Chapter 8
Nitrogenous Substrates: Nucleic Acids to Amino Excretion

Nitrogen (N) may be the most limiting nutrient for many populations of wildlife because nitrogenous compounds in crude protein are the basis for the structure and function of animals (Fig. 1.4) (White 1993). The organic N in plants and animals includes amino acids and the wide array of proteins they form, the genetic program in DNA and its transcripts in RNA, as well as numerous intermediary metabolites and vitamins. Protein is the largest fraction of N in the animal body, with contractile functions in all muscle fibers, structural functions in connective tissue, skin, tendons and arteries, and specialized functions such as hormones, enzymes, antibodies, transport molecules and clotting factors. Animals may use stored protein for energy during migration and hibernation, and in the production of milk and eggs. Both herbivores and carnivores use dietary proteins for new tissue and for energy, but herbivores also may need to recycle N to conserve the seasonally low supply of protein from their diets.

8.1 Amino Acids and Essentiality

Proteins are a sequence of amino acids linked by peptide bonds. The side chains of 20 amino acids give proteins their structural and functional properties (Fig. 8.1). Structural proteins have repeating sequences that polymerize into fibers (e.g., collagen). The sequences for enzymes (e.g., pancreatic lipase) may be subdivided into sections that fold into globules containing binding sites for cofactors (e.g., co-lipase) and catalytic pockets for substrates (e.g., triglycerides). In a globular protease, the charged side chain on lysine helps to dissolve the protein in water; the small side chain of glycine allows tight folds between helical sections; and the hydrocarbon side chains of valine and leucine line the interior of the protein where the large weakly charged side chain of histidine is used for catalytic reactions (Fig. 8.1) (Mathews and Van Holde 1996). Protein synthesis requires the full complement of the 20 common amino acids in varying proportions that depend on the amino acid sequence of the proteins being formed.

Animals cannot synthesize all the amino acids they require and must therefore rely on a dietary supply from prey, plants or microbes to sustain protein synthesis.

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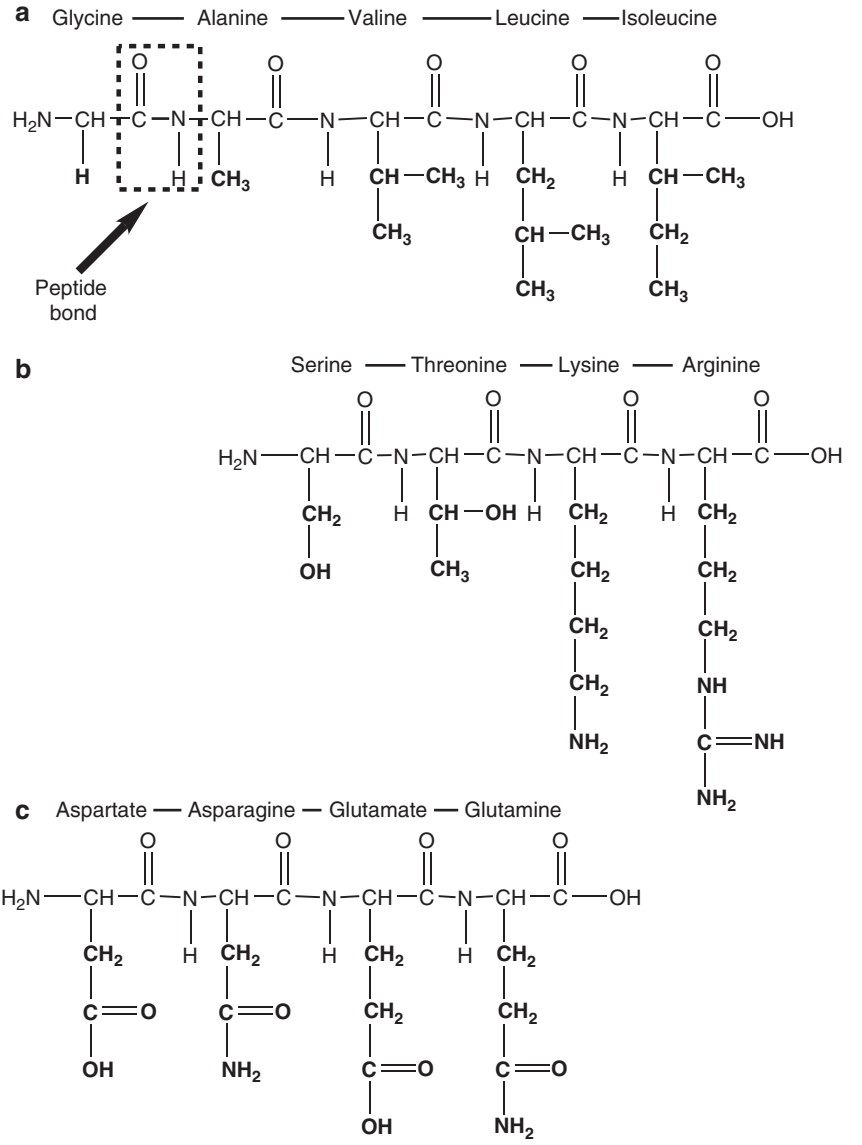

Fig. 8.1 Amino acids used by animals. Amino acids are grouped according to the structure of the side chain (*bold*) and are presented as a peptide. **a** Hydrocarbon side chains. **b** Side chains with hydroxyl (OH) and amine (NH_2) groups. **c** Amide (CONH) and acid (COOH) side chains. **d** Cyclic side chains. **e** Sulfur amino acids. Cystine consists of two cysteine side chains connected through a sulfhydryl bridge. Taurine is not found in proteins but is used as a conjugate for bile salts

Amino acids can be considered in two parts: the α-amino group used in the peptide bond, and a C skeleton that includes the side chain (Fig. 8.1). Non-essential or dispensable amino acids can be synthesized completely from a pool of C and

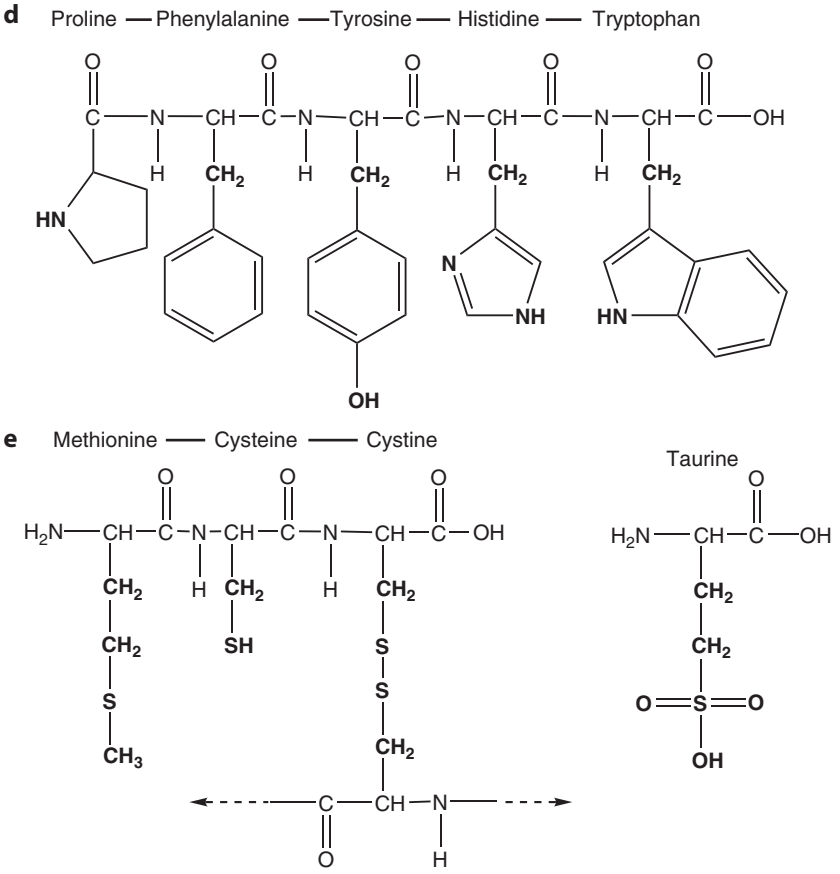

Fig. 8.1 (continued)

amino-N in the diet. A dietary supply of amino acid is required at all life stages when both parts of the amino acid cannot be synthesized (complete essentiality) or when the C skeleton cannot be formed (side-chain essential). Completely essential amino acids such as threonine and lysine are replaced quickly by the body because they cannot be reformed if the amino group is removed or the carbon skeleton is altered. These acids can be used as dietary markers because their isotopic composition tracks those of dietary proteins in growing animals (Fantle et al. 1999). Dietary amino acids also may be required when the synthetic pathways are inadequate for the demand (conditional essentiality). The rate of histidine synthesis in mammals, for example, is sufficient for maintenance of adults but not for growth (Table 8.1).

