Intestinal Nitrogen Recycling and Utilization in
Health and Disease

Werner G. Bergen and Guoyao Wu

Department of Animal Sciences, Auburn University, AL 36849 and Department of Animal Science, Texas A&M University, College Station, TX 77843

Abstract
The role of intestinal microflora in digestive and metabolic processes has received increasing attention from researchers and clinicians. Both enterocytes and small intestine luminal microorganisms can degrade peptides and amino acids (AA). Further, enterocytes can utilize ammonia via glutamate, glutamine, citrulline, and urea synthesis, whereas luminal microbes will deaminate AA, hydrolyze luminal urea, and recycle this ammonia by synthesis of new microbial cells. Although, undoubtedly, some indispensable AA may arise from N cycling and microbial synthesis in the intestinal lumen, the actual net impact on protein nutrition status appears to be limited in humans and animals. Moreover, potential contributions of the recycled N as colonic luminal microbial proteins to AA in blood depend on colonic protein digestion and AA absorption. Finally, new evidence indicates that gut microbial metabolism may be enhanced by prebiotics and probiotics, with the prospects of new treatment paradigms for eliminating undesirable secondary N metabolites and ameliorating complications in whole-body N metabolism under the conditions of intestinal stress, liver disease, and kidney failure.

Introduction
The role of the microflora in the physiology, nutrition, and health of the human gastrointestinal tract (GIT), as well as systemic and bowel-specific disease, has gained increasingly extensive attention by researchers (1). In all species, the GIT is colonized with a massive microflora; the DNA of all these organisms represents a huge combined “genome” or the “microbiome” (2). Intestinal bacteria and hosts are in a state of symbiotic mutualism and the genome of the human host and the “microbiome” (2). Intestinal bacteria and hosts are in a state of symbiotic mutualism and the genome of the human host and the “microbiome” (2). Certain animal and insect species harbor a pregastric microflora that produces indispensable amino acids (IAA) from simple and recycled nitrogenous compounds for the hosts (3). This advantage is not shared by humans and nonruminants, and the ability of distal small intestinal and colonic microbes to provide IAA is limited (3,4). Hence, consumption of diets deficient in IAA cannot sustain nonruminants for an indefinite period (5). Luminal microorganisms may also be useful as scavengers to eliminate deleterious N compounds from the body, crowd out opportunistic pathogenic organisms, and participate in many hitherto undefined processes related to proper digestive function (6). This review highlights the demarcation between N metabolism in eukaryotic cells of the GIT (enterocytes and colonocytes) and microbial cells that grow in the lumen of the small intestine and large intestine (luminal microorganisms).

Dietary sources and interconversions of N in the intestine
In nonruminant animals and humans, the diet is the major source of N in the lumen of the small intestine. The N can be in the form of proteins, peptides, amino acids (AA), urea, ammonia, and other nitrogenous substances (including glutathione, polyamines, purines, pyrimidines, nitrite, nitrate, uric acid, and nitrosylated products). Except for neonates in which nearly all of protein-bound AA in milk are absorbed by the small intestine (7), the true ileal digestibility values for AA in plant and animal protein-based diets are 80–90% in pigs (8), resulting in the entry of undigested protein into the large intestine (colon).

Free AA and small peptides are degraded by both enterocytes and luminal bacteria. Glutamine, glutamate, and aspartate in the lumen, as well as glutamine from the mesenteric artery circulation, are extensively oxidized by enterocytes of the porcine small intestine to generate ammonia, CO₂, alanine, proline, citrulline, and arginine (9). These cells also actively degrade enteral proline to produce ornithine, citrulline, and arginine. Moreover, arginine can be extensively hydrolyzed by enterocytes of postweaning mammals to ornithine plus urea (10). There is also emerging evidence indicating that arterial small peptides containing proline and arginine may be taken up by the small intestine where they are hydrolyzed to form proline and arginine (11). A new aspect of AA nutrition is the finding that 30–50% of essential AA in the diet may be degraded by the small intestine during the initial phase of protein digestion and AA absorption or first-pass AA metabolism (12) (Fig. 1). For example, in milk protein-fed piglets, 40% of leucine, 30% of isoleucine, and 40% of valine in the diet are extracted by the portal-drained viscera in the first pass, with <20% of the extracted branched-chain AA.

