Gross and Saviano, 1991). This may be a manifestation of a physiological requirement. Additionally, dietary adenine metabolism is unique compared to other purines in that a greater proportion is absorbed and stored into tissues during the fasted state (Saviano and Clifford, 1978).

6.4 Biosynthesis

De novo purine and pyrimidine nucleotide synthesis is a metabolically costly process requiring a significant amount of energy in the form of adenosine 5'triphosphate (ATP). However, preformed nucleosides and purine and pyrimidine bases can be converted into nucleotides via the salvage pathway (Rudolph, 1994). This pathway may spare energy and allow cells that are incapable of de novo synthesis to maintain their nucleotide pools (i. e., leukocytes, erythrocytes, bone marrow cells, intestinal mucosal cells, and lymphocytes).

The de novo and salvage pathways of both purine and pyrimidine synthesis leads to the production of nucleoside 5'monophosphate or nucleotides (**Figure 6.**) via the utilization of 5-phospho-D-ribosyl-1-pyrophosphate (PRPP) and phosphoribosyltransferase (PRT). The PRPP is generated from a reaction involving the Ribose-5-phosphate and ATP. This reaction requires 2 high-energy phosphate equivalents and is catalyzed by the enzyme PRPP synthetase.

6.5 Purine nucleotide synthesis

The liver is the major site for purine synthesis. The cytosol of the cell contains all the enzymes for purine synthesis and degradation (Carver and Walker, 1995). The synthesis of purine nucleotides starts with PRPP and through a series of steps, leads to the formation of inosine 5'monophosphate (IMP). Hypoxanthine is a purine base without the ribose moiety and is built upon a ribose by the amidotransferase enzymes and transformylation reactions. The synthesis of IMP requires glutamine, glycine, CO_2, aspartate, formate, and six high-energy phosphate groups of ATP (Carver and Walker, 1995). Inosine 5'monophosphate serves as a branch point for the conversion of both

AMP and GMP through two distinct pathways, wherein energy in the form of guanosine 5'triphosphate (GTP) and ATP, is required to form AMP and GMP, respectively.

Rate limiting steps occur during the synthesis of PRPP by PRPP synthetase, and this in turn is feedback inhibited by the AMP and GMP. The amidotransferase reaction that is catalyzed by PRPP amidotransferase is also feedback inhibited by the binding of ATP, adenosine diphosphate (ADP), and AMP at one inhibitory site, and GTP, guanosine diphosphate (GDP), and GMP at another.

6.6 Purine nucleotide catabolism and salvage pathways

Purine nucleotide degradation leads to the production of uric acid from the catabolism of hypoxanthine and xanthine via the xanthine oxidase enzyme (Carver and Walker, 1995). Molecular oxygen is a substrate and H_2O_2 is generated in the process (Carver and Walker, 1995). Uric acid is an insoluble complex formed and is excreted as sodium crystals in the urine. However, re-synthesis of nucleotides from purine bases and nucleosides is possible through a series of reactions in the salvage pathway. The purine bases (i. e., adenine, guanine, and hypoxanthine) are converted back to their corresponding nucleotides via phosphoribosylation. The enzymes involved in the salvage of purines are adenosine phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) that catalyzes reactions for adenine and hypoxanthine or guanine, respectively (Carver and Walker, 1995).

The de novo synthesis of AMP from IMP and the salvage of IMP via AMP catabolism have a net effect of deaminating aspartate to form fumarate. This process is called the purine nucleotide cycle (**Figure 7**), and is important in exercising muscle cells.

Muscle cells replenish TCA-cycle intermediates in the form of fumarate generated by the purine nucleotide cycle.

6.7 Pyrimidine nucleotide synthesis

The formation of pyrimidine bases requires ATP, glutamine, aspartate, and CO_2 (which is derived from carbamoyl phosphate). The enzymes involved in pyrimidine synthesis are located in the cytosol (Carver and Walker, 1995). Carbamoyl phosphate is derived from glutamine and bicarbonate within the cytosol. It is further catalyzed by the CPS-II enzyme, and condenses with aspartate in a reaction catalyzed by aspartate transcarbamoylase (ATCase), the rate-limiting enzyme of pyrimidine nucleotide biosynthesis.

Pyrimidine synthesis differs from purine synthesis in that the ring structure is assembled as a free base and not built upon PRPP (Carver and Walker, 1995). The PRPP is added to orotic acid, forming orotate monophosphate (OMP) that is subsequently decarboxylated to UMP. Four high-energy phosphate groups are utilized for the synthesis of UMP (Carver and Walker, 1995). Also, there is no branch in the pyrimidine synthetic pathway. Uridine 5'monophosphate is phosphorylated twice to yield uridine triphosphate (UTP) by uridylate kinase and nucleoside diphosphate kinase, for the first and second phosphorylations, respectively. The UTP is aminated by the cytidine triphosphate (CTP) synthase enzyme, forming CTP.

Thymine nucleotides are derived from either deoxyuridine monophosphate (dUMP) or deoxyuridine and deoxythymidine via the de novo and salvage pathways,

respectively. The dUMP is converted to deoxythymidine monophosphate (dTMP) by the thymidylate synthase enzyme and the methyl group is derived from tetrahydrofolate.

Regulation occurs at the first step that is catalyzed by the ATCase enzyme. This enzyme is inhibited by CTP and activated by ATP. The ATCase enzyme and the activity of CPS-II, is localized in the cytoplasm and utilizes glutamine as a substrate. Glycine also acts as a competitive inhibitor for glutamine binding sites in ATCase regulation. The CPS-II domain is activated by ATP, and inhibited by UDP, UTP, deoxyuridine triphosphate (dUTP), and CTP. The concentration of ATP also regulates pyrimidine biosynthesis via PRPP formation. An increase in the level of PRPP activates pyrimidine biosynthesis. The regulation of OMP decarboxylase is by competitive inhibition of UMP and CMP. Cytidine 5'triphosphate synthetase is feedback inhibited by CTP and activated by GTP.

6.8 Pyrimidine nucleotide catabolism and salvage pathways

Pyrimidine nucleotide degradation leads to β-alanine for both CMP and UMP, and to β-aminoisobutyrate for dTMP. Other pyrimidine degradation products are NH_3 and CO_2. However, re-synthesis of nucleotides from pyrimidine bases and nucleosides is possible through a series of reactions in the salvage pathway. Uracil can be salvaged to form UMP via two reactions involving uridine phosphorylase and uridine kinase resulting in the formation of uridine and UMP, respectively. Deoxyuridine can also be a substrate for uridine phosphorylase. The formation of dTMP requires thymine phosphorylase and thymidine kinase for the conversion of thymine to thymidine and the conversion of thymidine to dTMP, respectively. The salvage of deoxycytidine is catalyzed by

deoxycytidine kinase, which result in the formation of dCMP. The major function of the pyrimidine nucleoside kinases is to maintain a cellular balance between the level of pyrimidine nucleosides and pyrimidine nucleotides.

7. Physiological roles

Nucleotides act as building blocks of nucleic acids (i. e., DNA and RNA). In all cells, the concentration of RNA is approximately 1,000 times higher than the concentration of DNA. The concentration of RNA is relatively constant, while the concentration of DNA varies with the stage of the cell cycle (Barness, 1994). However, nucleotides also have physiological roles in the body.

7.1 Energy source

Carver and Walker (1995) reported that ATP is the most abundant nucleotide. The active transport of molecules and ions, synthesis of macromolecules, and mechanical work, are dependent on a constant supply of energy in the form of ATP. Adenosine 5'triphosphate is also called an energy carrier or energy transmitter (Voet and Voet, 1995). It also serves as a phosphate donor for nucleotide synthesis and as an allosteric effector in various metabolic pathways (Carver and Walker, 1995). Iwasa et al. (1997) reported that a well-balanced nucleoside-nucleotide mixture improved N metabolism and stimulated the synthesis of high-energy phosphate molecules in rats recovering from severe surgical stress.

7.2 Coenzyme components

A number of nucleotide derivatives play a role in physiological oxidation and reduction reactions such as flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD^+), and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Coenzyme A (CoA) is also a nucleotide derivative, however, it is not involved with redox reactions, but serves as a carrier of acyl groups rather than electrons (Voet and Voet, 1995). All of these nucleotide derivatives are involved with carbohydrate, protein, and fat metabolism.

7.3 Physiological regulators

Regulatory molecules such as cAMP and cyclic guanosine monophosphate (cGMP) are involved in information transmission from extracellular hormones to intracellular enzymes as secondary messengers (Carver and Walker, 1995).

7.4 Activated intermediate carriers

Nucleotides also serve as carriers of activated intermediates for many physiological reactions. Intermediates such as UDP-glucose, CMP-sialic acid, and cytidine diphosphate choline (CDP-choline) are involved in glycogen synthesis, glycoprotein synthesis, and phospholipid metabolism, respectively.

7.5 Protein synthesis

Iwasa et al. (1997) reported that after 70% hepatectomy in rats, whole body protein turnover increased in rats receiving total parenteral nutrition (TPN) with nucleotide supplementation compared to rats receiving normal unsupplemented TPN. Yamauchi et al. (1998) concluded that intraperitoneal administration of nucleoside-

nucleotide mixture increased small intestine RNA content during periods of protein deficiency in combination with infection in mice.

RNA synthesis occurs continuously during the cell cycle (Carver and Walker, 1995). RNA molecules are present in three different forms namely, messenger RNA (m-RNA), ribosomal-RNA (r-RNA), and transfer-RNA (t-RNA). Messenger RNA carries information from DNA to ribosomes, where proteins are synthesized. They account for only 3% of the total cellular RNA. Ribosomal RNA accounts for 80% of total cellular RNA, and is an important component of ribosomes. Transfer RNA carry activated AA to ribosomes for incorporation into peptide chains. Small RNA molecules are present in all cells and have catalytic activities in association with proteins.

7.6 Cell mitosis

Phases of the cell cycle include mitosis (M), gap1 (G_1), synthesis (S), and gap2 (G_2). Carver and Walker (1995) reported that DNA replication occurs during the S phase. During this time, enzyme activity for purine and pyrimidine synthesis, and nucleotide interconversion is increased (Carver and Walker, 1995). The activity of thymidine kinase, an enzyme responsible for nucleotide interconversion, is unique in that it fluctuates with the cell cycle. Its peak activity is attained during the phase of DNA synthesis. Cory (1992) and Tsujinaka et al. (1999) suggested that rapidly dividing tissues are geared toward DNA replication and RNA synthesis. Memory-deficient senescence-accelerated mice and mice with dementia showed improved memory with dietary nucleoside and nucleotide supplementation (Yamamoto et al., 1997).

7.7 Lipid metabolism

Dietary nucleotides have been associated with increases in omega-3 and omega-6 polyunsaturated fatty acids in lipid components of plasma and erythrocyte membranes in neonatal animals (Gil et al., 1985) and newborn infants (Delucci et al., 1987). Gil et al. (1985) speculated that dietary nucleotides may influence the synthesis of polyunsaturated fatty acids and the activity of desaturase in hepatocytes, erythrocytes, and enterocytes during the neonatal period. Carver and Walker (1995) suggested that dietary nucleotides play a role in the conversion of 18 carbon essential fatty acids to 20 and 22 carbon long chain polyunsaturated fatty acids by facilitating the increase in length of the carbon chain. However, in a previous study by Carver (1994), it was reported that restricting dietary nucleotides resulted in lipid accumulation. Nucleotide enriched diets fed to growing rats (Nishizawa et al., 1996) and infants (Sanchez-Poza et al., 1985) increased plasma HDL cholesterol and decreased LDL cholesterol. This suggests that nucleotides may have a physiological effect on lipoprotein metabolism during the neonatal period.

7.8 Hematology

A study conducted by Yamamoto et al. (1997) reported that nucleosides and nucleotides stimulate hematopoeisis. It was also concluded that in neonatal rats, dietary nucleotides increased erythrocyte 2,3 diphosphoglycerate concentration (Scopesi et al., 1999), thereby increasing the affinity of oxygen for hemoglobin. Nucleotide supplementation accelerated bone marrow and peripheral neutrophil proliferation in mice infected with methicillin resistant Staphylococcus aureus (Matsumoto et al., 1995) and in mice treated with cyclophosphoamide (Yamamoto et al., 1997). Yamauchi et al. (1998)

concluded that intraperitoneal administration of a nucleoside-nucleotide mixture stimulated bone marrow cell proliferation and increased DNA content during periods of relative protein deficiency in combination with infection in mice. Intravenous administration of adenosine was found to be a potent vasodilator (Berne et al., 1983) and had significant effects in neuronal, cardiac, and vascular tissues (Mosqueda-Garcia, 1992).

7.9 Gastrointestinal morphology

Uauy et al. (1990) and Carver (1994) reported that dietary nucleosides enhanced the growth and maturation of intestinal epithelial cells in weanling rats. This was demonstrated by an increased formation of mucosal protein, increased concentration of DNA, and taller villi in the small intestine. The authors reported that the maltase and lactase ratio was also increased with nucleoside-supplementation, hence, increased gut maturation. Parenteral supplementation of nucleic acids in rats supported mucosal cell proliferation and function (Tsujinaka et al., 1999). This was demonstrated by increased wet weight, protein and DNA contents, villous height, but not the crypt depth, and narrower tight junctions of jejunal mucosa width measurements. Kishibuchi et al. (1997) reported that nucleoside-nucleotide mixtures contribute to the improvement of gut mucosal barrier function, demonstrated by narrower intracellular spaces of mucosal cells, and a lack of increased intestinal cathepsin activity in the ileum. Nucleotide supplementation promoted rapid recovery of small intestine atrophy after food deprivation (Ortega et al., 1994), and diarrhea (Bueno et al., 1993) in rats. In humans, nucleotides enhance the expression of brush border enzymes in carcinoma cells when

stressed by glutamine deprivation (Sanderson and He, 1994). Under similar conditions, enzyme activity and enterocyte differentiation were enhanced by adding nucleotides to the culture medium.

7.10 Immunity

Dietary nucleotide supplementation has been associated with both humoral and cellular immunity, but the exact mechanism of action has not been elucidated. Kulkarni et al. (1994) suggested that 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 during transition to the S phase. Additionally, nucleotide deprivation caused a decrease in phagocytic activity, lymphokine production, and inhibits lymphocyte maturation (Paubert-Braquet et al., 1992). Dietary nucleotides contribute to the circulating pool of nucleosides (**Figure 6**) available to stimulate leukocyte production, which rapidly turn over, and thus have an increased nucleotide requirement (Kulkarni et al., 1994; Carver and Walker, 1995). These studies suggest that there may be a need for nucleotides in response to immunological challenges.

Dietary factors play a role in the antibody response of infants to immunization. In humans, infant formula fortified with nucleotides enhanced *H influenza* type b (Hib) and diphtheria humoral antibody responses (Fanslow et al., 1988; Pickering et al., 1998).

Polynucleotides significantly increased *in vitro* antibody production in response to T-cell antigens in mice (Jyonouchi et al., 1993; Jyonouchi, 1994). Navarro et al. (1996) reported that nucleotide-free diets supplemented with single nucleotides, AMP, GMP, or UMP, increased the immunoglobulin G (IgG) and Concanavalin A (Con A) driven

proliferative responses, whereas, CMP and IMP had no effect. The authors also reported that GMP was the only nucleotide that increased the hemolytic immunoglobulin M (IgM) -forming cell response. The effect of dietary nucleotides on delayed type hypersensitivity to sheep red blood cells (SRBC) was tested in mice (Rudolph et al., 1984). They reported a decrease in sensitivity of mice maintained on the nucleotide-free diet, which was consistent with a decrease in T-cell function and prolonged allograft survival. The effect of dietary nucleotide restriction in mice was also tested using two forms of stress: starvation and protein malnutrition (Pizzini et al., 1990). The authors reported that animals previously maintained on the nucleotide free diet supplemented with RNA, showed a significant increase in spontaneous Con A and phytohemagglutinin-stimulated blastogenesis. This was observed with a restoration of BW and popliteal lymph node immune reactivity.

Colonic myeloperoxidase (MPO) activity and interleukin-1 β concentration in rectal dialysate, increased during induced colitis in rats supplemented with nucleotides (Sukumar et al., 1999). This suggested that nucleotide supplementation ameliorates the severity of induced colitis in rats. Polynucleotide supplementation restored impaired interferon δ, interleukins 2, 4, and 5 production, and mRNA expression, in mice fed a nucleotide free diet (Jyonouchi et al., 1996). The restriction of dietary nucleotides in mice resulted in a decreased interlukin-2 production, natural killer cell cytotoxicity, and macrophage activation compared to those groups of animals fed nucleotide-supplemented diets (Carver, 1994).

Kulkarni et al. (1986) explored the effect of dietary nucleotides in response to a Staphylococcus aureus bacterial challenge. By 120-h post inoculation, all mice fed a nucleotide free diet and mice fed diets supplemented with adenine died. However, mortality was only 56% in mice fed diets supplemented with 0.25% RNA and in mice fed diets supplemented with uracil. Hence, dietary restriction increased mortality from staphylococcal sepsis and specific dietary nucleotide supplementation appeared to decrease bacterial challenge. Nucleotide supplementation increased bacterial resistance of mice inoculated with Candida albicans (Fanslow et al., 1988) and Staphylococcus aureus (Carver, 1994). Intraperitoneal administration of nucleotides and nucleosides decreased bacterial translocation, number of colony forming units, and increased survival against methicillin-resistant Staphylococcus aureus (Yamamoto et al., 1997).

In vitro immunity studies showed that nucleotides and nucleosides, increased delayed type cutaneous hypersensitivity, popliteal lymph node blastogenic response to antigens, allogens, and mitogens, and reduced endotoxin-induced bacterial translocation (Yamamoto et al., 1997). Kulkarni et al. (1984) examined the effect of a nucleotide free diet on immune function of mouse syngeneic bone marrow radiation chimeras. The graft-host disease mortality assay revealed a reduction in spleen cell activity from radiation chimeras, spleen cell proliferative response to phytohemagglutinin, and response to pokeweed mitogens in mice fed nucleotide-free diets. Additionally, the response to bacterial lipopolysaccharides remained virtually unaffected as compared with radiation chimeras in mice fed the control diet.

7.11 Hepatic tissue

Under normal physiological conditions, dietary nucleotides are required nutrients for the liver. An inadequate supply of nucleotides for a certain period of time may have transient negative effects on liver ultrastructure and function. Lopez et al. (1996) found that deprivation of dietary nucleotides in rats resulted in a reduction in hepatocyte nuclear and nucleolar areas, and nuclear chromatin condensation. In addition, the rough endoplasmic reticulum, ribosome association and abundance were also reduced, whereas, fat accumulated. In another study, the authors demonstrated that a 10-d dietary nucleotide deprivation caused an effect on liver RNA pool similar to the effect of starvation (Lopez et al., 1997). This indicated that dietary nucleotides may contribute to the liver RNA pool. However, this effect was only observed in young and adult rats, but not in old rats.

During a folate and methyl deficiency, there is an increase in dUMP/ deoxythymidine triphosphate (dTTP) ratio, reduced mitotic index (MI), and an increased ratio of proliferating cell index (PCI) to mitotic cells. All of which are indications of a delayed S-phase in mitotic cells. However, the addition of yeast RNA to folate, methyl, choline, and Met deficient hepatocytes, reversed these effects by approximately 50% (Jackson et al., 1997). A decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamic acid oxalacetic transaminase (GOT), and glutamic acid pyruvic transaminase (GPT) concentrations was observed in serum of rats fed nucleotide-supplemented diets. These rats also had faster recovery from hepatic injury compared to rats given standard nutrition (Ogoshi et al., 1988). When given parenterally, nucleotides promoted recovery from injuries caused by hepatotoxic agents in infants (Uauy, 1994).

8. Dietary supplementation

8.1 Humans

Infants fed nucleotide-supplemented formulas have accelerated body growth and neurological development, and favorable intestinal microflora associated with a lower rate of diarrhea (Yu, 1998). It was suggested that dietary nucleotides enhance intestinal absorption of Fe, affect lipoprotein and long chain polyunsaturated fatty acid metabolism, alter intestinal flora, have trophic effects on the intestinal mucosa and liver, reduce the incidence of diarrhea, and enhance growth in infants born small for normal gestational age (Cosgrove, 1998; Schlimme et al., 2000). Infants who received nucleotide supplementation in their formula, had less diarrhea during their first year of life, better vaccination responses, and produced more antibodies than those that did not receive nucleotides supplementation (Nucleotides, 2000). Carver et al. (1991) reported that in two-month-old infants, natural killer cell percent cytotoxicity and interlukin-2 production was higher in breast-fed and milk formula groups supplemented with 33 mg of nucleotides per liter compared with the standard unsupplemented milk formula group. It was demonstrated that fecal flora of infants fed a nucleotide-supplemented commercial formula had a predominance of bifidobacteria, which was similar to that seen in fecal flora of breast-fed infants (Tanaka and Mutai, 1980). In contrast, enterobacteria predominated in fecal flora of infants fed a commercial formula without nucleotide supplementation (Uauy, 1994).

Clinical studies in human patients were conducted to compare a new Arg, omega-3-fatty acids, and nucleotide-containing diet (Impact™, Sandoz Nutrition Berne,

Switzerland) to a standard high protein enteral food (Van-Buren et al., 1994). The authors found that in two separate double blind clinical studies, patients fed enteral diets containing nucleotides had improved immune function compared to patients receiving a nucleotide free diet. Infectious complications and length of hospital stay were also reduced in postoperative cancer patients fed Impact™ compared to the control group. In a more recent study, the influence of Impact™ on the incidence of systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) in patients after severe trauma was investigated (Weimann et al., 1998). The authors reported that their study supported the beneficial effect of an Arg, omega-3-fatty acids, and nucleotide supplemented enteral diet in critically ill patients.

8.2 Ruminants

Oliver et al. (2002) reported that dietary nucleotides from yeast extract in milk replacers, enhanced gut health and improved immune status, during the first five weeks of life. This was demonstrated by high fecal scores and an increased serum IgG concentration during the first week of life compared to the nonsupplemented milk replacer group. They conducted another experiment and reported that dietary supplementation of purified nucleotides at five times the level normally found in cow milk to milk replacers of newborn bull calves challenged with lipopolysaccharides (LPS) at 3 to 4 weeks of age, tended to have higher mean IgG levels compared to the unsupplemented milk replacer group. The authors concluded that dietary nucleotides do not affect metabolic status, but may enhance immunity in neonatal calves (Oliver et al., 2003).