The quality of a dietary protein is evaluated by comparing the proportions of essential amino acids in the food protein with those in the animal. Muscle proteins are consistent in composition among vertebrates and are generally similar to those of eggs and milk (Fig. 8.2). Consequently, carnivores can easily meet their requirements for essential amino acids because the diet closely matches the proteins synthesized

Table 8.1 Amino acids essential for protein synthesis in animals that lack a significant source of microbial protein from fermentation in the digestive tract

Side chain group	Amino Acid	Basis for essentiality
Hydrocarbon	Valine	Side chain – all life stages
Hydrocarbon	Leucine	Side chain – all life stages
Hydrocarbon	Isoleucine	Side chain – all life stages
Hydroxyl	Serine	Side chain – conditional on glycine and threonine supply in birds
Hydroxyl	Threonine	Complete amino acid – all life stages
Amine	Lysine	Complete amino acid – all life stages
Amine	Arginine	Side chain – conditional on N load for urea synthesis in mammals
Cyclic	Phenylalanine	Side chain – all life stages
Cyclic	Tyrosine	Side chain -conditional on phenylalanine supply
Cyclic	Histidine	Side chain – conditional on rate of growth
Cyclic	Tryptophan	Side chain – all life stages
Sulfhydryl	Methionine	Side chain – all life stages
Sulfhydryl	Cysteine	Side chain – conditional on methionine supply

by the animal. Plant proteins rarely match the composition of animal proteins. Plant leaves contain mostly photosynthetic proteins that are relatively well balanced, but seeds contain storage proteins that are often very imbalanced (Moir 1994). Animals may use an imbalanced protein from one food with a complementary source of amino acids from another item. Thus many primates obtain a balanced amino acid intake by supplementing a diet of fruits and leaves with prey such as insects, eggs and other vertebrates (National Research Council 2003). Alternatively, animals need to ingest more of an imbalanced protein to meet their daily requirement for a limiting amino acid. Low concentrations of lysine and methionine often limit the use of protein from a plant diet (Fig. 8.2). In herbivores, microbial protein may complement plant proteins in essential amino acid composition. Ruminal microbes synthesize proteins with higher proportions of lysine and methionine than many plant proteins (Fig. 8.2) by utilizing both protein and non-protein forms of N and sulfur (S) in plants (Nolan 1993). Grazing bison and kangaroos can therefore use the imbalanced proteins in senescent grasses because they are supplemented with the more balanced microbial protein synthesized in their foregut.

Dietary requirements for essential amino acids are also affected by interactions with other amino acids. Phenylalanine requirements are increased by low supplies of tyrosine because the cyclic side chain for phenylalanine is hydroxylated to form tyrosine (Fig. 8.1D). Conversely, high supplies of tyrosine save phenylalanine and thus lower the requirement for the latter amino acid. The high concentrations of the

Fig. 8.2 (continued) Research Council 2006); skin and wool of domestic sheep (National Research Council 2007b); feathers of domestic chicken (National Research Council 2003); whole eggs from mixed species of fish (Mambrini and Guillaume 2001); average of eggs from chickens, mallard ducks, turkeys and Japanese quail; average of milks from domestic cattle, sheep, goats and water buffalos (Agricultural Research Service 2007)

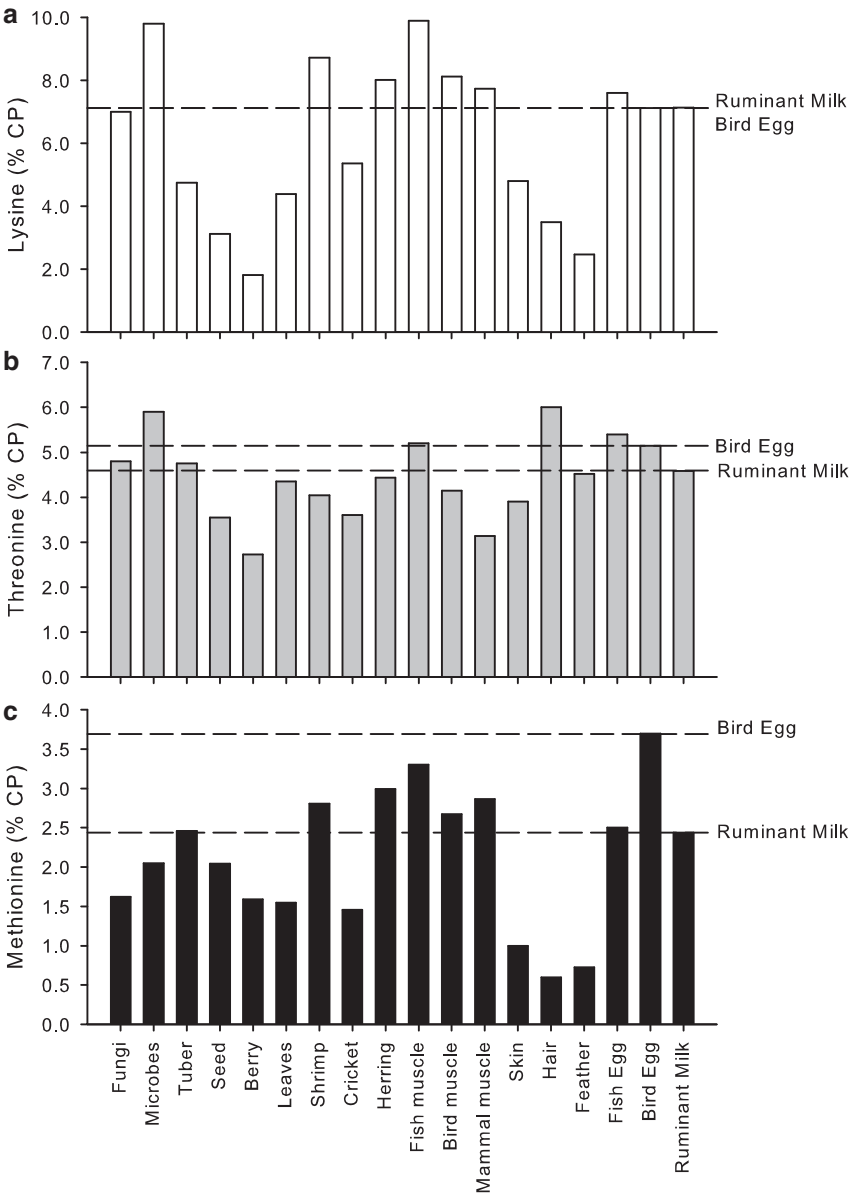

Fig. 8.2 Contribution of three essential amino acids to crude protein (CP) in the tissues of selected microbes, plants and animals. **a** Lysine. **b** Threonine. **c** Methionine. *Dashed horizontal lines* provide a reference for comparison with bird eggs or milk from ruminants. Microbes: yeast (National Research Council 2003) and mixed ruminal organisms from domestic sheep (National Research Council 2007b). Plants: tubers of sweet potato, seeds of corn, blueberries and leafy alfalfa (National Research Council 2003). Animal: muscle from mixed species of shrimp (National Research Council 2006); whole cricket and herring (National Research Council 2003); muscle from mixed species of fish (Mambrini and Guillaume 2001), domestic chicken and cattle (National

S-containing methionine in bird eggs reflect the requirements of the chick for both methionine and cysteine. Feathers, skin and hair are rich in cysteine, which forms the sulfhydryl bridges of cystine that crosslink the protein fibers of keratin (Fig. 8.1E). The requirements of birds during molt or egg laying are therefore considered to be the sum of both of these sulfur amino acids (Murphy and King 1984; Klasing 1998). Requirements for S-amino acids may also include the non-protein amino acid taurine that is mostly used to produce bile salts for lipid digestion and, in most animals, is synthesized from cysteine. However, domestic cats and red foxes require a dietary source of taurine (National Research Council 2006). In the wild, predators obtain taurine from the tissues of fish, birds and mammals. Cats (Family Felidae) are obligate carnivores because they have high requirements for taurine and other amino acids, both dispensable and essential; those requirements are most easily met by consuming other animals (Fig. 8.3).