Abbreviations used: AA, amino acid; BCAA, branched-chain amino acid; COS, chitosan oligosaccharide; GIT, gastrointestinal tract; IAA, indispensable amino acid.

1 Supported in part by the National Research Initiative Competitive grant (no. 2008-35206-18764) from the USDA Cooperative State Research Education and Extension Service, Auburn University, Alabama Agricultural Experiment Station (H-8200).
2 Author disclosures: W. G. Bergen and G. Wu, no conflicts of interest.
3 Department of Animal Sciences, Auburn University, AL 36849 and Department of Animal Science, Texas A&M University, College Station, TX 77843
4 To whom correspondence should be addressed. E-mail: bergewg@auburn.edu.

Reference
1. Supported in part by the National Research Initiative Competitive grant (no. 2008-35206-18764) from the USDA Cooperative State Research Education and Extension Service, Auburn University, Alabama Agricultural Experiment Station (H-8200).
FIGURE 1 N flows and interconversions in the small intestine and colon. Dietary proteins are digested in the small intestine lumen and AA are absorbed across mucosal and serosal cell lining. In addition, AA arising from digestion are deaminated/degraded and the NH₃ may be utilized for microbial cell synthesis. Ammonia arising from urea flux into the lumen from circulation (D) or enterocytes (B) will mix with other NH₃ pools. Ammonia may flow back into the enterocyte for carbamoyl phosphate (CP) synthesis and subsequently be used for enteric urea synthesis. Irrespective of origin, all NH₃ can mix within enterocytes, providing N for urea synthesis (E). This process is a cycling of N between the lumen, enterocytes, and N compounds in the circulation. First-pass metabolism is likely more extensive (microbial deamination of AA from protein digestion and subsequent carbon skeleton oxidation to SCFA and gasses; C) in the lumen than in enterocytes during AA uptake. Microbial cell synthesis depends on energy sources in both the small intestine and colon lumen. In the upper colon, luminal proteolysis is very limited. Urea and NH₃ may also flow from circulation into colonocytes and then the colon lumen. Colonocytes have limited urea synthesis and AA transporters. In the colon, microbial metabolism of AA results in the production of SCFA and gases (C) followed by repeated cell death, protein hydrolysis, deamination, and cell synthesis utilizing mainly β-glycosidic link polysaccharides (prebiotics) as the major energy source. Abbreviations: SPP, short peptide; EAA, essential AA; P5C, pyrroline-5-carboxylate; Ala, alanine; Pro, proline, Gln, glutamine; Glu, glutamate.

(BCAA) utilized for intestinal mucosal protein synthesis (12). This is consistent with a high activity of BCAA transaminase in enterocytes (13).

Stoll et al. (12) also reported that 50% of dietary lysine and methionine, 45% of dietary phenylalanine, and 60% of dietary threonine were extracted in the first pass by the portal-drained viscera of milk protein-fed pigs, with 30% of the extracted AA catabolized by the small intestine. In addition, van Goudoever et al. (14) found that intestinal oxidation of enteral lysine contributed one-third of total body lysine oxidation in growing pigs fed a high-protein diet. These in vivo findings point to potentially extensive oxidation of these IAA in the gut, principally implicating AA metabolism in enterocytes. Using the technique of viable enterocyte incubation, Chen et al. (13) reported, however, that there was no production of CO₂ or tricarboxylic acid cycle intermediates from carbon-1 or all carbons of lysine, histidine, threonine, and tryptophan. Likewise, oxidation of methionine and phenylalanine in porcine enterocytes was quantitatively negligible (13). In support of these metabolic data, there were no detectable activities of tryptophan dehydrogenase, threonine hydratase, histidine decarboxylase, or phenylalanine hydroxylase in porcine enterocytes (13).