8.3 Poultry

Broiler birds fed starter diets supplemented with yeast extract as a source of nucleotides, had better feed conversion during the first week of life compared to broiler birds fed starter diets not supplemented with yeast extract (E. Moran and B. Dozier, unpublished data). The authors concluded that yeast extract is an effective nutrient source during the first week of life. However, Tipa (2002) showed that broiler birds fed diets containing yeast extract as a source of nucleotides, had higher average live body weight, body weight gain, and better feed conversion from the first to the fourth week of life. A selective immune response was observed in the immune system of chickens fed diets with yeast extract supplementation as a source of nucleotides (M. A. Qureshi, unpublished data). This was demonstrated by an improvement in mononuclear phagocytic system function (i. e., macrophages), growth performance, and bursa and spleen development. However, there was no improvement in antibody response or in cell-mediated immunity.

8.4 Shrimp

Nucleotides or their precursors (i. e., purine and pyrimidine bases) are required in well-defined amounts in normal physiological conditions. However, the amount required for one base or another is dependent on the RNA and DNA composition of the cell to be built and on the efficiency of de novo synthesis and salvage pathways in shrimp tissues (Devresse, 2000). Nucleotides have been considered as “semi-essential” nutrients, and may become critical when the animal is under stress such as in sickness, injury, during infancy, and during growth spurts. Nucleotides may be a potential solution to reduce the

effects of devastating viral diseases in farmed shrimp (S. F. Dominy, personal communication).

Shrimp inoculated with a “white spot” virus and fed yeast extract at 10 kg/ ton of feed as a source of nucleotides, had better harvest weight, survival rate, production, and feed conversion rate compared to the control diet (S. Mendoza et al., unpublished data; Tibbets, 2002). The combination of yeast extract as a source of nucleotides, and a supplement known to modulate the immune response, produced 100 additional pounds of shrimp per hectare, improved survival rate by 5%, and had better feed conversion rates compared to the control diet (A. Solis, unpublished data).

8.5 Pigs

Bustamante et al. (1994) found that a mixture of nucleotides similar to those in human milk, exert a protective effect in the intestinal lumen of piglets against an inflammatory response to ischemia-reperfusion. However, the protective effects seen in the intestinal lumen were not due to nucleotides alone. Synthetic β-carotene and nucleotide addition increased lymphocyte stimulation to phytohaemagglutinin and Con-A in weanling piglets by 50 and 30%, respectively (Zomborsky-Kovacs et al., 1998). Piglets fed yeast RNA for 2 to 4 weeks improved lymphocyte function as evidence by their increased T-cell-mediated DTH responses to KLH, and in vitro proliferative responses to a non-specific T-cell mitogen (Cameron et al., 2001). Pigs fed diets supplemented with yeast extract as a source of nucleotides, performed similarly with the pigs fed diets with spray dried plasma protein in terms of ADG, ADFI or feed efficiency during phase-1 and -2 post-weaning (D. C. Mahan, unpublished data). Feeding nucleotide-rich yeast extract

protein improved gut health, growth rate of weanling pigs, and provided long-term improvement in growth rate of growing and finishing pigs comparable to that of feeding spray dried plasma protein (M. S. Carlson et al., unpublished data). Pigs with *E. coli* infection fed diets supplemented with yeast extract as a source of nucleotides at 4% (Maribo, 2003) and at 2.5% (P. Spring, unpublished data) improved weight gain, reduced diarrhea, and improved feed conversion compared to pigs fed the control diet. After stress induced by transport and slaughter, growing pigs fed a nucleotide mixture (2.1%) during the last 30 days of fattening, had lower serum creatine kinase, lactate dehydrogenase, and AST concentrations compared to animals fed a standard diet (M. Zomborsky, unpublished data).

9. Implication for swine nutrition

Research in human nutrition has been conducted to investigate the potential of nucleotide supplementation. Animal studies are currently being conducted to validate the numerous benefits that have been reported. The most significant finding attributed to nucleotide supplementation is its effect in modulating the immune system. Presently, an increasing number of antibiotics are being banned for use in the livestock industry. Because this is so, nucleotide supplementation may ultimately fall under the umbrella of non-antibiotic medication programs. This would give nutritionists and veterinarians an alternative to using antibiotics in animal feed and address the concerns of performance and disease challenge. Because of the nature of its metabolism, supplementation of

nucleotides would primarily benefit young pigs, but does not exclude potential benefits in adult animals.

10. Conclusion and perspectives

Nucleotides are molecules with considerable structural diversity and composed of a nitrogenous base linked to a sugar to which at least one phosphate group is attached. Feed or food ingredients containing cellular elements are potential sources of nucleotides. Nucleotide supplementation is reported to be involved in many important physiological roles in the body, and have effects upon hematology, gastrointestinal and hepatic tissue development, lipid metabolism, and immunity. In contrast to adult humans, the exact metabolism of nucleic acids ingested by infants is unknown. However, in adults, protease, endonuclease, phosphodiesterase, and nucleoside phosphorylase are the main enzymes involved in the process of breaking down dietary nucleoproteins to purine and pyrimidine bases. Synthesizing nucleotides de novo is metabolically costly compared to synthesis via the salvage pathway. Because catabolic enzymes predominate over anabolic enzymes for purines and pyrimidines, it has been suggested that exogenous nucleotides are not nutritionally significant. However, during periods of rapid growth and development, disease challenges, injury or stress, dietary nucleotide supplementation may be beneficial.

Literature cited

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

Berne, R. M., R. M. Knabb, S. W. Ely, and R. Rubio. 1983. Adenosine in the local regulation of blood flow: a brief overview. Federation Proc. 42:3136-3142.

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.

Bueno, J., A. Torres, A. Almendros, R. Carmona, M. C. Nunez, and A. Gil. 1994. Effect of dietary nucleotides on small intestinal repair after diarrhea. Histological and ultrastructural changes. Gut 35:926-933.

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.

Carlson, M. S., T. L. Veum, J. R. Turk, D. W. Bollinger, and G. W. Tibbets. 2001. A comparison between feeding either peptide or plasma proteins with or without a feed grade antibiotic on growth performance and intestinal health. University of Missouri research data. (Unpublished).

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. 12:144-148.

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

Chen M. H., and B. L. Larson. 1971. Pyrimidine synthesis pathway enzymes and orotic acid in bovine mammary tissue. J. Dairy Sci. 54:842-846.

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.

De Lucchi C., M. L. Pita, N. J. Faus, J. A. Molina, R. Uauy, and A. Gil. 1987. Effect of dietary nucleotides on fatty acid composition of erythrocyte membrane lipids in term infants. J. Pediatr. Gastroenterol. Nutri. 6:568-574.

Devresse, B. 2000. A forgotten but key nutrient for the immune system: Nucleotides. A review and current advances. Available: **http://bioxianleid.8u8.com/y365/3/untitled111.htm.** Accessed Jun. 16, 2003.

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

Fuke, S., and S. Konosu. 1991. Taste active components in some foods: a review of Japanese research. Physiol. Behav. 49:863-868.

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.

Gil, A., and F. Sanchez-Medina. 1982b. Effects of thermal industrial processing on acid soluble nucleotides of milk. J. Dairy Res. 49:295-300.

Gil, A., M. L. Pita, J. Martinez, J. A. Molina, and F. Sanchez-Medina. 1985. Effect of dietary nucleotides on the plasma fatty acids in at-term neonates. Hum. Nutr. Clin. Nutr. 40:185-195.

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.

Imondi, A. R., M. E. Balis, and M. Lipkin. 1969. Changes in enzyme levels accompanying differentiation of intestinal epithelial cells. Exp. Cell Res. 58:323-330.

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.

Jackson, C. D., C. Weis, B. J. Miller, and S. J. James. 1997. Dietary nucleotides: effects on cell proliferation following partial hepatectomy in rats fed NIH-31, AIN-76A, or folate/methyl- deficient diets. J. Nutri. 127:834-837.

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.

Jyonouchi, H., S. Sun, and S. Sato. 1996. Nucleotide-free diet suppresses antigen-driven cytokine production by primed T-cell: effects of supplemental nucleotides and fatty acids. Nutr. 12:608-615.

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, S. S., D. C. Bhateley, A. R. Zander, C. T. Van Buren, F. B. Rudolph, K. A. Dicke, and A. D. Kulkarni. 1984. Functional impairment of T-lymphocytes in mouse radiation chimeras by a nucleotide-free diet. Exp. Hematol. 12:694-699.

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.

Larson, B. L., 1969. Biosynthesis of milk. J. Dairy Sci. 52:737-747.

Larson, B. L., 1976. Comparative production of β-lactoglobulin and orotic acid with lactose in bovine mammary cell cultures: effects of cell density and constituent inhibition. J. Dairy Sci. 59:1881-1889.

Lassalas, B., J. P. Jouany, and L. Broudiscou. 1993. Dosage des bases puriques et pyrimidiques pcr chromatographie liqide a haunte performance. Annales de Zootechnie. 42:170-171.

Leach, J. L., J. H. Baxter, B. E. Molitor, M. B. Ramstack, and M. L. Masor. 1995. Total potentially available nucleosides of human milk by stage of lactation. Am. J. Clin. Nutr. 61:1224-1230.

Lerner, A., and R. Shamir. 2000. Nucleotides in infant nutrition: a must or an option. Israel Medical Association Journal 2:772-774.

Lopez, N. A. T., J. D. Bueno, A. Gil, and A. P. Sanchez. 1996. Morphological changes in hepatocytes of rats deprived of dietary nucleotides. British J. Nutr. 76:579-589.

Lopez, N. A., A. Gil, and A. P. Sanchez. 1997. Age related effect of dietary nucleotides on liver nucleic acid content in rats. Annal. Nutr. Metab. 41:324-330.

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., D. N. Peters, and H. H. Stein. 2004. Nucleotides in sow colostrum and milk at different stages of lactation. J. Anim. Sci. 82:1339-1342.

Matsumoto, Y., A. A. Adje, K. Yamauchi, M. Kise, Y. Nakasone, Y. Shinegawa, H. Yokoyama, and S. Yamamoto. 1995. Mixture of nucleosides and nucleotides increases bone marrow cell and peripheral neutrophil number in mice infected with methicillin- resistant Staphylococcus aureus. Biochemical and molecular roles of nutrients. J. Nutr. 125:815-822.

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.

Mosqueda-Garcia, R. 1992. Adenosine as a therapeutic agent. Clin. Invest. Med. 15:445-455.

Nagodawithana, T. W. 1995. Flavor enhancers. Pages 8-10 in Savory Flavors. Esteekay Associates, Inc., ed. Milwaukee, WI.

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.

Nishizawa N., Y. Harada, and M. Fujimoto. 1996. Effect of dietary nucleotides on cholesterol metabolism in mice. Page 15 in Proc. 70th Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry. Tokyo, Japan. (Abstr.)

Nucleotides in Formula. Ask Dr. Nathan. 2000. Available: **http://www.babyzone.com/drnathan/ask/nucleotides.htm**. Accessed Jun. 19, 2002.

Ogoshi, S., M. Iwasa, T. Yonezawa, and T. Tamiya. 1985. Effect of nucleotide and nucleoside mixture on rats given total parenteral nutrition after 70% hepatectomy. J. Paranter. Enter. Nutr. 9:339-342.

Ogoshi, S., M. Iwasa, S. Kitagawa, Y. Ohmori, S. Mizobuchi, Y. Iwasa, and T. Tamiya. 1988. Effects of total parenteral nutrition with nucleotide and nucleoside mixture on D-galactosamine-induced liver injury in rats. J. Paranter. Enter. Nutr. 12:53-57.

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. 16:5. (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. Dairy Sci. (accepted Abstr.)

Ortega, M. M., M. C. Nunez, A. Gil, and A. Sanchez-Pozo. 1994. Dietary nucleotides accelerate intestinal recovery after food deprivation in old rats. J. Nutr. 124(Suppl.):1413-1418.

Ott, F. G., and E. Schlimme. 1991. Thermisch induzierte bildung von N6-methyladenosin in milch. Kieler Milchwirtschaftliche Forschunngsberichte 43:213-217.

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.

Pizzini, R. P., S. Kumar, A. D. Kulkarni, F. B. Rudolph, and C. T. Van Buren. 1990. Dietary nucleotides reverse malnutrition and starvation-induced immunosuppression. Arch. Surg. 125:86-90.

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

Rudolph, F. B., A. N. Kulkarni, V. B. Schandle, and C. T. Van Buren. 1984. Involvement of dietary nucleotides in T lymphocyte function. Adv. Exp. Med. Biol. 165:175-178.

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. Nutri.114:753-760.

Sanchez-Pozo, A., M. L. Pita, A. Martinez, J. A. Molina, R. Sanchez-Medina, and A. Gil. 1985. Effect of dietary nucleotides upon lipoprotein pattern of newborn infants. Nutri. Res. 6:53-57.

Sanchez-Pozo, A., J. Lopez, M. L. Pita, A. Izquierdo, E. Guerrero, F. Sanchez-Medina, A. M. Valverde, and A. Gil. 1986. Changes in protein fractions of human milk. Ann. Nutri. Metab. 30:15-20.

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

Saviano, D. A., and A. J. Clifford. 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.

Schneehagen E., and E. Schlimme. 1992. Ribonucleoside: Minore Inhaltsstoffe der Humanmilch. Kieler Milchwirtschaftliche Forschungsberichte 44:67-74.

Scopesi, F., C. M. Verketse, D. Paola, D. Gazzalo, M. A. Pronzato, P. L. Bruschettini, and U. M. Marinari. 1999. Dietary nucleotide supplementation raises erythrocyte 2,3-diphosphoglycerate concentration in neonatal rats. J. Nutr. 129:662-665.

Skala, J. P., O. Koldovsky, and P. Hahn. 1981. Cyclic nucleotides in breast milk. Am. J. Clin. Nutr. 34:343-350.

Sukumar P., A. Loo, R. Adolphe, J. Nandi, A. Oler, and R. A. Levine. 1999. Dietary nucleotides augment dextran sulfate sodium-induced distal colitis in rats. J. Nutr. 129:1377-1381.

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

Thorell, L., L. B. Sjoberg, 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.

Tipa, C. 2002. Production performance of broilers fed yeast extract as partial or total replacement for fishmeal. D.V.M. Thesis, Univ. of the Philippines at Los Baños, Laguna.

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

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.

Van-Buren, C. T., A. D. Kulkarni, and F. B. Rudolph. 1994. The role of nucleotides in adult nutrition. J. Nutr. 124:160-164.

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.

Weimann, A., L. Bastian, W. E. Bischoff, M. Grotz, M. Hansel, J. Lotz, C. Trautwein, G. Tusch, H. J. Schlitt, and G. Regel. 1998. The influence of arginine, omega-3-fatty acids and nucleotide-supplemented enteral support on systemic inflammatory response syndrome and multiple organ failure in patients after severe trauma. Nutr. 14:165-172.

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. Nutri. 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, and G. Tornyos. 1998. The effect of β-carotene and nucleotide base supplementation on haematological, biochemical and certain immunological parameters in weaned pigs. J. Anim. and Feed Sci. 7:245-251.

Table 2.1. Nucleotide base concentrations in different protein sources (mg/g). Adapted from Lassalas et al. (1993)

Ingredients	Nucleotide base					Total bases
	Adenine	Cytosine	Guanine	Uracil	Thymine	
Complete fishmeal	1.66	1.21	9.43	1.24	0.53	14.07
Fish solubles	1.85	1.05	23.31	1.24	0.83	28.28
Press cake fishmeal	1.02	0.84	1.28	0.81	0.24	4.19
Single cell protein	1.73	1.00	8.75	1.73	3.72	16.93
Yeast	2.77	1.75	2.54	1.91	0.24	9.21
Yeast extract	7.32	1.35	6.24	7.33	0.33	22.57

Table 2.2. Purine concentration of different food sources (mg/g). Adapted from Clifford and Story (1976)

Item	Adenine	Guanine	Hypoxanthine	Xanthine	Total purines	RNA	Protein (%)
Organ meats							
Beef liver	6.20	0.74	0.61	0.00	1.97	2.68	20
Beef kidney	4.20	0.47	0.63	0.61	1.23	1.34	18
Beef heart	1.50	0.16	0.38	1.02	1.71	0.49	19
Beef brain	1.20	0.12	0.26	1.12	1.62	0.61	11
Pork liver	5.90	0.77	0.71	0.82	2.89	2.59	22
Chicken liver	7.20	0.78	0.71	0.22	2.43	4.02	20
Chicken heart	3.20	0.41	0.12	1.38	2.23	1.87	18
Fresh seafood							
Anchovies	0.80	1.85	0.06	2.12	4.11	3.41	20
Clams	1.40	0.24	0.12	0.86	1.36	0.85	17
Mackerel	1.10	0.26	0.05	1.52	1.94	2.03	23
Salmon	2.60	0.80	0.11	1.33	2.50	2.89	23
Sardines	0.60	1.18	0.06	2.15	3.45	3.43	23
Squid	1.80	0.15	0.24	0.78	1.35	1.00	15

Dried legumes							
Blackeye peas	10.40	0.82	0.20	0.16	2.22	3.06	22
Garbanzo bean	1.70	0.14	0.18	0.07	0.56	3.56	21
Lentils	5.40	0.51	0.15	0.42	1.62	1.40	28
Pinto beans	4.60	0.39	0.25	0.34	1.44	4.85	20
Split peas	8.80	0.74	0.11	0.22	1.95	1.73	21

Table 2.3. Concentration of adenosine 5'monophosphate (AMP), guanosine 5'monophosphate (GMP), inosine 5'monophosphate (IMP), cytidine 5'monophosphate (CMP), and uridine 5'monophosphate (UMP) in human (H), bovine (B), caprine (C), ovine (O), equine (E), and porcine (P) milk during lactation (μmoles/ 100ml) [a, b]

Nucleotide	Milk	Day of lactation					
		5-7	8	10-11	14-15	21	28-31
AMP	H	2.24[e]	-	-	2.60[e]	-	2.02[e]
	B	3.15[d]	1.80[c]	-	2.91[d]	1.81[d]	-
	C	11.00[d]	6.30[d]	12.20[c]	2.79[d]	-	4.07[d]
	O	-	15.67[d]	-	11.87[d]	-	8.47[d]
	E	-	-	0.50[c]	-	-	-
	P	12.80[f]	-	-	6.80[f]	4.30[f]	3.00[f]
CMP	H	3.10[e]	-	-	2.64[e]	-	1.87[e]
	B	3.02[d]	6.20[c]	-	4.90[d]	4.12[d]	-
	C	8.07[d]	5.86[d]	-	2.28[d]	-	3.55[d]
	O	-	23.30[d]	-	7.17[d]	-	8.70[d]
	E	-	-	1.50[c]	-	-	-
	P	7.10[f]	-	-	3.50[f]	2.30[f]	2.50[f]

GMP	H	0.50^{e}	-	-	-	-	0.32^{e}
	B	0.83^{d}	-	-	-	-	
	C	-	-	1.70^{c}	0.99^{d}	-	0.70^{c}
	O	-	1.50^{d}	-	-	-	-
	E	-	-	-	-	-	-
	P	14.00^{f}	-	-	10.20^{f}	6.00^{f}	7.10^{f}
IMP	H	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
	O	-	-	-	-	-	-
	E	-	-	-	-	-	-
	P	2.60^{f}	-	-	1.40^{f}	0.90^{f}	0.40^{f}
UMP	H	-	-	-	-	-	-
	B	2.87^{d}	1.30^{d}	-	-	-	-
	C	12.37^{d}	12.59^{d}	5.90^{c}	16.08^{d}	-	12.64^{d}
	O	-	65.16^{d}	-	20.07^{d}	-	26.08^{d}
	E	-	-	7.70^{c}		-	-
	P	263.10^{f}	-	-	144.00^{f}	122.80^{f}	104.00^{f}

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

[b] The analyses of nucleotides conducted were either 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. (2004).