8.2 Proteins and Digestion

The protein content of tissues is often estimated as 'crude protein' from total N content. Total N is measured by digesting the sample in strong acid to determine ammonia by the Kjeldahl method, or by combusting the sample in a furnace to

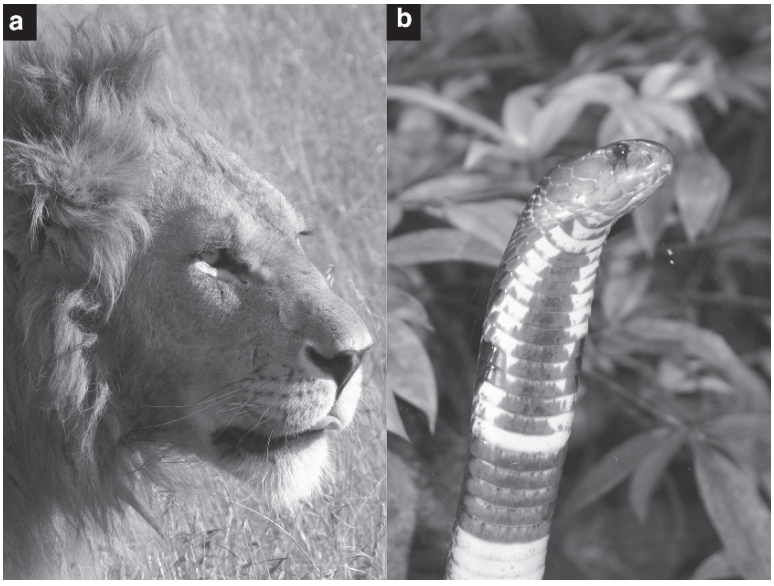

Fig. 8.3 Carnivores derive most of their dietary energy from the oxidation of amino acids in prey. **a** Felids such as the African lion may be considered obligate carnivores because of their requirements for specific amino acids such as arginine and taurine, and their high N requirement for maintenance. **b** Indian cobras use special salivary glands to inject neurotoxins (venom) and proteolytic enzymes to immobilize their prey

determine N_2 gas released in an elemental analyzer. The average N content of a wide variety of proteins is 16 g N·100 g^{-1} protein, and so crude protein is usually calculated as 6.25 g crude protein·g^{-1} N (Robbins 1993). However, the N content of a protein is affected by its amino acid composition; some legume and seed proteins, for example, are 18.9% N (5.29 g crude protein·g^{-1} N). Also, not all the N in the diet is associated with protein, but may be in the form of inorganic nitrates that are not available to animals.

Dietary proteins have a wide diversity of structures and amino acid sequences that must be degraded to peptides and amino acids for absorption. The simple peptide bond between two amino acids has 400 possible sequences (20 × 20 amino acids). Protein digestion (proteolysis) has broad specificity for amino acid sequences, which allows the same system to digest many proteins. Proteolysis is carefully controlled to avoid self-digestion; loss of these controls at death results in continued proteolysis that degrades the tissues of the digestive tract. Consequently, the digestive tract is quickly removed from harvested fish and game to avoid changes in flavor or microbial contamination of the meat as the viscera degrades. Proteolytic enzymes are secreted in the stomach and duodenum as zymogens, which are inactive enzyme precursors that are activated by acid or another enzyme in the digestive tract. Salivary glands of snakes also secrete proteases that are active only in venom and serve to increase the penetration of neurotoxins by degrading skin and muscle proteins (Zug 1993) (Fig. 8.3).

Hydrochloric acid secreted into the stomach denatures proteins by disrupting the weak bonds that fold and hold proteins in tissues, opens membranes by releasing embedded proteins, disaggregates fibrous proteins and precipitates soluble proteins. Acid secretion is stimulated by the presence of protein in the stomach and stopped by negative feedback from the mucosa when digesta pH falls below 1.5 (Fig. 8.4). Denaturation increases the time and surface area for enzymatic digestion by precipitating soluble proteins into a slower-moving solid phase and by opening the structure of all proteins to enzymes. Enzyme secretion is combined with acid secretion in the gastric glands. In mammals, inactive pepsinogen is produced by chief cells at the base of the gland and activated to pepsin with acid produced by parietal cells at the top of the gland (Fig. 8.4). Gastric glands in fish are simpler than those of mammals; carnivores such as pike (Family Esocidae) and sculpin (Family Cottidae) produce pepsinogen and acid together from a group of oxyntopeptic cells in the stomach (Rust 2002). The combination of acid and pepsin breaks the internal peptide bonds of the protein and prevents the molecule from reforming in the higher pH of the midgut.

Gastric proteolysis does not completely digest protein, but it increases the rate of digestion of denatured proteins and peptides in the midgut. Gastric proteolysis is most advantageous to carnivores such as snakes and lizards that consume large meals of protein and frogs that swallow whole prey. Acid secretion and the subsequent regulation of blood pH contribute to large increases in energy expended after a meal, and ultimately reduce the net gain of energy from the diet of these predators (Wang et al. 2005). Animals that consume smaller amounts of proteins that are more easily degraded require less gastric proteolysis and thus have lower costs of digestion. Most fish do not secrete acid or pepsin in the early larval stages, and some species

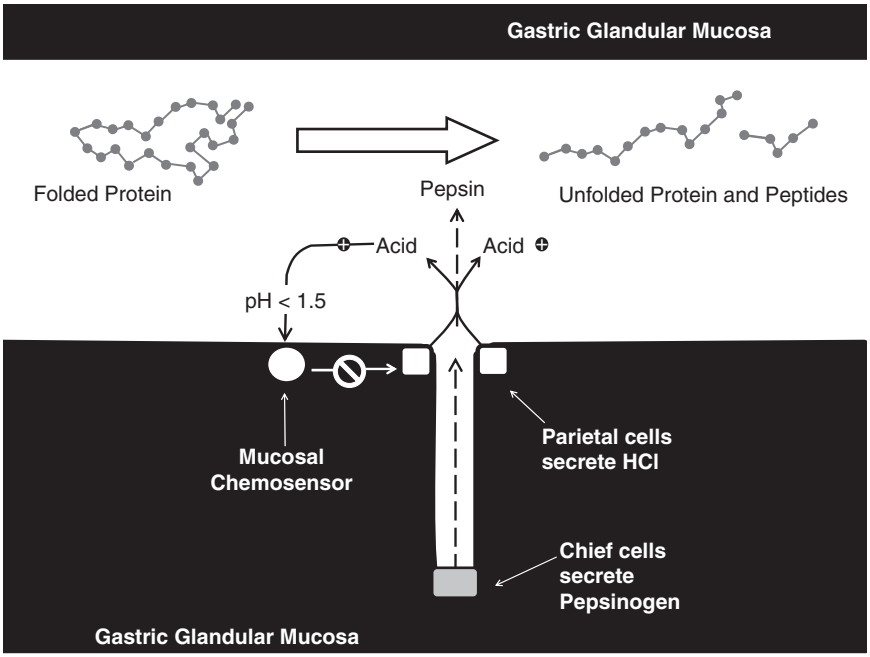

Fig. 8.4 Gastric secretion and its control by feedback inhibition in mammals. Pepsinogen is activated to pepsin by acid hydrolysis soon after the enzyme leaves the gastric gland. Low pH in the digesta inhibits further acid secretion. Proteins are unfolded and partly degraded to peptides by the combined effect of pepsin and acid

are agastric as adults (Chapter 5) (Guillaume and Choubert 2001). Larval fish may rely on easily digested proteins as well as free amino acids from zooplankton (Rønnestad and Conceição 2005). Young mammals also rely on readily digestible milk proteins (caseins) that can be denatured with less acid. Suckling mammals produce a form of pepsin (chymosin or rennett) that is active at a higher pH (pH 4–5) than adult pepsin (pH 2–3). Young animals may therefore expend less energy on protein digestion and direct those savings to deposition of dietary protein and growth. The costs of digestion and growth are discussed further in Chapter 10.

The flow of acid digesta into the duodenum stimulates the release of the hormone secretin into the blood and the secretion of mucus that protects the mucosa. Secretin stimulates the pancreas to release alkaline buffers that raise the digesta to pH 7–8. Pancreatic secretions also contain a series of zymogens that form an activation cascade. The key to the activation cascade is enterokinase, which is released from the duodenum when acid and protein enter the midgut (Fig. 8.5). Enterokinase activates trypsin, which subsequently activates other enzymes such as chymotrypsin and carboxypeptidase (Fig. 8.5). The cascade amplifies and accelerates proteolysis because enterokinase can activate multiple trypsin molecules. Each enzyme has a different specificity for amino acid side chains that complements the rest of the cascade, which results in the rapid degradation of a long chain of peptides from the

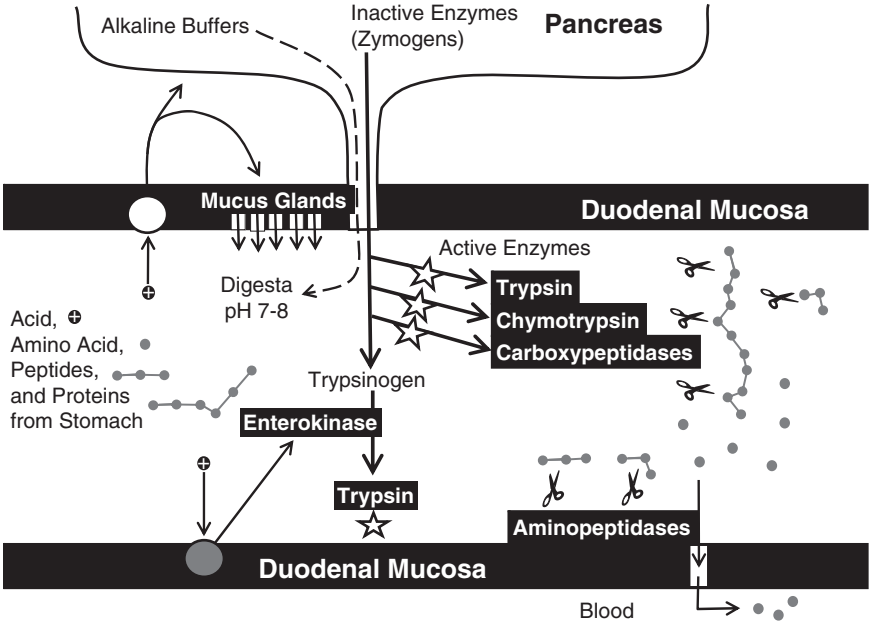

Fig. 8.5 Digestion of protein in the small intestine. Acidic digesta stimulate the release of buffers from the mucosa and from the pancreas. Pancreatic enzymes are activated by a cascade in the digestive tract. Enteropeptidase from the mucosa activates trypsinogen to trypsin. Trypsin (*star*) activates other trypsinogen molecules as well as chymotrypsinogen, pro-carboxypeptidases and other pancreatic zymogens. Amino peptidases at the surface of the mucosa degrade small peptides. Amino acids and small peptides are transported into the cells of the mucosa. Peptides are further cleaved in the cell before amino acids are released into the blood

center and both ends. Peptides are further cleaved by amino-peptidases that are bound to the mucosal surface.