First-pass metabolism is likely more extensive (microbial deamination of AA from protein digestion and subsequent carbon skeleton oxidation to SCFA and gasses; C) in the lumen than in enterocytes during AA uptake. Microbial cell synthesis depends on energy sources in both the small intestine and colon lumen. In the upper colon, luminal proteolysis is very limited. Urea and NH₃ may also flow from circulation into colonocytes and then the colon lumen. Colonocytes have limited urea synthesis and AA transporters. In the colon, microbial metabolism of AA results in the production of SCFA and gases (C) followed by repeated cell death, protein hydrolysis, deamination, and cell synthesis utilizing mainly β-glycosidic link polysaccharides (prebiotics) as the major energy source. Abbreviations: SPP, short peptide; EAA, essential AA; P5C, pyrroline-5-carboxylate; Ala, alanine; Pro, proline, Gln, glutamine; Glu, glutamate.

Endogenous sources of nitrogen and recycling in the intestine

The endogenous sources of N in the lumen of the small intestine include saliva, gastric secretions, sloughed cells, and cell debris originating from the stomach and intestinal epithelium, bile, small intestinal secretions, pancreatic secretions, mucus, microorganisms, and mesenteric arterial blood (15). There are also secretions from the circulation (urea, ammonia) into the small and upper large intestine. Flows of sloughed colonocytes and colon microbiota into the lumen of the large intestine, although the amounts are less than those from the upper gastrointestinal parts, also represent endogenous N. Most of the endogenous N can be reabsorbed before reaching the terminal ileum and, to a much lesser extent, in the large intestine; this process is referred to as N recycling (13) (Fig. 1). In growing pigs, endogenous N flow leaving the small intestine (determined by collecting digesta at the terminal ileum) represents 33% of dietary N intake and can be substantially increased in response to dietary antinutritional factors, high levels of fiber, and protein intake (16). Similar results have been reported for adult humans (17) and formula-fed infants (18). Approximately 75 and 15% of the N endogenously secreted from the upper GIT are reabsorbed by the small intestine and large intestine, respectively, into mucosal epithelial cells (18). Studies with pigs and humans indicate that 20–25% of the urea synthesized in the liver enters, via circulation, the lumen of the intestine (primarily the small intestine), where urea is hydrolyzed by microbial urease into ammonia and CO₂ (Fig. 1) (19). Interestingly, the in vivo kinetics data indicate equal returns of urea C and N moieties to the urea pool (19), suggesting the presence of a metabolically significant rate of urea resynthesis in the epithelial cells of the intestine. In support of this view, Wu (10) discovered the synthesis of urea from both extracellularly and intracellularly
generated ammonia in enterocytes of postweaning pigs (10). Urea resynthesis in enterocytes helps to explain the apparent discrepancy in urea recycling between isotope dilution and mass balance studies (15). In a recent human feeding study of tracer-enriched proteins, Fouillet et al. (20) reported that N cycling or salvage from the gut back into the body AA pool was approximately one-half of dietary N intake. Unfortunately, the composition of putative salvage N was not assessed and the potential of enterocyte ammonia utilization for dispensable AA and urea synthesis was not considered in the interpretation of the isotopomer data (10,11,15,20).

Among the endogenous AA in the terminal ileum, the arginine family of AA (proline, glutamate plus glutamine, aspartate plus asparagine, and arginine) are the most abundant, followed by: 1) serine and glycine; 2) BCAA; and 3) other AA (15). When [15N] ammonia was orally administered into humans, arginine was highly enriched with 15N (21). This phenomenon can now be explained by arginine synthesis from ammonia, bicarbonate, ornithine (derived from glutamine, glutamate, and proline), and aspartate in enterocytes (9). Poor 15N abundance of lysine and threonine may largely reflect the absence of catabolic pathways for their degradation initiated by a transaminase. Ammonia fixation initially involves glutamate dehydrogenase to generate glutamate, which reacts with another ammonia molecule to form glutamine by glutamine synthetase. These 2 enzymes are abundant in bacteria but have low activities in intestinal mucosal cells. Some of the ammonia is utilized by luminal microorganisms to grow (i.e. synthesize AA and microbial protein). To varying degrees, such cells may be subsequently digested and the arising peptides and AA absorbed, catabolized, or transported with the digesta flow to the large intestine (6) (Fig. 1). Evidence shows that the colonic epithelium is not a source of digestive enzymes and that protein/peptide hydrolyses in the colon are a principal function of the microorganism (22). Most likely, deamination and decarboxylation by colonic luminal microbes will out-compete any AA or peptide transporters in colonocytes for the substrates (6). Therefore, microorganisms in the intestinal lumen likely play a role in ammonia utilization through the synthesis of AA, some of which can enter the lumen of the large intestine (Fig. 1).