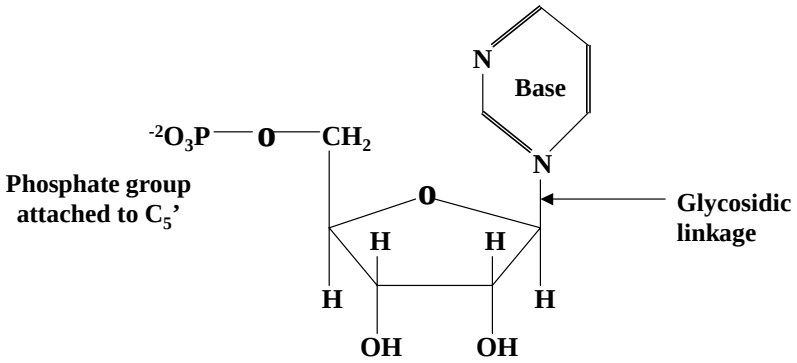


Figure 2.1. Structure of nucleotides

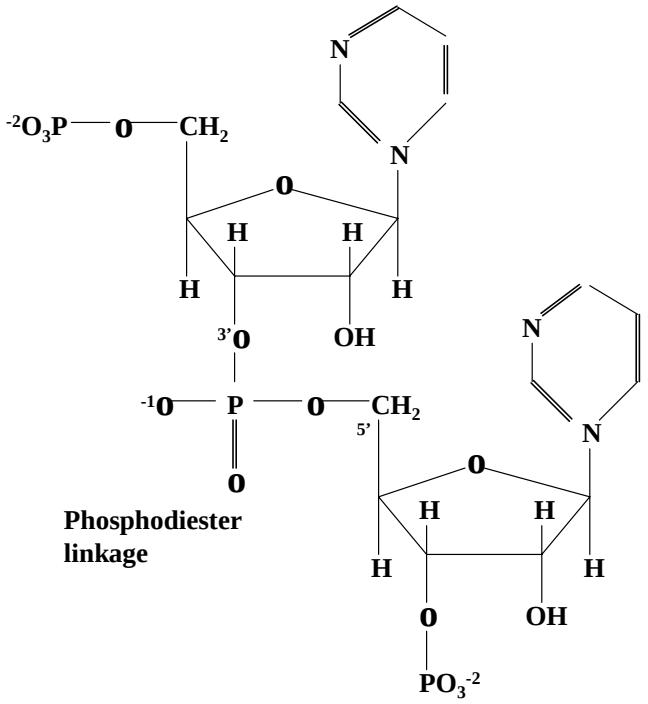


Figure 2.2. Phosphodiester linkage of a nucleic acid

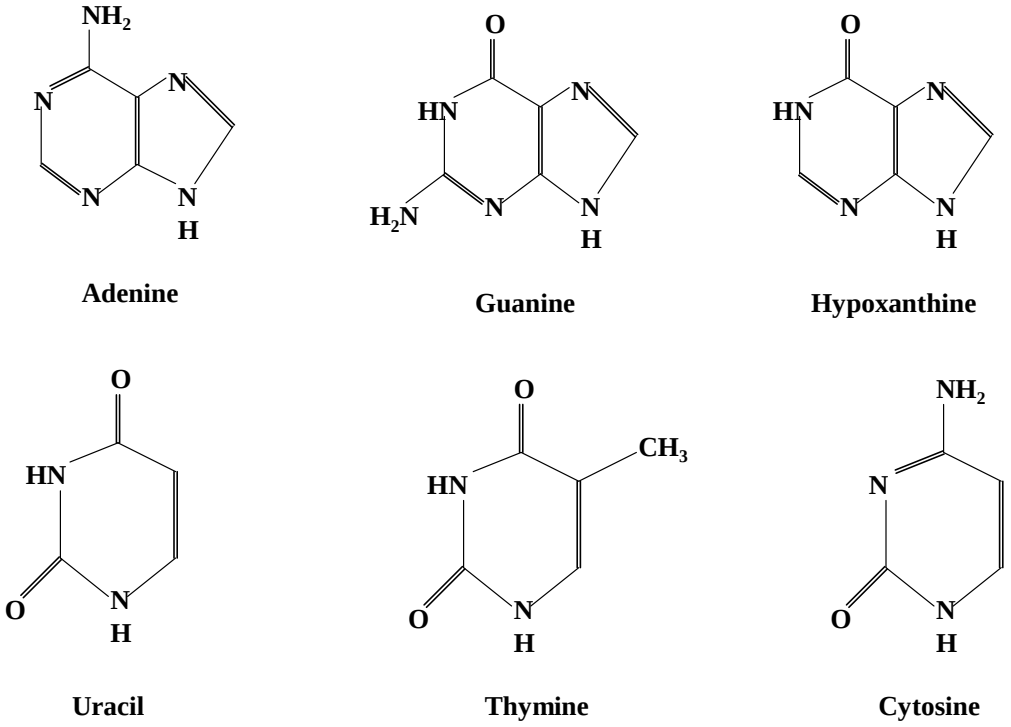
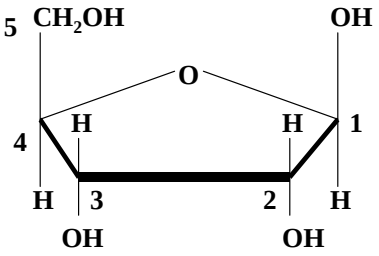
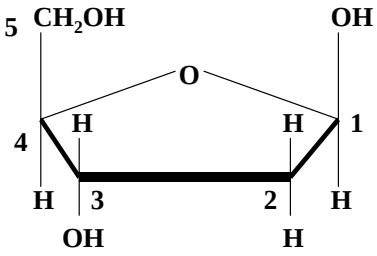


Figure 2.3. Purine and pyrimidine bases of nucleotides



ribose



2’-deoxyribose

Figure 2.4. Pentose sugars

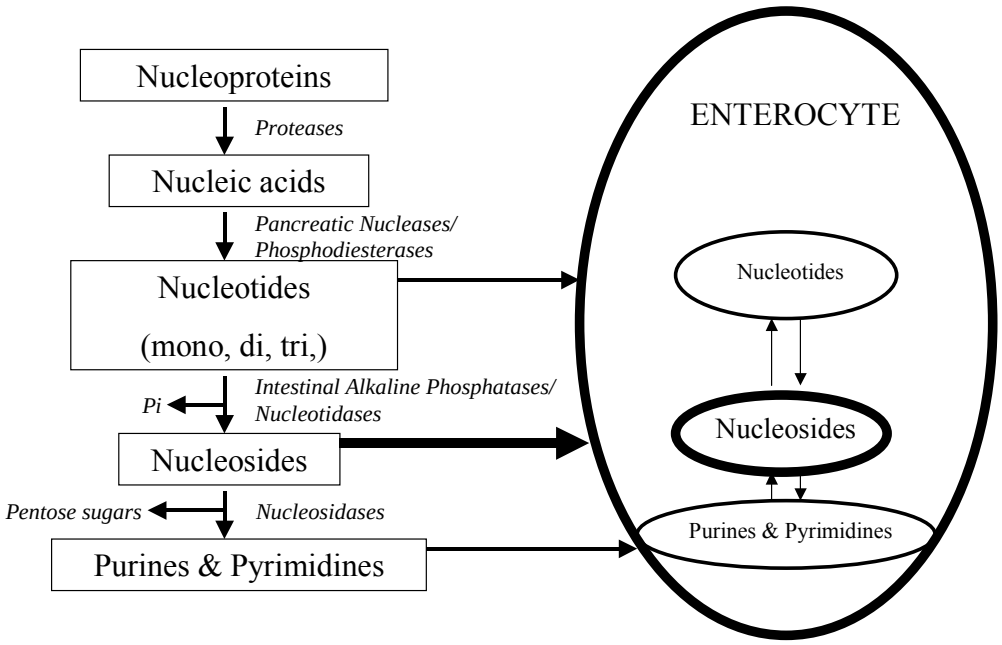


Figure 2.5. Digestion and absorption of nucleic acids and their related products. Adapted from Quan and Uauy (1991)

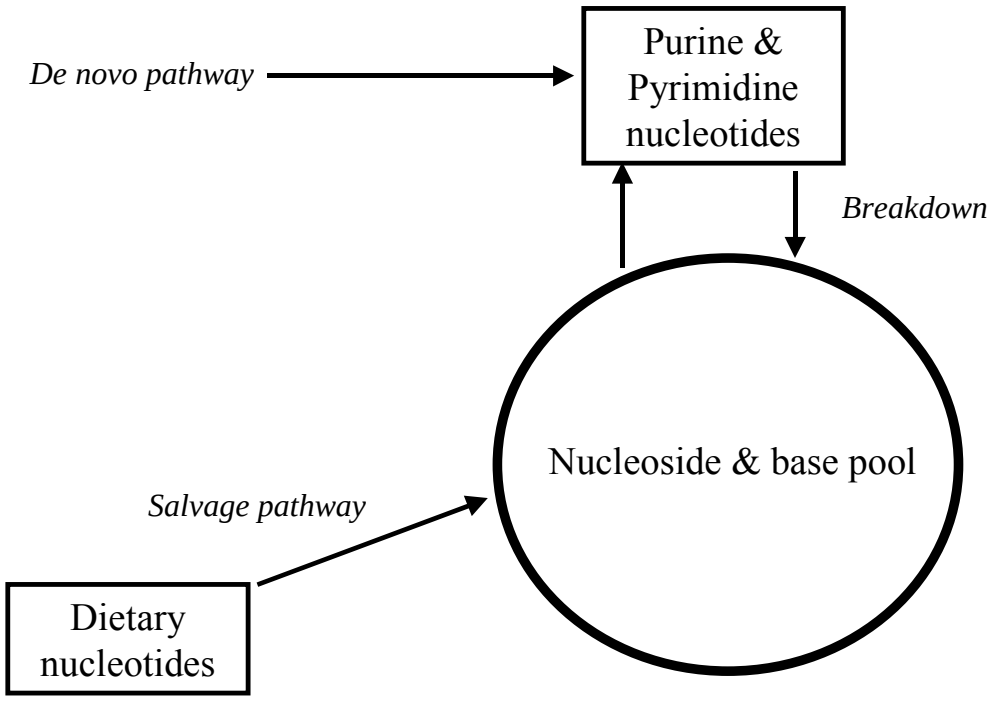


Figure 2.6. De novo and salvage synthesis of nucleotides

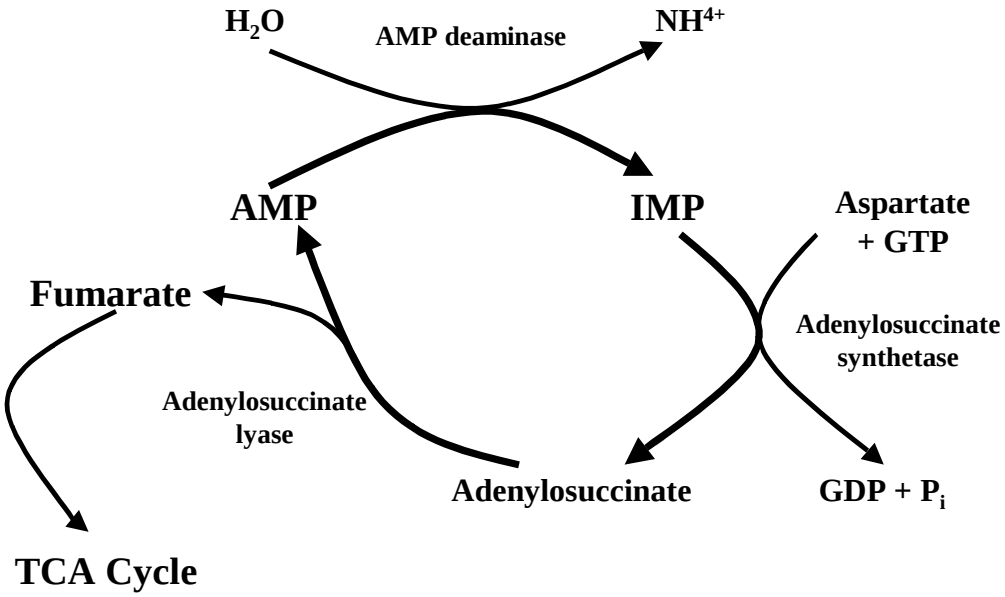


Figure 2.7. Purine nucleotide cycle. Adapted from Voet and Voet (1995)

CHAPTER 3

Nucleotides in sow colostrum and milk at different stages of lactation

ABSTRACT: An experiment was conducted with the objective of measuring the concentrations of total milk solids (TMS), CP, and 5'monophosphate nucleotides in sows' colostrum and milk. Twelve multiparous sows (Landrace x Yorkshire x Duroc) were used. Litter size was standardized at 11 piglets for all sows at farrowing. Sows were fed an 18% CP corn soybean meal-based diet throughout lactation. The experimental period comprised the initial 28 d of lactation with colostrum being collected within 12 h of farrowing and milk being collected on d-3, 7, 14, 21, and 28. Milk samples were analyzed for TMS, CP, adenosine 5'monophosphate (5'AMP), cytidine 5'monophosphate (5'CMP), guanosine 5'monophosphate (5'GMP), inosine 5'monophosphate (5'IMP), and uridine 5'monophosphate (5'UMP). Total milk solids decreased ($P < 0.05$) from 26.7% on d 0 to 23.1% on d-3. It further decreased ($P < 0.05$) to 19.3% on d-7, but after that, it remained constant at 18.2, 18.8, and 19.2% on d-14, 21, and 28, respectively. The concentration of CP decreased from 16.6% in colostrum to 7.7, 6.2, 5.5, 5.7, and 6.3% in milk collected on d-3, 7, 14, 21, and 28, respectively (linear and quadratic effect; $P < 0.05$). Concentrations of 5'AMP, 5'CMP, 5'GMP, and 5'IMP increased from d-0 to d-3 and d-7 and then decreased during the remaining lactation period (quadratic effect; $P < 0.05$). The concentration of 5'UMP decreased from d-0 to d 28 of lactation (linear and quadratic effects; $P < 0.05$). In colostrum, 5'UMP represented 98% of all

5’monophosphate nucleotides and in milk, 5’UMP accounted for 86-90% of all nucleotides, regardless of d of lactation. The results of this experiment suggest that the concentration of TMS and CP in sow mammary secretions change during the first week of lactation, but are constant thereafter. Likewise, the concentration of 5’monophosphate nucleotides change during the initial week post-partum, but during the last 2 weeks of a 4-wk lactation period, the concentration is constant.

Key Words: Colostrum, Milk, Nucleotides, Piglets, Sow

Introduction

The problems associated with antibiotic resistance in humans have led to a growing interest in antibiotic-free animal production worldwide. The dietary inclusion of additives that may act as alternatives to antibiotics have been suggested as ways to raise pigs without using in-feed antibiotics (Turner et al., 2001). One group of metabolites that may potentially act as an alternative to antibiotics is dietary nucleotides. The major nucleotides include adenosine 5’monophosphate (AMP), cytidine 5’monophosphate (CMP), guanosine 5’monophosphate (GMP), inosine 5’monophosphate (IMP), and uridine 5’monophosphate (UMP). Because of their role in cell division, cell growth, and modulation of the immune system, dietary nucleotides may help maintain intestinal health, and thus, reduce the incidence of enteric diseases. Indeed, dietary nucleotides were shown to reduce the prevalence of diarrhea in human infants (Carver et al., 1991;

Pickering et al., 1998). It is also recognized that nucleotides are needed by an animal to respond to immunological challenges (Kulkarni et al., 1994).

Very limited information is available about the young pig's needs for nucleotides, but because of the effectiveness of dietary nucleotides in improving intestinal health and in the development of the immune system in other species, it may be speculated that nucleotides are needed by young pigs during periods of stress and immunological challenges. Nursing pigs receive dietary nucleotides via colostrum and milk, but in contrast to most other livestock species, no data on the concentration of nucleotides in sows' colostrum and milk are available. Thus, to establish a starting point for future studies with nucleotide supplementation to diets for young pigs, it is necessary to measure the concentration of nucleotides in the colostrum and milk of sows. It was the objective of the present experiment to generate such data.

Materials and Methods

Animals and housing

Twelve multiparous sows (Yorkshire x Landrace x Duroc) were used in the experiment. Each sow was housed in a 1.52- x 2.13-m farrowing crate in an environmentally controlled room. A feeder and a nipple drinker were installed in front of each farrowing crate. Animal care procedures were approved by the South Dakota State University Animal Care and Use Committee (# 02-E021). The experimental period comprised the initial 28 d post-partum.

Diets and feeding

Corn soybean meal-based gestation and lactation diets (Table 1) were formulated to meet or exceed suggested requirements for all nutrients (NRC, 1998). Sows were fed 3 kg of the gestation diet once daily during the final 3 wk of gestation. The lactation diet was provided from d 110 of gestation and throughout lactation. During the initial 3 d of lactation, feed intake was restricted, but sows were allowed ad libitum access to feed thereafter. Water was available at all times.

Sample collection

The litter size was standardized at 11 piglets per litter within 24 h after farrowing. Mammary secretions were collected from all functional teats within 12 h of farrowing (d 0), and in the morning on d-3, 7, 14, 21, and 28 of lactation. An attempt was made to collect equal amounts of secretions from all teats from the most anterior teat to the most posterior teat on both sides of the sow. On d-0, samples were collected by hand-stripping the mammary glands, when colostrum was free flowing. On the remaining collection days, 1 mL of oxytocin (20 USP units/mL, VEDCO Inc., St. Joseph, MO) was administered intravenously prior to milking. A total of 50 mL of fluids were collected at each collection. The collected colostrum and milk samples were divided into two sub-samples and stored at -20^{o}C.

Chemical analysis

Colostrum and milk samples were analyzed in duplicate for their concentration of Kjeldahl N (AOAC, 2000). The values for N concentration were multiplied with the protein correction factor 6.38 to calculate the concentration of CP (AOAC, 2000). The

concentration of total solids (TMS) in colostrum and milk was determined by the Mojonnier method (Artherton and Newlander, 1977).

Nucleotide extraction was carried out in duplicate samples according to the methods of Paubert-Braquet et al. (1992). Whole milk samples were thawed and 10 mL of 0.6 N perchloric acid was added to 5 mL of milk and placed in a 30 mL centrifuge tube. The mixture was centrifuged at 17,000 x g for 10 min, the supernatant was filtered through filter paper, and fat was removed using a mosquito forcep. Ten mL of filtrate was added to 4 mL of 1 M KOH to neutralize the acid. Following centrifugation at 17,000 x g for 10 min, the supernatant was aliquoted into sample tubes and analyzed for 5'monophosphate nucleotides (i. e., AMP, CMP, GMP, IMP, and UMP) using a Waters 2690 HPLC system with a photodiode array detector. Internal standards (Sigma-Aldrich Co., St. Louis, MO) added to the defatted milk before the extraction procedures were used to correct for losses during extraction and analysis.

Statistical analysis

Data were analyzed by ANOVA using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included time as the main effect. Linear and quadratic effects of day of lactation on colostrum and milk concentrations of TMS, CP, and 5'nucleotides were analyzed using a contrast statement; an alpha level of 0.05 was used to assess significance. Correlation coefficients in the Proc Corr procedure of SAS were used to identify possible correlations between the concentrations of TMS, CP, and 5'monophosphate nucleotide values. In all analyses, a *P*-value of 0.05 or less was considered significant.

Results

Results from the experiment are shown in Table 2. The concentration of TMS in the colostrum and milk from sows decreased from parturition to d-28 of lactation (linear effect; $P < 0.001$). The concentration of CP also declined from d-0 to d-3 and 7 and then reached a plateau (linear and quadratic; $P < 0.001$).

The most abundant nucleotide in sows' colostrum and milk was 5'UMP. The concentration of this nucleotide decreased from 555.6 μmol/100 mL in colostrum to 104 μmol/100 mL in milk on d-28 (linear and quadratic effects; $P < 0.001$ and $P < 0.05$, respectively). The concentration of 5'UMP represented approximately 98% of all 5'monophosphate nucleotides in sow colostrum and 86 to 90% in milk. Compared with 5'UMP, much lower concentrations of 5'AMP, 5'CMP, 5'GMP, and 5'IMP were found. The concentrations of these nucleotides increased from d 0 to d 3 and d 7 and decreased between d-7 and d-14, but thereafter, the concentration remained constant (quadratic effect; $P < 0.05$).

There is a strong correlation ($P < 0.05$) between the concentration of TMS and CP in sows colostrum and milk (data not shown). However, no significant correlations between the concentration of CP and the analyzed 5'monophosphate nucleotides were detected.

Discussion

The concentration of TMS and CP found in colostrum and milk in the current experiment are in agreement with the findings of other researchers (Bowland et al., 1949;

Klobasa et al., 1987). This indicates that TMS and CP of colostrum and milk in swine have remained relatively constant over the last 50 years despite changes in genetics, housing systems, productivity, and feed formulations. Therefore, the nucleotide concentrations found in the current study are believed to be representative for colostrum and milk from swine in general.

The concentrations of 5'AMP and 5'CMP in sows' colostrum and milk were in the range of those seen in other species (Johke, 1963; Gil and Sanchez-Medina, 1981). However, 5'GMP and 5'UMP concentrations in porcine colostrum and milk observed in this study were higher than in other species (Johke, 1963). The concentration of 5'IMP has not been reported in other species. Inosine 5'monophosphate can be synthesized from AMP, and can further be transformed into other cellular metabolites such as inosine through nucleotidases or alkaline phosphatase enzymes (Thorell et al., 1996). The detection of 5'IMP in this study indicates either that the conversion of AMP to IMP via the enzyme AMP deaminase may be more efficient in sows than in other species or that the IMP-degradation enzymes are inefficient in swine.

The concentration of 5'UMP in sow colostrum and milk observed in this experiment is higher than in other species (Johke, 1963; Atwood et al., 1991). This suggests that species-specific secretory mechanisms may be present. Catabolic enzymes such as kinases for specific nucleotides may have been present during the process of secretion, accumulation, and storage within the mammary gland and may have contributed to the nucleotide concentration as well. This hypothesis was suggested by

Thorell et al. (1996), who identified the presence of catabolic enzymes in milk responsible for the conversion of purine nucleotides to uric acid.

The changes throughout lactation in the concentration of 5'UMP were similar to the changes in TMS. This may indicate that the concentration of 5'UMP in sows' milk is associated with lactose synthesis. During the synthesis of lactose and sialyllactose, which occurs in the Golgi vesicles, 5'UMP and 5'CMP are formed and may be released into the alveolar lumen during exocytosis (Arthur et al., 1991). Lactose and uridine diphosphate (UDP) are formed in the Golgi apparatus of the cell from glucose and UDP galactose. Uridine diphosphate is eventually broken down into UMP and inorganic phosphate. Therefore, an increased production of lactose produces an increase in UDP substrates that are enzymatically degraded to UMP. As a consequence, the concentration of 5'UMP is expected to be correlated to lactose production and thus to TMS. The results from the current experiment support this hypothesis.

The changes in the concentration of 5'AMP, 5'CMP, 5'GMP, and 5'IMP during lactation were different from those observed for CP, TMS, and 5'UMP. This observation suggests that the concentration of these nucleotides is not related to the production of milk lactose or protein.

Nucleotides in milk may originate from two different sources (i. e., dietary sources or nucleotides synthesized de novo). The reduced concentration of nucleotides in milk during the latter stages of lactation may reflect a reduced de novo synthesis because the diet was not changed and the feed intake of the sows was constant during this period (data not shown). This reduction in the de novo synthesis may be a response to a reduced

need for nucleotides by the nursing piglets. However, the reduction in milk nucleotide concentration could also be a response to an increased utilization of nucleotides within the mammary gland where they are used as substrate for the synthesis of DNA (Voet and Voet, 1995). Because the concentration of DNA within the mammary gland increases from d 5 to d 21 of lactation (Kim et al., 1999), more nucleotides are needed for DNA synthesis during this period, which might be the reason why the concentration of nucleotides in milk decreased.

From the above discussion follows that it is not clear if the nucleotides present in sows' milk are secreted in response to a specific need by the piglets, - or if they are secreted into the milk because the mammary gland has no other use for them. Nevertheless, the data from this experiment indicate the amounts of nucleotides that nursing piglets receive prior to weaning. Therefore, these data can serve as a starting point for future studies aimed at investigating if intestinal health and pig performance can be improved by the inclusion of dietary nucleotides in diets for pigs during the post-weaning period.

Implications

The relatively constant concentration of nucleotides in the milk of sows during the second half of lactation suggests that the requirement for nucleotides by young pigs may be relatively constant. Therefore, the 5'monophosphate nucleotide concentration found in this study may serve as a starting point for nucleotide supplementation studies in

weanling pigs. It has also been established that CP is not a good indicator of the nucleotide concentration in sows' colostrum and milk.

Literature Cited

AOAC. 2000. Official Methods of Analysis. 17th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Artherton, V. H. and J. A. Newlander. 1977. The chemistry and testing of dairy products. 4th ed. AVI Publ. Co., Inc., Westport, CT.

Arthur, P. G., J. C. Kent, P. E. Hartmann. 1991. Metabolites of lactose synthesis in milk from women during established lactation. J. Pediatr. Gastroenterol. Nutr. 13:260-266.

Atwood, C. S., J. K. Toussaint, and P. E. Hartmann. 1991. The concentration of cellular metabolites in sows' milk change during lactogenesis. Page 74 in Manipulating Pig Production III. B. S. Batterham, ed. Australasian Pig Science Society.