Mucosal cells absorb both individual amino acids and short peptides of two or three amino acid residues (dipeptides and tripeptides) (Breves and Wolffram 2006). Amino acids are absorbed through the mucosal cell and secreted into the blood by an array of transporters with different side-chain specificities. Amino acid transporters use ATP and can therefore oppose concentration gradients when an acid has a higher concentration in the blood than in the digesta; herbivores therefore can absorb amino acids even when the concentrations in the diet and the digesta are low. Over half the amino acids may be absorbed as short peptides by PEPT1 transporters (Breves and Wolffram 2006). Most of the short peptides are hydrolyzed to amino acids within the mucosal cell before being secreted into the blood. The number of amino acid and peptide transporters declines precipitously from the ileum to the hindgut, so that amino acids are poorly absorbed from the cecum and colon (Hume et al. 1993). Most microbial protein produced by fermentation in the hindgut is therefore lost in the feces. Small hindgut fermenters such as marsupial possums, hares, lemmings and grouse may recover some of this protein by practicing

cecotrophy (Chapter 5); the ingestion of cecal contents allows microbial protein to be digested in the foregut and the midgut in the same manner as dietary proteins (Stevens and Hume 1995).

A very small fraction of the proteins and peptides in digesta is absorbed when cell membranes invaginate and bring substances into the cells (pinocytosis) without degrading the amino acid sequences. The absorption of these proteins does not contribute significantly to the amino acid uptake of the animal but is an integral component of the immune system. Lymphoid cells are distributed in clusters throughout the digestive tract as follicles at the esophagus (tonsils), duodenum (Peyer's patches), ileum and cecum (Klasing 2005). The absorbed proteins (antigens) are used to produce antibody proteins that will recognize and bind to invading organisms. The antibodies are produced by immune response cells (lymphocytes) in the mucosa. Free antibodies (immunoglobulins) that are secreted in mucus bind to the surface proteins of organisms and make them more vulnerable to proteolysis and thus destruction. Infant mammals receive immunoglobulins in milk. Some of the milk immunoglobulins bind the proteins of organisms while others are absorbed by the infant for the developing library of antigens. Birds transfer immunoglobulins to their eggs for immune function in the chick (Klasing 2005). Infections and immune responses of young animals may slow growth because energy and nutrients are diverted to repairing tissue and to responses at the intestine that can impair absorption when the mucosa is infiltrated with lymphocytes. The cost of maintaining immune function and its relationship with trace minerals and vitamins are discussed in Chapters 9 and 10.

8.3 Intermediary Metabolism of Amino Acids

The organs of the body have different demands for amino acids because they synthesize proteins with different amino acid sequences. The C and N in amino acids are therefore exchanged within a cell and between organs. Transaminases catalyze reversible reactions that transfer amino groups between the C skeletons of all 20 common amino acids. For example, alanine aminotransferase (ALT) uses the amino acid alanine and the C skeleton keto-glutarate to produce a C skeleton pyruvate and another amino acid, glutamate. Hibernating bears use ALT to make glucose in the liver from alanine in muscle; alanine is exported from muscle to the liver where the amino acid is converted to pyruvate for gluconeogenesis (Koebel et al. 1991). The transaminases are intracellular enzymes that are elevated in blood serum when cells are damaged; serum ALT increases as liver cells are damaged in dogs but decreases during hibernation in bears, which indicates that the liver and other tissues are not damaged by the long fast (Blood and Studdert 1988; Barboza et al. 1997).

Amino acids can be oxidized for energy (ATP) or stored as lipid or glycogen once the amino group is removed by a transaminase. All 20 common amino acids can be converted to C skeletons that can enter the TCA cycle (Fig. 8.6). Consequently, the C from all amino acids can be used as a fuel during fasting or exercise. All amino acid C can be stored as fatty acids (ketogenic) in triglycerides (fat). Glucogenic amino acids

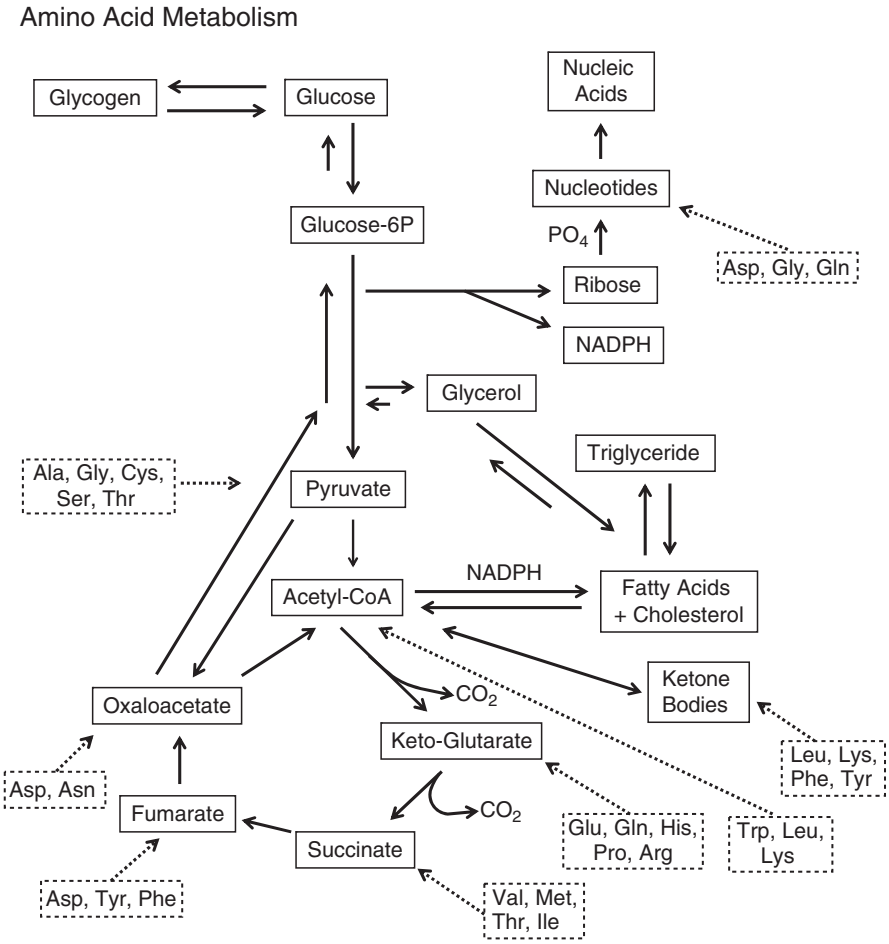

Fig. 8.6 Metabolic pathways for utilizing amino acids for energy or for storing the C as fat (triglyceride) or glycogen

can be used to produce glucose, glycerol and glycogen (Fig. 8.6). Body proteins are used to restore glucose reserves during prolonged fasting (e.g., hibernation in bears) or exercise (e.g., migratory flights of birds) because all but two of the amino acids are glucogenic. The use of body protein for energy is discussed in Chapter 10.

8.4 Nucleic Acids and Digestion

The bases of DNA and RNA contain N; bases with double rings are purines (adenine, guanine) whereas those with a single ring are pyrimidines (thymine, cytosine, uracil; Fig. 8.7). A sugar (ribose or deoxy-ribose) added to a base forms a nucleoside that

then becomes a nucleotide when phosphate is added to the sugar. Phosphate esters on each nucleotide (e.g., adenosine-mono-phosphate or AMP) are used as energy currencies in ADP and ATP. Phosphate esters between sugars form polynucleotide chains for the various forms of RNA (messenger, transcriptional, ribosomal) and DNA (nuclear, mitochondrial, cytosolic). Nucleic acids can be synthesized de novo from phosphate and amino acids because glucogenic acids can be used to produce ribose and dispensable amino acids are used to produce the bases (Fig. 8.6). Consequently, vertebrate animals do not require nucleic acids in their diet. On the other hand, biting flies and mosquitoes may require nucleic acids in their diet during egg production because they cannot produce sufficient DNA for their eggs. Biting insects that use the N from blood, muscle and skin of vertebrates subsequently affect the movements and energy expenditures of animals such as caribou and moose during summer (Renecker and Hudson 1990; Russell et al. 1993; Mörschel and Klein 1997).