AA uptake in the intestine require active absorptive processes. In the small intestine, short peptides are transported into enterocytes by H+ -dependent peptide transporters (e.g. Pep T1) and free AA are absorbed by basic, cationic, neutral AA, as well as leucine- and glycine-prefering transporters (23). All of these AA transporter families are characterized by extensive substrate overlaps and many are part of symport or antiport systems (24). Expression of low- and high-affinity leucine-prefering transporters (LAT-1; LAT-2) was studied in Caco-2 cells, IEC-6 cells, and rat jejunal, ileal, and colonic epithelial cells (25). Expression of LAT-2 was similar for all membranes studied; however, expression of the high-affinity LAT-1 protein was most abundant in the colon, with much lower expression in the ileum and jejunum. Others have characterized 150 transporter-related AA uptake in the mouse colon and shown that mouse colonic transporter mCATR0+ may absorb AA from colonic contents (26). Extensive expression screening has been performed for tissue-specific human solute carrier transporter superfamilies (27). Such efforts have identified intestinal solute transporter superfamilies for AA uptake. However, more work is needed to clarify the quantitative impact of colonic human solute (AA) transporters on the host’s protein nutrition.

Use of probiotics for gut health

Probiotics can be defined as dietary supplements containing beneficial live bacteria or yeast that confer a health benefit on the gut flora and the host. *Lactobacillus* and *Bifidobacterium* are the most widely used probiotic bacteria for maintaining the intestinal ecosystem and integrity. Of particular interest, probiotics increase the digestibility of dietary protein, absorption of AA into the portal circulation, and the efficiency of utilization of dietary AA (28,29), as well as intestinal metabolism of AA and ammonia (30). The possible underlying mechanisms may include enhancements of the release of digestive proteases from the GIT and pancreas and the absorption of small peptides and AA into enterocytes. However, some studies also report no benefits of *Lactobacillus* and *Bifidobacterium* on protein digestibility in pigs (31). The possible reasons for these divergent results may include differences in L animals (e.g. individual variations, developmental stage, basal growth rate, enteric pathogenic challenges, health status, intestinal microbial populations and numbers, intestinal fermentation products, and interactions of probiotics with the intestinal immune system and mucosa); 2) the probiotic products used (e.g. sources, strains, quality, shelf lives, and doses); 3) experimental diets (e.g. nutrient composition and balance, feeding frequency, palatability, the amount of feed consumption, presence of antinutritive factors, and water quality); and 4) environmental factors (sources and levels of stress, housing facilities, temperature, humidity, cleanliness, and management skills).

Several lines of evidence from animal and human studies indicate the efficacy of probiotic therapy in both prevention and treatment of intestinal and extraintestinal disorders. First, oral administration of the *Lactobacilli* preparation (consisting of *L. gasseri*, *reuteri*, *acidophilus*, and *fermentum* isolated from the porcine GIT mucosa) to weanling piglets enhanced feed intake, digestion of nutrients (protein, carbohydrate, and minerals), production of SCFA, growth performance, and resistance to *Escherichia coli* infection while improving microbial balance in the GIT and reducing the incidence of diarrhea (28). Similarly, Estrada et al. (32) reported a beneficial effect of *Bifidobacterium* on pig growth performance. Second, early oral administration of probiotics (*B. animalis* and *L. acidophilus*, casei, pentosus, and *plantarum*) to formula-fed preterm infants increased intestinal growth, villus height, brush border and aminopeptidase A and N activities, altered the colonization of a beneficial commensal microbiota, ameliorated the mucosal atrophy and dysfunction, and reduced the incidence and severity of necrotizing enterocolitis (33). Third, oral administration of *B. lactis* BB12 to preterm infants enhanced whole-body growth, intestinal production of acetate and lactate, and intestinal secretion of Ig (34). Additionally, results of a multicenter trial indicated that supplementing *B. bifidum* and *L. acidophilus* to breast milk or breast milk plus formula twice daily for 6 wk prevented necrotizing enterocolitis and reduced the incidence of death in very low-birthweight preterm infants (35). Similarly, daily oral administration of probiotics (*L. reuteri* ATCC 55730) for 30 d improved feeding tolerance, bowel habits, gastrointestinal motility, and gut function in preterm infants (36). Forth, consumption of *L. acidophilus*-SDC 2012, 2013 by adult patients with irritable bowel syndrome reduced abdominal pain and discomfort (37).