Bowland, J. P., R. H. Grummer, P. H. Phillips, and G. Bohstedt. 1949. The effect of plane of nutrition on the composition of sows' colostrum and milk. J. Anim. Sci. 8:199-206.

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.

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.

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

Kim, S. W., W. L. Hurley, I. K. Han, and R. A. Easter. 1999. Changes in tissue composition associated with mammary gland growth during lactation in sows. J. Anim. Sci. 77:2510-2516.

Klobasa, F., E. Werhahn, and J. E. Butler. 1987. Composition of sow milk during lactation. J. Anim. Sci. 64:1458-1466.

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. Nutr. 124:1442-1446.

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

Paubert-Braquet, M., N. Hedef, S. Picquot, and C. Dupont. 1992. Quantification of nucleotides in human milk and their effects on cytokine production by murine fibroblasts, J774A1 macrophages and human monocytes. Pages 22-34 in Foods, Nutrition and Immunity, vol. 1. M. Paubert-Braquet, C. Dupont, and R. Paoletti, eds. Dyn. Nutr. Res. Basel, Karger.

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.

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

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.

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.

Table 3.1. Composition and calculated analysis of gestation and lactation diets (as-fed basis)

ITEM	Gestation diet	Lactation diet
Ingredients, %		
Corn	80.05	64.93
Soybean meal, 44 %	15.75	27.50
Soybean oil	1.00	4.45
Limestone	0.90	0.90
Dicalcium phosphate	1.75	1.60
L-Lysine HCL	0.00	0.07
Salt	0.40	0.40
Vitamin premix [a]	0.05	0.05
Mineral premix [b]	0.10	0.10
Total	100.00	100.00
Calculated nutrient composition		
ME, kcal/kg	3,354	3,523
Crude protein, %	14.12	18.45
Calcium, %	0.79	0.80
Phosphorous, %	0.66	0.67
Arginine, %	0.84	1.19
Histidine, %	0.39	0.50

Isoleucine, %	0.56	0.77
Leucine, %	1.37	1.65
Lysine, %	0.68	1.05
Methionine, %	0.24	0.29
Methionine + cysteine, %	0.51	0.62
Phenylalanine, %	0.69	0.91
Threonine, %	0.52	0.70
Tryptophan, %	0.15	0.22
Valine, %	0.67	0.88

[a] Vitamin premix provided the following quantities of vitamins per kg of the diet: Niacin, 25 mg; Vitamin B_{12}, 25 mcg; Pantothenic acid, 13.2 mg; Riboflavin, 5.0 mg; Vitamin K, 2.2 mg; Biotin, 0.20 mg; Vitamin A, 5,000 IU; Vitamin D_3, 500 IU, and Vitamin E, 44 IU.

[b] Trace mineral premix provided the following quantities of minerals per kg of diet: Se, 0.30 mg as sodium selenite ; I, 0.30 mg as potassium iodate; Cu, 25 mg as copper sulfate; Mn, 25 mg as manganese sulfate; Fe, 120 mg as iron sulfate; Zn, 125 mg as zinc oxide.

Table 3.2. Concentration of total milk solids, CP (N x 6.38) and 5'monophosphate nucleotides in colostrum and milk from sows at different stages of lactation [a]

	Day of lactation							*P*-value	
Item	0	3	7	14	21	28	SEM	Linear effect	Quadratic effect
TMS [b]	26.7	23.4	19.4	18.2	18.8	19.2	0.71	<0.001	0.142
CP [c]	16.6	7.8	6.2	5.5	5.7	6.3	0.41	<0.001	<0.001
AMP [d]	4.0	11.3	12.8	6.8	4.3	3.0	2.19	0.320	0.003
CMP [e]	1.5	7.1	7.1	3.5	2.3	0.98	0.98	0.173	<0.001
GMP [f]	5.4	14.7	14.0	10.2	6.0	7.1	1.41	0.028	<0.001
IMP [g]	1.1	1.8	2.6	1.4	0.9	0.4	0.38	0.365	0.013
UMP [h]	555.6	305.6	263.1	144.0	122.8	104.0	27.05	<0.001	0.017

[a] n = 12

[b] Total milk solids, %

[c] Crude protein, %

[d] Adenine 5' monophosphate, umol/100 mL

[e] Cytidine 5' monophosphate, umol/100 mL

[f] Guanine 5' monophosphate, umol/100 mL

[g] Inosine 5' monophosphate, umol/100 mL

[h] Uridine 5' monophosphate, umol/100 mL

CHAPTER 4

Effects of dietary nucleosides on intestinal microbial activity of newly weaned pigs

ABSTRACT: Two experiments were conducted to determine the effects of adding nucleosides to starter diets for weanling pigs. In Exp. 1, 36 pigs were weaned at 18 ± 2 days of age and allotted to three treatment groups in a completely randomized design. Pigs allotted to treatment 1 were fed a corn casein lactose-based basal diet (Diet 1). Pigs allotted to treatments 2 and 3 were fed the basal diet supplemented with nucleosides (Diet 2 and Diet 3) in amounts that correspond to 30% and 150% of the quantities found in sow milk on d 14 of lactation, respectively. The concentration of IgG was determined in the serum while microbial concentration was determined in fecal samples on d-0, 7, and 14 post-weaning. On d 7, pigs fed Diet 3 had lower ($P = 0.05$; linear effect) fecal counts of *Cl. perfringens* compared with pigs fed Diet 1 (5.04 vs. 6.08 $\log_{10}$ cfu/g). On d 14, pigs fed Diet 2 and pigs fed Diet 3 had lower ($P = 0.01$; linear effect) fecal counts of *Cl. perfringens* compared with pigs fed Diet 1 (4.26 and 3.00 vs. 4.76 $\log_{10}$ cfu/g). On d 14, the fecal counts of *L. acidophilus* and of Bifidobacterium spp. were higher ($P = 0.01$ and $P = 0.05$; linear effect) in pigs fed Diet 2 compared with pigs fed Diet 1 (9.33 and 8.82 vs. 8.35 and 7.68 $\log_{10}$ cfu/g, respectively). Serum IgG concentrations linearly decreased ($P < 0.04$) with time after weaning, but there were no differences among treatment groups. Exp. 2 was an in-vitro study that was conducted to determine the antimicrobial and probiotic properties of nucleosides. Broths containing microflora from pigs were

prepared and either not supplemented with nucleosides (control) or supplemented with nucleosides. The bacterial growth in both broths was measured over a 16-h period. Results showed that *E. coli* was inhibited by the inclusion of nucleosides ($P < 0.01$; linear effect). In contrast, *Cl. perfringens* and Bifidobactrium spp. counts in nucleoside supplemented broth were higher ($P < 0.03$) compared with the control broth. The results of the two experiments indicate that nucleoside supplementation during the immediate post-weaning period may positively influence the gastrointestinal microflora by decreasing enterobacteria and increasing *L. acidophilus* and Bifidobacterium species.

Key Words: Immunoglobulins, Microflora, Nucleosides, Pigs

Introduction

Nucleotides may be synthesized de novo in an animal or they may be provided via the diet. Dietary nucleotides are digested in the small intestine by pancreatic nucleases to yield nucleosides and free phosphorus. Nucleosides are subsequently absorbed into the enterocytes (Carver and Walker, 1995). After absorption, nucleosides may be converted to nucleotides in the enterocyte via the salvage pathway. Synthesizing nucleotides via this pathway requires less energy compared to de novo synthesis of nucleotides (Kulkarni et al., 1994). Therefore, by providing nucleotides or nucleosides in the diet for pigs, the maintenance energy requirement may theoretically be reduced.

Feed ingredients are not routinely analyzed for their concentration of nucleotides or nucleosides and the requirement for nucleotides or nucleosides by newly weaned pigs

has not been reported. However, it has been suggested that the requirement for nucleotides is increased during periods of stress (Carver and Walker, 1995). Therefore, it is likely that the requirement for nucleotides is elevated during the immediate post-weaning period. Pigs often have a low energy intake during this period and the concentration of glutamine is low in starter diets compared to sow milk. This may reduce de novo synthesis of nucleotides because energy and glutamine are needed in the synthesis of both purines and pyrimidines (Rodwell, 2000). At the same time, the pig needs to develop its intestinal tract, immune system, and intestinal microflora and all of these processes also require nucleotides (Uauy, 1994; Yu, 1998, Cameron et al., 2001). As a consequence, the young pig is in a nucleotide dilemma during the post-weaning period because the need for nucleotides is increased, but the precursors needed for the synthesis of nucleotides (i. e., energy and glutamine) are reduced. Because dietary nucleotides are digested to nucleosides prior to absorption, we hypothesized that nucleosides may be more effective in supporting pig performance compared to nucleotides. Therefore, it was the objective of the present work to test the hypothesis that dietary nucleosides may improve the intestinal microflora and the development of the immune system in weanling pigs.

Materials and methods

Feed analysis and calculated concentration in starter diets

The nucleotide concentration in 12 feed ingredients (i. e., barley, casein, corn, dried whey, fishmeal, naked oats, non-fat dried milk, spray dried plasma protein, spray

dried red blood cells, soybean meal, soy protein concentrate, and whey protein concentrate), was measured (Table 1) according to the method of Paubert-Braquet et al. (1992). A starter diet calculated to contain 1.5% Lys and 3,493 kcal ME/kg was formulated to meet or exceed recommendations for all nutrients (NRC, 1998). The nucleotide concentration of this diet was calculated and compared to the concentration in sow milk (DM-basis) on d 14 of lactation (Table 2). The results of this comparison suggest that a standard starter diet contains an adequate amount of cytidine 5'monophosphate (5'CMP), but the concentration of adenosine 5'monophosphate (5'AMP), guanosine 5'monophosphate (5'GMP), inosine 5'monophosphate (5'IMP), and uridine 5'monophosphate (5'UMP) is considerably lower than in sow milk. Based on this information, two experiments were conducted.

Experiment 1

Animals, housing, and experimental design

Thirty-six pigs (Ausgene International, Gridley, IL) were weaned at 18 ± 2 days of age (BW: 6.70 ± 0.68 kg) and allotted to one of three treatment groups according to ancestry, sex, and BW in a completely randomized design. There were six replicate pens per treatment and two pigs per pen. Pigs were obtained from the South Dakota State University Swine Research Farm and housed in conventional nursery rooms containing 1.2- x 1.4-m pens with a plastic coat slatted floor. A feeder and a nipple drinker were installed in each pen. Room temperature was maintained at 30-32^{o}C. Animal care procedures were approved by the South Dakota State University Animal Care and Use Committee (#03-E013).

Diets and feeding

Three diets were prepared (Table 3). Diet 1 was a nucleoside deficient control diet. A diet that was supposed to be semi-deficient in nucleosides (Diet 2) and a diet that was believed to be adequate in nucleosides (Diet 3) were also formulated. Diets 2 and 3 contained 30% and 150% of the nucleosides in sow milk on d 14 of lactation, respectively (Mateo et al., 2004). Diets were formulated to meet or exceed recommendations for all nutrients (NRC, 1998) and they were provided in a meal form. All diets were provided on an ad libitum basis during 2 weeks post-weaning. Water was available at all times.

Data recording and sample collection

Individual pig weights were recorded at weaning, at the end of wk 1, and at the conclusion of the experiment on d-14 post-weaning. The amount of feed provided in each feeder was recorded daily and orts were subtracted from the total amount of feed supplied in the feeder to calculate total feed disappearance. Average daily weight gain and ADFI for each pen were summarized and used to calculate the G:F for each wk and for the entire experimental period. On the d of weaning and on d-7 and d-14 post-weaning, a 10-mL blood sample was collected from each of the 36 pigs via jugular vein puncture. Samples were allowed to clot overnight at 4^{o}C and centrifuged at 3000 rpm for 30 min the following day. Serum was harvested and placed in a freezer at -20^{o}C for long-term storage. On the d of weaning and on d-7 and d-14 post-weaning, a fresh fecal sample was also collected from each pen. The first pig that defecated in each pen on the d of weaning was identified as the pig to be collected in each pen throughout the experiment. The

sample was collected immediately after being voided and placed in an autoclaved glass vial. Vials containing fecal samples were placed on ice while sampling other pigs. Immediately following the collection of all samples, they were brought to the microbiology lab for microbial enumeration.

Sample analysis

Serum samples were thawed and analyzed for IgG concentration using a radial immunodiffusion kit (Bethyl Laboratories, Inc. Montgomery, TX). Reference standards and serum samples were applied into wells of the radial immunodiffusion (RID) plates. The RID plates were incubated at room temperature for a minimum of 18 hours. After incubation, the precipitin ring diameter of the reference standards and the serum samples were measured to the nearest tenth of a millimeter. A reference curve was constructed on a two-cycle semi logarithmic graph paper by plotting the concentration of each standard on the logarithmic scale vs. the precipitin ring diameter on the linear scale. A line was drawn connecting adjacent points. The IgG concentration of test samples was determined by locating each of the sample's ring diameter on the reference curve and reading IgG concentration on the logarithmic scale.

Serial dilutions of aseptically collected samples were prepared by mixing 1 g of fecal sample with 99 mL of peptone water, using a Stomacher blender (Ali and Fung, 1991; Dave and Shah, 1996; Fung, 2000). Subsequent dilutions up to 10^{-8} were prepared using 9-mL peptone water diluents mixed with 1 mL of the previous dilution. Sample dilutions were prepared within one h to minimize changes in the microbial populations in fecal samples. Total coliforms and *Escherichia coli* were enumerated using standard

3M™ petrifilms (Fung, 2000). Enumeration of *Clostridium perfringens* was carried out by using Fung's double tube system utilizing TSC (Tryptose-Sulfite-Cycloserine) agar (Ali and Fung, 1991). Numbers of *Lactobacillus acidophilus* and Bifidobacterium spp. were enumerated according to the protocol developed by Dave and Shah (1996) using De Man Rogosa Sharp-salicin agar (MRS-salicin agar) and MRS-NNPL agar (Neomycin sulfate, Nalidixic acid, Paromomycin sulfate, and Lithium chloride agar), respectively.

Experiment 2

Exp. 2 was an in-vitro experiment that comprised two phases. The first phase was microbial isolation and activation, and the second phase was a growth assay to determine the effects of supplementing nucleosides on bacterial growth over a 16-h period. In phase 1, two bacterial colonies of each bacterium (i. e., Bifidobacterium spp. *Cl. perfringens, E. coli,* and *L. acidophilus*) were isolated from the plates and double tube system previously used for microbial enumeration during the in vivo study using standard microbiological methods (Ali and Fung, 1991; Dave and Shah, 1996; Fung, 2000). These bacterial strains were further activated by transferring the colonies into 10-mL tubes containing microorganism-specific broth and placed in an incubator at 37^{o}C for 24 hours. De Man Rogosa Sharp (MRS) broth was used for both Bifidobacterium spp. and *L. acidophilus* bacteria (Dave and Shah, 1996). Reinforced Clostridial (RC) broth and Bacto Nutrient (BN) broth was used for *Cl. perfringens* and *E. coli* bacteria, respectively (Ali and Fung, 1991). Inoculated broth of Bifidobactrium spp., *L. acidophilus*, and *Cl. perfringens* were incubated anaerobically, whereas that of *E. coli* was incubated aerobically. This procedure was repeated three times over three days by pipetting 0.1 mL of each broth

solution containing the microorganisms into a new tube containing the same broth. The solution was mixed before and after transfer using a vortex mixer, and further incubated for 24 hours. All transfers were performed under a biological hood with high efficiency particulate filters to avoid contamination during activation and propagation. For *Cl. perfringens*, serial dilution and enumeration were performed after every transfer to verify viable growth using the method described in the in vivo study. When microbial growth was verified on the third d, phase 2 was initiated.

In phase 2, two activated and purified strains of each bacteria from phase 1 were used to determine the effects of supplementing nucleosides on bacterial growth over a 16-h period. Each strain was transferred into a bottle containing 200 mL of specific broth as described in phase 1, but devoid of yeast extract or beef extract to eliminate the presence of nucleosides or nucleotides. The contents of this bottle were divided into two sterilized bottles each containing 100 mL. The two bottles represented the treatment and control group for a specific microorganism. Powdered adenosine (0.2 g), cytidine (0.1 g), guanosine (0.3 g), inosine (0.03 g), and uridine (4.0 g) nucleosides (Sigma-Aldrich Co. St. Louis, MO) were dissolved in five separate 50-mL beakers containing 50 mL of deionized water. Three mL of adenosine, cytidine, and uridine solution was aspirated from their respective beakers, and mixed into the treatment bottle to provide 0.012%, 0.006%, and 0.236% of adenosine, cytidine, and uridine, respectively. Likewise, 3.2 mL of guanosine and 3.2 mL of inosine solution was aspirated and mixed into the treatment bottle to provide 0.019% and 0.002% of guanosine and inosine, respectively. The nucleoside inclusion rates used in this study were representative of 150% of the

concentration of nucleosides found in sow milk on d 14 of lactation on a DM basis (Mateo et al., 2004). This nucleoside mixture was added and mixed in each treatment group bottle for all four groups of bacteria, which represented 0 h. Serial dilution and subsequent plating of colonies for all the treatment and control bottles were carried out in duplicate using the method described for the in vivo study. Enumerations were carried out at 4, 8, 12, and 16 hours of incubation using the same protocol as used in the in vivo study. These five time periods were used to construct the growth curves for both treatment and control groups to validate normal growth patterns and determine the effects of nucleoside supplementation on the growth of the four bacterial groups.

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Stat Inc., Cary, NC). The model included time, treatment, and treatment x time interaction as the main effects and was used to evaluate ADG, ADFI, G:F, serum IgG concentration, and microbial count in Exp 1. In Exp 2., the model included time and treatment as the main effects to evaluate microbial count. In order to increase the normality of distribution and decrease variation, bacterial concentration was converted to a $\log_{10}$ basis before statistical analysis was performed. Least square means were calculated and separated using the PDIFF option of SAS. Contrast statements of SAS were used to determine linear and quadratic effects. The pen and plate was used as the experimental unit for Exp. 1 and Exp. 2, respectively. An alpha level of 0.05 was used to assess significance in both experiments.

Results

Experiment 1

Pig performance. No differences among treatment groups were observed for ADG, ADFI, and G:F during any of the time periods or overall for the entire experiment.

Microbial analysis. The total coliform count tended to decrease linearly from d 0 to 14 ($P = 0.08$) in the fecal samples of pigs fed the nucleoside adequate diet (Table 5). However, this was not the case for the control diet or the nucleoside semi-deficient diet. No significant differences between dietary treatments were observed for the total coliform count, regardless of the time after weaning. Likewise, the *E. coli* count was not affected by neither dietary treatment nor time after weaning.

The fecal count of *Cl. perfringens* linearly decreased from d-0 to d-14 post-weaning ($P < 0.01$) in pigs fed the nucleoside semi-deficient and nucleoside adequate diets. On d 14, the numbers of *Cl. perfringens* were different ($P = 0.01$; linear effect) across all treatment groups, with pigs fed the nucleoside deficient diet having the highest *Cl. perfringens* count followed by pigs fed the nucleoside semi-deficient diet and the nucleoside adequate diet (4.76, 4.26, and 3.00 $\log_{10}$ cfu/g, respectively). The fecal count of Bifidobacterium spp. tended to be higher ($P = 0.07$) on d-7 post-weaning in pigs fed the nucleoside semi-deficient and nucleoside adequate diets compared with pigs fed the nucleoside deficient diet. On d-14 post-weaning, the concentration of Bifidobacterium spp. linearly increased ($P = 0.05$) as nucleosides were included in the diet (7.68, 8.35, and 8.32 $\log_{10}$ cfu/g in the control, nucleoside semi-deficient, and nucleoside adequate diet, respectively). Likewise, on d 14, *L. acidophilus* fecal counts were higher ($P = 0.01$; linear

effect) in pigs fed the nucleoside semi-deficient diet and the nucleoside adequate diet compared with pigs fed the nucleoside deficient diet (9.33 and 9.21 vs. 8.82 $\log_{10}$ cfu/g).

Serum IgG concentration. The serum IgG concentration linearly decreased ($P < 0.04$) from d 0 to 14 across all treatment groups (Table 6). However, no differences between dietary treatments were observed during any of the time periods or overall for the entire experiment.

Experiment 2

The *E. coli* count increased during the initial 4 hours and then decreased linearly during the following 12 hours ($P < 0.01$), regardless of whether nucleosides were included in the broth or not. However, the *E. coli* count tended to be inhibited after 8, 12, and 16 hours of growth due to the inclusion of nucleosides in the broth ($P = 0.09$, $P = 0.09$, and $P = 0.05$, respectively).

The nucleoside-supplemented broth for *Cl. perfringens* had higher ($P < 0.01$) counts compared with the broth without the supplementation of nucleosides after 16 hours of incubation (6.82 vs. 6.30 $\log_{10}$ cfu/g). The growth curve (not shown) for *Cl. perfringens* suggested an inhibition (i. e., decrease) for both the control and treatment group during the initial 4 hours, however this inhibition tended to be greater in the treatment group in which nucleosides were added.

The Bifidobactrium spp. count increased linearly ($P < 0.01$) from 0 to 16 hours regardless of whether nucleosides were included in the broth or not. However, the broth supplemented with nucleosides had higher ($P = 0.02$) counts of Bifidobactrium spp. after

12 hours (8.57 vs. 8.33 $\log_{10}$ cfu/g) compared with the unsupplemented broth and a tendency ($P = 0.09$) for a higher Bifidobactrium spp. count was observed after 4 hours.

No effect of nucleoside supplementation was observed on the *L. acidophilus* count in this experiment during the 16-h experimental period.

Discussion

Pig performance. Previously, Fuke and Konosu (1991) and Nagodawithana (1995) reported that supplementing nucleotides (i. e., inosine 5'monophosphate and guanosine 5'monphosphate) in food for humans improved flavor. These ingredients enhanced the savory properties present in food and are thought to contribute to the unique Umami taste. The addition of nucleosides in the current experiment did not affect ADFI and there were no differences in pig performance among treatment groups. The reason we did not observe any effects of nucleoside supplementation on pig performance could be that the pigs used in this experiment were housed only 2 per pen and are not as diseased challenged as pigs kept under commercial conditions. Therefore, these pigs were likely less stressed than commercial pigs.

Microbial analysis. The decrease in the concentration of potentially pathogenic bacteria (i. e., *Cl. perfringens*) and the increase in beneficial bacteria (i. e., *L. acidophilus* and Bifidobacterium spp.) upon the addition of nucleosides that was demonstrated in this experiment are in agreement with previous findings (Tanaka and Mutai, 1980; Uauy, 1994). This indicates that dietary nucleosides may manipulate the intestinal microflora. The mechanism (i. e., inhibition or displacement) causing these changes remains unclear.