Nucleic acids are packaged with proteins and are contained within cell membranes. Acid in the foregut exposes the nucleic acids by disrupting membranes, by denaturing the proteins that wrap DNA and by disrupting the weak bonds between bases in DNA and ribosomal RNA. Pancreatic proteases continue the process of unraveling nucleic acids in the duodenum. Pancreatic endonucleases for RNA (ribonuclease) and DNA (deoxyribonuclease) cut sugar-phosphate esters at the

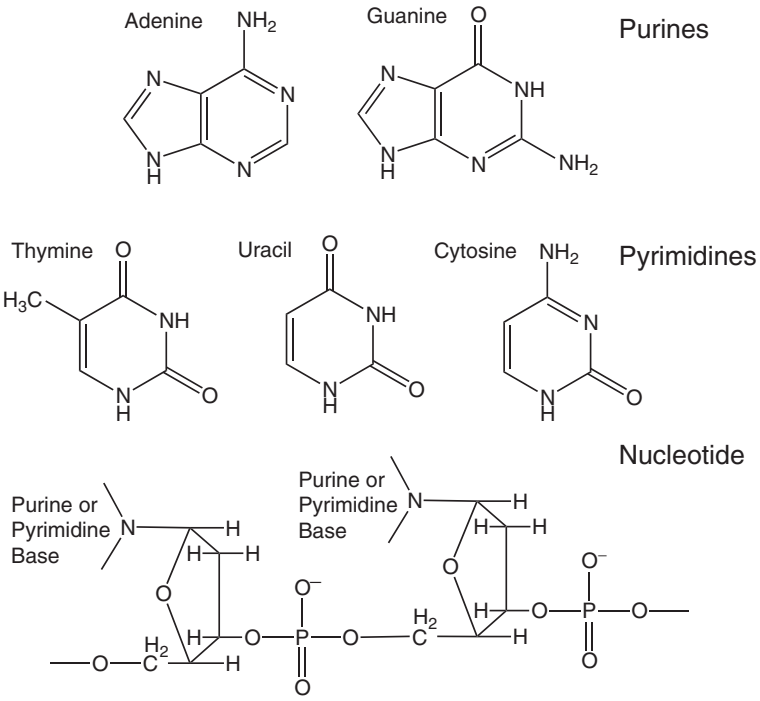

Fig. 8.7 Pyrimidine and purine bases used to form nucleotide chains in RNA and DNA with phosphates and either ribose (RNA) or deoxy-ribose (DNA)

center of the chains to produce polynucleotides. Intestinal phosphodiesterases cleave mononucleotides from the ends of the chains before nucleotidases remove the phosphate to produce nucleosides (base + sugar) (Mathews and Van Holde 1996). Nucleosides and inorganic phosphate are absorbed by active transport into the mucosa (Breves and Wolffram 2006).

Nucleic acids may account for 20% of the microbial N flowing into the duodenum of ruminants because every microbial cell produced by fermentation has a genome (Stevens and Hume 1995). Elk, bison, sheep, goats, cattle and kangaroos have high concentrations of endonuclease in the pancreas because nucleic acids are an important source of nucleosides as well as phosphate for foregut fermenters. Absorbed nucleosides are readily phosphorylated by tissues for incorporation into nucleotides. The sugar from excess nucleosides can be oxidized for energy or used for synthesis of glucose or triglycerides (Fig. 8.6). Excess purines and pyrimidines are oxidized and then excreted in the urine.

8.5 Nitrogen Metabolism

Proteins and nucleic acids are both forms of organic N that are linked by their functions and by the metabolism of the N they contain. Organic N flows through several interconnected metabolic pools in the body. Body protein is the largest pool of N, and is constantly turned over as cells maintain their function. Proteins are degraded and synthesized continuously; the difference between the two rates results in net synthesis or net loss (Fig. 8.8). Total protein turnover is similar between summer activity and winter hibernation of bears; body protein is gained in summer when the rate of synthesis exceeds degradation, but is lost during hibernation when the rate of degradation exceeds synthesis (Barboza et al. 1997). Protein turnover releases and consumes all 20 common amino acids that exchange C and N by transamination in a combined pool of amino acids. Cellular RNA and DNA are also turned over as proteins are synthesized and degraded throughout the life of cells, from growth and maintenance to their eventual death. Nucleic acid turnover therefore results in turnover of the subsidiary pools of purine and pyrimidine N in the body (Fig. 8.8).

Waste N is formed whenever the C in either protein or nucleic acids is oxidized for energy. Body N is excreted during normal cell turnover for maintenance of tissues and when cellular constituents are used during fasting or exercise. Dietary N is excreted when N intake exceeds the demand for cell replacement and growth, that is, when dietary protein is oxidized for energy. Waste N is routed to pathways that are already available for oxidizing the C from amino acids or purines (Fig. 8.8).

8.5.1 Ammonia

Amino acids are the most abundant form of organic N and the principal source of excretory N from the diet or from body tissue. The simplest route for using C from

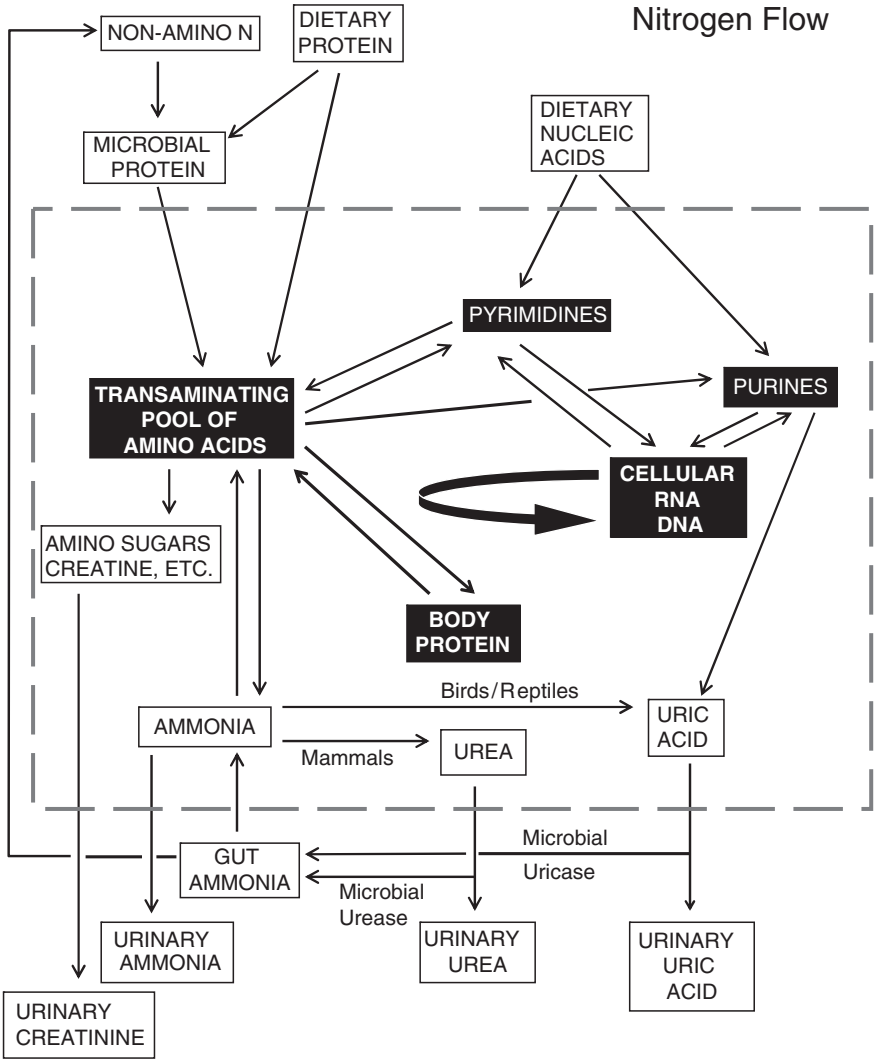

Fig. 8.8 Exchanges of N within the animal body (*broken line*) and their relationships to dietary intake and excretion of N metabolites

amino acids is to remove the amino groups as ammonia (NH_3). Amino groups are routed to a common amino acid such as glutamate by transamination; for example, the amino group from excess alanine is transferred to α keto-glutarate with ALT to produce glutamate (Figs. 8.1c, 8.6 and 8.8). The amino group is removed from glutamate by glutamate dehydrogenase to release ammonia (Fig. 8.9). Glutamine also carries an amino group on the side chain; an amino group is added to the side chain of glutamate to produce glutamine and subsequently removed to produce ammonia (Fig. 8.9). Ammonia is produced at the liver and the kidney from glutamate