Recent proteomics studies showed that the interactions between probiotics and the small intestine affected protein profiles. For example, after exposure of *L. fermentum* 15007 (a strain isolated from the porcine intestine) to the intestinal lumen (38), key enzymes involved in the microbe’s energy metabolism decreased, including lactate dehydrogenase, dihydrolipoamide
dehydrogenase, and nicotinate phosphoribosyltransferase and in AA metabolism, including arginyl-tRNA synthetase and aspartate-semialdehyde dehydrogenase, while increasing glyco-side hydrolase (an enzyme for mucin degradation) and fructose-6-phosphate phosphoketolase (an enzyme of the pentose phosphate pathway) (38). In response to an interaction with L. fermentum I5007, intestinal cells exhibited changes in proteins related to nutrient transport and antioxidative capacity that were beneficial for gut integrity, including voltage-dependent anion channel 1, glutathione transferase, and heat shock protein gp96 (38). These proteins serve as useful biomarkers for metabolic changes in Lactobacillus and intestinal epithelial cells in response to their interactions.

Use of prebiotics for gut health
Prebiotics can be defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of 1 or a limited number of the bacteria in the small and large intestines. Oligosaccharides (mainly fructooligosaccharides and mannanoligosaccharide) are commonly used prebiotics for livestock species, poultry, and humans. A salient example of prebiotics is their ability to: 1) improve the digestibility of nutrients (protein, dry matter, and minerals), absorption of AA by the small intestine, N economy in the intestine, and feed efficiency (39); 2) modulate the metabolism of urea, ammonia, and AA in the lumen of the large intestine (30); and 3) influence the intestinal microflora to selectively promote the growth and activity of beneficial bacteria (e.g. bifidobacteria) and therefore promote postnatal development of the intestine and whole-body immune systems (40).

Thus, there is evidence that dietary supplementation with mannanoligosaccharide supplementation enhanced the growth performance of young pigs (41,42), but fructo-oligosaccharides had no effect (32,43). Additionally, dietary supplementation with Chitosan oligosaccharides (COS) inhibited the growth of oral and intestinal pathogenic bacteria while increasing the density of small intestinal microvilli (44). In addition, dietary COS supplementation to mice reduced the levels of early preneoplastic markers for colon carcinogenesis while stimulating mucosal and systemic antibody responses against Bordetella pertussis filamentous hemagglutinin and recombinant pertussis (45). Moreover, COS supplementation improved the immune status in chickens and weanling pigs, as well as expression of genes in the small intestinal mucosa (44). Further, a mixture of neutral short-chain galactooligosaccharides and long-chain fructooligosaccharides reduced the incidence of atopic dermatitis, infection episodes, and allergic manifestations in infants. Finally, protective effects of prebiotics appear to persist beyond the intervention period primarily through modification of the intestinal flora (46). Utilization of the distal gut microbiota as a scavenger (and bowel elimination) for potentially pathogenic nitrogenous constituents in the digesta also has merit, but our understanding and ability to manipulate colonic fermentations for such purposes must clearly be extended to integrate these ideas into patient care. For example, during liver failure, gut microbial metabolism may actively contribute to hyperammonemia, whereas during kidney failure, uremia may increase intestine-luminal ammonia (47). To what degree modulation of AA metabolism in enterocytes and intestinal microbes may lower the ammonia and urea load to such patients is not well understood (48) and warrants further investigation.

Literature Cited