Metabolites such as acid and bacteriocins produced by Bifidobacterium spp. may have caused a decrease in the pH in the gastrointestinal tract that could have inhibited the growth of *Cl. perfringens* over a period of time, but this hypothesis needs to be experimentally verified. It is also possible that the reduction in the concentration of *Cl. perfringens* was a result of the increase in probiotic bacteria.

Serum IgG concentration. 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 with the unsupplemented control calves (Oliver et al., 2003). In contrast, nucleoside supplementation had no effect on serum IgG concentration in the current study. The absence of a response in serum IgG concentration may have been due to an absence of a challenge or due to a shorter period of supplementation. It has been demonstrated that challenged piglets fed diets supplemented with nucleotides for 2 to 4 weeks had better immune responses compared to piglets fed diets supplemented with nucleotides for a shorter period (Cameron et al., 2001).

Partial inhibition of *E. coli* from the 4^{th} to the 16^{th} h of growth in the in-vitro study may have been due to one or more of the nucleosides present in the growth medium that may have created unfavorable conditions for *E. coli*, and as a result decreased their counts after 4 hours. In contrast, the data for Bifidobacterium spp., *L. acidophilus,* and *Cl. perfringens* suggested that the growth of these bacteria respond positively to nucleoside supplementation. This indicates that these bacteria may use nucleosides as an additional nutrient for growth. The inhibition of *Cl. perfringens* during the initial 4 hours

may have been due to the oxygen content in the media because the decline was observed in both broths (i. e., with and without supplementation of nucleosides). The oxygen content in the media may have caused this initial decrease in growth because *Cl. perfringens* is an anaerobic microorganism. Because of the supplementation of nutrients provided by the nucleosides in the treatment group, these naturally slow growing bacteria were able to recover faster compared to the control group, and thus showed an earlier increase in growth.

Combining the findings from the in-vivo study and the in-vitro assay suggests that the hypothesis of displacement as a possible mechanism for increased probiotic microflora and a concomitant decline in pathogenic microflora with nucleoside supplementation is strengthened. An increase in growth rate of the non-pathogenic bacteria and increased production of their metabolites may have provided an environment that restricted the proliferation of the pathogenic strains. Nucleoside supplementation also increased the growth of *Cl. perfringens* in the in vitro study, but in the in vivo experiment, nucleosides reduced the concentration of *Cl. perfringens*. The reason for this discrepancy may be that clostridia are naturally slow growing bacteria. Therefore, more fastidious probiotic bacteria will displace them under in vivo conditions, which may have been the reason why we saw a decrease in *Cl. perfringens* in Exp. 1.

In conclusion, the results of the present experiments indicate that nucleoside supplementation positively influences the intestinal microflora by decreasing potentially pathogenic bacteria and increasing beneficial bacteria.

Implication

The findings from the present experiments indicate that pigs fed diets supplemented with nucleosides may have an elevated concentration of probiotic bacteria and a reduced concentration of pathogenic bacteria in the gastrointestinal tract. Under commercial conditions, this may improve the intestinal health, which may lead to an improvement in the immune system and reduced scouring during the post-weaning period.

Literature cited

Ali, M. S., and D. Y. C. Fung. 1991. Occurrence of *Clostridium perfringens* in ground beef and ground turkey evaluated by three methods. J. Food Safety 11:197-203.

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

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

Dave, R. I., and N. P. Shah. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, Lactobacillus delbrueckii ssp. bulgaricus, *Lactobacillus acidophilus* and Bifidobacteria. J. Dairy Sci. 79:1529-1536.

Fuke, S., and S. Konosu. 1991. Taste active components in some foods: a review of Japanese research. Physiol. Behav. 49:863-868.

Fung, D. Y. C. 2000. Handbook for rapid methods and automation in microbiology. International Workshop XX. Kansas State University, Manhattan, KS, USA, July 6-14, 2000.

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. Nutr. 124:1442-1446.

Mateo, C. D., D. N. Peters, and H. H. Stein. 2004. Nucleotides in sow colostrum and milk at different stages of lactation. J. Anim. Sci. 82:1339-1342.

Nagodawithana, T. W. 1995. Flavor enhancers. Pages 8-10 in Savory Flavors. Esteekay Associates, Inc., ed. Milwaukee, WI.

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

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.

Rodwell, V. W. 2000. Metabolism of purine and pyrimidine nucleotides. Pages. 386-401 in Harpers Biochemistry, 25th ed. R. K., Murray, D. K. Granner, P. A. Mayes, and V. W. Rodwell, ed. Appleton and Lange, Stamford, CT.

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

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

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

Table 4.1. Nucleotide concentration (ppm) in some commonly used feed ingredients (as-is basis)

Ingredient Nucleotide:	5'AMP [a]	5'CMP [a]	5'GMP [a]	5'IMP [a]	5'UMP [a]
Barley	1	2	1	1	0
Casein	0	1	0	0	0
Corn	2	3	3	1	0
Fish meal	11	26	2	35	1
Naked oats	3	3	3	1	1
Non-fat dried milk	0	65	0	195	106
Plasma protein, spray dried	2	2	2	1	0
Red blood cells, spray dried	44	0	3	6	2
Soybean meal, 44 %	8	16	3	2	9
Soy Protein Concentrate	1	0	2	1	0
Whey, dried	19	270	0	4	1
Whey protein concentrate	0	34	0	159	89

[a] Adenosine 5’monophosphate (5’AMP), cytidine 5’monophosphate (5’CMP), guanosine 5’monphosphate (5’GMP), inosine 5’monophosphate (5’IMP), and uridine 5’monophosphate (5’UMP).

Table 4.2. Comparison of the nucleotide concentration (ppm) of a starter diet for weanling pigs and of sow milk (DM-basis)

Item Nucleotide:	5'AMP [a]	5'CMP [a]	5'GMP [a]	5'IMP [a]	5'UMP [a]
Total in starter diet [b]	6	58	2	4	1
Sows milk[c]	117	56	185	23	2334
Difference	-111	2	-183	-19	-2333

[a] Adenosine 5'monophosphate (5'AMP), cytidine 5'monophosphate (5'CMP), guanosine 5'monphosphate (5'GMP), inosine 5'monophosphate (5'IMP), and uridine 5'monophosphate (5'UMP).

[b] 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, crystalline amino acids, 7.18%.

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

Table 4.3. Composition (as-fed basis) of experimental diets (Exp. 1)

Item Treatment:	Nucleoside deficient	Nucleoside semi-deficient	Nucleoside adequate
Ingredient, %			
Casein	19.00	19.00	19.00
Corn	38.33	38.28	38.06
Dextrose	10.00	10.00	10.00
Lactose	20.00	20.00	20.00
Sucrose	5.00	5.00	5.00
Soybean oil	3.00	3.00	3.00
Limestone	1.00	1.00	1.00
Dicalcium phosphate	2.50	2.50	2.50
L-lysine HCL	0.01	0.01	0.01
DL-methionine	0.25	0.25	0.25
L-threonine	0.14	0.14	0.14
L-tryptophan	0.02	0.02	0.02
Salt	0.40	0.40	0.40
Vitamin premix [a]	0.10	0.10	0.10
Trace mineral premix [b]	0.25	0.25	0.25
Adenosine [c]	-	0.002	0.012
Cytidine [d]	-	0.001	0.006
Guanosine [e]	-	0.004	0.019

Inosine [f]	-	0.0005	0.002
Uridine [g]	-	0.047	0.236
Total	100.00	100.00	100.00
Analyzed nutrient composition			
Gross Energy, kcal/kg	4,188	4,123	4,115
Crude Protein, %	23.29	21.58	22.14
Calcium, %	0.90	0.90	0.90
Phosphorous, %	0.73	0.73	0.73
Indispensable AA			
Arginine, %	0.85	0.78	0.78
Histidine, %	0.66	0.60	0.60
Isoleucine, %	1.09	1.01	0.99
Leucine, %	2.23	2.03	2.01
Lysine, %	1.62	1.50	1.48
Methionine, %	1.70	1.54	1.59
Phenylalanine, %	1.16	1.06	1.06
Threonine, %	1.37	1.27	1.29
Tryptophan, %	0.28	0.24	0.26
Valine, %	1.42	1.31	1.29
Dispensable AA			

Alanine, %	0.80	0.73	0.73
Aspartate, %	1.53	1.39	1.41
Cysteine, %	0.10	0.09	0.10
Glutamic acid, %	4.85	4.41	4.48
Glycine, %	0.48	0.44	0.44
Proline, %	2.35	2.13	2.13
Serine, %	0.90	0.85	0.87
Tyrosine, %	1.13	1.04	1.03
Nucleosides			
Adenosine, %	0.000008	0.0024	0.012
Cytidine, %	0.000010	0.0011	0.006
Guanosine, %	0.000010	0.0037	0.019
Inosine, %	0.000004	0.0005	0.002
Uridine, %	0.000000	0.0467	0.234

[a] Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 21,980 IU as vitamin A acetate; vitamin B_{12}, 88 mcg; vitamin D_3, 3,296 IU as D-activated sterol; vitamin E, 110 IU as DL-α-tocopheryl acetate; vitamin K_3, 8.8 mg as menadione dimethylpyrimidinol bisulfite; biotin, 0.34 mg; D-pantothenic acid, 66 mg as calcium pantothenate; folic acid, 2.2 mg; niacin, 110 mg; pyridoxine, 6.6 mg as pyridoxine hydrochloride; riboflavin, 19.8 mg; and thiamin, 6.6 mg as thiamine mononitrate.

[b] Provided the following quantities of trace minerals per kg of complete diet: Cu, 16.5 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.30 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

[c] Adenosine (9-β-D-Ribofuranosyladenine), minimum 99% purity (Sigma-Aldrich Co. St. Louis, MO).

[d] Cytidine (Cytosine β-D-riboside), minimum 99% purity (Sigma-Aldrich Co. St. Louis, MO).

[e] Guanosine (9-[β-D-Ribofuranosyl]guanine), minimum 98% purity (Sigma-Aldrich Co. St. Louis, MO).

[f] Inosine (Hypoxanthine 9-D-ribofuranoside), minimum 99% purity (Sigma-Aldrich Co. St. Louis, MO).

[g] Uridine (1-β-D-Ribofuranosyluracil), minimum 99% purity (Sigma-Aldrich Co. St. Louis, MO).

Table 4.4. Performance of weaned piglets fed different levels of nucleosides in the diet (Exp.1) [a]

Item	Treatment:	ND [b]	NSD [b]	NA [b]	SEM	Linear effect	Quadratic effect
ADG, kg							
d 0 to 7		0.057	0.060	0.044	0.009	0.80	0.66
d 7 to 14		0.135	0.124	0.128	0.016	0.63	0.65
d 0 to 14		0.096	0.092	0.086	0.010	0.79	0.87
ADFI, kg							
d 0 to 7		0.321	0.341	0.336	0.012	0.25	0.28
d 7 to 14		0.549	0.603	0.593	0.026	0.17	0.20
d 0 to 14		0.435	0.472	0.464	0.016	0.13	0.16
G:F, kg/kg							
d 0 to 7		0.177	0.173	0.129	0.024	0.95	0.87
d 7 to 14		0.245	0.200	0.216	0.024	0.21	0.23
d 0 to 14		0.221	0.191	0.185	0.019	0.31	0.38

[a] Means of six observations per treatment group.

[b] Nucleoside deficient (ND), Nucleoside semi-deficient (NSD), and Nucleoside adequate (NA).

Table 4.5. Microbial count (log_{10} cfu/g) in fecal samples of pigs fed different nucleoside levels in the diet (Exp 1) [a]

Item	Treatment:	ND [b]	NSD [b]	NA [b]	SEM	Linear effect	Quadratic effect
Total coliforms	d 0	7.99	8.16	8.04	0.152	0.45	0.44
	d 7	8.19	7.78	7.48	0.313	0.39	0.51
	d 14	8.25	7.71	7.60	0.296	0.23	0.30
SEM		0.244	0.338	0.186			
Linear effect		0.67	0.54	0.08			
Quadratic effect		0.82	0.71	0.16			
E. coli	d 0	7.49	7.46	7.51	0.213	0.92	0.90
	d 7	8.02	7.41	7.22	0.321	0.21	0.29
	d 14	7.98	7.47	7.54	0.341	0.31	0.35
SEM		0.261	0.393	0.206			
Linear effect		0.24	0.92	0.28			
Quadratic effect		0.39	0.91	0.25			
Cl. perfringens	d 0	7.51	7.58	7.86	0.230	0.86	0.99
	d 7	6.08	5.77	5.04	0.280	0.50	0.78
	d 14	4.76	4.26	3.00	0.105	0.01	0.29
SEM		0.266	0.204	0.172			

Linear effect		0.05	<0.01	< 0.01			
Quadratic effect		0.88	0.56	0.09			
Bifidobacterium spp.	d 0	7.87	8.04	7.95	0.281	0.68	0.68
	d 7	7.77	8.30	8.25	0.190	0.07	0.10
	d 14	7.68	8.35	8.32	0.217	0.05	0.08
SEM		0.294	0.154	0.228			
Linear effect		0.89	0.37	0.48			
Quadratic effect		0.98	0.58	0.68			
L. acidophilus	d 0	8.87	8.61	8.82	0.158	0.26	0.24
	d 7	8.72	9.06	9.06	0.167	0.18	0.22
	d 14	8.82	9.33	9.21	0.125	0.01	0.02
SEM		0.141	0.124	0.181			
Linear effect		0.45	0.10	0.54			
Quadratic effect		0.47	0.54	0.83			

[a] Means of six observations per treatment group.

[b] Nucleoside deficient (ND), Nucleoside semi-deficient (NSD), and Nucleoside adequate (NA).

Table 4.6. Serum IgG concentration in pigs fed different levels of nucleosides in the diet (mg/dl) [a]

Item	Treatment:	ND [b]	NSD [b]	NA [b]	SEM	Linear effect	Quadratic effect
d 0		944	827	942	103.9	0.43	0.40
d 7		693	653	699	65.9	0.67	0.64
d 14		558	586	618	62.5	0.77	0.84
SEM		78.91	75.08	84.65			
Linear effect		<0.01	0.03	0.01			

[a] Means of six observations per treatment group.

[b] Nucleoside deficient (ND), Nucleoside semi-deficient (NSD), and Nucleoside adequate (NA).

Table 4.7. Changes (log_{10} cfu/g) in probiotic and pathogenic microflora in broth without or with nucleoside supplementation (Exp. 2) [a]

Item	h	Broth:	Control	Nucleosides	SEM	*P* value
E. coli	0		6.45	6.26	0.087	0.26
	4		8.40	8.30	0.125	0.63
	8		8.39	8.18	0.046	0.09
	12		8.10	7.76	0.079	0.09
	16		8.15[x]	7.82[y]	0.059	0.05
SEM			0.091	0.076		
Linear effect			<0.01	<0.01		
Quadratic effect			<0.01	<0.01		
Cl. perfringens	0		6.28	6.23	0.026	0.30
	4		5.98	5.74	0.524	0.78
	8		5.60[x]	6.20[y]	0.000	<0.01
	12		6.00	6.48	0.336	0.42
	16		6.30[x]	6.82[y]	0.025	<0.01
SEM			0.014	0.394		
Linear effect			<0.01	0.58		
Quadratic effect			<0.01	0.30		
Bifidobacterium spp.	0		7.17	7.18	0.010	0.55

	4	7.63	7.83	0.046	0.09
	8	8.09	8.29	0.063	0.16
	12	8.33[x]	8.57[y]	0.022	0.02
	16	8.22	8.67	0.125	0.13
SEM		0.080	0.050		
Linear effect		<0.01	<0.01		
Quadratic effect		<0.01	<0.01		
L. acidophilus	0	5.93	6.05	0.363	0.84
	4	6.32	6.27	0.318	0.93
	8	6.19	6.32	0.388	0.83
	12	6.15	6.48	0.352	0.58
	16	6.74	6.98	0.531	0.78
SEM		0.449	0.337		
Linear effect		0.99	0.96		
Quadratic effect		0.75	0.56		

[a] Means of two observations per treatment group.

[x, y] Means within a row lacking a common superscript differ ($P < 0.05$).

CHAPTER 5

Effects of dietary nucleotides on intestinal microbial activity and performance of newly weaned pigs

ABSTRACT: Two experiments were conducted to determine the effects of adding nucleotides to starter diets for weanling pigs. In Exp. 1, 20 pigs were weaned at 19.4 ± 1 d of age and randomly allotted to two treatment groups in a completely randomized design. Pigs allotted to Treatment 1 were fed a conventional starter diet. Pigs allotted to Treatment 2 were fed this diet supplemented with nucleotides in amounts that correspond to 100% of the quantities found in sow milk (DM-basis) on d-14 of lactation. Five pigs from each treatment group were sacrificed on d-14 and the remaining 5 pigs were sacrificed on d-28 post-weaning. Pig performance, serum IgG concentration, intestinal morphology, intestinal microflora composition, and intestinal tissue DNA, RNA, and protein content were measured to determine the effects of nucleotide supplementation. No differences between treatment groups were observed for pig performance, serum IgG or intestinal tissue DNA, RNA, and protein content. Likewise, the concentration of Bifidobacterium spp., *Cl. perfringens*, and *L. acidophilus* in intestinal contents was not different between treatment groups. On d-14, duodenal villus height (VH) was lower (P = 0.03) in pigs fed the nucleotide-supplemented diet vs. pigs fed the control diet (353 vs. 426 μm), but ileal VH and villus height:lamina propria depth (VH:LPD) was higher (P = 0.01) in pigs fed the nucleotide-supplemented diet vs. pigs fed the control diet (321 vs.

239 μm and 1.56 vs. 1.23 μm, respectively). On d-28, duodenal lamina propria depth (LPD) was greater ($P = 0.02$) and ileal VH:LPD was higher ($P = 0.04$) in pigs fed the nucleotide-supplemented diet vs. pigs fed the control diet (398 vs. 326 μm and 1.78 vs. 1.38 μm, respectively). Exp. 2 was an in-vitro experiment that was conducted to determine bacterial growth in a broth without (control) or with nucleotide supplementation measured in duplicate over a 16-h period. Results showed that the nucleotide-supplemented broth had a higher ($P = 0.03$) total coliform count vs. the control after 8 h of incubation (10.22 vs. 10.12 $\log_{10}$ cfu/g). These observations show that nucleotides may serve as an energy source for microbes. After 16 h of incubation, the nucleotide-supplemented broth also had a lower *Cl. perfringens* count ($P < 0.01$) vs. the control (6.78 vs. 7.15 $\log_{10}$ cfu/g). Data from the two experiments indicate that nucleotide supplementation during the post-weaning period positively influences gastrointestinal morphology and microflora.

Key Words: DNA, Immunoglobulins, Microflora, Morphology, Nucleotides, Pigs

Introduction

It was recently reported that weanling pigs ingest lower quantities of nucleotides when they are fed post-weaning starter diets compared to what they obtain from sow milk (Mateo et al., 2005). Pigs can synthesize nucleotides but energy and glutamine are required for this synthesis. Because weanling pigs are often deficient in both energy and glutamine they may not be able to synthesize sufficient quantities to cover the needs for

all body tissues. Both an in vivo and an in vitro experiment were conducted to investigate the effects of dietary nucleoside supplementation during the post-weaning period (Mateo et al., 2005). The authors reported that dietary nucleoside supplementation during the immediate post-weaning period in pigs may positively influence the gastrointestinal microflora by decreasing enterobacteria and increasing *L. acidophilus* and Bifidobacterium species. Because dietary nucleotides are digested to nucleosides in the small intestine, it is possible that a similar response will be obtained by supplementing starter diets for weanling pigs with nucleotides. It has previously been reported that nucleotide supplementation to starter diets for pigs increased the development of the immune system (Zomborsky-Kovacs et al., 1998). It has also been reported that weanling pigs fed nucleic acids (i. e., yeast RNA) for 2 to 4 wk improved lymphocyte function and in-vitro proliferative responses to a non-specific T-cell mitogen (Cameron et al., 2001).

Based on previous research it was hypothesized that nucleotide supplementation of diets for weanling pigs may support the development of the immune system and the small intestine. The current experiments were conducted to test this hypothesis.

Materials and methods

Experiment 1

Animals, housing, and experimental design

Twenty pigs were weaned at 19.4 ± 1 d of age (BW: 6.3 ± 0.5 kg) and randomly allotted to one of two treatment groups according to ancestry, sex, and BW in a completely randomized design. There were 10 replicate pens per treatment and 1 pig per

pen. Pigs were obtained from the South Dakota State University Swine Research Farm and housed in 1.2- x 1.4-m nursery pens. A feeder and a nipple drinker were installed in each pen. Room temperature was maintained at 30 to 32^{o}C. Animal care procedures were approved by the South Dakota State University Animal Care and Use Committee (#04-E-002).

Diets and feeding

Two diets were prepared (Table 1). The control diet was a conventional pig starter diet. The nucleotide-supplemented diet was identical to the control diet, but was supplemented with nucleotides in amounts similar to that found in sow milk (DM-basis) on d-14 of lactation (Mateo et al., 2004). Diets were formulated to meet or exceed recommendations for all nutrients for 3- to 5-kg pigs (NRC, 1998) and they were provided in a meal form. All diets were provided on an ad libitum basis during four weeks post-weaning. Water was available at all times throughout the experiment.

Sample collection

On d-14 post-weaning, a 10-mL blood sample was collected from the jugular vein of each pig using a vacutainer tube and an 18-G needle. Blood samples were centrifuged at 3000 rpm for 30 minutes after collection. Serum was harvested and stored at -20^{o}C.

On d-14 post-weaning, five pigs per treatment were randomly selected, sacrificed, and intestinal tissues were recovered. Pigs were sacrificed by administering 1 mL per 4.5 kg BW of pentobarbital sodium (SP6 Euthanasia 6-Grain®, VETCO, Inc. St. Joseph, MO) intravenously. Confirmation of death was based on the cessation of the animals' heartbeat. Standard necropsy procedures were conducted. The small intestine was

separated from other organs and weighed. The mesenteric web was cut and the total length of the small intestine was measured and recorded.

A 10-cm small intestinal tissue sample was obtained from 33% (i. e., duodenum), 66% (i. e., jejunum), and 100% (i. e., distal ileum) of the length of the small intestine measured from the pyloric sphincter. The intestinal tissue section was tied with a cotton string on both ends and cut. Five to ten mL of 10% neutral buffered formalin was injected into each sample to obtain a natural size of the intestine and placed in a plastic bag containing formalin for sample fixation.

An additional tissue sample (2 cm in length) was obtained from the jejunum of each pig. This sample was split longitudinally and washed with phosphate buffer saline (PBS), wiped on paper towels, placed in Eppendorf tubes, and stored at -80^{o}C.