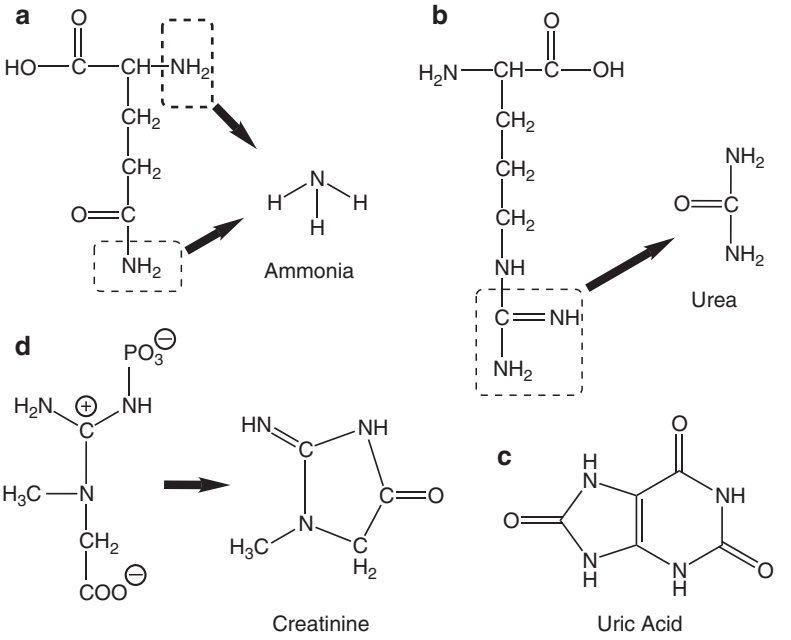

Fig. 8.9 Excretory metabolites of N. **a** Ammonia is formed from the amino groups of amino acids such as glutamine. **b** Urea is produced by hydrolysis of the arginine side chain. **c** Uric acid is produced from purines such as adenine, guanine and their amino acid precursors. **d** Creatinine is spontaneously formed from creatine phosphate in muscle

and glutamine. However, ammonia is toxic because it spontaneously forms ammonium in water (NH_4^+) and acts as a potent alkali that alters the pH of body fluids. Consequently, animals cannot tolerate accumulation of ammonia in the body. Most of the waste N from fish is excreted as ammonia at the gills where ammonium diffuses readily into the surrounding water; this is an inexpensive route for N excretion that allows larval fish to rely on amino acids as their primary source of C and energy (Mambrini and Guillaume 2001).

8.5.2 Urea

Mammals only use ammonium excretion to control urinary pH for acid–base balance. Mammals excrete most of their waste N as urinary urea, which is non-toxic and readily diffuses across membranes. Transaminated amino groups and ammonia are used to produce urea in the liver (Waterlow 1999). Urea synthesis is a modification of the pathway for synthesizing the amino acid arginine (Fig. 8.1B); the addition of one enzyme (arginase) hydrolyzes the side chain to produce urea (Fig. 8.9). Dietary arginine serves as a carrier for routing excess N from the diet to urea; obligate

carnivores such as cats have high demands for arginine and thus urea synthesis (National Research Council 2006) because they oxidize large amounts of protein for energy.

Urea is a primary osmolyte for marine sharks; urea is excreted at the gills and the kidney but accumulates in the body when filtered urea is returned to the blood as the salinity of water changes (Withers 1992; Pillans et al. 2005). Terrestrial mammals concentrate urea in the kidneys, especially as water availability declines; desert mammals increase the concentration of urea in both urine and blood. The ability to retain urea conserves water in camels and desert kangaroos because these animals can excrete less water in urine (Schmidt-Nielsen et al. 1957; Hume 1999). Urea also diffuses from the blood into the saliva and across the mucosa of the foregut and hindgut through urea transporters (Martin et al. 1996; Marini et al. 2006). Microbes in the gut degrade the urea with urease to produce ammonia, which can be used for microbial protein synthesis. Waste N that is recycled as urea in ruminants and kangaroos sustains the microbial community and its fermentation of low-protein fibrous forage (Kennedy et al. 1992). Urea recycling is also an important route for conserving N in winter when diets are both low in N and high in fiber. In reindeer and caribou, for example, 71% of the urea produced in winter is recycled to the gut when animals consume low-N diets such as lichens (Barboza and Parker 2006, 2008). In muskoxen, 87% of the urea produced on a fibrous diet of grass in winter is recycled to the gut, and 45% of this recycled N is returned to the amino acid pool in the body (Barboza and Peltier, unpublished data). Recycled urea-N can return to the pool of amino acids in two ways: when the microbial protein synthesized from the recycled N is digested and the amino acids are absorbed in the small intestine, or when the microbial amino acids are deaminated in the gut and the ammonia is absorbed and attached to C-skeletons in the liver (Fig. 8.8). In dormant bears, 97% of the urea-N produced in the body is recycled to the gut, and 100% of the recycled N is returned to the amino acid pool, which conserves body protein and allows young animals to survive their first winter (Barboza et al. 1997).

8.5.3 Uric Acid

Uric acid is produced from a pathway for degrading purine bases (adenine and guanine) in most tissues, especially the liver (Figs 8.8 and 8.9). All animals produce uric acid, but the compound is processed further to more soluble products such as urea in fish and allantoin in most mammals other than humans. The urinary excretion of purine metabolites such as allantoin can be used to indicate the digestion of nucleic acids, which is mainly associated with digestion of microbial cells in herbivorous mammals (Balcells et al. 1998). Purine excretion can be used to indicate the microbial yield and indirectly the relative metabolizable energy intake by domestic ruminants and free-ranging elk from urine samples collected in snow (Garrott et al. 1996).

In reptiles and birds, most waste N is excreted as uric acid. Excess N from amino acids is transaminated to aspartate, glycine and glutamine which are incorporated

into purines (Fig. 8.6). Uric acid is less soluble than urea and can therefore be safely stored in a solid form within the developing egg of birds and excreted with minimal water loss by desert birds and reptiles (Campbell 1994). Uric acid is secreted by active transport at the renal tubules rather than filtered like urea. Consequently, uric acid excretion allows carnivorous birds and reptiles to excrete large amounts of N in a semi-solid form even though their kidneys can only produce a dilute urine (Kirschner 1991). Uric acid is maintained in a colloidal form in the kidneys by forming microspheres coated with small amounts of protein; the acid would otherwise precipitate and block the tubules (a condition called renal gout) (Braun 1999). In reptiles, uric acid may also facilitate excretion of excess minerals such as Na or K that can be complexed with urate salts for safe storage in the urinary bladder (Minnich 1972). In ducks and grouse, urine from the cloaca is refluxed into the colon and ceca where water and ions are absorbed and where urates are degraded by microbial uricases (Braun 1999; Hughes et al. 1999). The ammonia so produced is subsequently absorbed into the blood and returned to the liver. Recycling of uric acid in birds is not as well researched as urea recycling in mammals; recycled urate-N could conserve body protein in birds such as grouse that consume low-N winter browse, but the proportions of urate-N recycled and returned to amino acids have not been measured (Laverty and Skadhauge 1999).

8.5.4 Creatinine

Creatinine is derived from the high-energy store of phosphate in muscle (creatine phosphate; Fig. 8.9). Creatine phosphate spontaneously forms creatinine, which cannot be re-used and is therefore excreted in the urine. Creatinine in blood and urine is derived from muscles in the diet and the body of carnivores. In herbivores, daily urinary excretion of creatinine (milligrams per day, $mg \cdot d^{-1}$) is proportional to muscle mass and is not affected by muscle activity. The concentration of creatinine (milligrams per milliliter, $mg \cdot mL^{-1}$) can therefore be used as a reference for concentrations (milligrams per milliliter, $mg \cdot mL^{-1}$) of other metabolites by calculating a ratio to creatinine (milligrams of metabolite per milligram of creatine, mg metabolite·mg $creatinine^{-1}$).

The urinary ratio method has been used to evaluate the relative condition of wild ruminants in winter where urine can be collected from snow (DelGiudice et al. 1989). Creatinine ratios have been applied to deer (*Odocoileus* sp.) for urea from amino acids, allantoin from purines, 3-methylhistidne from muscle, hydroxyproline from connective tissue, and cortisol and corticosterone from stress hormones (DelGiudice et al. 1988, 1996, 1998; Saltz and White 1991; Parker et al. 1993a; Vagnoni et al. 1996). Increases in the urinary ratio of urea to creatinine concentrations have been used to indicate the oxidation of amino acids from body protein in winter for white-tailed deer, elk and bison (Moen and DelGiudice 1997; DelGiudice et al. 2001). Creatinine concentrations do not remain constant in all species because clearance of creatinine at the kidneys changes with season in reindeer, caribou and muskoxen (Peltier et al. 2003; Parker et al. 2005; Barboza and Parker 2006). Also, urea to creatinine concentrations

may not accurately reflect body protein loss when renal function is altered such as by water availability or solute load, or when dietary N is excreted as urea (Barboza et al. 2004). One alternative to using urea to creatinine concentration ratios as a measure of body condition is to compare the N isotopes in urinary urea with those from dietary N and muscle creatinine or blood cells; in reindeer and caribou the isotopic similarity between urinary urea and body proteins increases as body N is oxidized to urea (Parker et al. 2005; Barboza and Parker 2006).