On d-28 post-weaning, a blood sample was collected from the remaining 10 pigs. These pigs were then sacrificed and samples were collected using procedures similar to that used for the pigs that were sacrificed on d-14. In addition, samples of intestinal contents were taken from the ileum, the cecum, and the distal colon by tying both ends of the intestinal section (10 cm in length) with a cotton string and separating it from the rest of the intestines. Each segment was placed in a plastic bag and stored at 4^{o}C prior to intestinal microflora composition analysis.

Data recording and sample analysis

Individual pig weights were recorded at the beginning of the experiment, at the end of the second wk, and at the end of the experiment. The amount of feed provided in each feeder was recorded daily and orts were subtracted from the total amount of feed

supplied in the feeder to calculate total feed disappearance. Average daily weight gain and ADFI for each pen were summarized and used to calculate G:F for the initial 2 weeks, the following 2 weeks, and for the entire experimental period.

Serum samples were thawed and analyzed for IgG concentration using a radial immunodiffusion kit (Bethyl Laboratories, Inc. Montgomery, TX) according to the method described by Mateo et al. (2005).

The villous height (VH) and lamina propria depth (LPD) was measured in the intestinal samples collected from the duodenum, the jejunum, and the ileum. The section of intestinal sample collected remained in formalin for a 2 to 4-d fixation period. A ring shaped section was cut from each intestinal section, placed in a tissue cassette, and stored in formalin. Standard paraffin sectioning was conducted on each sample and stained with hematoxylin and eosin (H and E). All samples were dehydrated with alcohol using a tissue processor. Pictures of selected slides from duodenum, jejunum, and ileum of the small intestine were taken at 10-X magnification with the aid of a stereomicroscope with a mounted video camera screen. From the pictures taken, five of the tallest well-oriented villi and their associated LPD were measured with the aid of image analysis software plugged into the photoshop software (Adobe Photoshop™ San Jose, CA). Intestinal section samples were evaluated based on changes in VH, LPD, and villus height to lamina propria depth ratio (VH:LPD).

Frozen jejunum tissue samples were ground in liquid N for approximately 5 minutes using a frozen mortar and pestel. Proteins were extracted by adding 0.5 mL of M-PER®-Mammalian Protein Extraction Reagent (Pierce Rockford, IL) + NaCl to the

pulverized jejunum tissue sample (30 to 50 mg). After homogenization, the solution was shaken for 10 minutes at room temperature, and then subjected to centrifugation (14,000 g for 15 minutes at room temperature). The supernatants were diluted with the addition of 20-μL extracted supernatant to 80 μL of M-PER® and analyzed by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Tissue RNA and DNA were extracted from the jejunum tissue and analyzed by the method of Uni et al. (1955) using TRI REAGENT®-RNA/DNA Isolation Reagent (Molecular Research Center, Inc. Cincinnati, OH).

Contents of the stomach, the ileum, the cecum, and the distal colon were collected, mixed, and the pH was measured using a pH-meter (PerpHecT® model 330 Thermo Orion Beverly, MA).

Microbial analysis for Bifidobacterium spp., *Cl. perfringens*, and *L. acidophilus* was conducted after 24 h as described by Mateo et al. (2005). Briefly, serial dilutions of aseptically collected samples were prepared by mixing 1 g of intestinal content with 99 mL of peptone water, using a Stomacher blender (Ali and Fung, 1991; Dave and Shah, 1996; Fung, 2000). Subsequent dilutions of up to 10^{-8} were prepared using 9-mL peptone water diluents mixed with 1 mL of the previous dilution. Sample dilutions were prepared within an hour to minimize changes in the microbial populations of intestinal content samples.

Data analysis

An analysis of variance using the PROC MIXED procedure of SAS (SAS Stat Inc., Cary, NC) was used in this experiment. The model included time and treatment as

the main effects to evaluate ADG, ADFI, G:F, serum IgG concentration, intestinal weight, length, and weight:length, VH, LPD, VH:LPD, intestinal protein, RNA, DNA, RNA:DNA, DNA:protein, RNA:protein, and pH. To evaluate intestinal microflora composition on d-28, treatment was used as the main effect. In order to increase the normality of distribution and decrease variation, bacterial concentration was converted to a $\log_{10}$ basis before statistical analysis was performed. The pig was used as the experimental unit for the analysis of ADG, ADFI, G:F, serum IgG concentration, intestinal weight and length, intestinal weight:length, intestinal tissue protein, RNA, DNA, RNA:DNA, DNA:protein, RNA:protein, pH, and intestinal microflora composition. Individual villi and their associated LPD were used as the experimental unit for the analysis of VH, LPD, and VH:LPD. Least square means were calculated and separated using the PDIFF option of SAS. A *P*-value of less than 0.05 was used to assess significance between treatments.

Experiment 2

Exp. 2 was an in-vitro experiment that comprised two phases. The first phase was microbial isolation and activation, and the second phase was a growth assay to determine the effects of supplementing nucleotides on bacterial growth over a 16-h period. Measurements were taken every 4 h for Bifidobacterium spp., *Cl. perfringens*, *E. coli*, *L. acidophilus*, and total coliform growth in broth without (control) and with nucleotide supplementation. Phase 1 was conducted as described by Mateo et al. (2005). In Phase II, however, nucleotides were added instead of nucleosides. Powdered adenosine 5'monophosphate (0.2 g) (Fisher Scientific Hampton, NH), cytidine 5'monophosphate

(0.1 g), guanosine 5'monophosphate (0.3 g), inosine 5'monophosphate (0.03 g), and uridine 5'monophosphate (4.0 g) nucleotides (Sigma-Aldrich Co. St. Louis, MO) were dissolved in five separate 50-mL beakers containing 50 mL of deionized water. Three mL of adenosine 5'monophosphate (5'AMP), cytidine 5'monophosphate (5'CMP), and uridine 5'monophosphate solution (5'UMP) was aspirated from their respective beakers, and mixed into the treatment bottle to provide 0.012%, 0.006%, and 0.236% of 5'AMP, 5'CMP, and 5'UMP, respectively. Likewise, 3.2 mL of guanosine 5'monophosphate (5'GMP) and 3.2 mL of inosine 5'monophosphate (5'IMP) solution was aspirated and mixed into the treatment bottle to provide 0.019% and 0.002% of 5'GMP and 5'IMP, respectively. The nucleotide inclusion rates used in this study were similar to that found in sow milk on d-14 of lactation on a DM basis (Mateo et al., 2004).

Data analysis

An analysis of variance using the PROC MIXED procedure of SAS (SAS Stat Inc., Cary, NC) was used to analyze the data. The model included time and treatment as the main effects to evaluate microbial count. In order to increase the normality of distribution and decrease variation, bacterial concentration was converted to a $\log_{10}$ basis before statistical analysis was performed. The plate was used as the experimental unit. Contrast statements of SAS were used to determine linear and quadratic effects. Least square means were calculated and separated using the PDIFF option of SAS. An alpha level of less than 0.05 was used to assess significance between treatments.

Results

Experiment 1

Pig performance. No differences between treatment groups were observed for ADG, ADFI, and G:F during the initial 2 weeks, the final two weeks, or for the entire experiment (Table 2).

Serum IgG concentration. No differences between dietary treatments were observed for serum IgG concentration on d-14 or on d-28 post-weaning (Table 3).

Weight and length of the small intestine. No differences between treatment groups were observed for weight, length, and weight:length of the small intestine on d-14 (Table 4). On d-28, pigs fed the nucleotide-supplemented diet tended to have a longer ($P = 0.06$) small intestine compared to the intestine of pigs fed the control diet (12.3 vs. 10.9 m), but no differences in weight or weight:length were observed.

Intestinal morphology. On d-14, duodenal VH was lower ($P = 0.03$) in pigs fed the nucleotide-supplemented diet compared to pigs fed the control diet (353 vs. 426 μm) (Table 5). In contrast, ileal VH was higher ($P = 0.01$) in pigs fed the nucleotide-supplemented diet compared to pigs fed the control diet (321 vs. 239 μm). No differences between treatment groups were observed for duodenal and ileal VH on d-28. Likewise, no differences between treatment groups were observed for VH in the jejunum on d-14 or on d-28.

On d-28, duodenal LPD was greater ($P = 0.02$) in pigs fed the nucleotide-supplemented diet compared to pigs fed the control diet (398 vs. 326 μm). In contrast,

jejunal LPD was lower ($P = 0.05$) in pigs fed the nucleotide-supplemented diet compared to pigs fed the control diet on d-14 (235 vs. 279 μm). No differences between treatment groups were observed for duodenal LPD on d-14 and jejunal LPD on d-28. Likewise, no differences between treatment groups were observed for LPD in the ileum on d-14 or on d-28. Similarly, no differences between treatment groups were observed for VH:LPD in the duodenum and jejunum on d-14 or on d-28. However, VH:LPD in the ileum was greater ($P = 0.01$) for pigs fed the nucleotide-supplemented diet vs. pigs fed the control diet (1.56 vs. 1.23 μm) on d-14. The VH:LPD in the ileum was greater ($P = 0.04$) for pigs fed the nucleotide-supplemented diet vs. pigs fed the control diet (1.78 vs. 1.38 μm) on d-28.

Using data pooled from both treatment groups, intestinal VH and LPD progressively decreased ($P < 0.01$) from the duodenum to the jejunum, and from the jejunum to the ileum on d-14 and on d-28 post-weaning (Table 6). In contrast, the VH:LPD did not differ from the duodenum to the jejunum or from the jejunum to the ileum.

DNA, RNA, and protein concentration. No differences between treatment groups were observed for DNA, RNA, protein concentration, RNA:DNA, DNA:protein or RNA:protein in jejunal intestinal tissues on d-14 or on d-28 post-weaning (Table 7).

Measurement of pH. No differences between treatment groups were observed for pH in the contents of the stomach, the distal ileum, the cecum, or the distal colon on d-14 or on d-28 (Table 8). However, pH was lower ($P < 0.01$) in the stomach (pH ranged from

2.67 to 3.34) compared to the distal ileum, the cecum, and the distal colon (pH ranged from 6.01 to 6.82).

Microbial analysis. No differences between treatment groups were observed for Bifidobacterium spp., *Cl. perfringens*, and *L. acidophilus* counts in intestinal contents collected from the ileum, the cecum, and the colon (Table 9).

Experiment 2

No differences between treatment groups were observed for Bifidobacterium spp. count from 0 to 12 h of incubation (Table 10). However, broth supplemented with nucleotides tended to decrease ($P = 0.08$) Bifidobacterium spp. count compared to broth without nucleotide supplementation (10.45 vs. 10.54 $\log_{10}$ cfu/g) after 16 hours of incubation. The Bifidobacterium spp. count linearly increased ($P < 0.01$) from 0 to 16 h in both treatment groups. No differences between treatment groups were observed for *L. acidophilus* count. However, the *L. acidophilus* count linearly increased ($P < 0.01$) from 0 to 16 h in both treatment groups.

The broth supplemented with nucleotides increased ($P = 0.03$) total coliform count compared to the broth without nucleotide supplementation after 8 h (10.22 vs. 10.12 $\log_{10}$ cfu/g). Total coliform count tended to further increase after 12 h ($P = 0.08$) and 16 h ($P = 0.07$) in the broth supplemented with nucleotides compared to the broth without nucleotide supplementation (10.43 vs. 10.35 and 10.54 vs. 10.31 $\log_{10}$ cfu/g, respectively). The total coliform count linearly increased ($P < 0.01$) from 0 to 16 h in both treatment groups.

The broth supplemented with nucleotides tended to increase ($P = 0.05$) the *E. coli* count compared to the broth without nucleotide supplementation (9.86 vs. 9.62 $\log_{10}$ cfu/g) after 4 h of incubation (Table 10). The *E. coli* count linearly increased ($P < 0.01$) from 0 to16 h in both treatment groups.

After 16 h of incubation, the broth supplemented with nucleotides had a lower ($P < 0.01$) *Cl. perfringens* count compared to the broth not supplemented with nucleotides (6.78 vs. 7.15 $\log_{10}$ cfu/g). Similarly, the broth treated with nucleotides tended to have a lower ($P = 0.08$) *Cl. perfringens* count compared to the broth not supplemented with nucleotides after 12 h (7.61 vs. 7.72 $\log_{10}$ cfu/g). The *Cl. perfringens* count linearly increased ($P < 0.01$) from 0 to 16 h in both treatment groups.

Discussion

Pig performance. The reason why there was no effect of nucleotide supplementation on pig performance may be that the pigs used in this experiment were housed individually and were not as disease challenged as pigs kept under commercial conditions. Therefore, these pigs were likely less stressed than commercial pigs. It has been suggested that the positive effect of nucleotides or nucleosides on growth performance may be seen under conditions of infection pressure (Maribo, 2003).

Serum IgG concentration. A regulatory role of dietary nucleotides in immunohematopoiesis has been reported (Kulkarni et al., 1992). Dietary supplementation of purified nucleotides to milk replacers of newborn bull calves challenged with lipopolysaccharides (LPS), resulted in calves that tended to have higher mean IgG

concentration compared with the unsupplemented control calves (Oliver et al., 2003). In contrast, nucleotide supplementation had no effect on serum IgG concentration in the current study. The absence of a response in serum IgG concentration to dietary nucleotide supplementation may have been due to an absence of a pathogenic challenge.

Weight and length of the small intestine. Pigs fed the nucleotide-supplemented diet tended to have an increased small intestinal length compared to the small intestine of pigs fed the control diet. Because nucleotides are the building blocks of nucleic acids and growth of new tissue requires RNA and DNA, this observation may be due to an increase in intestinal tissue growth with the addition of nucleotides in the diet. The weight and the weight:length of the small intestine did not differ between treatment groups. These results disagree with the findings of Carver (1994) who reported that feeding 0.21% of dietary nucleotides to weanling mice was associated with an increase in small intestinal weight (as a percent of BW) and weight:unit length.

Intestinal morphology. Proliferation of enterocytes as reflected by VH is assumed to be indicative of the growth of intestinal absorptive tissue. The results of the current study indicate that ileal VH and VH:LPD, but not ileal LPD, was increased by nucleotide supplementation. The increase in VH:LPD in the ileum of pigs fed diets supplemented with nucleotides suggest that nucleotides increase cell proliferation and growth (Tsujunaka et al., 1993). This may indicate that nucleotides have trophic effects on the intestinal mucosa.

DNA, RNA, and protein concentration. In a chick study conducted by Uni et al. (1955), DNA concentration, DNA:protein, RNA:DNA, and RNA:protein were used to

estimate the rate of mitosis, cell size, tissue activity, and ribosomal capacity, respectively. The absence of differences between treatment groups in the current study contradicts the findings of other researchers (Nunez et al., 1990; Uauy et al., 1990) who found increased mucosal protein and DNA concentration in the intestine of rats fed diets supplemented with dietary nucleotides. In the current investigation, nucleotide supplementation increased VH:LPD in the ileum of pigs, but this was not complimented by an increase in intestinal DNA, RNA, protein, RNA:DNA, RNA:protein, and DNA:protein concentration. This may be because the values for intestinal DNA, RNA, and protein concentration were taken from whole intestinal sections and not just the villi in the jejunum. In future experiments, it may be helpful to look at the DNA and RNA concentrations specifically in the villi or in the crypts in the small intestine.

pH measurement. The differences in pH in the gastrointestinal tract contents between the stomach and the small and the large intestine were expected. Production of HCL by parietal cells of the fundic glands decreased the pH in stomach contents compared to that found in the small and the large intestine contents. Bacterial growth is highly dependent on environmental pH. Therefore, a change in pH is an indication of a possible increase or decrease in a specific microbial population. The results in this study indicate that pH was not affected by the addition of nucleotides to the diet. Therefore, bacterial growth was similar between treatment groups. This hypothesis was verified with the findings in the in vitro experiment that showed no differences between treatment groups after 16 h of incubation for Bifidobacterium spp., *E. coli*, and *L. acidophilus* counts. In contrast, differences between treatment groups were observed for total

coliforms and *Cl. perfringens* counts. This finding may suggest that the total coliforms and *Cl. perfringens* are more responsive to nucleotide supplementation in pure culture compared to Bifidobacterium spp., *E. coli*, and *L. acidophilus.*

Microbial analysis. The absence of differences in intestinal content microbial count between treatment groups in this study contradicts the findings of other researchers (Tanaka and Mutai, 1980; Uauy, 1994), who reported a predominance of probiotic bacteria compared to pathogenic bacteria when nucleotides or nucleosides were supplemented in the diet. However, their findings where based on stool samples from infants who consumed infant milk formula supplemented with nucleotides and not intestinal content samples used in this study. A predominance of probiotic bacteria compared to pathogenic bacteria was observed in the feces of pigs when nucleosides were supplemented to their diet (Mateo et al., 2005). This observation may suggest that dietary nucleotides influence gastrointestinal microflora in the distal end of the gastrointestinal tract and not in the proximal end or that nucleotides are metabolized differently from nucleosides. A lack of enzymes that cleave off and digest the P group from the nucleotide molecule may also explain this finding, however, these speculations need to be experimentally proven. Results of the microbial analysis in Exp. 1 were further strengthened with the findings in Exp. 2 that showed no differences between treatment groups after 16 h of incubation for Bifidobacterium spp., *E. coli*, and *L. acidophilus* counts.

The increase in total coliform count from 8 to 16 h in broth supplemented with nucleotides indicates that these bacteria may use nucleotides as an additional nutrient for

growth. Partial inhibition of *Cl. perfringens* from the 12th to the 16th h of growth may have been due to one or more of the nucleotides present in the growth medium that created unfavorable conditions for *Cl. perfringens* and as a result decreased their counts after 12 h of incubation.

Combining the findings from the in vivo and the in vitro studies suggest that nucleotides may restrict the proliferation of *Cl. perfringens* and may provide a more favorable environment for total coliforms. These results are similar to the findings of Mateo et al. (2005) who demonstrated a decrease in the concentration *Cl. perfringens* count in the feces of pigs fed diets supplemented with nucleosides.

Implications

The findings from the present experiments indicate that pigs fed diets supplemented with nucleotides may decrease potentially pathogenic bacteria that may reduce scouring during the post-weaning period. In addition, nucleotide supplementation may increase VH:LPD in the ileum, which may result in improved intestinal morphology and improved nutrient uptake.

Literature cited

Ali, M. S., and D. Y. C. Fung. 1991. Occurrence of *Clostridium perfringens* in ground beef and ground turkey evaluated by three methods. J. Food Safety 11:197-203.

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

Carver, J. D. 1994. Dietary nucleotides: cellular immune, intestinal, and hepatic system effects. J. Nutr. 124(Suppl. 1):144-148.

Dave, R. I., and N. P. Shah. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, Lactobacillus delbrueckii ssp. bulgaricus, *Lactobacillus acidophilus* and Bifidobacteria. J. Dairy Sci. 79:1529-1536.

Fung, D. Y. C. 2000. Handbook for rapid methods and automation in microbiology. International Workshop XX. Kansas State University, Manhattan, KS, USA, July 6-14, 2000.

Kulkarni, A. D., W. C. Fanslow, F. B. Rudolph, and C. T. Van Buren. 1992. Immunohematopoietic effects of dietary nucleotide restriction in mice. Transplantation 53:467-472.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with phenol reagent. J. Biol. Chem. 139:265-275.

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.

Mateo, C. D., D. N. Peters, and H. H. Stein. 2004. Nucleotides in sow colostrum and milk at different stages of lactation. J. Anim. Sci. 82:1339-1342.

Mateo, C. D., D. N. Peters, R. I. Dave, and H. H. Stein. 2005. Effects of dietary nucleosides on intestinal microbial activity of newly weaned pigs. J. Anim. Sci. (In press).

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

Nunez, M. C., M. V. Ayudarte, D. Morales, M. D. Suarez, and A. Gil. 1990. Effect of dietary nucleotides on intestinal repair in rats with experimental chronic diarrhea. J. Parenter. Enter. Nutr. 14:598-604.

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.)

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

Tsujunaka, T., S. Iijima, Y. Kido, T. Homma, C. Ebisui, K. Kan, I. Imamura, H. Fukui, and T. Mori. 1993. Role of nucleoside and nucleotide mixture in intestinal mucosal growth under total parenteral nutrition. Nutrition 9:532-535.

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.

Uni, Z., Y. Noy, and D. Sklan. 1955. Development of the small intestine in heavy and light strain chicks before and after hatching. Br. Poultry Sci. 36:63-71.

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

Table 5.1. Composition of the experimental diets (as-fed basis)

Item	Treatment:	Control	Nucleotides
Ingredient, %			
Corn		47.82	47.54
Whey, dried		20.00	20.00
Soybean meal, 44%		14.00	14.00
Fish meal		8.00	8.00
Spray dried plasma protein		3.00	3.00
Soybean oil		5.00	5.00
Limestone		1.30	1.30
L-lysine HCL		0.09	0.09
DL-methionine		0.04	0.04
Salt		0.40	0.40
Vitamin premix [a]		0.10	0.10
Trace mineral premix [b]		0.25	0.25
Adenosine 5'monophosphate [d]		-	0.012
Cytidine 5'monophosphate [e]		-	0.005
Guanosine 5'monophosphate [f]		-	0.019
Inosine 5'monophosphate [g]		-	0.002
Uridine 5'monophosphate [h]		-	0.238
Total		100.00	100.00

Analyzed nutrient composition		
Gross Energy, kcal/kg	4,238	4,251
Crude Protein, %	23.28	22.74
Calcium [c], %	0.92	0.92
Phosphorous [c], %	0.66	0.66
Indispensable AA		
Arginine, %	1.24	1.20
Histidine, %	0.56	0.53
Isoleucine, %	0.90	0.88
Leucine, %	1.90	1.85
Lysine, %	1.53	1.46
Methionine, %	0.89	0.88
Phenylalanine, %	0.98	0.95
Threonine, %	1.15	1.10
Tryptophan, %	0.26	0.26
Valine, %	1.10	1.07
Dispensable AA		
Alanine, %	1.18	1.18
Aspartate, %	2.07	1.98
Cysteine, %	0.28	0.28

Glutamic acid, %	3.48	3.33
Glycine, %	1.00	0.98
Proline, %	1.20	1.16
Serine, %	0.84	0.82
Tyrosine, %	0.76	0.74
Nucleotides		
Adenosine 5'monophosphate, %	0.038	0.114
Cytidine 5'monophosphate, %	0.000	0.054
Guanosine 5'monophosphate, %	0.000	2.008
Inosine 5'monophosphate, %	0.150	0.152
Uridine 5'monophosphate, %	0.000	0.130

[a] Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 21,980 IU as vitamin A acetate; vitamin B_{12}, 88 mcg; vitamin D_3, 3,296 IU as D-activated sterol; vitamin E, 110 IU as DL-α-tocopheryl acetate; vitamin K_3, 8.8 mg as menadione dimethylpyrimidinol bisulfite; biotin, 0.34 mg; D-pantothenic acid, 66 mg as calcium pantothenate; folic acid, 2.2 mg; niacin, 110 mg; pyridoxine, 6.6 mg as pyridoxine hydrochloride; riboflavin, 19.8 mg; and thiamin, 6.6 mg as thiamine mononitrate.

[b] Provided the following quantities of trace minerals per kg of complete diet: Cu, 16.5 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.30 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

[c] Values for Ca and P were calculated while all other values were analyzed.

[d] 5'AMP disodium salt purchased from Fisher Scientific Hampton, NH.

[e] 5'CMP ($C_9H_{14}N_3O_8P$; FW: 323.2), minimum 98-100% purchased from Sigma-Aldrich Co. St. Louis, MO.

[f] 5'GMP ($C_{10}H_{12}N_5O_8PNa_2$; FW: 407.2), minimum 99% purchased from Sigma-Aldrich Co. St. Louis, MO.

[g] 5'IMP ($C_{10}H_{13}N_4O_8P$; FW: 348.2), minimum 98-100% purchased from Sigma-Aldrich Co. St. Louis, MO.

[h] 5'UMP ($C_9H_{11}N_2O_9PNa_2$; FW: 368.1), minimum 98% purchased from Sigma-Aldrich Co. St. Louis, MO.

Table 5.2. Performance of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item	Treatment:	Control	Nucleotides	SEM	*P* value
Average daily gain, kg					
d 14		0.136	0.145	0.020	0.75
d 28		0.469	0.487	0.053	0.82
d 0 to 28		0.263	0.299	0.046	0.60
Average daily feed intake, kg					
d 14		0.218	0.206	0.025	0.71
d 28		0.676	0.644	0.059	0.71
d 0 to 28		0.419	0.424	0.048	0.94
Gain:Feed, kg/kg					
d 14		0.624	0.842	0.197	0.42
d 28		0.688	0.753	0.035	0.24
d 0 to 28		0.598	0.702	0.057	0.25

[a] Means of 10 and 5 observations per treatment group on d-14 and on d-28 post-weaning, respectively.

Table 5.3. Serum IgG concentration (mg/dL) of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item	Treatment:	Control	Nucleotides	SEM	*P* value
Serum IgG					
d 14		959	748	133.5	0.28
d 28		513	619	94.1	0.46

[a] Means of 10 and 5 observations per treatment group on d-14 and on d-28 post-weaning, respectively.

Table 5.4. Weight, length, and weight:length ratio of the small intestine of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item	Treatment:	Control	Nucleotides	SEM	*P* value
Weight, g					
d 14		519	455	96.0	0.65
d 28		849	846	75.4	0.98
Length, m					
d 14		9.3	8.5	0.73	0.45
d 28		10.9	12.3	0.44	0.06
Weight:length, g/m					
d 14		55.8	53.5	9.6	0.98
d 28		77.8	68.7	4.2	0.20

[a] Means of 5 and 4 observations per treatment group on d-14 and on d-28 post-weaning, respectively.

Table 5.5. Villus height (VH), lamina propria depth (LPD), and VH:LPD ratio of the small intestine in weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item	Treatment: Control	Nucleotides	SEM	*P* value
VH, (μm)				
Duodenum				
d 14	426 [x]	354 [y]	22.6	0.03
d 28	490	476	22.6	0.66
Jejunum				
d 14	333	330	22.6	0.91
d 28	393	435	22.6	0.20
Ileum				
d 14	239 [x]	322 [y]	22.6	0.01
d 28	349	396	22.6	0.14
LPD, (μm)				
Duodenum				
d 14	269	261	15.8	0.72
d 28	327 [x]	398 [y]	21.4	0.02
Jejunum				
d 14	279 [x]	235 [y]	15.8	0.05
d 28	309	307	21.4	0.96
Ileum				
d 14	205	216	15.8	0.62

d 28	279	240	21.4	0.20
VH:LPD				
Duodenum				
d 14	1.64	1.41	0.10	0.09
d 28	1.70	1.45	0.14	0.22
Jejunum				
d 14	1.31	1.49	0.10	0.19
d 28	1.29	1.46	0.14	0.37
Ileum				
d 14	1.23 [x]	1.56 [y]	0.10	0.01
d 28	1.38 [x]	1.78 [y]	0.14	0.04

[a] Means of 75 and 60 observations per treatment group on d-14 and on d-28 post-weaning, respectively.

[x, y] Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 5.6. Villus height (VH) and lamina propria depth (LPD), and VH:LPD ratio in the duodenum (D), the jejunum (J), and the ileum (I) of the small intestine of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item	Small intestine:	D	J	I	SEM	*P* value
d 14						
VH, (μm)		390 [x]	332 [y]	280 [z]	29.3	<0.01
LPD, (μm)		265 [x]	257 [x]	210 [y]	23.8	<0.01
VH:LPD		1.52	1.40	1.41	0.09	0.35
d 28						
VH, (μm)		483 [x]	414 [y]	373 [y]	16.0	<0.01
LPD, (μm)		363 [x]	308 [y]	260 [z]	15.1	<0.01
VH:LPD		1.58	1.37	1.58	0.10	0.24

[a] Means of 150 and 120 observations on d-14 and on d-28 post-weaning, respectively.

[x, y] Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 5.7. DNA, RNA, protein concentration, RNA:DNA ratio, DNA:protein ratio, and RNA:protein ratio in the jejunum of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item Treatment:	Control	Nucleotides	SEM	*P* value
Day 14				
DNA, ng/mg	6,332	6,070	579	0.76
RNA, ng/mg	2,331	2,118	231	0.54
Protein, mcg/mg	99.25	106	8.38	0.56
RNA:DNA	0.37	0.36	0.04	0.83
RNA:protein	23.74	19.77	1.94	0.19
DNA:protein	64.32	57.07	4.21	0.26
Day 28				
DNA, ng/mg	6,186	6,221	446	0.95
RNA, ng/mg	2,429	2,684	141	0.23
Protein, mcg/mg	94.94	105	4.72	0.14
RNA:DNA	0.40	0.44	0.03	0.42
RNA:protein	25.77	25.45	1.46	0.87
DNA:protein	66.11	59.09	5.63	0.40
Overall				
DNA, ng/mg	6,251	6,154	334	0.84
RNA, ng/mg	2,385	2,432	140	0.81
Protein, mcg/mg	96.86	106	4.26	0.14

RNA:DNA	0.39	0.40	0.03	0.70
RNA:protein	24.87	22.93	1.33	0.31
DNA:protein	65.32	58.19	3.44	0.16

[a] Means of 5 and 4 observations per treatment group on d-14 and on d-28 post-weaning, respectively.

Table 5.8. pH measurements of the contents collected from the stomach, the distal ileum, the cecum, and the distal colon of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item	Treatment: Control	Nucleotides	SEM	*P* value
Stomach				
d 14	2.81	2.67	0.428	0.83
d 28	3.12	3.34	0.207	0.48
Ileum				
d 14	6.71	6.61	0.246	0.80
d 28	6.69	6.82	0.114	0.44
Cecum				
d 14	6.29	6.01	0.249	0.45
d 28	6.27	6.22	0.108	0.76
Colon				
d 14	6.49	6.39	0.255	0.78
d 28	6.59	6.41	0.103	0.24

[a] Means of 5 and 4 observations per treatment group on d-14 and on d-28 post-weaning, respectively.

Table 5.9. Microbial count (log_{10} cfu/g) in intestinal contents of the jejunum, the cecum, and the colon of weaned pigs fed diets without or with nucleotides (Exp.1) [a]

Item Treatment:	Control	Nucleotides	SEM	*P* value
Ileum				
Bifidobacterium spp.	8.62	9.21	0.229	0.12
L. acidophilus	8.70	8.10	0.593	0.50
Cl. perfringens	8.15	7.02	0.532	0.19
Cecum				
Bifidobacterium spp.	8.56	7.67	0.314	0.09
L. acidophilus	8.89	8.98	0.149	0.68
Cl. perfringens	7.61	7.31	0.407	0.62
Colon				
Bifidobacterium spp.	7.61	7.46	0.418	0.81
L. acidophilus	8.69	9.06	0.189	0.22
Cl. perfringens	7.02	7.47	0.383	0.44

[a] Means of 4 observations per treatment group.

Table 5.10. Microbial count (log_{10} cfu/g) in broth without or with nucleotide supplementation (Exp.2) [a]

Item	h	Broth: Control	Nucleotides	SEM	*P* value
Bifidobacterium spp.					
	0	8.77	8.76	0.020	0.76
	4	9.28	9.23	0.040	0.47
	8	10.00	9.92	0.032	0.20
	12	10.34	10.28	0.043	0.43
	16	10.54	10.45	0.021	0.08
SEM		0.034	0.031		
Linear effect		<0.01	<0.01		
Quadratic effect		<0.01	<0.01		
L. acidophilus					
	0	8.15	8.04	0.050	0.26
	4	8.34	8.29	0.035	0.42
	8	8.73	8.55	0.107	0.36
	12	8.85	8.90	0.147	0.82
	16	8.74	8.91	0.090	0.31
SEM		0.101	0.088		
Linear effect		<0.01	<0.01		

Quadratic effect		0.05	0.26		
Total coliforms					
	0	8.30	8.26	0.018	0.23
	4	10.02	10.14	0.046	0.21
	8	10.12 [x]	10.22 [y]	0.013	0.03
	12	10.35	10.43	0.018	0.08
	16	10.31	10.54	0.043	0.07
SEM		0.032	0.029		
Linear effect		<0.01	<0.01		
Quadratic effect		0.05	<0.01		
E. coli					
	0	7.97	7.99	0.007	0.18
	4	9.62	9.86	0.040	0.05
	8	9.76	9.93	0.063	0.20
	12	10.16	10.16	0.018	1.00
	16	10.16	10.11	0.028	0.34
SEM		0.047	0.021		
Linear effect		<0.01	<0.01		
Quadratic effect		<0.01	<0.01		

Cl. perfringens					
	0	6.09	6.17	0.178	0.77
	4	7.51	7.64	0.173	0.66
	8	7.85	7.65	0.076	0.18
	12	7.72	7.61	0.025	0.08
	16	7.15 [x]	6.78 [y]	0.000	<0.01
SEM		0.148	0.071		
Linear effect		<0.01	<0.01		
Quadratic effect		<0.01	<0.01		

[a] Means of 2 observations per treatment group.

[x, y] Means within a row lacking a common superscript letter differ ($P < 0.05$).

CHAPTER 6

Apparent and standardized ileal digestibility of amino acids in yeast extract and spray dried plasma protein by weanling pigs

ABSTRACT: The objective of this experiment was to measure the apparent (AID) and standardized (SID) ileal digestibility coefficients of AA and CP in yeast extract and spray dried plasma protein (SDPP) by weanling pigs. A casein-based diet, an SDPP-based diet, a yeast extract-based diet, and a N-free diet were formulated. Casein was also included in the SDPP-based diet and the yeast extract-based diet. Eight barrows (13 d of age; BW: 5.0 ± 0.8 kg) were equipped with a T-cannula in the distal ileum and returned to their dams for the remaining nursing period. The pigs were weaned at 21 d of age and allotted to four treatment groups in a repeated 4 X 4 Latin square design with four periods and four animals. Each experimental period lasted 7 d. The initial 5 d of each period was considered an adaptation period to the diet. Ileal samples were collected on d-6 and on d-7 of each period. The AID of AA and CP in all diets were calculated using the direct procedure. The ileal endogenous losses of AA and CP were measured in pigs fed the N-free diet. By subtracting the endogenous losses of AA and CP from the total ileal output, the SID of AA and CP were calculated for each diet. The AID and SID in yeast extract and SDPP were calculated using the difference procedure. The results indicate that the AID for CP and all AA with the exception of Cys and Ser are similar between yeast extract and SDPP. Likewise, no differences in SID for AA or CP were observed between

yeast extract and SDPP. The current data demonstrates that the AID and SID of CP and the majority of AA in yeast extract are comparable to that of SDPP. In addition, yeast extract and SDPP contain protein that is relatively well digested by young pigs.

Key Words: Amino acids, Digestibility, Pigs, Spray dried plasma protein, Yeast extract

Introduction

Spray dried plasma protein (**SDPP**) is a slaughter plant by-product and is a commonly used protein source in diets for weanling pigs (Cho et al., 1997; Chae et al., 1999). It is produced by adding an anticoagulant to the blood from slaughtered animals. The erythrocytes are separated from the plasma by centrifugation. The plasma obtained is then spray-dried to produce the final product called SDPP (Howell and Lawrie, 1983). Diets for weanling pigs often contain SDPP, but because of a perceived risk of contamination with pathogens such as BSE, Salmonella, and *E. coli*, animal protein sources have been scrutinized by the public. In the EU, all protein sources originating from land-animals have been banned. This has prompted swine producers and nutritionists to search for alternative sources of protein to replace the use of animal proteins.

A potential non-animal protein source that is currently being evaluated is yeast extract, which is derived from yeast cell contents. Yeast extract contains a relatively high quantity of AA and nucleotides (Tibbets, 2002). Pigs that were infected with *E. coli* and fed starter diets supplemented with yeast extract at 4% had improved weight gain, less

diarrhea, and improved feed conversion compared to pigs fed a control diet without yeast extract (Maribo, 2003). Therefore, yeast extract is a potential protein source in starter diets. However, the digestibility coefficients of AA and CP in yeast extract has not been measured. To accurately establish the feeding value of yeast extract in pig starter diets, it is necessary to measure the contents of digestible AA and CP in this feed ingredient. It was the objective of this experiment to measure the apparent (**AID**) and standardized (**SID**) ileal digestibility coefficients of AA and CP in yeast extract by weanling pigs and compare these values to the digestibility of AA and CP in SDPP.

Materials and methods

Animals, housing, and experimental design

Eight nursing barrows (13 d of age; BW 5.0 ± 0.8 kg) originating from the matings of SP-1 boars to Line 13 sows (Ausgene International Gridley, IL) were obtained from the South Dakota State University Swine Research Farm and equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). Following the surgery and after a recuperation period of 12 h, pigs were returned to their dams and weaned at 21 d of age. Following weaning, pigs were housed individually in 1.2- x 1.8-m pens with solid sidings and a slatted floor. The pen was equipped with a feeder and a nipple drinker. Room temperature was maintained at 22^{o}C. A heating lamp was installed in each pen allowing pigs to maintain a local temperature of 28-30^{o}C. The experiment was reviewed and approved by the Institutional Animal Care and Use Committee at

South Dakota State University (#04-A026). Pigs were arranged in a repeated 4 X 4 Latin square design with four periods and four animals in each square.

Diets and feeding

Three protein-containing ingredients (i. e., casein, SDPP, and yeast extract) were analyzed for their nutrient composition and used to formulate the experimental diets (Table 1). Four experimental diets were prepared (Table 2). These diets were formulated to contain 0.9% Ca and 0.7% P (Table 3). The casein-based diet contained 22.4% casein (Acid Casein®, Murray Goulburn Co-operative Co. Ltd. Melbourne, Australia). The SDPP-based diet contained 11.2% casein and 13% SDPP (Appetein®, American Protein Corporation, Inc. Ankeny, IA). The yeast extract-based diet contained 11.2% casein and 20.3% yeast extract (NuPro™, Alltech, Inc. Nicholasville, KY). A cornstarch-based N-free diet was formulated as well. In all four diets, Solka floc (3%), dextrose (5%), sucrose (10%), and lactose (20%) were also included. Chromic oxide (0.4%) was included in all diets as an inert marker; vitamins and minerals were included at levels that met or exceeded the NRC requirements for 3- to 5-kg pigs (NRC, 1998).

Feed was provided to each pig on an ad libitum basis. At the conclusion of each period, orts were removed from the feeders and weighed before new feed was supplied to the pigs. Water was available at all times throughout the experiment.

Following weaning, pigs were allowed a 1-wk recuperation period before the experiment was initiated. During this time, they were fed a casein-based experimental diet (20% CP).

Sample collection

Each experimental period lasted 7 d. The initial 5 d of each period was considered an adaptation period to the diet. Ileal digesta were collected for 10 h on d-6 and on d-7 of each period as described by Stein et al. (1999a). Briefly, cannulas were opened, a plastic bag was attached to the cannula barrel, and ileal digesta that flowed into the bag were collected. Bags were removed whenever they were filled with ileal digesta. Samples were stored at –20^{o}C to prevent bacterial degradation of the digesta AA. Upon the completion of one experimental period, pigs were deprived of feed overnight and the following morning, a new experimental diet was offered.

Chemical analysis

At the conclusion of the experiment, ileal digesta samples were thawed and pooled within animal and diet, and a sub-sample was taken for chemical analysis. All ileal digesta samples were lyophilized and finely ground prior to chemical analysis. Dry matter was analyzed in the ingredients, diets, and ileal digesta samples according to AOAC procedures (AOAC, 2002, procedure 39.1.02). The concentration of CP was analyzed in ingredients, diets, and ileal samples (Thiex et al., 2002). Amino acids were also analyzed in these samples on a Thermo Quest HPLC (Thermo Separation Products, Inc. San Jose, CA), using ninhydrin for post-column derivatization and nor-leucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 *N* HCL for 24 h at 110^{o}C (AOAC, 1998, procedure 4.1.11; alternative III). Methionine and Cys were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight prior to hydrolysis with 6 *N* HCL for 24 h at 110^{o}C (AOAC, 1998, procedure

4.1.11; alternative I). Tryptophan was determined after exposing the samples to nitrogenous gas and 6 *N* NaOH hydrolysis for 22 h at 110°C (AOAC, 1998, procedure 45.4.04). The chromium concentration of diets and ileal digesta samples were determined using spectrophotometry as described by Fenton and Fenton (1979).

Calculations

The AID for AA and CP in the three protein-containing diets were calculated using Eq. **[1]** (Stein et al., 1999a):

$$AID = (1 - [(AAd / AAf) \times (Crf / Crd)]) \times 100 \quad \textbf{[1]}$$

where AID is the apparent ileal digestibility coefficient of an AA (%), AAd is the AA content in the ileal digesta DM (g/kg), AAf is the AA content in the feed DM (g/kg), Crf is the chromium content in the feed DM (g/kg), and Crd is the chromium content in the ileal digesta DM (g/kg). The CP content in diets and digesta was used instead of the AA content in calculating the AID of CP.

The basal endogenous losses (**EAL**) to the distal ileum of AA and CP were determined based on the flow obtained after feeding the N-free diet using Eq. **[2] (**Stein et al., 1999b):

$$EAL = [AAd \times (Crf / Crd)] \quad \textbf{[2]}$$

where EAL is the basal endogenous loss of an AA (g/kg DMI). The EAL of CP was calculated using the same equation.

By correcting the AID for EAL of AA and CP, standardized ileal digestibility coefficients (SID) for AA and CP in the casein, the SDPP, and the yeast extract-containing diets were calculated using Eq. **[3]** (Stein et al., 2001):

$$SID = [AID + (EAL / AAf)] \quad \textbf{[3]}$$

where SID is the standardized ileal digestibility coefficient (%). The SID of CP was calculated using the same equation.

The AID and SID of AA and CP in SDPP were calculated using the difference procedure according to Eq. **[4] (**Fan and Sauer, 1995):

$$D_A = (D_D - [D_B \times S_B]) / S_A \quad \textbf{[4]}$$

where D_A is the AID or SID of AA and CP in SDPP (%), D_D is the AID or SID of AA and CP in the SDPP-based diet (%), D_B is the AID or SID of AA and CP in the casein-based diet (%), S_B is the contribution level of AA and CP from casein to the SDPP-based diet (in decimal %), and S_A is the contribution level of AA and CP from SDPP to the SDPP-based diet, $S_A = 1 - S_B$ (in decimal %). The AID and SID in yeast extract (%) was calculated using the same equation.

Statistical analysis

An ANOVA using the PROC MIXED procedure in SAS (Littell et al., 1996) was used in this experiment. Pig and period were used as random effects and diet was used as the fixed effect. The model included diet as the independent variable, and AA and CP as the dependent variables. This model was used to compare the AID and SID for AA and CP in the casein-, the SDPP-, and the yeast extract-based diets. The pig was used as the experimental unit. Least square means were calculated and separated using the PDIFF option of SAS. A *P*-value of less than 0.05 was used to assess significance between treatments.

An ANOVA using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was used to compare the AID and SID of AA and CP in SDPP and yeast extract. The model included ingredient as the main effect. The pig was used as the experimental unit. Least square means were calculated and separated using the PDIFF option of SAS. A *P*-value of less than 0.05 was used to assess significance between treatments.

Results and discussion

AA composition of protein sources. The concentration of CP and most AA in the protein sources used in the current study was similar to previous reports (Van der Peet-Schwering and Binnendijk, 1997; NRC, 1998). The exception is Cys in casein that was found in a lower concentration than indicated in previous studies (NRC, 1998; CVB, 1999). The concentrations of Phe, Trp, and Cys in SDPP were also lower than previous estimates (NRC, 1998).

Apparent ileal digestibility coefficients of diets. The AID of most AA and CP in the casein-based diet were lower than those previously reported (Walker et al. 1986; NRC, 1998; CVB, 1999). The reason why lower AID for AA and CP were obtained for the casein-based diet may be that the pigs used in this experiment were younger than the pigs used in other studies. Younger pigs have a less mature gastrointestinal tract and therefore, would digest the casein-based diet less efficiently compared to older pigs. An immature gastrointestinal tract may also cause less reabsorption of endogenous AA and CP and, thereby, cause an increase in endogenous losses. An increase in endogenous losses will lead to a decrease in the AID (Pedersen and Boisen, 2002).

With the exception of Trp, no differences in the AID for CP or the indispensable AA were observed among the three diets (Table 4). The AID for Trp in the yeast extract-based diet was lower ($P = 0.02$) than the AID for Trp in the casein-based diet (77.2 vs. 84.8%). In contrast, no difference in AID for Trp was observed between the casein- and the SDPP-based diets or between the SDPP- and the yeast extract-based diets.

Except for Ala and Cys, no differences were observed for the AID of the dispensable AA, the mean of the dispensable AA, and the mean of all AA among the experimental diets. The AID for Ala was higher ($P = 0.02$) in the casein-based diet compared to the AID for Ala in the SDPP- and the yeast extract-based diets (80.4 vs. 75.0% and 71.6%, respectively). However, the AID for Cys was higher ($P < 0.01$) in the SDPP-based diet compared to the AID for Cys in the casein- and the yeast extract-based diets (72.9 vs. 56.3% and 58.8%, respectively).