8.6 Nitrogen Balance and the Requirement for N

N balance is the net gain or loss of N from the body of an animal, which is usually measured by mass balance in a metabolism cage or tank (Chapter 4). The minimum requirement for dietary N is the intake that supports zero N balance, that is, when the rates of protein synthesis and degradation are equal (Fig. 8.10). Positive N balance reflects deposition of dietary N in tissues for either short-term stores in the liver or for growth and seasonal gain of tissue such as muscle. N balance does not continue to increase with intake of N because mass gain is controlled by the genetic program for

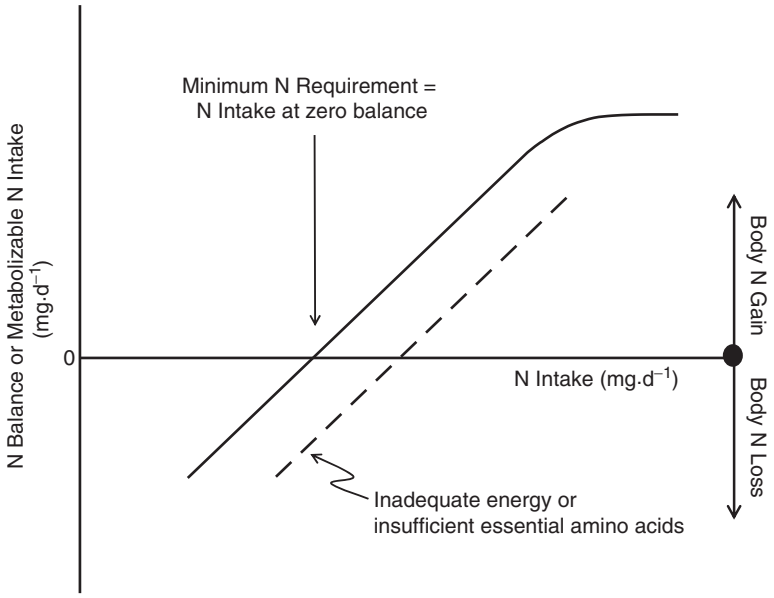

Fig. 8.10 Estimating the minimum requirement for N from the relationship between N balance and N intake (*solid line*). Animals retain less N when supplies of energy or essential amino acids are inadequate (*broken line*). Inadequate intakes of energy increase N loss because dietary and body proteins are used for energy. Inadequate supplies of essential amino acids decrease N gain by limiting the synthesis of body protein. N balance reaches an asymptote when intakes of N exceed the maximum for protein deposition in the body

tissue turnover and synthesis. Protein synthesis requires a complete supply of amino acids; diets that are low in one or more essential amino acids will increase the apparent N requirement because the animal must consume more N to meet the demand for the limiting amino acids (Fig. 8.10). Animals in negative N balance use stores of body protein to sustain the amino acid pool for protein synthesis in critical tissues. Labile proteins such as albumins from the liver are used to sustain the amino acid pool between meals, whereas proteins in muscle may be used during prolonged fasting or exercise (Wannemacher and Cooper 1970; Waterlow 1999; Bordel and Haase 2000).

The minimum N requirement for maintenance of an animal can be estimated from endogenous losses in urine and feces. Daily and seasonal requirements for N are minimized by reducing losses of N in both the urine and the feces (Table 8.2). Those losses are lowest when energy intakes are adequate for protein turnover, that is, when digestible energy intakes are sufficient to maintain body mass. The interactions between energy demands and body protein of fasting, growing and reproducing animals are discussed further in Chapter 10.

8.6.1 Endogenous Urinary N

The minimum urinary N loss that is predicted at zero N intake is called endogenous urinary N (EUN). The principal source of N in EUN is urea or uric acid from amino acids that were released by normal protein turnover (Fig. 8.8). Amino N is excreted when the C skeleton is used for energy (ATP) or for glucose synthesis (Fig. 8.6). Carnivores have higher EUN than herbivores and consequently require more dietary N at zero balance. High EUN in cats and other obligate carnivores reflects their inability to down-regulate amino acid oxidation when protein intakes decline

Table 8.2 Calculating N balance in a ruminant (e.g., caribou)

Parameter	Calculation	Measure
Ingested N ($g \cdot d^{-1}$)	A	56
Fecal N ($g \cdot d^{-1}$)	B	12
Digestible N intake ($g \cdot d^{-1}$)	C = A − B	44
N digestibility ($g \cdot g^{-1}$)	D = C ÷ A	0.79
Metabolic fecal N (MFN) loss ($g \cdot d^{-1}$)	E	9
Truly digestible N intake ($g \cdot d^{-1}$)	F = A − (B − E) = C + E	53
True N digestibility ($g \cdot g^{-1}$)	G = F ÷ A	0.95
Urinary N loss ($g \cdot d^{-1}$)	H	8
Metabolizable N retained ($g \cdot d^{-1}$) (N balance)	I = C − H	36
N metabolizability ($g \cdot g^{-1}$)	J = I ÷ A	0.64
Endogenous urinary N (EUN) loss ($g \cdot d^{-1}$)	K	4
Truly metabolizable N intake ($g \cdot d^{-1}$)	L = C − (H − K) = I + K	40
True N metabolizability ($g \cdot g^{-1}$)	M = L ÷ A	0.71
Biological value ($g \cdot g^{-1}$)	N = L ÷ F	0.75

(Hendriks et al. 1997). High energy demands may contribute to increasing EUN if amino acids are used for energy. Eutherian mammals such as deer have higher EUN than marsupials (kangaroos), partly because their basal rates of energy metabolism are higher than those of marsupials (Hume 1999). High glucose availability reduces EUN because amino acids are rarely needed for gluconeogenesis. Nectarivorous marsupials (e.g., honey possums) and birds (e.g., hummingbirds) have very low EUN losses and thus low N requirements (Brice and Grau 1991; Bradshaw and Bradshaw 2001). Recycling of waste N further reduces EUN and N requirements. Herbivores with extensive fermentation systems such as grazing kangaroos, wombats and camels have low EUN because they can re-use a large proportion of the urea they produce (Hume 1999).

8.6.2 Fecal N Losses

Losses of N in feces depend on the N content and structure of the diet and the function of the digestive tract (Chapter 5). Fecal N losses are lowest for nectarivores because their liquid diet is highly digestible. Diets of fibrous plants result in more feces and a greater loss of indigestible N and unresorbed N. Indigestible N is mainly associated with proteins and non-amino N bound to the cell walls of plants. Some soluble dietary proteins can also escape digestion if they are bound to tannins, a group of PSMs found in the leaves of woody plants (Chapter 3). A standard approach to measure protein binding of forage tannins is to measure the ability of the food to precipitate protein from cattle blood (bovine serum albumen or BSA). This quantification of tannin-binding capacity is used to more accurately calculate the reduction in digestible protein and digestible dry matter consumed by ruminants (Hanley et al. 1992). Tannins may also bind to proteins in the secretions of some animals such as saliva (Robbins et al. 1987) which reduces the impacts of tannins as digestion-reducing agents.

The unresorbed fraction of fecal N that is not of immediate food origin is called metabolic fecal N (MFN) because it is comprised of mucus secretions and cells. MFN increases with food intake and with abrasive dietary components such as plant fiber consumed by herbivores and the particles of sand ingested by omnivores that feed on underground fungi, insects and plant tubers (Young and Hume 2005). MFN also includes microbes that are associated with the solute phase as well as those bound to the fiber matrix in the particulate phase of digesta (Mason 1969). Hindgut fermenters such as horses lose most of the microbial N produced from fermentation in their feces, whereas cecotrophy in ringtail possums recovers enough microbial N from the feces to halve their dietary N requirement (Chilcott and Hume 1984). The fecal loss of microbial N is influenced by the structure and composition of the diet, and the composition of the microbial community (Chapter 6). Modified amino acids can serve as bacterial markers in the feces (e.g., 2,6-diaminopimelic acid or DAPA) of herbivores. DAPA can be used as an index of the dietary energy available to the foregut fermentation in ruminants such as white-tailed deer (Brown