The differences detected in the AID for Trp, Ala, and Cys among experimental diets is likely due to the low concentration of these AA in some of the ingredients used in formulating these diets. The lower the AA concentration in an ingredient, the lower the AID of that AA will be in the diet because of a relatively larger contribution of AA of endogenous origin to the ileal output (Fan et al., 1994). Casein has a low concentration of Cys and yeast extract has a low concentration of Trp and Cys. These low concentrations explain why low values for AID were observed for these two AA.

Standardized ileal digestibility coefficients of diets. The SID of most AA and CP in the casein-based diet obtained in this study (Table 5) were lower than those reported previously (Kies et al., 1986; Walker et al., 1986; Nyachoti et al., 1997; Pedersen and

Boisen, 2002). With the exception of Trp, no differences among the three diets in the SID of the indispensable AA and for the mean of the indispensable AA were observed. The SID for Trp was higher ($P = 0.02$) in the casein-based diet compared to the SID for Trp in the SDPP- and the yeast extract-based diets (93.4 vs. 86.7% and 86.3%, respectively). However, no differences in the SID for Trp were observed between the SDPP- and the yeast extract-based diets.

Except for Ala, no differences were observed for the SID of the dispensable AA, the mean of the dispensable AA, and the mean of all AA among the experimental diets. The SID for Ala was higher ($P < 0.01$) in the casein-based diet compared to the SID for Ala in the SDPP- and the yeast extract-based diets (91.1 vs. 83.4% and 81.4%, respectively). No differences in the SID for Ala were observed between the SDPP- and the yeast extract-based diets.

Endogenous losses. The EAL for AA and CP obtained in the current study were similar to those reported previously (De Lange et al., 1989; Jansman et al., 2002; Pedersen and Boisen, 2002).

Apparent ileal digestibility of AA and CP in SDPP and yeast extract. With the exception of Cys and Ser, no differences in AID between SDPP and yeast extract were observed for any AA (Table 6). The AID obtained for most AA in SDPP were similar to those previously reported (Cho et al., 1997; NRC, 1998; Chae et al., 1999). However, the AID of Met obtained in the current study was higher than previous estimates (NRC, 1998).

Except for Cys and Ser, no differences were observed in the AID for the dispensable AA, the mean of the dispensable AA, and the mean of all AA between SDPP and yeast extract. The AID of Cys and Ser in SDPP was higher ($P < 0.05$) than the AID of Cys and Ser in yeast extract (73.7 vs. 58.3% and 77.8 vs. 67.1%, respectively). The low AID for Cys and Ser in yeast extract are probably caused by the low concentration of these AA in yeast extract.

Standardized ileal digestibility of AA and CP in SDPP and yeast extract. No differences were observed in the SID for any AA or CP between SDPP and yeast extract (Table 7). This suggests that AA and CP digestibility in yeast extract is similar to SDPP.

Implication

Data from the current experiment provide information on the apparent and standardized ileal digestibility coefficients in spray dried plasma protein and yeast extract by weanling pigs. The results indicate that the apparent ileal digestibility for crude protein and all amino acids except Cys and Ser are similar between spray dried plasma protein and yeast extract. Both ingredients contain protein that is relatively well digested by young pigs. Both ingredients, therefore, are well suited for use in diets for weanling pigs.

Literature cited

AOAC. 1998. AOAC International. 16th ed. P. Cunniff, ed. Assoc. Offic. Anal. Chem. International, Gaithersburg, MD.

AOAC. 2002. AOAC International. 17th ed. W. Horwitz, ed. Assoc. Offic. Anal. Chem. International, Gaithersburg, MD.

Chae, B. J., In K. Han, J. H. Kim, C. J. Yang, J. D. Hancock, I. H. Kim, and D. A. Anderson. 1999. Effects of dietary protein sources on ileal digestibility and growth performance for early-weaned pigs. Livestock Production Science 58:45-54.

Cho, S. B., J. H. Kim, In K. Han, H. K. Moon, B. J. Chae, and W. T. Cho. 1997. Apparent digestibility of amino acids, energy and proximate nutrients in dietary protein sources and tapioca for young pigs. Asian-Australasian Journal of Animal Sciences 10:643-651.

CVB. 1999. Data about chemical composition, digestibility and feedstuffs. Veevoeder Tabel, Centraal Veevoeder Bureau, Lelystad.

De Lange, C. F. M., W. C. Sauer, R. Mosenthin, and W. B. Souffrant. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. 67:746-754.

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: Effect of amino acid level. J. Anim. Sci 72:2851-2859.

Fan, M. Z., and W. C. Sauer. 1995. Determination of apparent ileal amino acid digestibility in barley and canola meal for pigs with the direct, difference and regression methods. J. Anim. Sci. 73:2364-2374.

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631-634.

Howell, N. K., and R. A. Lawrie. 1983. Functional aspects of blood plasma proteins. I. Separation and characterization. J. Food Technol. 18:747-762.

Jansman, A. J. M., W. Smink, P. van Leeuwen, and M. Rademacher. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Animal Feed Science and Technology 98:49-60.

Kies, A. K., P. J. Moughan, and W. C. Smith. 1986. The apparent and true ileal digestibility of nitrogen and amino acids in lactic casein for the growing pig. Anim. Feed Sci. Technol. 16:169-178.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS Systems for mixed models. SAS Inst. Inc., Cary, NC.

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.

NRC. 1998. Nutrient requirements of swine. 10th ed. National Academy Press, Washington, DC.

Nyachoti, C. M., C. De Lange, H. Schulze, and L. C. De. 1997. Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. J. Anim. Sci. 75:3206-3213.

Pedersen, C., and S. Boisen. 2002. Establishment of tabulated values for standardized ileal digestibility of crude protein and essential amino acids in common feedstuffs for pigs. Acta Agric. Scand. 52:121-140.

Stein, H. H., S. Aref, and R. A. Easter. 1999a. Comparative protein and amino acid digestibilities in growing pigs and sows. J. Anim. Sci. 77:1169-1179.

Stein, H. H., S. W. Kim, T. T. Nielsen, and R. A. Easter. 2001. Standardized amino acid digestibilities in growing pigs and sows. J. Anim. Sci. 79:2113-2122.

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical Note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436.

Stein, H. H., N. L. Trottier, C. Bellaver, and R. A. Easter. 1999b. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J. Anim. Sci. 77:1180-1187.

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.

Thiex, N. J., H. Manson, S. Anderson, and J. Persson. 2002. Determination of crude protein in animal feed, forage, grain, and oil seeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. J. of AOAC International 85:309-317.

Van der Peet-Schwering, C. M. C., and G. P. Binnendijk. 1997. Spray dried porcine and bovine plasma and animal and plant protein in diets of weaned piglets. In: Report P1.185. Praktijkonder-zoek Varkenshouderij, Rosmalen.

Walker, W. R., C. V. Maxwell, F. N. Owens, and D. S. Buchanan. 1986. Milk vs. soybean protein sources for pigs. II. Effects on amino acid availability. J. Anim. Sci. 63:513-524.

Table 6.1. Analyzed nutrient composition of feed ingredients (DM-basis)

Item Ingredient:	Casein	Spray dried plasma protein	Yeast extract
Dry matter, %	91.60	91.76	93.65
Crude protein, %	93.07	82.44	48.79
Indispensable AA			
Arginine, %	3.43	4.59	2.03
Histidine, %	3.01	2.52	1.07
Isoleucine, %	4.96	2.69	2.10
Leucine, %	8.96	7.59	3.64
Lysine, %	7.32	6.95	2.42
Methionine, %	3.28	1.03	0.91
Phenylalanine, %	4.94	4.34	2.06
Threonine, %	5.47	5.34	2.30
Tryptophan, %	1.12	1.29	0.41
Valine, %	6.56	5.60	2.38
Dispensable AA			
Alanine, %	2.65	3.86	2.55
Aspartic acid, %	6.35	7.73	3.52
Cysteine, %	0.23	2.04	0.45
Glutamic acid, %	20.27	10.71	7.93
Glycine, %	1.69	2.82	1.96

Proline, %	10.38	4.12	2.75
Serine, %	3.27	4.31	1.66
Tyrosine, %	5.21	4.04	1.60

Table 6.2. Ingredient composition of experimental diets (as is basis)

Item Diet:	Casein	Spray dried plasma protein	Yeast extract	N-free
Ingredients, %				
Casein	22.40	11.20	11.20	-
Cornstarch	31.90	30.20	23.10	53.30
Dextrose	5.00	5.00	5.00	5.00
Lactose	20.00	20.00	20.00	20.00
Sucrose	10.00	10.00	10.00	10.00
Spray dried plasma protein	-	13.00	-	-
Yeast extract	-	-	20.30	-
Soybean oil	3.00	3.00	3.00	3.00
Limestone	0.40	1.00	1.20	0.50
Dicalcium phosphate	2.80	2.10	1.70	3.70
Salt	0.40	0.40	0.40	0.40
Trace mineral premix [a]	0.15	0.15	0.15	0.15
Vitamin premix [b]	0.05	0.05	0.05	0.05
Chromic oxide	0.40	0.40	0.40	0.40
Solka floc	3.00	3.00	3.00	3.00
Magnesium oxide	0.10	0.10	0.10	0.10
Potassium carbonate	0.40	0.40	0.40	0.40

[a] Provided the following quantities of trace minerals per kg of complete diet: Cu, 16.5 mg as copper sulfate; Fe, 165 mg as iron sulfate; I, 0.36 mg as potassium iodate; Mn, 44 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 165 mg as zinc oxide.

[b] Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 10,990 IU as vitamin A acetate; vitamin B_{12}, 44 mcg; vitamin D_3, 1,648 IU as D-activated sterol; vitamin E, 55 IU as DL-α-tocopheryl acetate; vitamin K_3, 4.4 mg as menadione dimethylpyrimidinol bisulfite; biotin, 0.17 mg; D-pantothenic acid, 33 mg as calcium pantothenate; folic acid, 1.1 mg; niacin, 55 mg; pyridoxine, 3.3 mg as pyridoxine hydrochloride; riboflavin, 9.9 mg; and thiamin, 3.3 mg as thiamine mononitrate.

Table 6.3. Analyzed nutrient composition of diets (DM basis)

Item Diet:	Casein	Spray dried plasma protein	Yeast extract	N-free
Metabolizable Energy, kcal/kg [a]	3,541	3,467	3,403	3,602
Dry matter, %	92.12	92.11	92.60	92.53
Crude protein, %	23.00	21.04	18.46	0.17
Calcium [a], %	0.91	0.93	0.91	0.93
Phosphorous [a]	0.70	0.71	0.71	0.71
Indispensable AA				
Arginine, %	0.85	0.98	0.71	0.00
Histidine, %	0.67	0.62	0.49	0.00
Isoleucine, %	1.17	0.88	0.91	0.00
Leucine, %	2.19	1.99	1.63	0.01
Lysine, %	1.80	1.75	1.32	0.00
Methionine, %	0.76	0.50	0.51	0.00
Phenylalanine, %	1.19	1.09	0.90	0.00
Threonine, %	1.48	1.39	1.08	0.00
Tryptophan, %	0.19	0.25	0.15	0.00
Valine, %	1.51	1.40	1.13	0.00
Dispensable AA				
Alanine, %	0.68	0.82	0.69	0.01
Aspartic acid, %	1.60	1.75	1.33	0.00

Cysteine, %	0.06	0.28	0.08	0.00
Glutamic acid, %	5.15	3.87	3.83	0.01
Glycine, %	0.43	0.54	0.47	0.00
Proline, %	2.44	1.75	1.69	0.00
Serine, %	0.84	0.95	0.67	0.00
Tyrosine, %	1.17	1.02	0.84	0.00

[a] Values for ME, Ca, and P were calculated (NRC, 1998) while all other values were analyzed.

Table 6.4. Apparent ileal digestibility coefficients (AID) of AA and CP (%) for experimental diets by weanling pigs [a]

Item	Diet: Casein	Spray dried plasma protein	Yeast extract	SEM	*P*-value
n	5	8	6		
Crude protein	82.5	80.6	79.0	2.99	0.77
Indispensable AA					
Arginine	84.7	86.9	83.3	2.97	0.64
Histidine	89.8	87.5	84.4	1.96	0.21
Isoleucine	85.7	85.2	82.3	2.52	0.57
Leucine	89.1	87.6	86.6	2.21	0.72
Lysine	88.2	85.7	84.8	2.35	0.57
Methionine	91.8	90.0	90.0	1.69	0.67
Phenylalanine	89.0	87.8	86.7	2.24	0.75
Threonine	84.0	81.0	76.7	2.37	0.18
Tryptophan	84.8 [x]	80.9 [xy]	77.2 [y]	2.02	0.02
Valine	85.3	82.2	80.9	2.40	0.47
Mean, indispensable AA	87.2	85.5	83.3	2.04	0.57
Dispensable AA					
Alanine	80.4 [x]	75.0 [y]	71.6 [y]	2.48	0.02
Aspartic acid	81.0	77.0	75.2	3.27	0.53
Cysteine	56.3 [x]	72.9 [y]	58.8 [x]	2.44	<0.01

Glutamic acid	88.5	83.6	84.9	2.04	0.18
Glycine	64.7	65.8	64.1	2.49	0.65
Proline	90.3	87.8	85.1	1.55	0.11
Serine	79.2	78.3	73.0	2.88	0.26
Tyrosine	90.5	88.4	88.3	2.03	0.69
Mean, dispensable AA	78.9	78.6	75.1	2.63	0.49
Mean, total AA	83.5	82.4	79.7	2.42	0.60

[a] AID = 1 - (AA or CP in digesta / AA or CP in feed) x (Cr in feed / Cr in digesta) x 100%.

[x, y] Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 6.5. Standardized ileal digestibility coefficients (SID) of AA and CP (%) for experimental diets by weanling pigs [a]

Item	Diet: Casein	Spray dried plasma protein	Yeast extract	SEM	*P*-value
n	5	8	6		
Crude protein	89.9	88.2	87.6	2.95	0.87
Indispensable AA					
Arginine	90.6	91.7	90.0	3.05	0.91
Histidine	93.5	91.3	89.2	1.95	0.33
Isoleucine	90.2	90.7	87.6	2.48	0.57
Leucine	92.8	91.4	91.3	2.19	0.84
Lysine	91.8	89.3	89.4	2.34	0.66
Methionine	94.0	93.2	93.1	1.68	0.90
Phenylalanine	93.0	91.9	91.5	2.22	0.88
Threonine	89.0	85.9	83.5	2.21	0.30
Tryptophan	93.4 [x]	86.7 [y]	86.3 [y]	2.00	0.02
Valine	89.7	87.1	86.9	2.40	0.67
Mean, indispensable AA	91.8	89.9	88.8	2.11	0.81
Dispensable AA					
Alanine	91.1 [x]	83.4 [y]	81.4 [y]	2.42	<0.01
Aspartic acid	87.2	82.4	82.2	3.31	0.56
Cysteine	85.2	79.2	78.7	3.52	0.10

Glutamic acid	90.9	86.9	88.1	1.99	0.28
Glycine	91.4	87.4	86.3	2.58	0.11
Proline	99.1	99.7	97.8	2.64	0.64
Serine	87.3	84.7	82.2	2.70	0.38
Tyrosine	94.0	92.1	92.7	1.98	0.77
Mean, dispensable AA	90.8	87.0	86.2	2.58	0.63
Mean, total AA	91.3	88.6	87.7	2.41	0.75

[a] SID = apparent ileal digestibility of the diet + (endogenous losses / intake) x 100%. Endogenous losses (g/ kg DMI) of AA and CP were calculated as the following quantities: CP, 17.19; Arg, 0.51; His, 0.26; Ile, 0.53; Leu, 0.83; Lys, 0.66; Met, 0.17; Phe, 0.49; Thr, 0.76; Trp, 0.16; Val, 0.74; Ala, 0.74; Asp, 1.03; Cys, 0.19; Glu, 1.32; Gly, 1.20; Pro, 2.27; Ser, 0.66; Tyr, 0.41; n = 6.

[x, y] Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 6.6. Apparent ileal digestibility coefficients (AID) of spray dried plasma protein and yeast extract (%)

Item Ingredient:	Spray dried plasma protein	Yeast extract	SEM	*P*-value
n	8	6		
Crude protein, %	78.8	75.9	4.16	0.62
Indispensable AA				
Arginine, %	88.3	82.1	3.29	0.20
Histidine, %	85.5	77.7	3.37	0.12
Isoleucine, %	84.3	77.9	5.02	0.38
Leucine, %	86.0	83.1	3.43	0.55
Lysine, %	83.5	78.6	3.62	0.35
Methionine, %	85.1	86.3	5.53	0.87
Phenylalanine, %	86.7	83.4	3.19	0.47
Threonine, %	78.1	68.4	4.39	0.14
Tryptophan, %	79.1	70.6	3.08	0.07
Valine, %	79.4	74.7	4.36	0.46
Mean, indispensable AA	83.6	78.3	2.25	0.14
Dispensable AA				
Alanine, %	72.0	68.2	3.30	0.43
Aspartic acid, %	74.1	70.4	4.33	0.55
Cysteine, %	73.7 [x]	58.3 [y]	2.46	<0.01

Glutamic acid, %	75.8	81.6	5.26	0.45
Glycine, %	66.7	63.5	3.36	0.51
Proline, %	82.3	75.1	5.05	0.33
Serine, %	77.8 [x]	67.1 [y]	3.33	0.04
Tyrosine, %	86.4	84.5	3.14	0.66
Mean, dispensable AA	76.1	71.1	3.64	0.35
Mean, total AA	80.3	75.1	2.95	0.23

Table 6.7. Standardized ileal digestibility coefficients (SID) of spray dried plasma protein and yeast extract (%)

Item Ingredient:	Spray dried plasma protein	Yeast extract	SEM	*P*-value
n	8	6		
Crude protein, %	86.5	85.4	4.01	0.85
Indispensable AA				
Arginine, %	92.4	89.3	3.41	0.53
Histidine, %	89.4	83.7	3.33	0.25
Isoleucine, %	91.4	84.0	4.85	0.30
Leucine, %	90.0	88.8	3.33	0.80
Lysine, %	86.9	84.7	3.58	0.67
Methionine, %	90.9	91.0	5.42	0.99
Phenylalanine, %	91.0	89.3	3.11	0.70
Threonine, %	82.6	76.5	4.18	0.31
Tryptophan, %	83.7	80.4	2.78	0.41
Valine, %	84.6	82.8	4.27	0.77
Mean, indispensable AA	88.3	85.0	2.55	0.60
Dispensable AA				
Alanine, %	78.9	77.7	3.14	0.79
Aspartic acid, %	78.8	77.9	4.28	0.88
Cysteine, %	79.0	78.9	2.34	0.97

Glutamic acid, %	80.2	85.6	5.10	0.46
Glycine, %	83.7	85.3	3.49	0.74
Proline, %	100.8	96.4	7.91	0.69
Serine, %	83.0	77.1	3.13	0.20
Tyrosine, %	90.4	90.2	2.99	0.97
Mean, dispensable AA	84.4	83.7	3.54	0.53
Mean, total AA	86.5	84.4	3.01	0.50

CHAPTER 7

Conclusion

The current work provides basic data on nucleotide nutrition in young pigs. In the initial experiment, the concentration of 5'monophosphate nucleotides that are present in sow colostrum and milk were measured. Such data have never been previously reported. The concentration of 5'UMP in sow colostrum and in sow milk represent 98% and 86-90% of all 5'monophosphate nucleotides, regardless of the d of lactation. This suggests that of the five nucleotides considered in this study, 5'UMP may be the primary nucleotide of physiological importance to the suckling pig. Further studies need to be conducted to investigate this particular nucleotide and its role in the nutrition of young pigs. The lack of a correlation between the nucleotide concentration and the CP concentration in sow colostrum and milk lead to the conclusion that the nucleotide concentration in sow milk cannot be predicted from the CP concentration. This suggests that ingredients with a high concentration of CP do not necessarily have a high nucleotide concentration.

In the second study, the nucleotide concentrations in 10 commonly used feed ingredients were measured and the concentration in a standard starter diet was calculated. It was concluded that a standard starter diet fed to weanling pigs contains an adequate amount of 5'CMP, but the concentration of 5'AMP, 5'GMP, 5'IMP, and 5'UMP is considerably lower than in sow milk. Therefore, newly weaned pigs that are expected to have an elevated need for nucleotides are ingesting fewer nucleotides than prior to weaning. At the same time, the supply of major precursors for nucleotide synthesis (i. e.,

energy and glutamine) is reduced. This observation led to the hypothesis that supplementation of starter diets with nucleosides or nucleotides may be beneficial. Both an in-vivo and an in-vitro experiment were conducted to investigate the effects of supplementing a starter diet with nucleosides. It was concluded that nucleoside supplementation of starter diets during the immediate post-weaning period may elevate the concentration of probiotic bacteria and reduce the concentration of pathogenic bacteria in the gastrointestinal tract of the pigs. Under commercial conditions, this may improve the intestinal health, which may lead to an improvement in the immune system and reduced scouring during the post-weaning period.

In the third study, nucleotides were added to a starter diet. Results of this experiment indicated that dietary nucleotides may also positively influence the gastrointestinal microflora. It was also demonstrated that nucleotide supplementation may increase VH:LPD in the ileum, which may be a result of improved intestinal health. Both the second and the third experiments were conducted with healthy pigs that were only minimally stressed. Because the tissue needs for nucleotides are believed to increase during periods of stress, future studies should be aimed at investigating responses to nucleotide intake during periods of stress. It may also be beneficial to look at individual nucleotides or a different combination of nucleotides than what was used in the present experiments.

In the final study, AID and SID coefficients of AA and CP in yeast extract and spray dried plasma protein by weanling pigs were measured using the difference method. Results indicate that the AID for CP and all AA with the exception of Cys and Ser are

similar between yeast extract and SDPP. Likewise, no differences in SID for AA or CP were observed between yeast extract and SDPP. The current data demonstrates that the AID and SID of CP and the majority of AA in yeast extract are comparable to that of SDPP. In addition, yeast extract and SDPP contain protein that is relatively well digested by young pigs. This information will allow swine producers and nutritionists formulating diets for weanling pigs on the basis of apparent or standardized ileal AA digestibility to include yeast extract in their formulas and therefore, take advantage of the benefits nucleotide supplementation.

In conclusion, the findings from the work reported in this thesis indicate that the dietary supply of nucleotides is reduced during the post-weaning period for pigs compared to the supply from sow milk. It is also shown that dietary nucleosides and possibly nucleotides may benefit the intestinal microflora and stimulate intestinal health. More research is required to further investigate the role of dietary nucleotides in weanling pigs.