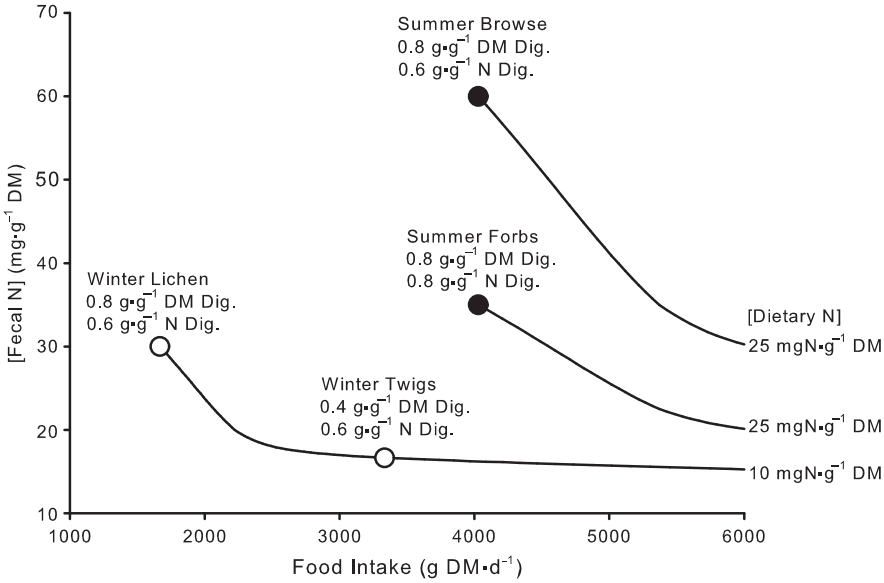

Fig. 8.11 A seasonal model of fecal N concentration (mg N·g^{-1} DM) in relation to dietary N for caribou. Food intakes are based on meeting energy demands of 24 and 58 MJ·d^{-1} in winter and summer respectively. Food intakes vary as the energy digestibility of the diet changes from 0.8 to 0.2 kJ·kJ^{-1} when the gross energy content is 18 kJ·g^{-1} DM (digestible energy contents of 3.6–14.4 kJ·g^{-1} DM). Sources of fecal N include metabolic fecal N (MFN) as well as the undigested residues from the food; MFN is calculated as 1 g N·100 g^{-1} fecal DM. This calculation may underestimate fecal N because abrasion can increase MFN at low digestibilities and high intakes of DM

et al. 1995); increasing energy intake provides more energy for microbial growth and the production of more DAPA in the rumen (Van Soest 1994).

Fecal concentrations of N have been used to indicate the N content of food for herbivores, especially ruminants such as deer and sheep. However, the concentration of any metabolite in feces is affected by the DM digestibility of the diet. Less digestible diets result in more feces that are mainly associated with plant fiber or ash which lower the concentration of all N fractions. Fecal N concentration declines as an animal eats more food to compensate for the decreasing digestibility of energy in the diet, that is, indigestible DM dilutes fecal N as food intakes increase. The model in Fig. 8.11 predicts a decline in fecal N when caribou switch from a highly digestible food such as lichen to less digestible forage such as twigs that contain the same concentration of N. Increases in the N content of the diet will increase fecal N concentration because more N is lost with the indigestible fraction. Fecal N concentrations typically increase when herbivores switch from senescent winter twigs to new summer forbs because DM digestibility and dietary N content increase together (Fig. 8.11) (McKinney et al. 2006). Tannins, which often tend to be higher in summer browse, may enhance the indigestible N and thus the concentration of N in feces (Osborn and Ginnett 2001). Variation in the energy and N

digestibility of foods as well as changes in food intake and digestive function (Chapter 5) alter the relationship between fecal N and dietary N concentrations (Fig. 8.11). The concentration of fecal N from white-tailed deer does not increase with increasing dietary protein when diets are highly digestible but does increase with dietary protein on low-energy diets (Brown et al. 1995). Similarly, the model in Fig. 8.11 predicts that fecal N concentration would change very little when caribou switch from winter lichen to summer forbs because both diets are high in digestible energy.

Thus fecal N may be more useful as an indicator of dietary N for some species and habitats than others. Long-term data sets on fecal N reflected annual variation in forage quality for growth of bighorn sheep but patterns of fecal N were less indicative of forage growth and body condition of mule deer (Kucera 1997; Blanchard et al. 2003). The relationship between fecal N concentration and dietary N varies because digestive responses may enhance or diminish the rate at which fecal N increases with dietary N. Fecal N is just one of several indicators that may be used to relate food quality and abundance to the body condition of herbivores; these complex interactions are best evaluated with multiple markers and an appreciation of the underlying physiology. Body composition of animals is further discussed in Chapter 10.

8.6.3 Protein Quality

The quality of dietary N for an animal is related to the efficiency of digesting and retaining the ingested N. Fecal and urinary losses of N include dietary N as well as MFN and EUN. The apparent or net uptake of N from the diet is therefore the digestible N intake, and the apparent or net retention of that dietary N is the metabolizable N intake or N balance (Table 8.2). The gross uptake of N from the diet is the truly digestible N intake, that is, the apparent digestible N intake plus MFN (Table 8.2). Similarly, the addition of EUN to apparent metabolizable N intake is the true retention of N from the diet (Table 8.2).

The proportion of MFN in total fecal N is highest when foods are low in N. Consequently, apparent digestibilities of N decline with dietary N content and therefore underestimate the quality of low-N foods (Robbins 1993). True N digestibilities are a better indicator of the availability of protein for digestion, which is estimated as $1.00\,g \cdot g^{-1}$ for prey consumed by bears (Pritchard and Robbins 1990) and $0.93\,g \cdot g^{-1}$ for grasses and legumes consumed by mule and white-tailed deer (Robbins et al. 1987). High true digestibilities reflect the wide specificity and speed of proteolysis in the small intestine (Fig. 8.5). True N digestibilities may be reduced by PSMs that form complexes with dietary proteins (e.g., tannins in woody browse) or with gut proteases (e.g., protease inhibitors in seeds) (Harborne 1993).

The proportion of the absorbed N that is retained by the animal is the biological value, which is calculated as the ratio of truly metabolizable to truly digestible N intakes (Table 8.2). Nitrogen metabolizabilities and biological values are highest

Table 8.3 The effect of dietary protein quality on a growing carnivore (e.g., the American mink)

Parameter	Calculation	Low-quality protein	High-quality protein
Biological value ($mg \cdot mg^{-1}$)	A	0.75	0.95
Ingested N ($mg \cdot d^{-1}$)	B	1,200	1,200
N digestibility ($mg \cdot mg^{-1}$)	C	0.9	0.9
Digestible N intake ($mg \cdot d^{-1}$)	$D = B \times C$	1,080	1,080
Metabolic fecal N (MFN) loss ($mg \cdot d^{-1}$)	E	6	6
Truly digestible N intake ($mg \cdot d^{-1}$)	$F = D + E$	1,086	1,086
Truly metabolizable N intake ($mg \cdot d^{-1}$)	$G = F \times A$	815	1,032
Endogenous urinary N (EUN) loss ($mg \cdot d^{-1}$)	H	200	200
Metabolizable N retained ($mg \cdot d^{-1}$) (N balance)	$I = G - H$	615	832
Maintenance requirement for N ($mg \cdot d^{-1}$)	J	560	560
Body protein gain ($mg \cdot d^{-1}$)	$K = (I - J) \times 6.25$	341	1,698

when the supply of amino acids from the diet closely matches the requirement for tissue synthesis. Large differences between the composition of animal tissues and plant proteins may result in biological values as low as 0.58 for pollen (Bradshaw and Bradshaw 2001). Egg and milk proteins have high biological values at or near 1, which indicates that very little amino-N is lost by oxidation of these proteins. High biological values for egg or milk protein in growing animals reflect natural selection for maximizing the transfer of N from mother to offspring. In the example in Table 8.3, a growing mink is projected to gain body protein four times faster on high-quality egg protein than on the same amount of lower-quality plant protein (i.e., with a biological value of 0.95 vs. 0.75). Growth and reproduction may be limited by the biological value of proteins available to small animals with limited home ranges. Habitat disturbances that reduce the proportions of forbs with high N content and DM digestibility lower the biological value of available proteins for growth of cottontail rabbits (Lochmiller et al. 1995). Similarly, granivorous birds may rely upon the high biological value of some seed proteins to meet their demands for reproduction (Allen and Hume 1997; Valera et al. 2005). The energy and protein requirements of growing and reproducing animals are further discussed in Chapter 10.

8.7 Summary: Nitrogen

Nitrogen is often the most limiting nutrient during growth and reproduction because nitrogenous compounds (proteins, nucleic acids) are the basis for the structure and function of animals. Proteins consist of 20 common amino acids, of which approximately half cannot be synthesized by the animal and so must be supplied by the diet or by microbial synthesis in the digestive tract. The quality of food protein is therefore determined by its amino acid composition; high-quality food proteins (high

biological value) closely match those synthesized by the animal. Amino acids are deaminated before the carbon is used for energy or glucose production. The N released from amino acid catabolism is potentially toxic. Ammonium is highly toxic but easily excreted by fish into the surrounding water. Urea and uric acid are non-toxic forms of waste N that are produced by terrestrial animals. Herbivores that consume low-N diets can recycle waste N by degrading urea or uric acid with microbes in the digestive tract. Nitrogen recycling minimizes the loss of fecal and urinary N, which reduces the requirement for N.